4 Individual Comments and Responses

This Page Intentionally Left Blank

I-Dic

Comment from Pat Dickens

I would like to register a comment not to use chemicals in West Marin. We have so many organic gardens and farms here. 1

Pat Dickens
Bolinas

Comment Letter I-Dic — Dickens, Pat

Response 1

For many years, the District has cooperated closely with organic growers and businesses throughout the Service Area. As a result, no adverse consequences to organic agriculture have been reported. As part of the Proposed Program, the District will continue its consultation and cooperation with organic growers and businesses. Vector control materials certified and labeled for organic use will be used as appropriate in conjunction with organic operations and accompanying organic operation plans.

The District uses several biorational formulations of mosquito larvicides that contain three bacterial active ingredients that are found in nature. Certain formulations containing these active ingredients are labeled for use with organic crops by the Organic Materials Review Institute and the USEPA. Examples of bacteria pathogenic to mosquitoes are *Bacillus sphaericus* (Bs), the several strains of *Bacillus thuringiensis israelensis* (Bti), and *Saccharopolyspora spinosa*. Two bacteria, Bs and Bti, produce proteins that are toxic to most mosquito larvae, while *Saccharopolyspora spinosa* produces compounds known as spinosysns, which effectively control all larval mosquitoes. Bs can reproduce in natural settings for some time following release. The Bti materials the District applies do not contain live organisms but only spores made up of specific protein molecules. All three bacteria are naturally occurring soil organisms that are also commercially produced for use as mosquito larvicides. These are the only three active ingredients approved for use in controlling larval mosquitoes when organic production is in progress.

One adult mosquito control product used by the District (Merus 2.0) is approved (by OMRI and the USDA National Organics Program) for use in conjunction with organic operations.

With respect to the issue raised by the commenter about chemical treatments for vector control in West Marin, the District operates under the terms of an agreement with a group known as the West Marin Mosquito Council (WMMC). The most recent version of the Agreement was approved by the District and WMMC in May 2016. The Agreement recognizes that the District may apply all the bacterial products discussed above, plus certain non-organic products, namely Agnique MMF, S-methoprene, and mosquito larvicide oils such as CoCoBear.

As discussed initially in the PEIR Program Description (Section 2.3.5.1.1), Agnique MMF is a very thinly applied (monomolecular) film product that acts as a larvicide and pupicide. Similarly, mosquito larvicide oils such as CoCoBear are applied as larvicides and pupicides where needed and appropriate. The WMMC Agreement also permits the use of s-methoprene, a mosquito growth regulator discussed in the PEIR Section 2.3.5.1.1. In the geographical region referred to by the commenter (West Marin), s-methoprene is applied only when necessary to Onsite Wastewater Treatment Systems (OWTS), also known as septic systems, to control larval mosquitoes. Under the District's policies and practices, Agnique, s-methoprene and larvicide oils are always used judiciously and in such a manner as not to interfere in any way with organic agriculture or production.

I-Fra1

Comment 1 from Mary Fraser

Dear District Board of Directors and Staff,
Please choose the No Chemical option from your draft PEIR. Pesticides drift, so you cannot guarantee anyone that you will not affect them. Pesticides get into our air and our water. I am attaching a speech that I recently wrote about pesticides, drift and their threat to the $45 million dollar agricultural market in Marin. 1

My name is Mary Fraser and I am a resident of Marin County. I am here today to ask you to ban the use of pesticides on Marin County Open Spaces and Parks. I am asking Parks and Open Space to become organic.

I want to share this graphic with you. For those of you who can't read it, it says: It's Raining Roundup. With a picture of a little girl playing in the rain. Below it, it says Monsanto's Roundup in 75% of Air and Rain Samples. This graphic has been created from a study done by the US Geological Survey. The following quotes are from their study.

"Pesticides can become airborne through volatilization and wind erosion of particles both during and after their application. Volatilization from treated areas is a continuous process and can be a major dissipative route for many pesticides. Airborne pesticides can be carried by wind and deposited in unintended areas by dry (gas and particle) and wet (rain and snow) deposition." This is what is commonly known as drift. 2

Marin County has organic agriculture that is worth $46 million dollars per yr. $46 million dollars per yr. There are 66 registered organic operations. The drift from the use of pesticides on Open Space and Parks may jeopardize that $46 million dollars or it may drift into more populated areas and contribute to health problems. No one knows where it will go. We just know that pesticides drift.

Coincidentally, I also just happened to sign up for the Marin Organic newsletter. I want to read you the reply I received.

Hi Mary,

I just wanted to send a quick note to thank you for signing up. 3

When the founders of Marin Organic first met in the mid-1990's they envisioned a county free of herbicides and pesticides and hoped that organic would become the new norm. Well, today we're proud to say that the majority of productive farmland in Marin County is certified

I-Fra1

organic. We're still here carrying on that mission, and part of that is educating folks through our newsletters.

Jeffrey Westman-Executive Director Marin Organic

Please join Marin Organics vision of Marin County as a place free of herbicides and pesticides.

Attached are documents regarding the USGS studies that I referenced above.

Mary Fraser

[redacted]

[redacted]

3

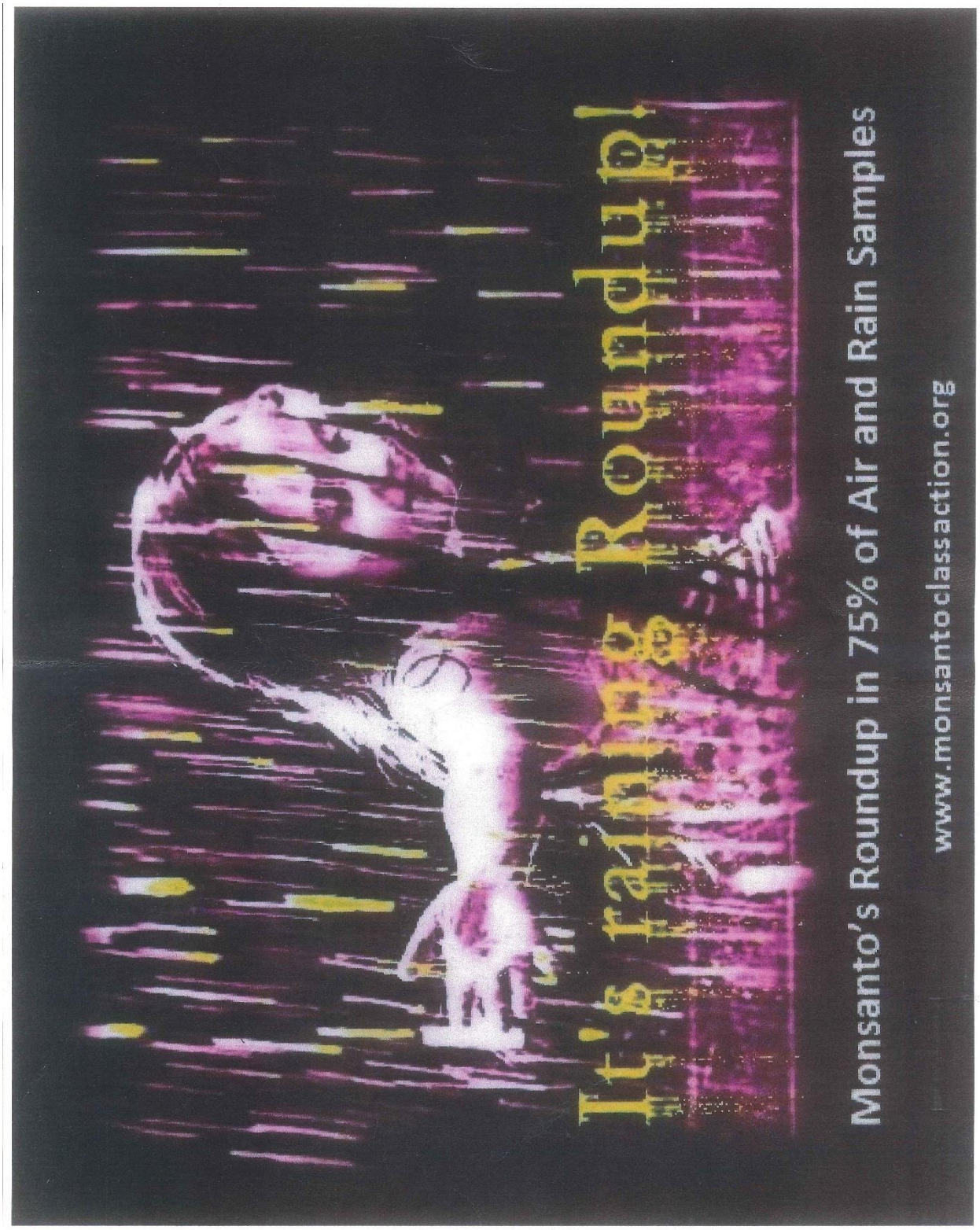
http://www.usgs.gov/newsroom/article.asp?ID=2909#.VfnIlNVViko

1. http://www.greenmedinfo.com/blog/roundup-weedkiller-found-75-air-and-rain-samples-gov-study-finds

   http://www.ncbi.nlm.nih.gov/pubmed/24549493

   http://www.ncbi.nlm.nih.gov/pubmed/18453431

   http://www.ncbi.nlm.nih.gov/pubmed/21128261


It's raining Roundup!
Monsanto's Roundup in 75% of Air and Rain Samples
www.monsantoclassaction.org

Comment Letter I-Fra1 Fraser, Mary

Response 1

The District minimizes the potential for drift from larvicide and adulticide applications by following the best management practices listed below in addition to meeting all label requirements:

> Postpone or cease application when predetermined weather parameters exceed product label specifications, when wind speeds exceed the velocity as stated on the product label, or when a high chance of rain is predicted and rain is a determining factor on the label of the material to be applied. (H6)

> Applicators will remain aware of wind conditions prior to and during application events to minimize any possible unwanted drift to waterbodies, and other areas adjacent to the application areas. (H7)

> Spray nozzles will be adjusted to produce larger droplet size rather than smaller droplet size. Use low nozzle pressures where possible (e.g., 30 to 70 pounds per square inch). Keep spray nozzles within a predetermined maximum distance of target weeds (e.g., within 24 inches of vegetation for hand application) or vectors. Adjusting droplet size would only apply to larvicides, herbicides, and non-ULV applications. Use ULV applications that are calibrated to be effective and environmentally compatible at the proper droplet size (about 10-30 microns). (H8)

The No Chemical Program was reviewed in Section 15.4.2 of the Draft PEIR. This comment will be considered by the District's Board of Trustees in its consideration whether to approve the Program as proposed, or with modifications.

Response 2

The District uses several formulations of mosquito larvicides which contain three bacterial active ingredients that are found in nature. Certain formulations containing these active ingredients are labeled for use with organic crops by the Organic Materials Review Institute and the USEPA. Examples of bacteria pathogenic to mosquitoes are *Bacillus sphaericus* (Bs), the several strains of *Bacillus thuringiensis israelensis* (Bti), and *Saccharopolyspora spinosa*. Two bacteria, Bs and Bti, produce proteins that are toxic to most mosquito larvae, while *Saccharopolyspora spinosa* produces compounds known as spinosyns, which effectively control all larval mosquitoes. Bs can reproduce in natural settings for some time following release. Bti materials applied by the District do not contain live organisms, but only spores made up of specific protein molecules. All three bacteria are naturally occurring soil organisms that are commercially produced for use as mosquito larvicides. These are the only three active ingredients referenced as being acceptable for use in mosquito habitat present on organic farms. Therefore, the District's use of these materials does not threaten the organic farming industry.

Concerning drift, see Response 1 above. There is potential for some infrequent applications of adulticides to impact residents and/or recreationists with objectionable odors. The materials have been used in the current Program, and people have not complained about odors. However, it is possible that complaints could occur in the future. As discussed in Section 10.2.7 (page 10-32):

> **Impact AQ-25:** The Chemical Control Alternative could subject people to objectionable odors. Impacts could be **potentially significant but mitigable**, even with BMPs implemented.

To mitigate Impact AQ-25, the District and its contractors may implement any of the following measures as applicable to the specific application situation to reduce drift towards human populations/residences from the ground and aerial applications of odorous treatment compounds:

Mitigation Measure AQ-25a: Whenever possible and practicable, defer application of treatment compounds until such time that favorable wind conditions would reduce or avoid the risk of drift into populated areas.

Mitigation Measure AQ-25b: Utilize equipment such as wind meters and global positioning system (GPS) tracking devices when applicable that assist in documenting site-specific compliance with all label requirements for drift mitigation.

Mitigation Measure AQ-25c: Use precision application technology to reduce drift and the total amount of material applied. This measure can include (1) Precision guidance systems that minimize ground or aerial spray overlap (e.g., GPS and Real Time Kinetics – GPS/RTK) and (2) Computer-guided application systems that integrate real-time meteorological data and computer model guidance to reduce drift from aerial application (e.g., trade names "AIMMS," "Wingman™ GX," and "NextStar™ Flow Control").

Use of any one of these measures would reduce the impact to **less than significant**.

The impact to human health is less than significant as explained in Section 7.2.5 for herbicides and Section 7.2.7 for the other chemical control options. For more information on glyphosate, see our responses to your comments I-Fra3.

Response 3

Comment noted and considered. No response is necessary, as the letter was provided for informational purposes and does not address the PEIR.

Concerning the hyperlinks to material referenced as being from USGS, the following responses are provided.

> http://www.usgs.gov/newsroom/article.asp?ID=2909#.VfnIlNVViko (USGS 2016)

This is a link to the primary USGS website that contains hundreds of links to research and other reports addressing numerous issues in health and environmental contamination. Although there are several reports listed that address glyphosate, they are generally addressing the same issues as the reports used in the PEIR including Appendix B. In the absence of a list of specific entries and links in the website, we can neither feasibly nor practically address or comment on any except for the specific ones where we have links provided by the commenter that are listed below. These studies appear to be focused on chemicals associated with agricultural uses and are not applicable to vector control.

> Sayer, Ji. 2014. Roundup Weedkiller Found In 75% of Air and Rain Samples, Gov. Study Finds. http://www.greenmedinfo.com/blog/roundup-weedkiller-found-75-air-and-rain-samples-gov- study-finds.

This represents an overview of the monitoring studies evaluating the chemicals detected in rain during several annual periods in Mississippi between 1995 and 2007 associated with agricultural pesticide spraying. Although there are numerous detected chemicals in the collected rainwater, the reports (this one and the two others by the same authors that focus on glyphosate) indicate the detections are very low (less than about 7 µgm per cubic meter, as an example). Using this estimated air concentration, the authors estimate that an individual breathing this level of chemical continuously might be subjected to about 27 billionths of a gram over a 24 hour period. The bulk of the summaries suggest substantial differences in chemicals detected between and among years of the study. They report that certain pesticides, but not always the same ones, seem to be dominate in the samples with detected chemicals present. It appears that these traces of chemicals were associated with specific agricultural practices. This is a summary of the studies conducted to evaluate the concentration of detectible chemicals in rain and air associated with agricultural pesticide spray events. The following report also addresses this monitoring technique.

> Majewski M.S. R.H. Coupe, W.T. Foreman, and P.D. Capel. 2014. Pesticides in Mississippi air and rain: a comparison between 1995 and 2007. *Environ Toxicol Chem.* Jun;33(6):1283-93. Available online at http://www.ncbi.nlm.nih.gov/pubmed/24549493.

  This is another publication using the same data as those in the prior citation. The assumptions and summaries are similar. It documents chemicals in collected in rain and attributes them to agricultural uses.

> Vogel, J.R., M.S. Majewski, and P.D. Capel. 2008. Pesticides in rain in four agricultural watersheds in the United States. *J Environ Qual.* May 2;37(3):1101-15. Available online at http://www.ncbi.nlm.nih.gov/pubmed/18453431.

  This is yet another publication using some of the same data as those in the prior citation but extending the region to more states. The assumptions and summaries are similar. It documents chemicals collected in rain and attributes them to agricultural uses.

> Chang, F.C., M.F. Simcik, and P.D. Capel. 2011. Occurrence and fate of the herbicide glyphosate and its degradate aminomethylphosphonic acid in the atmosphere. *Environ Toxicol Chem.* Mar;30; (3):548-55. Available online at http://www.ncbi.nlm.nih.gov/pubmed/21128261.

  This is another report on the ambient levels of glyphosate in air and rain. Air and rain samples were collected during two growing seasons in agricultural areas in Mississippi and Iowa. The frequency of glyphosate detection ranged from 60 to 100 percent in both air and rain. The concentrations of glyphosate ranged from <0.01 to 9.1 ng/cubic meter and from <0.1 to 2.5 µg/L in air and rain samples, respectively. The frequency of detection and median and maximum concentrations of glyphosate in air were similar or greater to those of the other high-use herbicides observed in the Mississippi River basin. It was estimated that up to 0.7 percent of the application is removed from the air in rainfall, although there is no metric to determine the initial percentage released. The authors indicated that glyphosate is efficiently removed from the air; it is estimated that an average of 97 percent of the glyphosate in the air is removed by a weekly rainfall ≥ 30 mm. The assumptions and summaries are similar. It documents chemicals in collected rain and attributes it to agricultural uses.

This information in the cited reports is not relevant to the PEIR's analysis or impact determinations because it does not address the specific methods of chemical use by the District in its Service Area, and the commenter does not explain its relevance to any specific analysis or determination in the PEIR.

Additional References

Chang, F-C., M.F. Simcik, and P.D. Capel. 2011. Occurrence and fate of the herbicide glyphosate and its degradate aminomethylphosphonic acid in the atmosphere. *Environ Toxicol Chem.* 30; (3):548-55.

Ji, Sayer. 2014. Founder, GreenMedInfo. Roundup Weedkiller Found In 75% of Air and Rain Samples, Gov. Study Finds. Available online at http://www.greenmedinfo.com/blog/roundup-weedkiller-found-75-air-and-rain-samples-gov-study-finds.

Majewski M.S., R.H. Coupe, W.T. Foreman, and P.D. Capel. 2014. Pesticides in Mississippi air and rain: a comparison between 1995 and 2007. *Environ Toxicol Chem.* Jun;33(6):1283-93. Available online at http://www.ncbi.nlm. nih.gov/pubmed/24549493.

US Geological Survey (USGS) 2016. Science News. Available online at http://www.usgs.gov/newsroom/article.asp?ID=2909#.VfnIlNVViko.

Vogel, J.R., M.S. Majewski, and P.D. Capel. 2008. Pesticides in rain in four agricultural watersheds in the United States. *J Environ Qual.* May 2;37(3):1101-15. Available online at http://www.ncbi.nlm.nih.gov/pubmed/18453431.

I-Fra2

Comment 2 from Mary Fraser

I want the Directors of the District to approve the No Chemicals option in the Draft PEIR. Here is a copy of a speech I did on why pesticides should be banned along with its references. Please use this as one of my comments. 1

WHY PESTICIDES SHOULD BE BANNED.

9/16/15

Thank you Mr. President, Fellow Toastmasters and Welcome Guests,

I want to share this graphic with you. For those of you who can't read it, it says: It's Raining Roundup. With a picture of a little girl playing in the rain. Underneath it says: Monsanto's Roundup found in 75% of air and rain samples. (1) I find this very disturbing. I would think you would find it disturbing too. Pesticides raining on our heads.

I am the mother of three children and have had the unfortunate experience of having a child in the intensive care unit for 35 days. Since I never, ever want to have that experience again, I've educated myself on health hazards. Today I want to share my findings about pesticides with you. I come from a large family of attorneys, so all of my facts in this speech have been verified. I have a copy of this speech available with references to all the facts that I quote.

This graphic was created from data found in official gov't studies. The United States Geographical Survey has done testing in several states and this is what they have consistently found. 75% of the air and rain samples had Roundup in it. (2)

Let me give you a little background on pesticides.

The word Pesticide means death to pests. It is an umbrella term. Under the umbrella, we have herbicides or death to plants, insecticides or death to animals and fungicides or death to fungi. Many people think that a more apt term for pesticide is ecocide. Death to the environment.

Now imagine that this jar is a can of pesticide. Inside this jar, there is the main ingredient or what is technically called the 'active principle'. This is the chemical that the EPA requires manufacturer's to do testing on. The manufacturer's do the testing. The EPA only reviews the tests. Along with the active principle, there are inerts and/or adjuvants added to the formula. Under EPA rules, the inerts and adjuvants do not need to be tested. They do not need to be

2

I-Fra2

disclosed to the public as they can be considered to be trade secrets or proprietary business information. So what you have, is a can of chemicals of completely unknown toxicity, untested and undisclosed. (3) Now many of you have gone to your doctor and when he wants to write you a prescription, he always asks about other drugs you are taking. He doesn't want any unforeseen chemical reactions. I guess the EPA didn't get that memo. This is the dirty little secret of pesticides and one of the dirty little secrets about the EPA. Now, after the manufacturer has created this pesticide of unknown toxicity, they are often patented. Once patented, no one can do research on the formula without the patent holders permission. Fat chance.

Let me go back to the graphic. " It's Raining Roundup." The active principle or main ingredient in Roundup is glyphosate. The World Health Organization has just declared that glyphosate causes cancer in animals. (4) They declared it to be a 'probable human carcinogen' only because they could not find enough enough scientific studies on humans and glyphosate. It is unethical to try to induce cancer in humans and the few studies they had just didn't have enough data.

The patent has run out on Roundup and a group of independent scientists did studies on it. What they found was that Roundup was 1000x more toxic than its main ingredient glyphosate. 1000x time more toxic. (5) Roundup is the most widely used pesticide in this country. We use millions of pounds of it per year. (6) It's what genetically modified crops like corn, soy , canola, sugar beets, alfalfa and cotton are designed to withstand heavy doses of. (7) 80% of all processed foods contain GMO ingredients. (8) It's allowed on 160 different food crops in this country. (9) Farmers spray it on many crops right before harvest to eliminate weeds and dry out the crops. Right before harvest. So many of us are now gluten intolerant. Well, Roundup is oftentimes sprayed on wheat right before harvest. You're not gluten intolerant. You're being poisoned. (9) And no one tests for residues of Roundup on our food. That's another dirty little secret about the EPA. (10) We test for other pesticides but not Roundup.

2

So what are the public health consequences of all this toxicity? According to the American Cancer Society, the official rate of cancer in 2015 is now 1 in 2. That's for men. 1 in 2. For women its 1 in 3. (11) And that is just cancer. Roundup has been linked to 32 other chronic diseases (12) and has been found in breast milk, urine (13) and now it's raining Roundup.

So what can you do? If you have Roundup at home, carefully take it to the hazardous waste facility. Eat only organic food and you will avoid about 80% of pesticides. This is particularly important for children as they eat more per lb. of body weight than adults do and their bodies do not have the same capacity to detox pesticides as adults do. (14)

I-Fra2

And exercise your democratic rights. The Marin Co Board of Supervisors has the issue of banning pesticides on its agenda on Oct. 6th. Let them know what you think. The CA EPA has opened comments about adding Roundup to its list of Prop. 65 chemicals and our Mosquito abatement program is proposing to use Roundup. I have notices on all of these actions and I have information on eating organic food .

Let me close with another graphic. This is a quote from Dr. Don Huber, a professor emeritus of Plant Pathology, Purdue University.

"Future generations will judge us- not on how many millions of tons of pesticides we sprayed, but on how willing we were- how perfectly willing we were, to sacrifice our children". (16)

2

Thank you. You have my permission to share this speech with anyone or to contact me if you wish a copy of this speech in electronic form.

Mary Fraser

[redacted]

References:

1. https://www.facebook.com/pages/Monsanto-Class-Action/992106394134272?sk=photos_stream

   Monsanto class action lawsuit Facebook photos

2. http://www.usgs.gov/newsroom/article.asp?ID=2909#.VfnIlNVViko

   http://www.greenmedinfo.com/blog/roundup-weedkiller-found-75-air-and-rain-samples-gov-study-finds

   http://www.ncbi.nlm.nih.gov/pubmed/24549493

   http://www.ncbi.nlm.nih.gov/pubmed/18453431

   http://www.ncbi.nlm.nih.gov/pubmed/21128261

3. **Poison Spring: The Secret History of Pollution and the EPA Hardcover – April 8, 2014**

I-Fra2

by E.G. Vallianatos (Author), McKay Jenkins (Author)

1. International Agency for Research on Cancer, **IARC Monographs Volume 112: evaluation of five organophosphate insecticides and herbicides.**

2. Hindawi Publishing Corporation

   BioMed Research International

   Volume 2014, Article ID 179691, 8 pages

Major Pesticides Are More Toxic to Human Cells Than Their Declared Active Principles

RobinMesnage,[1] Nicolas Defarge,[1] Joël Spiroux de Vendômois,[2] and Gilles-Eric Séralini[1]
http://dx.doi.org/10.1155/2014/179691

https://d3n8a8pro7vhmx.cloudfront.net/yesmaam/pages/680/attachments/original/1407922431/2012._Mesnage_et_al._Ethoxylated_adjuvants_of_glyphosate-based_herbicides_are_active_principles_of_human_cell_toxicity.pdf?1407922431

Ethoxylated adjuvants of glyphosate based herbicides are active principles of human cell toxicity.

1. **http://www.sciencedirect.com/science/article/pii/S027869151530034X**

Potential toxic effects of glyphosate and its commercial formulations below regulatory limits

7. https://d3n8a8pro7vhmx.cloudfront.net/ncap/pages/26/attachments/original/1428423381/glyphosate.pdf?1428423381 **JOURNAL OF PESTICIDE REFORM/ WINTER 2004 • VOL. 24, NO. 4 HERBICIDE FACTSHEET**

I-Fra2

1. Ibid

2. http://d3n8a8pro7vhmx.cloudfront.net/yesmaam/pages/680/attachments/original/1393210109/Glyphosate_II_Samsel-Seneff_Toxicology_FNL.pdf?1393210109

   Glyphosate, pathways to modern diseases II: Celiac sprue and gluten intolerance

   Anthony SAMSEL 1 and Stephanie SENEFF 2 1 Independent Scientist and Consultant, Deerfield, NH 03037, USA 2 Computer Science and Artificial Intelligence Laboratory, MIT, Cambridge, MA, USA ITX060413R01 • Received: 24 September 2013 • Revised: 10 November 2013 • Accepted: 12 November 2013

3. http://www.ecfr.gov/cgi-bin/text-idx?SID=195f8b224b4d7d5ab8cfff3bf0f92f68&node=se40.24.180_1364&rgn=div8

   Electronic Code of Federal Regulations 180.364 Glyphosate, tolerances for residues.

4. www.consumerreports.org/cro/news/2015/03/glyphosate-in-your-diet/index.htm

   Consumer Reports: Is there glyphosate in your diet? No one knows how much of this pesticide is in the produce we eat.

5. **American Cancer Society publication: Cancer Facts & Figures 2015, page 14.**

   http://www.cancer.org/acs/groups/content/@editorial/documents/document/acspc-044552.pdf

6. **http://people.csail.mit.edu/seneff/Swanson_et_al_2014.pdf**

   Journal of Organic Systems, 9(2), 2014 ORIGINAL PAPER

   Genetically engineered crops, glyphosate and the deterioration of health in the United States of America

   Nancy L. Swanson1 , Andre Leu2 *, Jon Abrahamson3 and Bradley Wallet4

I-Fra2

http://www.examiner.com/article/data-trends-show-correlation-between-increase-organ-disease-and-gmos

7. **Glyphosate Testing Report: Findings in American Mothers' Breast Milk, Urine and Water.**

   Conducted by Moms Across America and Sustainable Pulse

   April 7, 2014.

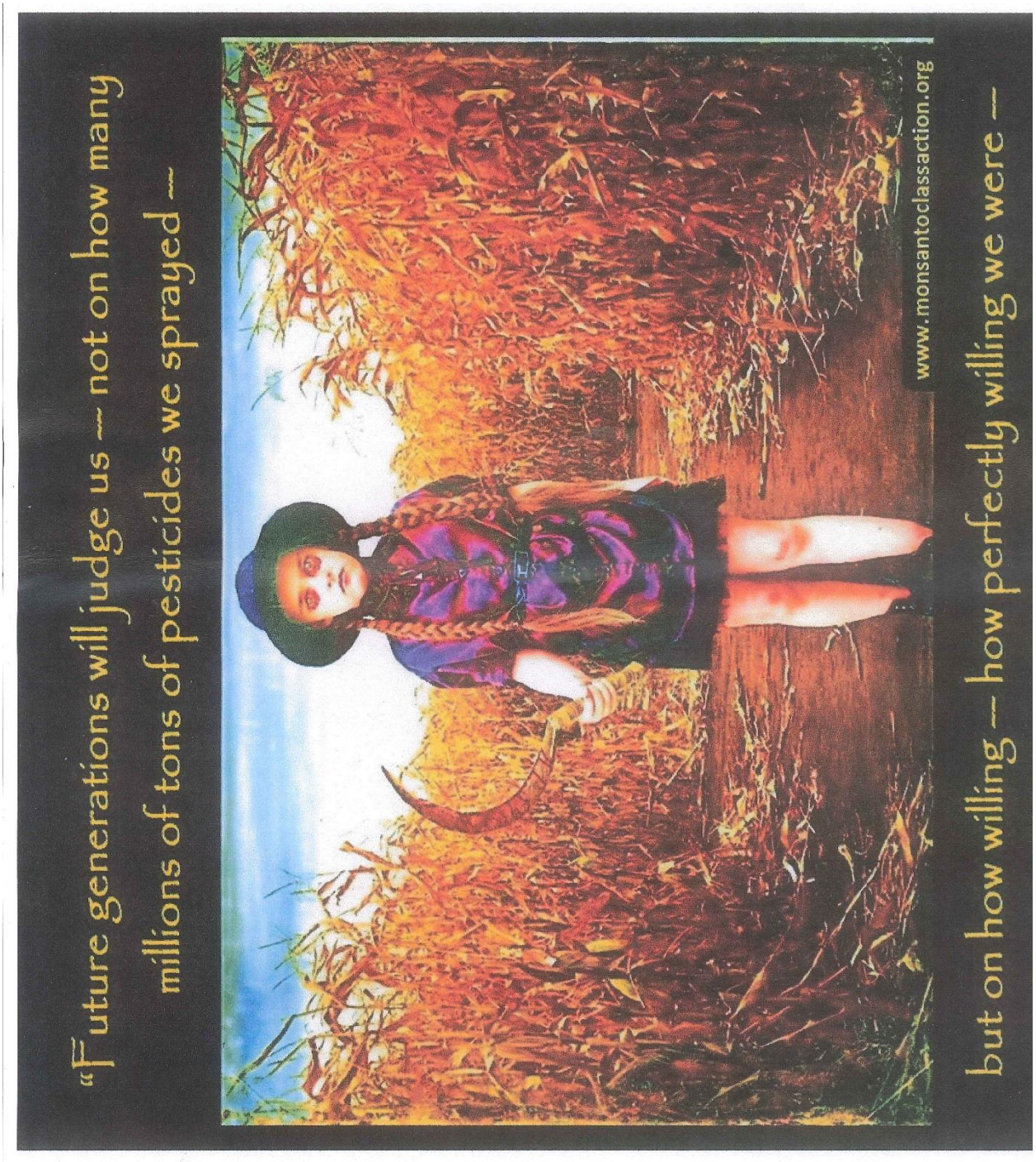
   Zen Honeycutt, Moms Across America, Henry Rowlands, Sustainable Pulse

8. Pesticide Action Network North America, **"A Generation in Jeopardy, How pesticides are undermining our children's health & intelligence".**
   http://www.panna.org/publication/generation-in-jeopardy

Sincerely,

Mary Fraser

[redacted]


"Future generations will judge us — not on how many
millions of tons of pesticides we sprayed —
but on how willing — how perfectly willing we were —
www.monsantoclassaction.org

Comment Letter I-Fra2 — Fraser, Mary

Response 1

The No Chemical Program was reviewed in Section 15.4.2 of the Draft PEIR. This comment will be considered by the District's Board of Trustees in its consideration whether to approve the Program as proposed, or with modifications.

Response 2

The copy provided of Ms. Fraser's speech to Toastmasters shows that is about why she feels that pesticides should be banned. It does not address the District's PEIR. Comments are noted and considered, and no further analysis of references for a speech to others is required. The commenter does not explain how the references on the general subject of glyphosate relate to the PEIR analysis.

See Responses I-Fra3-3 through I-Fra3-6 on the subject of glyphosate. Responses to Ms. Fraser's subsequent written comments cover the issues she has raised and the studies she has provided as attachments or as links to websites and are not duplicated herein.

I-Fra3

Comment 3 from Mary Fraser

Dear Marin Sonoma Mosquito and Vector Control,
I am writing to inform you of my concerns about your Draft PEIR. My concerns are many and I will be sending you multiple emails.
1. You have given the public inadequate time and notice of these hearings and their content. You published a 'legal notice' only on August 28th and expect the public to be able to read and prepare comments no later than Oct. 2, 2015 on a document that is over 500 pages. You made the document available in libraries in CD form. Most libraries have reservation systems in order to use a computer, time is limited to one hour per reservation and to print out any pages is a ten cent fee per page. All of this combined makes for great difficulty in reading and analyzing your Draft PEIR. I am requesting that you extend the official notification period to at least 120 days, that you take out 1/4 page 'display' ads in newspapers and that you send notifications of the Draft PEIR to multiple NGO's and non-profits. I also want you to supply copies of the Draft PEIR to interested individuals at no charge. I have not had adequate time to analyze your entire PEIR. 1

2. I attended your Public Hearing on Sept. 12, 2015 and watched a power point presentation that was misleading. The presentation stated that you use chemicals approved for organic food production and that you consulted beekeepers. During a very brief review of the Draft PEIR in my one hour computer time allotment at the public library, I found that you propose to use glyphosate. That is a chemical pesticide that is not allowed in organic food production. The World Health Organization thru its research arm, The International Agency for Research on Cancer has declared glyphosate to be carcinogenic to animals, giving glyphosate a 1 rating for animals and a 2A rating for humans. 2

In reviewing the Draft PEIR I noted that the information on glyphosate is inaccurate in the following ways: a. "Section 4.6.2.3 Human Toxicity. The shikimic acid pathway is specific to plants and some microorganisms; therefore, glyphosate is thought to have very low toxicity to mammals (USEPA 1993)" This is old information. Dr. Stephanie Seneff, MIT and Anthony Samsel have found that the shikimic pathway is present in bacteria in the human gut. Here is their peer reviewed, published paper.
http://people.csail.mit.edu/seneff/Entropy/entropy-15-01416.pdf
Dr. Seneff and Anthony Samsel have published numerous other articles about glyphosate. Here are other articles:
http://people.csail.mit.edu/seneff/ITX_2013_06_04_Seneff.pdf
http://surgicalneurologyint.com/surgicalint_articles/glyphosate-pathways-to-modern-diseases-iii-manganese-neurological-diseases-and-associated-pathologies/ 3

I-Fra3

http://people.csail.mit.edu/seneff/Hoy_wildlife_2015.pdf
http://people.csail.mit.edu/seneff/glyphosate/Chen_I_wan_Reference_info_glyphosate_June18_2014.pdf

3

Glyphosate is rarely used alone. It is almost always used in combination with inerts and adjuvants. A team of independent scientists did studies on the complete formulation of Roundup, which is the most widely used pesticide that has glyphosate as its main ingredient. The testing found that the complete formulations had toxicity levels that were up to 1000x more toxic. Here are two of the studies:

Major Pesticides Are More Toxic to Human Cells Than Their Declared Active Principles

RobinMesnage,[1] Nicolas Defarge,[1] Joël Spiroux de Vendômois,[2] and Gilles-Eric Séralini[1]
http://dx.doi.org/10.1155/2014/179691

https://d3n8a8pro7vhmx.cloudfront.net/yesmaam/pages/680/attachments/original/1407922431/2012._Mesnage_et_al._Ethoxylated_adjuvants_of_glyphosate-based_herbicides_are_active_principles_of_human_cell_toxicity.pdf?1407922431

Ethoxylated adjuvants of glyphosate based herbicides are active principles of human cell toxicity.

4

In Section 4.6.2.3 it is stated " There is currently no published scientific. evidence indicating that glyphosate is carcinogenic or mutagenic". I am attaching a copy of the WHO report that disputes this and that labels glyphosate as carcinogenic to animals and a probable human carcinogen. I don't understand why this report has not been included in the Draft PEIR. The initial release was in March 2015 and the entire monograph was released in July 2015. There have been numerous media reports about the WHO finding!
In Section 4.6.2.1 there is a table of the degradation of glyphosate. Monsanto, the manufacturer of glyphosate has been sued by both the State of New York and the county of France over falsely advertising that their product, Roundup, (which has glyphosate as its active principle) is biodegradable. The county of France won their case, even though it was appealed to the highest court in France and the State of New York settled the case with Monsanto. The degradation of glyphosate is dependent on the chemical nature of the environment it is in. In some cases it can take up to 20 years or more to biodegrade.

5

All of my referenced attachments should be included in my comments.

6

Please do not use glyphosate. I want the Directors of the District to approve the No Chemical option that is proposed in your Draft PEIR.
Mary Fraser
[redacted]

7

Entropy **2013**, *15*, 1416-1463; doi:10.3390/e15041416

OPEN ACCESS

entropy
ISSN 1099-4300
www.mdpi.com/journal/entropy

Review

Glyphosate's Suppression of Cytochrome P450 Enzymes and Amino Acid Biosynthesis by the Gut Microbiome: Pathways to Modern Diseases

Anthony Samsel [1] and Stephanie Seneff [2,*]

[1] Independent Scientist and Consultant, Deerfield, NH 03037, USA;
E-Mail: anthonysamsel@acoustictracks.net
[2] Computer Science and Artificial Intelligence Laboratory, MIT, Cambridge, MA 02139, USA

***** Author to whom correspondence should be addressed; E-Mail: Seneff@csail.mit.edu;
Tel.: +1-617-253-0451; Fax: +1-617-258-8642.

Received: 15 January 2013; in revised form: 10 April 2013 / Accepted: 10 April 2013 / Published: 18 April 2013

Abstract: Glyphosate, the active ingredient in Roundup®, is the most popular herbicide used worldwide. The industry asserts it is minimally toxic to humans, but here we argue otherwise. Residues are found in the main foods of the Western diet, comprised primarily of sugar, corn, soy and wheat. Glyphosate's inhibition of cytochrome P450 (CYP) enzymes is an overlooked component of its toxicity to mammals. CYP enzymes play crucial roles in biology, one of which is to detoxify xenobiotics. Thus, glyphosate enhances the damaging effects of other food borne chemical residues and environmental toxins. Negative impact on the body is insidious and manifests slowly over time as inflammation damages cellular systems throughout the body. Here, we show how interference with CYP enzymes acts synergistically with disruption of the biosynthesis of aromatic amino acids by gut bacteria, as well as impairment in serum sulfate transport. Consequences are most of the diseases and conditions associated with a Western diet, which include gastrointestinal disorders, obesity, diabetes, heart disease, depression, autism, infertility, cancer and Alzheimer's disease. We explain the documented effects of glyphosate and its ability to induce disease, and we show that glyphosate is the "textbook example" of exogenous semiotic entropy: the disruption of homeostasis by environmental toxins.

Keywords: glyphosate; cytochrome P450; eNOS; obesity; cardiovascular disease; cancer; colitis; shikimate pathway; gut microbiome; tryptophan; tyrosine; phenylalanine; methionine; serotonin; Alzheimer's disease; Parkinson's disease; autism; depression

PACS Codes: 87.19.xj; 87.19.xr; 87.19.xv; 87.19.xw; 87.19.xb; 87.19.xp

1. Introduction

The foodstuffs of the Western diet, primarily grown by industrial agriculture, are increasingly being produced using a two-part system of engineered plant seeds and toxic chemical application. Novel bacterial genes are incorporated through genetic engineering, and toxic chemical residues are readily taken up by the engineered plants. Research indicates that the new bacterial RNA and DNA present in genetically engineered plants, providing chemical herbicide resistance and other traits, have not yet fully understood biological effects. This paper however, will only examine the effects of the chemical glyphosate, the most popular herbicide on the planet.

Glyphosate (N-phosphonomethylglycine), the active ingredient in the herbicide Roundup®, is the main herbicide in use today in the United States, and increasingly throughout the World, in agriculture and in lawn maintenance, especially now that the patent has expired. 80% of genetically modified crops, particularly corn, soy, canola, cotton, sugar beets and most recently alfalfa, are specifically targeted towards the introduction of genes resistant to glyphosate, the so-called "Roundup Ready® feature" In humans, only small amounts (~2%) of ingested glyphosate are metabolized to aminomethylphosphonic acid (AMPA), and the rest enters the blood stream and is eventually eliminated through the urine [1]. Studies have shown sharp increases in glyphosate contamination in streams in the Midwestern United States following the mid 1990s, pointing to its increasing role as the herbicide of choice in agriculture [2]. A now common practice of crop desiccation through herbicide administration shortly before the harvest assures an increased glyphosate presence in food sources as well [3–5]. The industry asserts that glyphosate is nearly nontoxic to mammals [6,7], and therefore it is not a problem if glyphosate is ingested in food sources. Acutely, it is claimed to be less toxic than aspirin [1,6]. As a consequence, measurement of its presence in food is practically nonexistent. A vocal minority of experts believes that glyphosate may instead be much more toxic than is claimed, although the effects are only apparent after a considerable time lapse. Thus, while short-term studies in rodents have shown no apparent toxicity [8], studies involving life-long exposure in rodents have demonstrated liver and kidney dysfunction and a greatly increased risk of cancer, with shortened lifespan [9].

Glyphosate's claimed mechanism of action in plants is the disruption of the shikimate pathway, which is involved with the synthesis of the essential aromatic amino acids, phenylalanine, tyrosine, and tryptophan [10]. The currently accepted dogma is that glyphosate is not harmful to humans or to any mammals because the shikimate pathway is absent in all animals. However, this pathway *is* present in gut bacteria, which play an important and heretofore largely overlooked role in human physiology [11–14] through an integrated biosemiotic relationship with the human host. In addition to aiding digestion, the gut microbiota synthesize vitamins, detoxify xenobiotics, and participitate in immune system homeostasis and gastrointestinal tract permeability [14]. Furthermore, dietary factors modulate the microbial composition of the gut [15]. The incidence of inflammatory bowel diseases such as juvenile onset Crohn's disease has increased substantially in the last decade in Western Europe [16] and the

United States [17]. It is reasonable to suspect that glyphosate's impact on gut bacteria may be contributing to these diseases and conditions.

However, the fact that female rats are highly susceptible to mammary tumors following chronic exposure to glyphosate [9] suggests that there may be something else going on. Our systematic search of the literature has led us to the realization that many of the health problems that appear to be associated with a Western diet could be explained by biological disruptions that have already been attributed to glyphosate. These include digestive issues, obesity, autism, Alzheimer's disease, depression, Parkinson's disease, liver diseases, and cancer, among others. While many other environmental toxins obviously also contribute to these diseases and conditions, we believe that glyphosate may be the most significant environmental toxin, mainly because it is pervasive and it is often handled carelessly due to its perceived nontoxicity. In this paper, we will develop the argument that the recent alarming increase in all of these health issues can be traced back to a combination of gut dysbiosis, impaired sulfate transport, and suppression of the activity of the various members of the cytochrome P450 (CYP) family of enzymes. We have found clear evidence that glyphosate disrupts gut bacteria and suppresses the CYP enzyme class. The connection to sulfate transport is more indirect, but justifiable from basic principles of biophysics.

In the remainder of this paper, we will first provide evidence from the literature that explains some of the ways in which glyphosate adversely affects plants, microbes, amphibians and mammals. Section 3 will discuss the role that gut dysbiosis, arguably resulting from glyphosate exposure, plays in inflammatory bowel disease and its relationship to autism. Section 4 argues that the excess synthesis of phenolic compounds associated with glyphosate exposure represents a strategy to compensate for impairments in the transport of free sulfate. Section 5 will provide evidence that glyphosate inhibits CYP enzymes. Section 6 explains how obesity can arise from depletion of serum tryptophan due to its sequestering by macrophages responding to inflammation. Section 7 shows how extreme tryptophan depletion can lead to impaired nutrient absorption and anorexia nervosa. Section 8 provides a brief review of all the roles played by CYP enzymes in metabolism. Section 9 discusses a likely consequence to glyphosate's disruption of the CYP-analog enzyme, endothelial nitric oxide synthase (eNOS). Section 10 shows how glyphosate's effects could plausibly lead to brain-related disorders such as autism, dementia, depression, and Parkinson's disease. Section 11 mentions several other health factors that can potentially be linked to glyphosate, including reproductive issues and cancer. Section 12 discusses the available evidence that glyphosate is contaminating our food supplies, especially in recent years. Following a discussion section, we sum up our findings with a brief conclusion.

2. Glyphosate's Pathological Effects: Controlled Studies

It is well established that glyphosate, a member of the general class of organophosphates, inhibits the enzyme 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSP synthase), the rate-limiting step in the synthesis of aromatic amino acids in the shikimate pathway in plants [18]. This pathway, while not present in mammals, is present in algae, Archaea, bacteria, fungi, and prokaryotes, and unicellular eukaryotic organisms [19]. Indeed, corn and soy crops have both been shown to accumulate excess shikimate in response to glyphosate exposure [20]. However, a study comparing glyphosate-tolerant and glyphosate-sensitive carrot cell lines identified several pathologies beyond the inhibition of

aromatic amino acids following glyphosate exposure [21]. It was determined that, in addition to abnormally low levels of tryptophan, phenylalanine and tyrosine, the glyphosate-sensitive cells also had 50 to 65% reduced levels of serine, glycine and methionine. The reduction in methionine can have many adverse consequences, as methionine is an essential sulfur-containing amino acid that has to be supplied from the diet. In addition, there was evidence of excess ammonia in the glyphosate-sensitive but not the glyphosate-adapted cells. Both cell types readily absorbed glyphosate from the medium, with a rapid linear uptake observed during the first eight hours following exposure. This demonstrates that glyphosate would be present in food sources derived from glyphosate-exposed plants.

The excess ammonia observed in glyphosate-treated plants could be due to increased activity of phenylalanine ammonia lyase (PAL), an enzyme found in plants, animals, and microbes, that catalyzes the reaction that converts phenylalanine to trans-cinnamate, releasing ammonia [22]. In studies on transgenic tobacco, it was demonstrated that a decrease in the aromatic amino acid pool sizes (a direct consequence of glyphosate exposure) results in an enhancement of metabolic flux through the shikimate pathway, which leads to a rise in PAL activity as well as a doubling of the levels of chlorogenic acid, a polyphenolic compound related to cinnamate [23]. It has been proposed that glyphosate achieves part if not all of its growth-retardation effects on plants through induction of PAL activity [24]. The growth disruption could be due either to toxicity of the derived phenolic compounds [25] or to direct toxicity of the ammonia. A study of olive trees showed that there is a direct relationship between the total phenol concentration and PAL activity, suggesting that PAL is a major producer of phenolic compounds [26]. Glyphosate has been shown to increase PAL activity in both soybean seedlings [27] and in corn [28].

Under stress-inducing environments, the secondary metabolites derived from certain protein synthesis pathways become disproportionately important, and enzyme regulation induces dramatic shifts in the production of the amino acids *versus* the secondary metabolites. A study comparing glyphosate exposure with aromatic protein deprivation in plants found several effects in common, but there was a striking anomaly for glyphosate in that it caused a 20-fold increase in the synthesis of the rate-limiting enzyme for a pathway leading to flavonoid synthesis, as a side branch of the tryptophan synthesis pathway [29]. More generally, there is substantial evidence that glyphosate induces the synthesis of monophenolic compounds as well as the polyphenolic flavonoids, in both plants [30] and microbes [31], with concurrent depletion of aromatic amino acid supplies. When carrots are exposed to high doses of glyphosate, they produce significant amounts of various phenolic compounds as well as shikimic acid [32]. The significance of this will become apparent later on in Section 4 on sulfate transport. Elevated amounts of shikimate-derived benzoic acids such as protocatechuate and gallate are also found in plants exposed to glyphosate [29]. Strains of nitrogen-fixing bacteria in the soil produce hydroxybenzoic acids in the presence of glyphosate [31]. This digression towards the competing pathways to produce phenolic and benzoic acid compounds may well explain the suppression of aromatic amino acid synthesis by glyphosate.

Even Roundup Ready® crops typically experience slowed growth following glyphosate applications, and this has been attributed to glyphosate's role as a chelator of micronutrients. In early work, glyphosate was shown to interfere with the uptake of the divalent cations, calcium and magnesium, through soybean roots [33]. Glyphosate severely reduced calcium content in the mitochondria of both root and leaf cells. Since magnesium was also affected, but potassium was not, the authors

suggested that this property might hold for all divalent cations. More recent greenhouse experiments demonstrated that glyphosate application to the root system decreased the levels of calcium, magnesium, iron and manganese in the seeds of the plants [34]. It was proposed that glyphosate binds to and immobilizes all of these divalent micronutrients, impairing their uptake by the plant. These glyphosate-induced deficiencies would carry over to the food supply, leading to deficiencies in these nutrients in humans who consume foods derived from glyphosate-exposed crops.

Evidence of disruption of gut bacteria by glyphosate is available for both cattle and poultry. It has recently been proposed that glyphosate may be a significant factor in the observed increased risk to *Clostridium botulinum* infection in cattle in Germany over the past ten to fifteen years [35]. Glyphosate's demonstrated toxicity to *Enterococcus* spp. leads to an imbalance in the gut favoring overgrowth of the toxic *Clostridium* species. Glyphosate has been shown to have remarkable adverse effects on the gut biota in poultry [36], by reducing the number of beneficial bacteria and increasing the number of pathogenic bacteria in the gut. Highly pathogenic strains of *Salmonella* and *Clostridium* were found to be highly resistant to glyphosate, whereas beneficial bacteria such as *Enterococcus*, *Bacillus* and *Lactobacillus* were found to be especially susceptible. Due to the antagonistic effect of the common beneficial bacterium *Enterococcus* spp. on *Clostridia*, toxicity of glyphosate to *E*. spp could lead to overgrowth of *Clostridia* and resulting pathologies.

Pseudomonas spp. is an opportunistic pathogen and an antibiotic-resistant Gram-negative bacterium that has been shown to be able to break down glyphosate to produce usable phosphate and carbon for amino acid synthesis, but a toxic by-product of the reaction is formaldehyde [37], which is neurotoxic, and low levels of formaldehyde can induce amyloid-like misfolding of tau protein in neurons, forming protein aggregates similar to those observed in association with Alzheimer's disease [38].

A recent genome-wide study of the effect of glyphosate on *E. coli* revealed metabolic starvation, energy drain, and other effects involving genes that are poorly understood [39], in addition to suppression of the shikimate pathway. For example, half of the eight genes encoding ATP synthase were downregulated, suggesting an impairment in mitochondrial ATP synthesis. At the same time, genes involved in importing sugars were upregulated, which suggests a switch to anaerobic fermentation, producing pyruvate (a much less efficient solution) rather than oxidizing glucose for full breakdown to carbon dioxide and water. A switch to anaerobic metabolism is also suggested from a study showing that, in soil treated with glyphosate, the total count of fungi was significantly increased, while oxygen consumption was significantly inhibited [40].

Research conducted by exposing an outdoor aquatic mesocosm (approximating natural conditions) to two pesticides and two herbicides revealed a unique effect (among the four toxins studied) of the herbicide, glyphosate, to destroy tadpoles. Following only a two-week exposure period, two species of tadpoles were completely eliminated and a third one was nearly exterminated, resulting in a 70% decline in the species richness of tadpoles [41]. Other experiments on bullfrog tadpoles showed that prior glyphosate exposure reduced the survival rates of tadpoles exposed to the fungal pathogen, *Batrachochytrium dendrobatidis* (Bd). [42]. It is thus conceivable that glyphosate may be instrumental in the worldwide decimation of frogs currently taking place [43]. This also suggests that glyphosate disrupts embryonic development, a topic to which we will return later.

An insidious issue with glyphosate is that its toxic effects on mammals take considerable time to be overtly manifested. Studies on Wistar rats exposed to the highest levels of glyphosate allowed in water

for human consumption for 30 or 90 days showed enhanced lipid peroxidation and glutathione peroxidase activity, indicators of oxidative stress [44]. A long-term study conducted on rats showed remarkable pathologies that became apparent only after the three-month period that is usually allotted for toxicity trials. In this experiment, rats were monitored over their entire lifespan, while being fed either genetically modified (GM) or non-GM maize that had been optionally treated with Roundup® [9]. The rats that were chronically exposed to Roundup® developed several pathologies over the course of their lifespan, including large mammary tumors in the females and gastrointestinal, liver and kidney pathologies, especially in the males. The males developed both skin and liver carcinomas. Premature death in the treated male rats was mostly due to severe hepatorenal insufficiencies. Other researchers have shown that oral exposure to glyphosate in drinking water can induce DNA damage to mouse cells drawn from blood and liver [45].

Researchers have discovered that Roundup® is sometimes much more toxic than glyphosate by itself, and this discrepancy can be explained by the fact that Roundup® includes a surfactant which greatly enhances cytotoxic effects of glyphosate [46]. Specifically, the surfactant, TN-20, commonly found in glyphosate-based herbicides, was studied for its effect on glyphosate toxicity to rat cells *in vitro*. The results showed that the combination of the surfactant and glyphosate led to mitochondrial damage, apoptosis, and necrosis, under conditions where neither substance working alone achieved this effect. It was proposed that TN-20 disrupts the integrity of the cellular barrier to glyphosate uptake.

A study on three microorganisms commonly used as starters in dairy technologies demonstrated that Roundup®, but not glyphosate, inhibited microbial growth at lower concentrations than those recommended in agriculture [47]. This result illustrates an amplified effect of glyphosate's toxicity through the adjuvants found in Roundup®. The authors also suggested that a recent loss of microbiodiversity in raw milk may be explained through the same toxic mechanisms.

In humans, a prolonged accidental skin exposure to a glyphosate-surfactant herbicide has been shown to produce local swelling, bullae, and exuding wounds, followed by osteopenia, neurological impairment, and reduced nerve conduction [48]. Similarly oral exposure to glyphosate produces chemical burns and ulceration of the oral cavity [49].

3. Gut Dysbiosis, Autism and Colitis

It is now well established that autism spectrum disorder (ASD) is associated with dysbiosis in the gut [50], and, indeed, this is viewed by many as an important contributor to ASD [51]. An increase in short chain fatty acids and ammonia in the gut has been found in association with autism [52,53]. Since these are by-products of anaerobic fermentation, this suggests an overgrowth of anaerobic bacteria such as *Clostridia*, *Bacteriodetes*, and *Desulfovibrio*. *Clostri*dia have indeed been found in excess in the feces of autistic children [54]. By-products of fermentation by anaerobes, such as phenols, amines, ammonia, and hydrogen sulfide, can be toxic to the large bowel [1,8]. A strong link between autism and hepatic encephalitis has been identified [55], where the key underlying pathology may be excess ammonia in the blood stream. Ammonia plays an important role in the etiology of hepatic encephalopathy associated with both acute and chronic liver dysfunction [56,57]. The source of the ammonia is believed to be intestinal bacteria, including those in both the small and large intestine [58]. Impaired liver function prevents detoxification of ammonia via the urea pathway. Thus, the increased activity of

PAL induced by glyphosate [27,28] could play a role in creating a hyperammonemic environment in the gut and initiating subsequent pathology.

Indeed, there is now evidence that gut microbes can produce ammonia from phenylalanine via PAL [59]. A unique mouse phenotype has recently been identified that is defined by the behavior of its gut bacteria [60], and the authors suggest that this phenotype can be explained through increased metabolism of phenylalanine via the PAL pathway. Furthermore, this unique phenotype is also associated with excess synthesis of p-cresol, via a pathway involved in tyrosine breakdown. These authors go on to propose that the known sulfate deficiency associated with autism [61,62] may be explained by the depletion of sulfate through sulfation of *p*-cresol produced from tyrosine by *Clostridium difficile* in the gut [63,64], in order to detoxify it. As we will explain in the next section, we believe that, in fact, p-cresol and other phenolic compounds are part of the *solution* rather than the *cause*, with respect to impaired sulfate transport.

C. difficile is a well-established causal factor in colitis [65]. The incidence of *C. difficile*-associated disease has increased significantly in North America in recent years, and research into the association of this increase with inflammatory bowel disease has borne fruit [66]. In an observational study involving patients in a hospital in Wisconsin between 2000 and 2005, it was shown that *C. difficile* infection was almost nonexistent in patients with inflammatory bowel disease prior to 2003, but the rate grew from 4% to 7% to 16% in 2003, 2004, and 2005. One hypothesis presented was antibiotic use disrupting the beneficial gut bacteria, but it is conceivable that increased exposure to glyphosate is contributing to this increase.

A higher level of *p*-cresol in the urine has been associated with lower residual sulfonation [67] and with autism [68]. *p*-Cresol, formed via anaerobic metabolism of tyrosine by bacteria such as *C. difficile* [64], is a highly toxic carcinogen, which also causes adverse effects on the central nervous system, the cardiovascular system, lungs, kidney and liver [69]. A recent paper found that formula-fed infants had an overrepresentation of *C. difficile* in the gut bacteria [70]. In a case-control study, children with autism were found to be significantly more likely to have been formula-fed rather than breast-fed [71]. The study did not distinguish between organic and non-organic formula, but one can surmise that non-organic soy formula might be contaminated with glyphosate, and this could be a contributing factor to both the autism and the *C. difficile*. Urinary bacterial metabolites of phenylalanine, such as benzoic and phenylacetic acids, and of tyrosine (*p*-hydroxybenzoic acid and *p*-hydroxyphenylacetic acid) have been found to be elevated in association with several different diseases reflecting impaired intestinal resorption, including coeliac disease, cystic fibrosis, and unclassified diarrhoea [72]. It was proposed that these metabolites were produced by the gut bacteria. High concentrations of an abnormal phenylalanine metabolite have been found in the urine of people with autism and schizophrenia, up to 300x normal adult values, which is likely due to multiple species of anaerobic bacteria in the *Clostridium* genus [73]. Others have detected abnormally high concentrations of hippurate in the urine in association with autism [74]. Hippurate is a liver metabolite of benzoic acid [75]. Thus a variety of different compounds representing a deflection of aromatic amino acid synthesis towards oxidized benzene derivatives have been found in association with various digestive disorders and neurological disorders.

Studies have convincingly shown an inflammatory mucosal immunopathology in children with regressive autism characterized by infiltration of intestinal epithelial lymphocytes [76]. The infiltration of immune system cells like lymyphocytes and eosinophils is a direct response to the impaired barrier

function. As will be seen in the next section, we propose that this dysbiosis is caused principally by impaired sulfate supply to the mucosa, and that the toxic phenolic compounds both assist in correcting this deficiency and induce inflammatory responses due to their oxidizing effects.

4. Sulfate Transport Impairment and Phenol Synthesis

Autism is a disorder involving impaired social skills and neurodevelopmental delay that has reached epidemic proportions in recent years, with one in 50 children born in the United States today now classified on the autism spectrum, according to the U.S. Centers for Disease Control and Prevention. Impaired sulfur oxidation and low levels of serum sulfate have been established in association with autism since 1990, as evidenced by the following quote from [77]: “These results indicate that there may be a fault either in manufacture of sulphate or that sulphate is being used up dramatically on an unknown toxic substance these children may be producing” (p. 198).

In this section, we develop a novel hypothesis for the effect of glyphosate on aromatic amino acids in plants and microbes. Our arguments depend upon the observation that glyphosate, a short carbon-nitrogen chain with a carbonyl group and a phosphate group, is a strong anionic kosmotrope, since both carbonate and phosphate have this property. Sulfate is also a kosmotrope, whereas nitrate is a chaotrope. Kosmotropes and chaotropes represent opposite extremes on the Hofmeister series [78,79], where kosmotropes tend to structure the water surrounding them and to desolubilize proteins, whereas chaotropes destructure the water and solubilize proteins. Studies on fatalities due to acute over-exposure to glyphosate reveal hemodynamic disturbances, including intravascular disseminated coagulation (DIC) and multiple organ failure, associated with high serum concentrations of glyphosate (over 800 mg/L) [80]. We suspect this has to do with glyphosate's effect as a potent kosmotrope, causing a "salting out" of blood proteins and resultant coagulation and a “no-flow” situation [81].

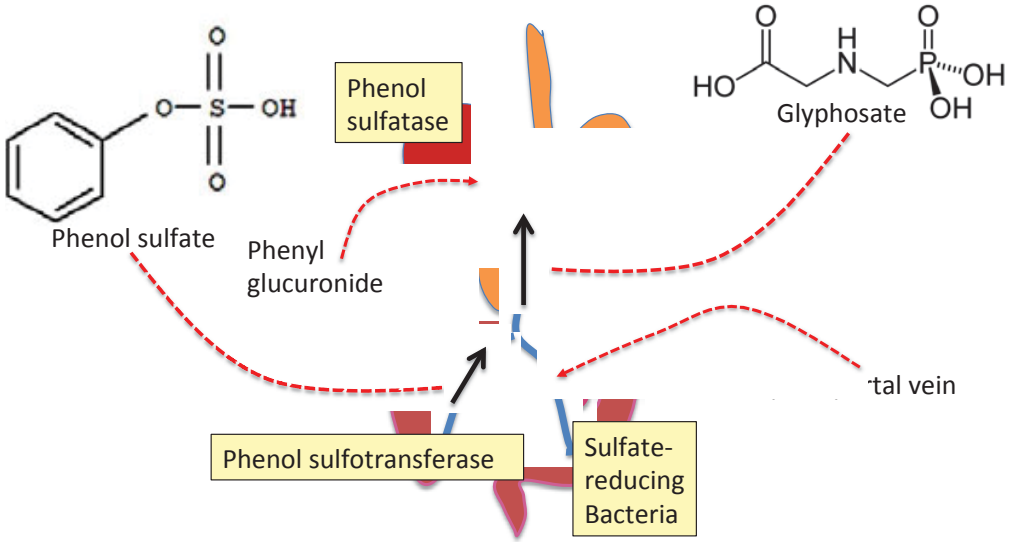
Molecules with a carbon ring and an available attachment site for sulfate (*e.g.*, phenolic compounds) are attractive for the purpose of transporting sulfate through the bloodstream when the kosmotropic load is elevated. Phenolic compounds like *p*-cresol can be readily sulfated in the gut, and this provides an opportunity to transport sulfate through the hepatic portal vein in the presence of glyphosate. The ring supports a charge distribution that diffuses the negative charge and suppresses the water-structuring properties of sulfate, thus preventing the vascular disturbances. A single phenol can perform this feat multiple times, as the sulfate can be attached to the phenol in the colon via a phenol sulfotransferase, and the liver utilizes sulfatases and sulfotransferases to transfer the sulfate moiety from the phenol to an available substrate, typically a xenobiotic or a sterol [82]. Thus, phenols could be responsible for supplying the sulfate critically needed to detoxify xenobiotics and bile acids and to produce various sterol sulfates, as well as supplying sulfate to the pancreas to be incorporated into mucopolysaccharides being released into the gut along with proteases by acinar cells [83].

In this scenario, the glyphosate itself, due to its kosmotropic properties, is disrupting the transport of free sulfate, and therefore the aromatic amino acids are oxidized into various phenolic compounds in order to compensate for this problem. Unfortunately, once they are unsulfated, the phenols become toxic, as they will react destructively with phospholipids and DNA through one-electron transfer [84].

Although flavonoids are generally considered to be beneficial to health, the biological mechanisms behind their benefit are not yet established. In [85], it is stated that “the potential role of microbial

metabolism in the gastrointestinal tract is often overlooked". These authors propose that monophenolic derivatives are likely produced through ring fission of flavonoids by the gut microflora in the colon. Thus, flavonoids can promote sulfate transport to the liver via this process. Furthermore, flavonoids themselves can be both glucuronylated and sulfated [85,86], especially at the 4'-OH position [87], so they could serve directly as sulfate transporters without being broken down. In fact, we hypothesize that their role in sulfate transport is the reason for their abundant synthesis in plants in the presence of glyphosate at the expense of tryptophan [29]. Since they are less toxic than monophenols, they become attractive for sulfate transport in the presence of glyphosate.

Figure 1. Schematic of cyclical process that could be utilized to transport sulfate from the gut to the liver in the face of glyphosate contamination in the hepatic portal vein. Phenolic compounds derived from aromatic amino acids would be cycled back and forth between the gut and the liver, sulfated during transport from the gut to the liver and glucuronylated during transport back from the liver to the gut. Ultimately, a sulfate reducing bacterium could metabolize the phenol, consuming sulfate.



The fact that glyphosate suppresses both alkaline and acid phosphatase activity in *in vitro* assays [88] as well as extracellular alkaline phosphatase synthesis in algae [89] suggests that phosphate faces the same problem as sulfate in plants, in the presence of glyphosate, and hence enzymatic activity that produces free phosphate is suppressed. It is interesting to note that autism is associated with elevated serum levels of pyridoxal phosphate (vitamin B_6) even in the absence of supplements [90]. Despite this, supplemental B6 has been shown to alleviate symptoms of autism [91,92]. We hypothesize that vitamin B6 is exploited to transport phosphate safely in the presence of glyphosate. The pyridoxal ring distributes the negative charge on the phosphate anion in the same way that phenols distribute the charge on sulfate, thus allowing phosphate to be transported in a non-kosmotropic form.

Glyphosate's kosmotropic effects can be counteracted through buffering of chaotropes in the blood, and this could be a factor in the increased levels of both ammonia [93] and various oxides of nitrogen, including nitric oxide, nitrite, and nitrate [94–96] observed in association with autism.

Thus, autism is associated with dysbiosis in the gut [50,51], along with impaired sulfate metabolism and a significantly reduced level of free sulfate in the blood stream (only one-third of the normal level) [63,97–101], excess production of nitric oxide [94–96], overgrowth of phenol-producing bacteria like C. difficile [101], and increased urinary levels of the toxic phenol, *p*-cresol [68]. Autism is also associated with a decreased ability to sulfate and hence detoxify acetaminophen, which aligns with insufficient sulfate bioavailability. A genetic defect in the phenol sulfotransferase gene is associated with autism [77]: this enzyme becomes more essential in the context of glyphosate contamination. All of these observations can potentially be explained by the effects of glyphosate on the gut bacteria and on the blood stream.

Both colitis and Crohn's disease are associated with sulfate depletion in the gut [102], which could be caused by the impaired sulfate transport problem induced by glyphosate exposure. An overgrowth of the sulfur-reducing bacterium, Desulfovibrio, has been found in association with autism [103]. Sulfate-reducing bacteria can utilize aliphatic and aromatic hydrocarbons as electron donors, and therefore they can play an important role in detoxifying toxic phenolic compounds [104–108]. Thus, the presence of *Desulfovibrio* in the gut may serve a dual purpose by metabolizing phenolic compounds while also disposing of free sulfate, which could be problematic if allowed to enter the blood stream in the presence of glyphosate. Thus, we hypothesize that, in the context of glyphosate in the vasculature, aromatic amino acids are diverted into phenolic compounds which can safely transport sulfate from the gut to the liver. The liver can then transfer the sulfate to another metabolite, such as a steroid, and then ship the phenol back to the digestive system for another round via the bile acids following glucuronidation [108]. Possibly after multiple rounds, the phenol is finally metabolized by a sulfate-reducing bacterium in the colon. This idea is schematized in Figure 1.

5. Evidence that Glyphosate Inhibits CYP Enzymes

The evidence that glyphosate inhibits CYP enzymes comes from several directions. There are studies showing inhibition of aromatase, a CYP enzyme that converts testosterone to estrogen, and studies showing enhancement of retinoic acid, which could be achieved by suppressing the CYP enzyme involved in its catabolism. Finally, there are studies that directly show inhibition of detoxifying CYP enzymes in both plants and animals.

Two studies point to a disruption of aromatase activity [109,110]. In [109], as little as 10 ppm. of glyphosate disrupted aromatase activity in human liver HepG2 cells, a well-established cell line to study xenobiotic toxicity. In [110], it was shown that aromatase activity is disrupted in human placental cells at a concentration 100 times lower than that recommended in agricultural use. Furthermore, even small amounts of the adjuvants present in Roundup® could substantially enhance this effect of glyphosate, probably by enhancing the ease with which it gains access to the membrane-bound protein. In experiments with oyster larvae, Roundup® was found to be toxic at less than 1/20 the concentration of glyphosate needed to induce toxicity, thus exhibiting the enormous enhancing effect of Roundup®'s adjuvants [111].

Retinoic acid plays a key role in embryonic development, where its tightly-regulated concentration levels impact developmental stages [112]. Due to reports of neural defects and craniofacial malformations in children born in regions where glyphosate-based herbicides are used, a group of

researchers investigated the effects of low doses of glyphosate (1/5,000 dilutions of a commercial glyphosate-based herbicide) in development of African clawed frog embryos and chick embryos [113]. The treated embryos were highly abnormal: the frog embryos developed into tadpoles with cranial deformities, and microcephaly was observed in the chick embryos. They traced this effect to an increase in endogenous retinoic acid (RA) activity, and showed that cotreatment with an RA antagonist prevented the deformities.

This increase in RA activity can be explained via inhibition of a CYP enzyme. A novel member of the CYP family has been discovered which is induced by retinoic acid and involved in its catabolism [114,115]. It is present in mammalian embryos and in the brain. Thus, if this enzyme is suppressed by glyphosate, it would explain the observed effect that glyphosate enhances levels of retinoic acid in embryonic development.

A study conducted in 1998 demonstrated that glyphosate inhibits cytochrome P450 enzymes in plants [116]. CYP71s are a class of CYP enzymes which play a role in detoxification of benzene compounds. An inhibitory effect on CYP71B1l extracted from the plant, Thlaspi arvensae, was demonstrated through an experiment involving a reconstituted system containing E. coli bacterial membranes expressing a fusion protein of CYP71B fused with a cytochrome P450 reductase. The fusion protein was assayed for activity level in hydrolyzing a benzo(a)pyrene, in the presence of various concentrations of glyphosate. At 15 microM concentration of glyphosate, enzyme activity was reduced by a factor of four, and by 35 microM concentration enzyme activity was completely eliminated. The mechanism of inhibition involved binding of the nitrogen group in glyphosate to the haem pocket in the enzyme.

A more compelling study demonstrating an effect in mammals as well as in plants involved giving rats glyphosate intragastrically for two weeks [117]. A decrease in the hepatic level of cytochrome P450 activity was observed. As we will see later, CYP enzymes play many important roles in the liver. It is plausible that glyphosate could serve as a source for carcinogenic nitrosamine exposure in humans, leading to hepatic carcinoma. N-nitrosylation of glyphosate occurs in soils treated with sodium nitrite [118], and plant uptake of the nitrosylated product has been demonstrated [119]. Preneoplastic and neoplastic lesions in the liver of female Wistar rats exposed to carcinogenic nitrosamines showed reduced levels of several CYP enzymes involved with detoxification of xenobiotics, including NADPH-cytochrome P450 reductase and various glutathione transferases [120]. Hence this becomes a plausible mechanism by which glyphosate might reduce the bioavailability of CYP enzymes in the liver.

Glyphosate is an organophosphate. Inhibition of CYP enzyme activity in human hepatic cells is a well-established property of organophosphates commonly used as pesticides [121]. In [122], it was demonstrated that organophosphates upregulate the nuclear receptor, constitutive androstane receptor (CAR), a key regulator of CYP activity. This resulted in increased synthesis of CYP2 mRNA, which they proposed may be a compensation for inhibition of CYP enzyme activity by the toxin. CYP2 plays an important role in detoxifying xenobiotics [123].

Beginning in around 2006, an alarming die-off of honeybees became apparent in the United States, and researchers are still struggling to understand what is causing this die-off [124]. Since the application of glyphosate also reached record levels that year, and has continued to increase since then, with no abatement in the bee colony collapse disorder, glyphosate could be playing a role in the bees'

plight. While correlation does not necessary imply causation, there are strong reasons why glyphosate might interfere with bees' resistance to other environmental toxins. At first glance, pesticides might be more highly suspect, since bees are, after all, an insect. However, honeybees have an innate resistance to most pesticides, which unfortunately depends upon several CYP enzymes. For example, metabolic detoxification mediated by CYPs contributes significantly to honey bee tolerance of pyrethroid insecticides [125]. Thus, the fact that glyphosate disrupts CYP enzymes would suggest that exposure to glyphosate would leave bees especially vulnerable to pesticides in their environment, resulting in a synergistic effect. A 2005 study in Alberta (Canada) revealed a reduced wild bee abundance and highly-correlated reduced pollination in GM canola compared with organically grown canola [126], with Roundup-treated non-GM canola coming in at an intermediate level. A study comparing bees exposed to glyphosate and/or Roundup® against a control population demonstrated a significantly higher mortality rate in the glyphosate-exposed bees ($p < 0.001$) [127]. Neonicotinoids such as imidacloprid and clothianidin can kill bees, and have been implicated in colony collapse disorder [128]. However, this toxic effect is likely synergistic in combination with glyphosate, as would occur with bees ingesting herbicide-contaminated pollen. Glyphosate is an organophosphate, and a study of human self-poisoning has demonstrated that organophosphate ingestion synergistically greatly enhances the toxicity of ingested neonicotinoids [129].

6. The Path to Obesity

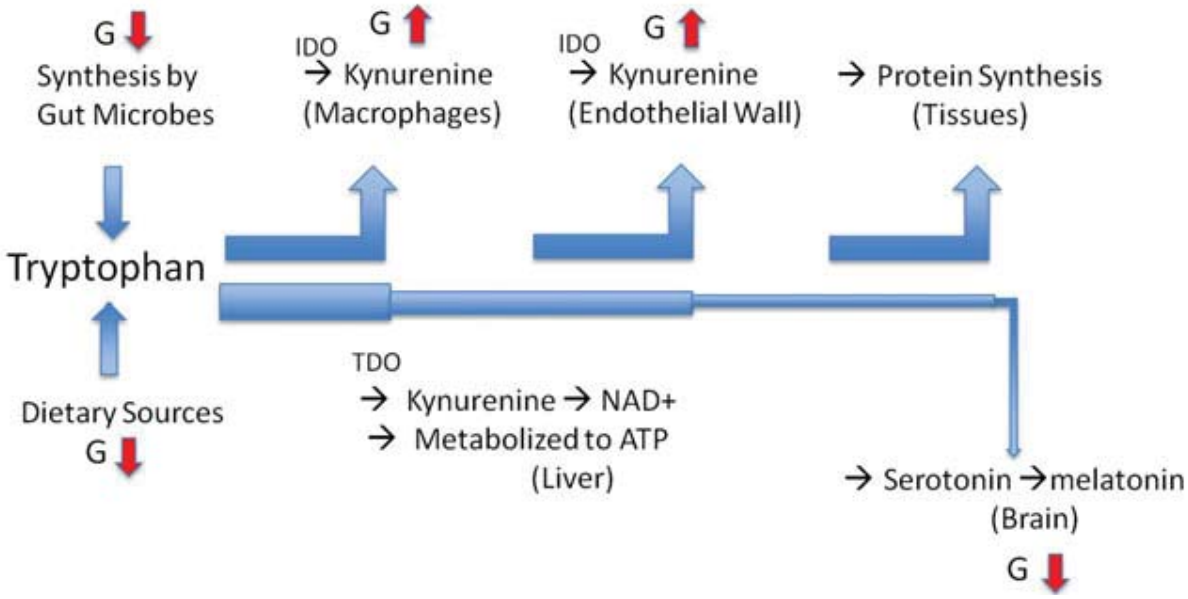
Having established a plausible mechanism whereby glyphosate's effects on gut bacteria would lead to depleted sulfate supplies in the gut with resulting inflammatory bowel disease, we now turn our attention towards the likely consequences of the resulting "leaky gut syndrome." It has been proposed that the exponential increase in the production of synthetic organic and inorganic chemicals may be causal in the current world-wide obesity epidemic, due to alterations in body chemistry that promote weight gain [130]. These chemicals are better known for causing weight loss at high exposure levels, and this apparent paradox can be explained with respect to glyphosate, by invoking its known effect of depleting tryptophan in plants and microbes. Its effect on CYP enzymes in the liver will compound the problem, due to the impaired ability to detoxify synthetic chemicals, which are increasingly present in the environment. In this section we will explain how glyphosate's depletion of tryptophan bioavailability can lead to obesity, and in Section 6 we will provide evidence that extreme depletion of tryptophan in the absence of obesity can cause severe impairment of the intestinal barriers, resulting in weight loss and anorexia, due to an inability to transport critical micronutrients across the damaged gut barrier.

Tryptophan is an essential amino acid, meaning that mammalian cells cannot synthesize it. Serum tryptophan depletion leads to serotonin and melatonin depletion in the brain [131]. Since serotonin (derived from tryptophan) is a potent appetite suppressant [132], it follows that serotonin deficiency would lead to overeating and obesity. As we have seen, tryptophan supplies could be depleted both in plant-based food sources and through impaired tryptophan synthesis by gut bacteria as direct effects of glyphosate. The observed 20-fold increase in the synthesis of tryptophan-derived polyphenolic flavonoids in the context of glyphosate provides strong evidence of impaired tryptophan synthesis [29].

Tryptophan has several important roles in the body. Ordinarily, dietary tryptophan (aside from its role as an essential amino acid in protein synthesis) is taken up by the liver and either fully

metabolized to produce ATP or processed through the enzymatic action of tryptophan dioxygenase (TDO) and indole amine dioxygenase (IDO), via a pathway involving kynurenine and quinolinate as intermediaries to produce NAD+, an essential cofactor in ATP synthesis and DNA repair [133] (see Figure 2). Any tryptophan not taken up by the liver circulates in the blood, and is transported across the blood brain barrier (BBB). It becomes the (sole) precursor to the synthesis of the neurotransmitter serotonin and the hormone melatonin [131]. A low ratio of tryptophan to competing proteins in the blood stream leads to reduced transport of tryptophan across the BBB and subsequent impaired serotonin and melatonin synthesis in the brain. Thus, low serum tryptophan levels translates into a tendency towards weight gain due to suppressed serotonin signaling [132].

Figure 2. Illustration of tryptophan pathways in the body and the adverse effect of glyphosate on tryptophan bioavailability. IDO: indole amine dioxygenase; TDO: Tryptophan dioxygenase; G: glyphosate.



However, under inflammatory conditions, and in response to pathogenic stimuli such as the lipopolysaccharide (LPS) in bacterial cell walls, tryptophan is converted into kynurenine by lymphoid tissues at the site of inflammation [134] and stockpiled by *in situ* macrophages and neutrophils [135–137] as kynurenine. Therefore, it is expected that inflammation in the gut would lead directly to serum tryptophan depletion, thus further reducing the bioavailability of tryptophan to the liver. There are several reasons why macrophages need to sequester kynurenine, the most important of which is likely to be the assurance of a localized resource to regenerate NAD+ following its depletion through the synthesis of poly-ADP ribose by poly(ADP-ribose)polymerase (PARP) [138–140]. Poly-ADP ribose plays an important role in the DNA repair mechanisms that are required following DNA damage, induced by the reactive oxygen and nitrogen species (ROS and RNS) released by macrophages to fight infection – superoxide, nitric oxide, and their reaction product, peroxynitrite. Superoxide is induced from oxygen in the artery wall by transfer of an electron from cytosolic NADPH to oxygen, and its synthesis is essential for killing invasive pathogens, but collateral exposure also leads to tissue damage.

Both the inflammatory cytokine interferon-γ (IFN-γ) and superoxide itself induce IDO synthesis, and IDO detoxifies superoxide by using it to break the pyrrole ring in tryptophan [141]. The DNA in the cell nucleus is highly vulnerable to superoxide exposure, which can lead to strand breaks. The synthesis of kynurenine from tryptophan by IDO results in replenishing the supply of NAD+ and NADP+, which has been depleted due to the activities of PARP as part of the DNA repair process.

Studies have confirmed that serum tryptophan levels are low in association with obesity [142,143]. In [143], plasma tryptophan levels were monitored several times over the course of a twenty-four hour period, and it was confirmed that serum tryptophan levels were chronically depressed, and the levels of other competing large neutral amino acids were elevated, in obese subjects compared to controls. This pathology persisted even after weight reduction through intense dieting.

A recent experiment involving transferring a strain of endotoxin-producing bacteria from the gut of an obese human to the sterile gut of germ-free mice demonstrated the dramatic obesogenic effect that over-production of endotoxin by gut bacteria can have [144]. These mice became obese over a 16-week trial period, when concurrently placed on a high-fat diet, and the obesity was associated with a low-grade chronic inflammatory state. Control germ-free mice on the same diet but without the infective agent did not become obese. It was hypothesized that chylomicrons produced for fat transport became a vehicle for endotoxin delivery to blood serum and subsequently to the liver and body fat stores, since inflammatory cytokines were found predominantly in the liver and epididymal fat pad rather than in the ilium. Since glyphosate induces a shift in gut bacteria towards endotoxin-producers, this effect can conceivably explain the association of a high-fat diet with obesity [145].

The obesity epidemic began in the United States in 1975, simultaneous with the introduction of glyphosate into the food chain, and it has steadily escalated in step with increased usage of glyphosate in agriculture (see Figure 1 in [146]). While it is common knowledge that Americans are continuing to grow more and more obese with each passing year [147,148], there may be less awareness that obesity aligns with glyphosate usage elsewhere in the world [149]. For example, South Africa arguably has the highest obesity rates in all of Africa [150], and it is also the African country that has most heavily embraced glyphosate usage since the 1970's and has freely adopted genetically modified crops with little regulation [151,152]. According to World Health Organization statistics [153], only 2.7% of adults in the United Kingdom were obese in 1972, a number that rose to 25.8% in 1999. Today, two thirds of U.K. citizens are either overweight or obese.

7. The Path to Inflammatory Bowel Disease and Anorexia Nervosa

We have seen how obesity can develop following the depletion of tryptophan through its diversion into polyphenolic flavonoids as well as aggressive uptake into macrophages, to provide assurance of DNA repair mechanisms in the face of excess ROS and RNS. Subsequent impaired serotonin synthesis stimulates overeating behaviors. Here, we argue that severe tryptophan deficiency without sufficient fat stores to harbor toxins and supply sterol sulfates can result in an inability to control microbial invasion as a consequence of impaired release of antimicrobial peptides. This can lead, paradoxically, to anorexia nervosa, resulting in a highly inflamed digestive system, pathogenic penetration through leaky intestinal epithelium, uncontrollable diarrhea, and subsequent anorexia.

Obesity offers protection against gastrointestinal inflammation, in part because the endotoxin can be stored in adipose tissue, sparing the gut barrier from inflammatory damage. However, a more important factor may be the ability of adipose tissue to directly supply sulfated steroids. The sulfotransferase that sulfates serotonin, thus inactivating it, is found in many tissues, including brain, heart, liver, lung, kidney and spleen [154]. Insufficient sulfate supply would likely compromise this function, leading to poor serotonin regulation. There is an interesting connection between levels of serotonin and sterol sulfates in the blood serum. DHEA sulfate is the most prominent sterol sulfate in the serum besides cholesterol sulfate [155]. Patients with autism have anomalously low DHEA sulfate levels along with anomalously low serotonin [156]. Serum DHEA sulfate levels are inversely associated with visceral fat [157], and DHEA sulfate supplements can induce weight loss in morbidly obese postmenopausal women [158]. We hypothesize that DHEA sulfate levels are a hormonal signal of sulfate bioavailability, and low bioavailability leads to low serotonin which induces overeating, in order to produce visceral fat. Visceral fat is a source of estrone sulfate [159], which, we hypothesize, may compensate for some deficiencies in DHEA sulfate and alleviate the burden on the adrenal glands to produce sterol sulfates. This would also reduce the demand on phenols to transport sulfate and therefore alleviate the inflammatory gut disorder, restoring homeostasis.

An important study elucidating the processes leading to inflammatory bowel disorder was conducted on male Ace2 knockout mice (Ace2−/y) [13]. Ace2 induces expression of the tryptophan transporter in the gut epithelium. Thus, these mice suffered from severe tryptophan deficiency. They responded to dextran sodium sulfate exposure with a much more severe colitis attack than their control littermates, leading to enhanced infiltration of inflammatory cells, increased intestinal bleeding, severe diarrhea, and weight loss. A series of further experiments revealed that a similar response could be provoked in the control mice by providing them with a diet that was specifically deficient in tryptophan. It was confirmed that the acute response was associated with impaired synthesis of antimicrobial peptides by macrophages, mediated by impaired mTOR (mammalian target of rapamycin) signaling. It is conceivable that the severe deficiency in tryptophan led to restricted protein synthesis in macrophages, preventing the synthesis of the antimicrobial peptide. Furthermore, the distribution of gut bacteria was profoundly affected by the Ace2−/y phenotype and by tryptophan deprivation.

Thus, severe tryptophan deficiency, as might be induced by glyphosate's interference with tryptophan synthesis in plants and microbes, can lead to an extreme inflammatory bowel disease that would severely impair the ability to absorb nutrients through the gut, due to inflammation, bleeding and diarrhea. This could easily explain the alarming increases that have been seen recently in inflammatory bowel diseases [16,17,160].

8. Cytochrome P450 Enzymes

The cytochrome P450 (CYP) enzymes are a diverse, ancient class of enzymes that date back to three billion years ago, and play an important role in plant, animal, and microbial biology [161]. These enzymes participate in oxidation, peroxidation and reduction of compounds ranging from pharmaceutical drugs to environmental chemicals to endogenous bioactive molecules [123]. There are at least 18 distinct CYP families in humans, which are classified as a series of numerical "CYP" classes. In humans, CYP1, CYP2, CYP3, and CYP4 P450 enzymes in the liver are essential for detoxification of

many xenobiotics [162]. Members of the CYP5 and CYP7 classes are essential for the formation of water-soluble bile acids from cholesterol in the liver. Bile acids act as powerful detergents to aid in the digestion of fats, and also provide a pathway for disposal of oxysterols. A loss-of-function mutation in CYP7B1 leads to liver failure in children, associated with high serum levels of oxysterols [163].

Both cholesterol and vitamin D3 synthesis and degradation depend upon various CYP enzymes. At least seven CYP enzymes have a role in converting acetate into sterols. Lanosterol 14α-desmethylase (CYP51A1) is pivotal in cholesterol synthesis. Two CYP enzymes in the liver catalyze 25-hydroxylation of vitamin D3 to its active form, and two other CYP enzymes catalyze the breakdown of vitamin D3 in the liver [164,165].

There is a growing epidemic of vitamin D deficiency in the United States. In a study on serum 25-hydroxyvitamin D levels obtained from the National Health and Nutrition Examination Survey (NHANES) data, it was found that vitamin D3 levels fell sharply in the interval from 2001 to 2004 compared to the interval from 1988 through 1994 [166]. While this problem is in part due to overaggressive sun avoidance practices, glyphosate's interference with CYP proteins may play a role in disrupting vitamin D3 activation in the liver.

Several CYP enzymes participate in steroid synthesis. Cytochrome P450 oxidoreductase deficiency (POR) is a newly described disorder of steroidogenesis [167]. Five crucial lipid hormones, aldosterone, androstenedione, cortisol, corticosterone and dehydroepiandrosterone (DHEA), are produced in the adrenal glands, testes and ovaries, and in the adrenal cortex. All steroid hormones are produced from cholesterol by these CYP enzymes, contained within the inner mitochondrial membrane. The lipophilic nature of these steroids allows them to diffuse across the lipid bilayers. CYP19A1 (aromatase), whose inhibition has been confirmed in association with glyphosate [109,110] converts androgenic precursors into estrogen. Suppressed aromatase synthesis has been found in the brain in association with autism [168], leading to the "super-male" profile associated with this condition [169].

CYP26A1 catabolizes retinoic acid; hence, its suppression would lead to excess retinoic acid bioavailability. CYP26A1 is induced by retinoic acid during neural differentiation, and its action leads to the degradation of retinoic acid, a necessary step towards maturation of the developing neurons [114]. The aryl hydrocarbon receptor (Ahr) gene induces CYP1B expression, leading to degradation of retinoic acid. Ahr-knockout mice accumulate excess retinoic acid in the liver [170]. Thus, if liver CYP1B expression were disrupted by glyphosate, it would lead to excess retinoic acid. Retinoic acid suppresses the synthesis of cholesterol sulfate, a crucial step in bile acid synthesis [171]; thus, excess retinoic acid in the liver should lead to impaired synthesis of bile acids and impaired fat metabolism.

Mutations in CYP7A1 are associated with high serum LDL and high hepatic cholesterol content, along with deficient bile acid excretion [172]. Human CYP7B1 mutations lead to both defects in bile acid synthesis and spastic paraplegia, involving impaired myelin sheath in the spinal cord and uncontrolled movement disorders. The drug, clopidogrel (Plavix), administered to suppress life-threatening stent thrombosis following cardiovascular surgery, depends upon a liver CYP enzyme, CYP2C19, to transform it into an activated metabolite. Patients with a loss-of-function mutation in this CYP enzyme have significant risk of an adverse event following surgery [173,174].

Glyphosate from food sources or as a contaminant in water would be likely to reach the liver in high concentrations through direct transport from the digestive system via the hepatic portal vein. It could be anticipated that glyphosate would disrupt many of the diverse CYP enzymes that are

bioactive in the liver, involved in cholesterol synthesis and metabolism, vitamin D3 synthesis and metabolism, the detoxification of xenobiotics, and regulation of retinoic acid.

Glyphosate would also be expected to travel throughout the blood stream, disrupting any CYP enzymes it comes in contact with. Of particular concern are the two that regulate blood clotting (thromboxane A2 synthase: CYP5A1) and hemorrhaging (prostacyclin synthase: CYP8A1). CYP5A1 stimulates platelet aggregation, whereas CYP8A1 inhibits platelet aggregation. The elderly often face instabilities in hemorrhaging and clotting leading to Disseminated Intravascular Coagulation (DIC) and life-threatening destabilization of the blood [175], which could be due to impaired function of these two enzymes.

9. Glyphosate's Potential Role in eNOS Dysfunction

Thus far, we have developed a plausible argument for how glyphosate could disrupt gut microbiota, leading to inflammation, depletion of tryptophan, and subsequent obesity, or, in the extreme case, anorexia nervosa. We have also discussed the many roles of CYP enzymes, and proposed that glyphosate's interference with CYP expression could lead to many pathologies that are commonly occurring today, such as vitamin D3 deficiency and abnormal blood clotting.

Endothelial nitric oxide synthase (eNOS) is an orphan member of the CYP family. It is present in endothelial cells that synthesize nitric oxide (NO), where it induces vessel relaxation and therefore enhanced blood flow [176]. Both eNOS and CYP enzymes are heme-thiolate proteins with the same redox partner, a diflavoprotein reductase. However, eNOS, unlike the other CYP enzymes, requires tetrahydrobiopterin (BH4) as a cofactor for the synthesis of NO, and no other member of the CYP family is capable of synthesizing NO.

It has recently been proposed that eNOS is a dual-purpose enzyme, producing NO when it is bound to calmodulin in the cytoplasm, and producing sulfate when it is bound to caveolin at the plasma membrane [177]. While no other CYP enzyme produces NO, this class is known to oxidize sulfur [178], an important aspect of their ability to detoxify sulfur-containing drugs. Red blood cells (RBCs) contain membrane-bound eNOS, and this has presented a puzzle to researchers, because the synthesis of NO by RBCs would be counterproductive, due to its high reactivity with hemoglobin to form a nitrosylated compound that is impaired in oxygen transport. Indeed, RBCs have mechanisms to maintain a very low concentration of the substrate L-arginine. However, it is highly plausible that RBCs use their eNOS to produce sulfate, which can then be combined with cholesterol to form cholesterol sulfate, known to be present in large amounts in RBC plasma membranes, where it has a stabilizing effect.

A significant adverse effect of glyphosate is its hypothesized disruption of sulfate synthesis by eNOS in the endothelium. This effect contributes to the inflammation already present due to the escape of pathogenic bacteria through the impaired gut barrier. In fact, the two effects are synergistic, because the sulfate depletion incurred by eNOS dysfunction further compromises the gut barrier, where sulfate deficiencies due to transport problems are already present. Due to its homology with the CYP enzymes, eNOS is predicted to be susceptible to disruption by glyphosate, but only in its sulfate-synthesis function. The result will be endothelial damage due to superoxide exposure, along with sulfate deficiency. We hypothesize that such disruption is a significant heretofore overlooked component of glyphosate's toxicity in mammals.

If, as proposed in [177], RBCs use eNOS to produce sulfate, then the sulfate can be combined with cholesterol to produce cholesterol sulfate, which, unlike cholesterol itself, is amphiphilic. RBCs are well positioned to deliver both cholesterol and sulfate to the tissues, supplying them with these essential nutrients. In [177], it was further proposed that endothelial cells produce sulfate catalyzed by eNOS, using superoxide as the oxidizing agent, a reaction that is catalyzed by sunlight exposure, and that the sulfate serves to replenish sulfate supplies to the glycocalyx, which is constructed from highly sulfated proteoglycans. Accumulation of sulfate deficiencies in the endothelial glycocalyx contributes significantly to vascular dysfunction [179]. Colitis is less prevalent in areas with a sunny climate [180], suggesting that sunlight improves intestinal health by increasing sulfate supply.

Ingested glyphosate readily enters the vasculature, and hence membrane bound eNOS in RBCs and the endothelial wall is vulnerable to the disabling effects of glyphosate on the P450 active site. This, over time, would result in cholesterol and sulfate deficiencies, manifested as multiple disease states. It would also explain the pathology where eNOS synthesizes superoxide in an "uncoupled" mode [181], a pathology that has been proposed as a major source of inflammatory ROS and subsequent endothelial dysfunction. We hypothesize that the superoxide is prevented from oxidizing sulfur by the glyphosate, and thus becomes a destructive agent in the artery wall.

9.1. Lysosomal Dysfunction

In [177], it was proposed that lysosomal dysfunction could be predicted to follow long-term impairment of eNOS' sulfate synthesis. Lysosomes, the "digestive system" of the cell, require substantial membrane cholesterol both to prevent hydrogen ion leaks and to protect membrane lipids from oxidative damage. Lysosomes also depend upon internalized sulfate, derived from heparan sulfate proteoglycans (HSPGs), to catalyze hydrolytic enzymes. Severe neurological dysfunction associated with lysosomal storage diseases involving impaired heparan sulfate homeostasis attest to the importance of sulfate in lysosomal function [182].

It has become increasingly apparent that lysosomal dysfunction is a major factor in Alzheimer's disease and Parkinson's disease [183], as well as in cardiovascular disease [184] and heart failure [185]. Mitochondria are ordinarily constantly broken down and renewed by lysosomal processes, and, when these become impaired, large aged mitochondria become a source of reactive oxygen species that contribute significantly to neuronal damage. Cardiomyocytes, like neurons, are long-lived postmitotic cells that are especially susceptible to lysosomal disrepair [186].

9.2. Tetrahydrobiopterin

The research literature has identified the cofactor tetrahydrobiopterin (BH4) as a significant player in eNOS function [187,188]. BH4 shifts the heme iron in eNOS to a high spin state, as well as increasing arginine binding, thus catalyzing the synthesis of NO by eNOS [187]. The synthesis of BH4 from its substrate GTP is induced by IFN-γ, which, in turn, is induced by bacterial lipopolysaccharides (LPS) [189]. Thus, a bacterial infection will induce NO synthesis by eNOS. However, an excess of exogenous NO (as might be expected to occur through iNOS synthesis of NO during a bacterial infection) causes a decrease in NO synthesis by eNOS with a simultaneous increase in superoxide synthesis, an effect that can lead to severe hypertension in infants with congenital heart disease treated

with inhaled NO [187]. Superoxide's reaction with NO to produce the highly toxic peroxynitrite ($ONOO^-$), a potent bacteriocidal agent, is likely a critical factor. The subsequent oxidation of BH_4 disrupts its ability to act as a cofactor [188], and causes "eNOS uncoupling," leading to superoxide synthesis in a highly disruptive feedback loop.

We hypothesize that glyphosate's nitrosylation of the active P450 site has derailed eNOS' ability to synthesize sulfate in a contained environment at the caveolar sites in the membrane, thus requiring an alternative method to synthesize sulfate that exposes the cell to ROS. This method, as previously described in [177,190], involves the oxidation of homocysteine thiolactone, catalyzed by ascorbic acid (vitamin C) and retinoic acid (vitamin A). Since glyphosate enhances the bioavailability of retinoic acid through its suppression of the CYP enzyme that metabolizes it [115], this will help to promote the alternative reaction leading to sulfate synthesis in the artery wall from homocysteine thiolactone, but also requiring the inflammatory agent, superoxide, which over time destroys the artery wall, leading to endothelial dysfunction and cardiovascular disease.

Elevated serum homocysteine is a strong risk factor in cardiovascular disease [191], in heart failure [192], in dementia [193], and in kidney failure [194,195]. We propose that sulfur-containing amino acids are deflected towards homocysteine synthesis in order to supply substrate for the critically-needed sulfate synthesis from superoxide in the artery wall. This also explains both the inflammation in the artery wall associated with atherosclerosis [196] and the deficiency in methionine associated with glyphosate, due to its depletion through its role as a substrate for homocysteine synthesis.

10. Involvement of the Brain

Impairment in the homeostasis of serotonin, an important neurotransmitter that regulates mood, appetite and sleep, has been linked to depression [197], autism [198], and Alzheimer's disease [199,200], as well as obesity [132]. We have already seen how glyphosate's induction of tryptophan-derived flavonoids and tryptophan's incorporation into macrophages as kynurenine via IPO can explain reduced brain serotonin levels. Vitamin D3 deficiency can also contribute to mood disorders, and is hypothesized to be a key factor in the syndrome, Seasonal Affective Disorder (SAD), manifested as depressed mood specifically during the winter months [201]. Excess ammonia and zinc deficiency are also implicated in neuronal disorders, particularly Alzheimer's disease, attention deficit hyperactivity disorder (ADHD), and autism. DNA methylation impairment is a factor in neuronal diseases, and glyphosate's depletion of methionine could contribute to this defect. Below, we elaborate on the effects of serotonin depletion, excess ammonia, zinc depletion, and methylation impairments on disorders of the brain. We conclude this section with specific mention of a possible role for glyphosate in two other diseases of the brain: multiple sclerosis and Parkinson's disease.

10.1. Serotonin, Mood Disorders, and Autism

Defects in serotonin transport are associated with a wide range of mood disorders. Major depression is accompanied by immune system activation, and the term "inflammatory and neurodegenerative (I&ND) hypothesis" has been used to describe this complex [202]. A demonstrated increased production of cytokines and immunoglobulins against bacterial-derived lipopolysaccharides points to increased gut permeability as a feature in depression [203]. Patients with depression and sleep disorders exhibit significantly lower

serum levels of tryptophan along with serum markers of inflammation such as IL-6 and IL-8 [204]. Selective serotonin reuptake inhibitors (SSRI's) are a popular class of drugs to treat depression: they work by impairing serotonin reuptake in the synapse, effectively increasing its bioavailability for neuronal signaling. This strongly suggests that insufficient serotonin in the synapse could be a factor in depression. In fact, dietary tryptophan depletion leads to relapse in recovering depressed patients [197].

Defects in the serotonin transporter gene, 5-HTT, have been associated with antisocial personality disorder and violent behavior [205]. There has been a marked increase in the rate of irrational school-associated violent deaths in the United States since 1990 [206], and glyphosate may play a role in this pattern through depletion of serotonin bioavailability. Disturbances in serotonin function in the brain are known factors in impulsive aggression, violence, and criminal behavior [207]. Farmers in India experienced anomalously high suicide rates following adoption of Western agricultural methods based on extensive use of Roundup® [208]. While an explanation based on economic stress has been proposed, suicide victims in general have low serotonin levels in the brain [209], so it is conceivable that serotonin suppression via depletion of tryptophan by glyphosate played a role in the suicides among farmers in India.

Genetic mutations in serotonin transporter genes have been found in association with both obsessive compulsive disorder and autism [210]. A study comparing 40 children with idiopathic infantile autism with normal controls showed a significantly lower serum ratio of tryptophan to large neutral amino acids [211]. 35% of the children with autism had a ratio that was at least two standard deviations below the mean value from the control group. It has been shown that dietary tryptophan depletion exacerbates anxiety and repetitive ritualistic behaviors in autistic subjects [198], an effect that was surmised to be due to impaired serotonin synthesis. Researchers have studied a mouse model of a defective serotonin transporter gene which results in a decrease in the bioavailability of serotonin for neuronal signaling in the brain, and have shown that the genetically modified mice exhibit autism-like behaviors [212]. This strongly suggests that impaired serotonin supply in the brain is a feature of autism.

Melatonin, produced from serotonin, is secreted by the pineal gland, primarily at night, and is a potent antioxidant and regulator of redox reactions [213,214]. Its neuroprotective role in aging and many neurodegenerative conditions such as Alzheimer's disease and Parkinson's disease is most likely due to its antioxidant effects [215–218]. Thus, it is anticipated that glyphosate would lead to impaired antioxidant protection, due to the suppression of melatonin synthesis, following the depletion of tryptophan as substrate, as previously discussed. Since melatonin is also a regulator of the wake/sleep cycle, impaired melatonin supply will lead to sleep disorders.

10.2. Ammonia, Autism and Alzheimer's Disease.

As stated previously, glyphosate enhances ammonia synthesis in susceptible plants via activation of the enzyme PAL [22], and gut microbes could produce excess ammonia through enhanced PAL synthesis under the influence of glyphosate. A parallel between autism and hepatic encephalitis has been made, emphasizing the role that ammonia plays as a toxin in the brain in both cases [219,220]. Ammonia has also been proposed to play a critical role in the etiology of Alzheimer's disease [221]. Thus, excess ammonia synthesis by gut bacteria under the influence of glyphosate could be a factor in both autism and Alzheimer's disease.

10.3. A Role for Zinc Deficiency.

Zinc deficiency is a major problem worldwide, particularly in the developing world, where limited access to zinc-rich foods such as shellfish and excess dietary exposure to phytates both contribute to the problem [222]. Phytates, found in many nuts and grains, bind to dietary minerals and interfere with their absorption. *Lactobacilli* and other beneficial gut bacteria produce the enzyme phytase, which catalyses the release of phosphate from phytates and improves the intestinal absorption of important minerals such as iron and zinc [223]. Because glyphosate reduces the number of these types of bacteria in the gut, it should enhance the chelating potential of phytates. This is likely a protective measure to avoid excess bioavailability of free phosphate, which is problematic in transport in the presence of glyphosate. Glyphosate's known ability to itself chelate divalent cations is likely a factor as well. Zinc deficiency increases the risk of diarrhea, pneumonia and malaria in infants and young children.

Zinc is the most abundant trace metal in the brain [224]. Most of the amyloid-β degrading enzymes are zinc metalloproteases, and zinc is also critical in the nonamyloidogenic processing of the amyloid precursor protein. Hence, zinc deficiency in the brain would be expected to lead to the build-up of amyloid-β, a key factor in the development of Alzheimer's disease. Zinc deficiency has also been implicated in autism [225] and ADHD [226,227]. Zinc is released into the synapse along with the neurotransmitter glutamate, and it is required for memory function and the maintenance of synaptic health as we age [228]. In [225], anomalously low zinc levels in hair analyses were found in children on the autism spectrum. In [226], zinc sulfate supplements improved ADHD symptoms, an effect that could be attributed to the supply of sulfate as well as zinc.

In [229], it was proposed that zinc deficiency along with excess exposure to copper may be a key factor in Alzheimer's disease. A study conducted in South Africa revealed that zinc supplementation was not effective in raising plasma levels of zinc in zinc-deficient Alzheimer's patients unless both vitamin A and vitamin D were simultaneously supplemented [230]. Hence, vitamin D3 deficiency (which could be caused by glyphosate's impairment of liver CYP enzymes) may interfere with zinc absorption, further depleting the supplies to the tissues.

10.4. Methylation Impairment

Methylation impairment has been observed in association with autism [231] and Alzheimer's disease [232], and this is caused by an inadequate supply of the substrate, methionine. While human cells are unable to synthesize methionine, it can be synthesized by many enteric bacteria, for example from cysteine via the transsulfuration pathway or through de novo synthesis from inorganic sulfur [233]. Glyphosate has been shown to significantly impair methionine synthesis in plants [21], and it may therefore be anticipated that it would have a similar effect in gut bacteria, which could then impair methionine bioavailability in humans. A further factor is the depletion of methionine through its deflection towards the transsulfuration pathway, as a precursor for homocysteine, which will be consumed to supply sulfate to the endothelial wall when eNOS' sulfate synthesis is impaired. Since methionine is the source of methyl groups in methylation pathways, this effect of glyphosate could contribute directly to methylation impairment.

10.5. Molecular Mimicry and Multiple Sclerosis

An increased incidence of inflammatory bowel disease has been found in association with multiple sclerosis (MS) [234]. This could be explained by the hypothesis that gut bacteria leaking into the vasculature cause an immune reaction, and that molecular mimicry leads to an autoimmune disorder resulting in destruction of the myelin sheath. A systematic search comparing reported sequences from all known human bacterial and viral agents against three known encephalitogenic peptides identified matching mimics predominantly in gut bacteria [235]. This could explain why no infective agents have been found in association with MS, but would also suggest that the recent increase in the incidence of MS [236] may be traced to inflammatory bowel disease, and, hence, to glyphosate.

10.6. Dopamine and Parkinson's Disease

Since dopamine is synthesized from tyrosine and its precursor phenylalanine, tyrosine and phenylalanine depletion by glyphosate in both plants and microbes would be expected to reduce their bioavailability in the diet. It has been demonstrated that dietary reductions of phenylalanine and tyrosine induce reduced dopamine concentrations in the brain [237]. Impaired dopaminergic signaling in the substantia nigra is a key feature of Parkinson's disease, and Parkinson's risk has been associated with exposure to various pesticides, including the herbicide paraquat [238], although, to our knowledge, glyphosate has not yet been studied in this respect. However, exposure of C. elegans to glyphosate led to pathology in the nervous system in the region analogous to the nigrostriatal dopamine system associated with Parkinson's disease [239].

Sulfate deficiency in the brain has been associated with Parkinson's disease, as well as Alzheimer's disease and Amyotrophic Lateral Sclerosis (ALS) [240]. We have argued above that glyphosate disrupts sulfate transport from the gut to the liver, and may interfere with sulfate synthesis by eNOS in the arterial wall and in RBCs and platelets, leading over time to severe sulfate deficiency throughout all the tissues. This would further impact these devastating diseases of the elderly, all of which are currently on the rise.

11. Other Adverse Health Effects

In this section, we will briefly mention several other pathologies in which we suspect that glyphosate may play a role in the observed increases in incidences in recent times. These include liver disease, cancer, cachexia, and developmental and fertility problems.

11.1. Liver Disease

Cytokines like TNF-α have been identified as a key factor in fatty liver disease, which has emerged as a growing public health problem worldwide [241]. In the extreme case, liver pathology develops into nonalcoholic steatohepatitis (NASH), which can lead to cirrhosis and liver failure. Cytokines induce inflammation which damages the liver. TNF-α inhibits insulin signaling [242,243], and cytokines can induce hepatic lipid overloading as well as liver fibrosis. Glyphosate's role in inducing cytokines has already been developed in this paper. Obesity is associated with an increased expression of membrane-associated TNF-α in adipose tissue [244].

11.2. Development and Fertility

Cholesterol sulfate plays an essential role in fertilization [245] and zinc is essential to the male reproductive system [246], with a high concentration found in semen. Thus, the likely reduction in the bioavailability of these two nutrients due to effects of glyphosate could be contributory to infertility problems. Furthermore, glyphosate's suppression of CYP protein activity would be expected to disrupt steroidogenesis. Inflammation leads to excess ROS and RNS exposure, which can damage DNA during cell replication, thus disrupting embryonic development. Glyphosate is capable of crossing the placental barrier [247]. Preeclampsia, a life-threatening condition for both the mother and the fetus that develops during the third trimester, is on the rise in America, and it has been proposed that this may be due to impaired sulfate supply [248], directly attributable to glyphosate exposure. For all of these reasons, glyphosate exposure would lead to infertility problems.

According to the World Bank, the fertility rate in Argentina peaked at 3.39 in 1978, and has been declining steadily since then. The rate of decline accelerated during the last five years of the twentieth century. Social pressures certainly explain some of the drop in birth rate, but it is possible that environmental factors, such as glyphosate, also play a role. 1994 was the year that the FDA authorized the sale of Roundup Ready® (RR) soybeans in the North American market [249], and Argentina followed suit two years later. "After they were authorized in 1996, RR soybeans spread through Argentina at an absolutely unprecedented speed in the history of agriculture: an average of more than two million acres a year." [249]. Argentina now exports 90% of its soybeans, which have become a monoculture crop and a cash cow.

The fertility rate in Brazil has also dropped dramatically over the past several decades from six children per woman on average to fewer than two, now lower than that of the United States. Brazil is the second largest producer and exporter of soybeans in the world behind Argentina, and it has embraced genetically modified soybeans engineered to be glyphosate-tolerant as a means to increase production since the mid 1990's. A rapidly evolving glyphosate-resistant weed population in Brazil due to genetically engineered glyphosate-tolerant crops is leading to increased use of glyphosate in recent years [250], the same time period in which a rapid drop in birth rates was observed. A steady increase in the rate of preterm births in Brazil over the past two decades has been noted, although the cause remains elusive. For instance, the rate increased from 6% in 1982 to 15% in 2004 in the town of Pelotas [251]. It is conceivable that increased exposure to glyphosate is contributing to this problem. This idea is in line with a study of an Ontario farm population, which revealed that glyphosate exposure any time during pregnancy was associated with a statistically significant increased risk of a late-pregnancy spontaneous abortion [252].

The birth rates in Western Europe have been declining for decades, with Germany's rate now being 1.36 children per woman. Birth rates have also been declining in the U.S since 2007, and are now at 1.9 children per woman, according to the 2011 government statistics [253].

Testicular Leydig cells produce testosterone, and thus play a crucial role in male reproductive function. The protein StAR (steroidogenic acute regulatory protein) mediates the rate-limiting step in steroidogenesis. In a recent *in vitro* study of a mouse tumor Leydig cell line, Roundup was shown to disrupt StAR expression, thus interfering with testosterone synthesis [254]. It was shown that

Roundup® interferes with testosterone synthesis even at very low environmental doses, and higher doses were associated with necrosis and apoptosis in rat testicular cells.

In [255], the *in vitro* effects of several different pesticides and herbicides on the synthesis of progesterone in testicular Leydig cells were investigated. Comparing eight different pesticides (Ammo®, Banvel®, Cotoran®, Cyclone®, Dual®, Fusilade® and Roundup®), it was found that, among these eight, Roundup® uniquely disrupted the cells' ability to produce progesterone, reducing synthesis levels by up to 94% in a dose-dependent manner, without reducing total protein synthesis.

For steroidogenesis, in addition to StAR, the side chain cleavage enzyme (P450scc) is required as well. The authors in [255] found that Roundup® inhibited both P450scc activity and StAR activity. Through formal measurements, it was determined that Roundup® reduced StAR protein levels by 90%, and inhibited P450scc activity by 71%. Glyphosate acting alone did not decrease steroidogenesis, suggesting that one or more of the adjuvants in Roundup® work in concert with glyphosate to suppress synthesis levels, *e.g.*, by enabling glyphosate entry into the cell through a surfactant effect or perhaps acting on their own to inhibit synthesis. StAR plays an important role in steroid production not only in the reproductive organs but also in the adrenal glands. Thus, Roundup® exposure would be expected to adversely affect fertility and impair the synthesis of glucocorticoids and mineralocorticoids in the adrenal glands.

Sea urchins are a popular model for studying mitosis in development. During mitosis, DNA damage or replication errors (for example due to excess exposure to ROS and RNS) leads to cell cycle arrest at certain "checkpoints" in G1, S, or G2 phase [256]. Cyclin-dependent protein kinases (CDKs) are important regulators of these checkpoints, signaling the "go-ahead" to transition to the next phase. Glyphosate in combination with the adjuvants in Roundup® experimentally induced a cell cycle delay in the transition from G2 to M phase in sea urchin embryos [257,258]. CDK1, acting on cyclin B, universally regulates the M-phase of the cell cycle, and Roundup® was shown to delay activation of CDK1/cyclin B via tyrosine 15 dephosphorylation *in vivo*, the likely means by which it interferes with cell cycle progression.

11.3. Cancer

While glyphosate is not generally believed to be a carcinogen, a study on a population of professional pesticide applicators who were occupationally exposed to glyphosate revealed a substantial increased risk to multiple myeloma [259]. Myeloma has been associated with agents that cause DNA damage [260], and DNA damage is a known consequence of chronic exposure to inflammatory agents, which, we have argued, are induced by glyphosate acting on the gut bacteria and suppressing CYP activity. Depleted supply of tryptophan as a substrate for poly-ADP ribose also contributes to DNA damage.

Multiple myeloma accounts for around 15% of all lymphatohematopoietic cancers and around 2% of all cancer deaths each year in the United States [261]. Symptoms include bone destruction, hypercalcemia, anemia, kidney damage and increased susceptibility to infection. Obesity is a known risk factor [261], so one way in which glyphosate could increase risk indirectly is through its potential role as an obesogen.

Virtually all multiple myelomas involve dysregulation of a cyclin D gene [262]. Overexpression of cyclin D protein releases a cell from its normal cell-cycle control and could cause a transformation to a malignant phenotype. The fact that glyphosate suppresses cyclin-dependent kinase could be a factor in inducing pathological overexpression of the substrate, cyclin D.

Another type of cancer that may be implicated with glyphosate exposure is breast cancer. The strongest evidence for such a link comes from the studies on rats exposed to glyphosate in their food supply throughout their lifespan, described previously, where some of the female rats succumbed to massive mammary tumors [9]. The incidence of breast cancer has skyrocketed recently in the United States, to the point where now one in three women is expected to develop breast cancer in their lifetime.

Breast cancer risk is associated with certain polymorphisms of the CYP gene CYP1A2 and the sulfotransferase, SULT1A1 [263], and this in turn is associated with altered estrogen and testosterone expression, along with increased premenopausal breast density, a risk factor for breast cancer [264]. In [263], it was suggested that impaired sulfation capacity could lead to slower metabolism of sex hormones and subsequent increased breast density, as well as increased risk to breast cancer. This suggests that disruption of CYP1A2 and/or of sulfate bioavailability by glyphosate could lead to a similar result. A high body mass index (BMI) is associated with low CYP1A2 activity in premenopausal women (p = 0.03) [265], and, as we have seen, the low CYP1A2 activity may be a reflection of glyphosate suppression of CYP enzymes, in association with glyphosate depletion of tryptophan as an obesogenic influence, and glyphosate disruption of sulfate synthesis by eNOS.

Obese postmenopausal women are at increased risk to breast cancer compared with lean postmenopausal women [266]. Studies on Zucker rats exposed to 7,12-dimethylbenz(a)anthracene, a chemical procarcinogen known to produce mammary adenocarcinoma in rats, demonstrated a much stronger susceptibility in obese rats compared to lean rats [267]. By the end of the study, obese rats had a 68% tumor incidence, compared to only 32% in lean rats. Subcutaneous fat expresses aromatase, and this increased expression has been suggested to play a role in inducing the increased risk, through the resulting increased estrogen synthesis [268,269]. It has been shown that inflammation increases aromatase expression in the mammary gland and in adipose tissue. Since we have developed an argument that glyphosate can lead to inflammation, this again suggests a link between glyphosate and breast cancer.

11.4. Cachexia

Cachexia (muscle wasting) is a frequent debilitating complication of cancer, AIDS, and other chronic inflammatory diseases. The loss of muscle mass arises from accelerated protein degradation via the ubiquitin-proteasome pathway, which requires ubiquitin conjugating of designated proteins prior to their disposal [270]. The ubiquitin-conjugating pathway is stimulated by TNF-α, thus promoting muscle breakdown. In [271], it was shown that TNF-α upregulates expression of the ubiquitin ligase atrogin1/MAFbx in skeletal muscle, via the mitogen-activated kinase (MAPK) pathway. Thus increased TNF-α expression as a consequence of the inflammatory response associated with glyphosate exposure could be a factor in cachexia.

12. Glyphosate in Food Sources

Following its successful commercial introduction in 1974 in the U.S., glyphosate has now become the dominant herbicide worldwide [6]. In large part this is due to its perceived lack of toxicity in humans. Since becoming generic in 2000, the dramatic drop in cost has encouraged global use of the generic version. Today, it is estimated that 90% of the transgenic crops grown worldwide are glyphosate resistant. The rapidly growing problem of glyphosate-resistant weeds is reflected in steady increases in the use of glyphosate on crops.

Table 1. Estimated range for glyphosate usage in agriculture in the U.S. as a function of year. Range is estimated in units of millions of pounds. These data were obtained from the EPA [272].

Glyphosate usage for the USA (Range in millions of pounds)				
Year	2001	2003	2005	2007
Range	85–90	128–133	155–160	180–185

Today, Americans spray more than 100 million pounds of Roundup®, the most popular among Monsanto's chemicals, on their yards and farms every year. According to the most recent statistics from the U.S. Environmental Protection Agency (EPA) [272], the U.S. currently represents 25% of the total world market on herbicide usage. Glyphosate, first registered for use in 1974, has been the most common herbicide used in the United States since 2001, and the amount of glyphosate usage has increased steadily since then, as shown in Table 1. In 2007, the most recent year for which such numbers are available, the U.S. used an estimated 180 to 185 million pounds of glyphosate, more than doubling the amount used just six years before.

The Western diet is a delivery system for toxic chemicals used in industrial agriculture. The diet consists primarily of processed foods based on corn, wheat, soy and sugar, consumed in high quantities. Chemical residues of insecticides, fungicides and herbicides like glyphosate contaminate the entire diet. Over the last decade, there has been widespread adoption in the U.S. of Roundup Ready® (RR) crops, particularly for the major productions of soy, beet sugar, and corn that supply the processed food industry. The recent alarming rise in type-2 diabetes has been attributed to excess intake of high fructose corn syrup, which has increased to unprecedented levels in the last decade [273]. This refined sugar is now usually derived from glyphosate-exposed GM corn. GM cotton is also increasingly being used as a source for cottonseed oil, widely present in processed foods such as potato chips, due to its low cost. A recent comparison between glyphosate-sensitive and glyphosate-resistant soybean crops revealed that the resistant plants took up much higher levels of glyphosate into their leaves [274]. A corollary is that these plants would be expected to yield much higher glyphosate concentrations in derived food sources, compared to their non-GMO counterparts.

Confined animal feeding operations (CAFOs) are used to produce dietary animal protein for a mostly non-agrarian population [275]. Cows, pigs, sheep, goats, chickens and even farm-raised fish and shrimp are fed a diet primarily of genetically engineered grains and forage materials laced with herbicide. As a consequence, animal products like, eggs, butter, cheese and milk are also contaminated with these residues. The highest levels of glyphosate are found in grain and sugar crops. The herbicide

is not only used with RR crops, but also, as previously mentioned, it is used as a preharvest desiccant on sugar cane, wheat and also RR sugar beets, canola, and cottonseed for oils, among others.

It is difficult to get information on actual amounts of glyphosate present in foods, due to the perception that it is nontoxic to humans [1,6]. The USDA Pesticide Data Program (PDP) is a voluntary program which randomly monitors agricultural chemical residues in the food supply. A search of the most recent data for 2010, published in May 2012, found statistics for the most popular agricultural chemicals except for glyphosate and glufosinate, another organophosphate. Residue data for the most popular herbicide on the planet were not available, but, interestingly, information on atrazine and other herbicides were readily available. Communication with USDA revealed that no data were available due to lack of monitoring. However, in 2013, for the first time, the USDA will be releasing a small amount of data for glyphosate residues only in soy. Lack of program funding was cited as the reason for this lack of data.

Recently, residue levels have been on the rise, due to higher rates and frequency of application, which in turn is due to increasing weed resistance. This has led the chemical and biotech industry to demand approvals for higher residue standards. In 1999 both the European Union (EU) and the UK raised the maximal glyphosate levels allowed in soy for industry from 0.1 ppm to 20 ppm. Both the USA and Argentina supply glyphosate-laden grains to European markets, so one could expect to find similar levels in the U.S.

The European Union's current standard for glyphosate in lentils is 0.1 mg/kg but a new industry proposal seeks to raise the standard by more than 100 times to 10 mg/kg or even 15 mg/kg [276]. This is not due to safety considerations, but rather to levels that are anticipated, following usage of the herbicide as a preharvest desiccant. The action will ignore the possible effects to public health. The effects of animal health from ingestion of glyphosate residues have also been ignored. Current standards for residues in feed and forage materials are totally out of line with those of humans. Tolerances for animal grass and corn forage are 300 and 400 ppm respectively. It is apparent that the EPA standard is being ignored on a global scale for industry at the expense of public health and the environment.

13. Discussion

Glyphosate is today the most popular herbicide in use in agricultural practices in the U.S., and, increasingly, throughout the world. Its usage rate has accelerated significantly in the last decade due mainly to two factors: (1) the patent expiration in 2000 led to greatly reduced cost, and (2) the adoption of genetically modified crops that are resistant to its toxic effects allows for higher exposure with little loss in harvest yield. The notion that glyphosate has minimal toxicity in humans, widely popularized by Monsanto, has prevented farmers from using caution in their application of this chemical to their crops.

The recent rise in the rates of autism diagnoses in the United States is a cause for alarm. We have recently proposed that autism can be characterized as a chronic low-grade encephalopathy, where the cascade of events taking place in the brain is a process that enables the renewal of severely depleted sulfate supplies to the brain [277]. We identified a dysbiosis in the gut as a source of ammonia that initiates the encephalytic response, and we proposed glyphosate as one of the many environmental toxins that might be responsible for the dysbiosis and for sulfate depletion. A review of the literature

on glyphosate has confirmed our suspicions that glyphosate might play a role, and, further, have led us to believe that glyphosate may be the most significant environmental toxin contributing to autism. While it is pervasive in our food supply, the fact that it is deemed by most regulators to be nontoxic makes it especially insidious. The key pathological biological effects of glyphosate -- disruption of the gut bacteria, impairment of sulfate transport, and interference with CYP enzyme activity—can easily explain the features that are characteristic of autism.

The term "developmental immunotoxicity" has been coined to describe permanent modifications to the immune function that take place early in life, leading to later development of allergies, asthma, and autoimmune diseases [278–280]. These authors have argued that prenatal and/or early life exposure to environmental toxins can lead to a phenotype that includes a hyperinflammatory response and disruption of cytokine networks, and they propose that an increased exposure to environmental toxins early in life may contribute to the increased incidence of these conditions observed today. It is significant that these problems often occur in association with autism [281].

Contrary to the current widely-held misconception that glyphosate is relatively harmless to humans, the available evidence shows that glyphosate may rather be the most important factor in the development of multiple chronic diseases and conditions that have become prevalent in Westernized societies. In addition to autism, these include gastrointestinal issues such as inflammatory bowel disease, chronic diarrhea, colitis and Crohn's disease, obesity, cardiovascular disease, depression, cancer, cachexia, Alzheimer's disease, Parkinson's disease, multiple sclerosis, and ALS, among others. While glyphosate is obviously not the only environmental toxin to contribute to these diseases and conditions, glyphosate's ability to disrupt the gut bacteria, to impair serum transport of sulfate and phosphate, and to interfere with CYP enzymes, logically progresses to this multitude of diseased states, through well-established biological processes. And glyphosate's disruption of the body's ability to detoxify other environmental toxins leads to synergistic enhancement of toxicity. While genetics surely play a role in susceptibility, genetics may rather influence *which* of these conditions develops in the context of glyphosate exposure, rather than *whether* any of these conditions develops.

We have explained the logical sequence of events leading to serotonin deficiency and subsequent pathologies, following glyphosate's disruption of tryptophan synthesis by gut bacteria [10,29], and its further sequestration into macrophages that infiltrate the intestinal tissues in order to detoxify lipopolysaccharides released from pathogenic bacteria, whose overgrowth is induced by glyphosate [35]. Sulfate depletion arises in the gut, both because of impaired transport of free sulfate in the bloodstream and impaired sulfate synthesis by eNOS [63,64]. Disruption of gut bacteria, exposure to toxic phenolic compounds necessary to enable sulfate transport, and deficient sulfate supply to the mucopolysaccharides in the gut all contribute to the leaky gut syndrome that is a common feature in autism [51]. The evidence shows that glyphosate can interfere with development through its suppression of aromatase synthesis [110] and through its interference with the breakdown of retinoic acid [113] and its interference with CDKs and sulfate supplies. Glyphosate could also be a factor in the current epidemic in vitamin D3 deficiency [166] through its disruption of the CYP enzymes that activate this hormone in the liver [164,165]. The kosmotropic property of the glyphosate molecule combined with its disruption of CYP enzymes in the blood stream can lead to excess thrombosis and hemorrhaging, common problems today among the elderly.

We propose that glyphosate's disruption of the synthesis of sulfate by the CYP orphan enzyme, eNOS, leads to widespread deficiencies in cholesterol and sulfate in the blood stream and all the tissues. We have previously described how disruption of eNOS' synthesis of sulfate would lead to diabetes and cardiovascular disease [177]. Glyphosate's induction of excess synthesis of ammonia in the gut, combined with depletion of zinc through impaired absorption, depletion of serotonin through dysbiosis of its substrate, tryptophan, depletion of dopamine through impaired synthesis of its substrate, tyrosine, depletion of vitamin D3, due to impairments in the CYP enzyme responsible for its activation, and depletion of sulfate through interference with its synthesis, can all lead to a multitude of pathologies in the brain, including autism, Alzheimer's disease, ADHD, Parkinson's disease, multiple sclerosis and ALS.

There is a substantial alignment among countries, worldwide, with low or decreasing birth rates, emerging obesity problems, and an increasing glyphosate burden. Given the arguments presented here, it is plausible that glyphosate is causal in these trends. It may also be possible to demonstrate strong correlations between glyphosate usage and both autism and breast cancer. Formal epidemiological studies should be conducted to look at these issues more closely.

In our opinion, it is imperative that governments around the globe unite in investing significant research funds to support independent studies evaluating the long-term effects of glyphosate. Other researchers should try to reproduce the results obtained in [9] showing tumorigenesis and premature death in rats with life-long exposure to glyphosate. The study on the gut microbiome of chickens [35] needs to be reproduced in other species, and the gene array study on *E. coli* [39] needs to be reproduced for other common gut bacteria. The novel idea that glyphosate disrupts sulfate transport through its kosmotropic effects, as predicted given biophysical laws, needs to be verified in specific studies among a variety of species. This could be done by comparing the levels of free sulfate in the blood under conditions of glyphosate exposure against controls. The study on glyphosate's effects on bees [126] should be reproduced by other researchers, along with further studies examining the impact of prior exposure to glyphosate on bees' resistance to pesticides. More refined and economical methods for detecting glyphosate in the food supply, such as in [0,283], and in the water supply [284], need to be developed, and then applied to a variety of different food items. Most critical in our view are the vegetable oils derived from GM crops □ canola oil, soybean oil, corn oil, and cottonseed oil, as well as soy-derived protein, beet sugar, and high fructose corn syrup – ingredients that are pervasive in processed foods. Glyphosate is likely also present in meat, eggs, cheese, and other dairy products derived from animals fed glyphosate-contaminated grass, alfalfa, corn, and soy [285,286].

14. Conclusion

This paper presents an exhaustive review of the toxic effects of the herbicide, glyphosate, the active ingredient in Roundup®, in humans, and demonstrates how glyphosate's adverse effects on the gut microbiota, in conjunction with its established ability to inhibit the activity of cytochrome P450 enzymes, and its likely impairment of sulfate transport, can remarkably explain a great number of the diseases and conditions that are prevalent in the modern industrialized world. Its effects are insidious, because the long-term effects are often not immediately apparent. The pathologies to which glyphosate could plausibly contribute, through its known biosemiotic effects, include inflammatory bowel disease,

obesity, depression, ADHD, autism, Alzheimer's disease, Parkinson's disease, ALS, multiple sclerosis, cancer, cachexia, infertility, and developmental malformations. Glyphosate works synergistically with other factors, such as insufficient sun exposure, dietary deficiencies in critical nutrients such as sulfur and zinc, and synergistic exposure to other xenobiotics whose detoxification is impaired by glyphosate. Given the known toxic effects of glyphosate reviewed here and the plausibility that they are negatively impacting health worldwide, it is imperative for more independent research to take place to validate the ideas presented here, and to take immediate action, if they are verified, to drastically curtail the use of glyphosate in agriculture. Glyphosate is likely to be pervasive in our food supply, and, contrary to being essentially nontoxic, it may in fact be the most biologically disruptive chemical in our environment.

Acknowledgements

This work was funded in part by Quanta Computers, Taipei, Taiwan, under the auspices of the Qmulus Project. We thank three reviewers whose valuable comments led to a much improved version of this paper.

References

1. Williams, G.M.; Kroes, R.; Munro, I.C. Safety evaluation and risk assessment of the herbicide roundup and its active ingredient, glyphosate, for humans. *Regul. Toxicol. Pharm*. **2000**, *31*, 117–165.
2. Battaglin, W.A.; Kolpin, D.W.; Scribner, E.A.; Kuivila, K.M.; Sandstrom, M.W. Glyphosate, other herbicides, and transformation products in midwestern streams, 2002. *J. Am. Water Resour. Assoc.* **2005**, *41*, 323–332.
3. Shaw, D.R.; Barrentine, W.L. Herbicide combinations for preharvest weed desiccation in early maturing soybean (Glycine max). *Weed Technol.* **1998**, *12*, 157–165.
4. Baur, J.R.; Miller, F.R.; Bovey, R.W. Effects of preharvest desiccation with glyphosate on grain sorghum. *Seed* **1977**, *69*, 1015–1018.
5. Baig, M.N.; Darwent, A.L.; Harker, K.N.; O'Donovan, J.T. Preharvest applications of glyphosate affect emergence and seedling growth of field pea (Pisum sativum). *Weed Technol.* **2003**, *17*, 655–665.
6. Duke, S.O.; Powles, S.B. Glyphosate: A once-in-a-century herbicide. *Pest. Manag. Sci.* **2008**, *64*, 319–325.
7. Weed Science Society of America Committee. In *Herbicide Handbook of the Weed Science Society of America*, 4th ed.; Weed Science Society of America: Champaign, IL, USA, 1979.
8. Smith, E.A.; Oehme, F.W. The biological activity of glyphosate to plants and animals: A literature review. *Vet. Hum. Tocicol.* **1992**, *34*, 531–543.
9. Séralini, G.-E.; Clair, E.; Mesnage, R.; Gress, S.; Defarge, N.; Malatesta, M.; Hennequin, D.; Spiroux de Vendˆomois, J. Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. *Food Chem. Toxicol.* **2012**, *50*, 4221–4231.
10. Herrmann, K.M.; Weaver, L.M. The shikimate pathway. *Annu. Rev. Plant. Physiol. Plant. Mol. Biol*. **1999**, *50*, 473–503.

11. Moco, S.; Martin, F.-P.J.; Rezzi, S. Metabolomics view on gut microbiome modulation by polyphenol-rich foods *J. Proteome Res.* **2012**, *11*, 4781–4790.
12. Ganal, S.C.; Sanos, S.L.; Kallfass, C.; Oberle, K.; Johner, C.; Kirschning, C.; Lienen-klaus, S.; Weiss, S.; Staeheli, P.; Aichele, P.; *et al*. Priming of natural killer cells by nonmucosal mononuclear phagocytes requires instructive signals from commensal microbiota. *Immunity* **2012**, *37*, 171–186.
13. Hashimoto, T.; Perlot, T.; Rehman, A.; Trichereau, J.; Ishiguro, H.; Paolino, M.; Sigl, V.; Hanada, T.; Hanada, R.; Lipinski, S. *et al.* ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature* **2012**, *487*, 477–483. (The same with ref.160)
14. Littman, D.R.; Pamer, E.G. Role of the commensal microbiota in normal and pathogenic host immune responses. *Cell. Host Microbe* **2011**, *10*, 311–323.
15. Holmes, E.; Loo, R.L.; Stamler, J.; Bictash, M.; Yap, I.K.; Chan, Q.; Ebbels, T.; De Iorio, M.; Brown, I.J.; Veselkov, K.A. *et al.* Human metabolic phenotype diversity and its association with diet and blood pressure. *Nature* **2008**, *453*, 396–400.
16. Ashorn, M. Gastrointestinal diseases in the paediatric age groups in Europe: epidemicology and impact on healthcare. *Aliment. Pharmacol. Ther.* **2003**, *18*, 80–83.
17. Bewtra, M.; Su, C.; Lewis, J.D. Trends in hospitalization rates for inflammatory bowel disease in the United States. *Clin. Gastroenterol. Hepatol*. **2007**, *5*, 597–601.
18. de María, N.; Becerril J.M.; García-Plazaola, J.I.;Ndez, A.H.; de Felipe, M.R.; Fernández-Pascual, M. New insights on glyphosate mode of action in nodular metabolism: Role of shikimate accumulation. *J. Agric. Food Chem*. **2006**, *54*, 2621–2628.
19. Richards, T.A.; Dacks, J.B.; Campbell, S.A.; Blanchard, J.L.; Foster, P.G.; McLeod, R.; Roberts, C.W. Evolutionary origins of the eukaryotic shikimate pathway: Gene fusions, horizontal gene transfer, and endosymbiotic replacements. *Eukaryot. Cell.* **2006**, *5*, 1517–1531.
20. Henry, W.B.; Koger, C.H.; Shaner, D.L. Accumulation of shikimate in corn and soybean exposed to various rates of glyphosate. *Crop. Management* **2005**. Available online: http://www.plantmanagementwork.org/sub/cm/research/2005/shikimate/ (accessed on 10 February 2013)
21. Nafziger, E.D.; Widholm, J.*M.;* Steinrcken, H.C.; Killmer, J.L. Selection and Characterization of a Carrot Cell Line Tolerant to Glyphos*ate. Plant. Physiol. **1984,** 76,* 571–574.
22. Howles, P.A.; Sewalt, V.J.H.; Paiva, N.L.; Elkind, Y.; Bate, N.J.; Lamb, C.; Dixon, R.A. Overexpression of L-phenylalanine ammonia-lyase in transgenic tobacco plants reveals control points for flux into phenylpropanoid biosynthesis. *Plant. Physiol*. **1996**, *112*, 1617–1624.
23. Guillet, G.; Poupart, J.; Basurco, J.; De Luca, V. Expression of tryptophan decarboxylase and tyrosine decarboxylase genes in tobacco results in altered biochemical and physiological phenotypes. *Plant. Physiol*. **2000**,*122*, 933–943.
24. Duke, S.O.; Hoagland, R.E.; Elmore, C.D. Effects of glyphosate on metabolism of phenolic compounds V. L-aminooxy-phenylpropionic acid and glyphosate effects on phenylalanine ammonia-lyase in soybean seedlings. *Plant Physiol.* **1980**, *65*, 17–21.
25. Michalowicz, J.; Duda, W. Phenols sources and toxicity. *Polish J. Environ. Stud*. **2007**, *16*, 347–362.
26. Ortega-García, F.; Peragón, J. Phenylalanine ammonia-lyase, polyphenol oxidase, and phenol concentration in fruits of *Olea europaea* L. cv. Picual, Verdial, Arbequina, and Frantoio during ripening. *J. Agric. Food Chem.* **2009**, *57*, 10331–10040.

27. Hoagland, R.E. Effects of glyphosate on metabolism of phenolic compounds: VI. Effects of glyphosine and glyphosate metabolites on phenylalanine ammonia-lyase activity, growth, and protein, chlorophyll, and anthocyanin levels in soybean (Glycine max) seedlings. *Weed Sci.* **1980**, *28*, 393–400.
28. Duke, S.O.; Hoagland, R.E. Effects of glyphosate on metabolism of phenolic compounds I. Induction of phenylalanine ammonia-lyase activity in dark-grown maize roots. *Plant Sci. Lett.* **1978**, *11*, 185–190.
29. Zhao, J.; Williams, C.C.; Last, R.L. Induction of Arabidopsisl tryptophan pathway enzymes and camalexin by amino acid starvation, oxidative stress, and an abiotic elicitor. *Plant Cell* **1998**, *10*, 359–370.
30. Hernandez, A.; Garcia-Plazaola, J.I.; Becerril, J.M. Glyphosate effects on phenolic metabolism of nodulated soybean (*Glycine max* L. Merr.). *J. Agric. Food Chem*. **1999**, *47*, 2920–2925.
31. Moorman, T.B.; Becerril, J.M.; Lydon, J.; Duke, S.O. Production of hydroxybenzoic acids by Bradyrhizobium japonicum strains after treatment with glyphosate. *J. Agric. Food Chem*. **1992**, 289–293.
32. Becerra-Moreno, A.; Benavides, J.; Cisneros-Zevallos, L.; Jacobo-Velázquez, D.A. Plants as biofactories: Glyphosate-induced production of shikimic acid and phenolic antioxidants in wounded carrot tissue. *J. Agric. Food Chem*. **2012**, *60*, 11378–11386
33. Duke, S.O.; Vaughn. K.C.; Wauchope, R.D. Effects of glyphosate on uptake, translocation, and intracellular localization of metal cations in soybean (Glycine max) seedlings. *Pestic. Biochem. Phys.* **1985**, *24*, 384–394.
34. Cakmak, I.; Yazici, A.; Tutus, Y.; Ozturk, L. Glyphosate reduced seed and leaf concentrations of calcium, manganese, magnesium, and iron in non-glyphosate resistant soybean. *Eur. J. Agron.* **2009**, *31*, 114–119.
35. Krüger, M.; Shehata, A.A.; Schrödl, W.; Rodloff, A. Glyphosate suppresses the antagonistic effect of *Enterococcus* spp. on Clostridium botulinum. *Anaerobe* **2013**, *20*, 74–78.
36. Shehata, A.A.; Schrödl, W.; Aldin, A.A.; Hafez, H.M.; Krüger, M. The effect of glyphosate on potential pathogens and beneficial members of poultry microbiota *in vitro*. *Curr. Microbiol*. **2013** *66*, 350–358.
37. Shinabarger, D.L.; Braymer, H.D. Glyphosate catabolism by Pseudomonas sp. strain PG2982. *J. Bacteriol*. **1986** , *168*, 702–707.
38. Nie, C.L.; Wang, X.S.; Liu, Y.; Perrett, S.; He, R.Q. Amyloid-like aggregates of neuronal tau induced by formaldehyde promote apoptosis of neuronal cells. *BMC Neurosci*. **2007**, *8*, 9.
39. Lu, W.; Li, L.; Chen, M.; Zhou, Z.; Zhang, W.; Ping, S.; Yan, Y.; Wang, J.; Lin, M. Genome-wide transcriptional responses of Escherichia coli to glyphosate, a potent inhibitor of the shikimate pathway enzyme 5-enolpyruvylshikimate-3-phosphate synthase. *Mol. Biosyst.* **2013**, *9*, 522–530.
40. Abdel-Mallek, A.Y.; Abdel-Kader, M.I.; Shonkeir, A.M. Effect of glyphosate on fungal population, respiration and the decay of some organic matters in Egyptian soil. *Microbiol. Res.* **1994**, *149*, 69–73.
41. Relyea, R.A. The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecol. Appl.* **2005**, *15*, 618–627.

42. Paetow, L.J.; McLaughlin, J.D.; Pauli, B.D.; Marcogliese, D.J. Mortality of American bullfrog tadpoles lithobates catesbeianus infected by Gyrodactylus jennyae and experimentally exposed to Batrachochytrium dendrobatidis. *J. Aquat. Anim. Health* **2013**, *25*, 15–26.
43. Crawford, A.J.; Lips, K.R.; Bermingham, E. Epidemic disease decimates amphibian abundance, species diversity, and evolutionary history in the highlands of central Panama. *PNAS* **2010**, *107*, 13777–13782.
44. Larsen, K.; Najle, R.; Lifschitz, A.; Virkel, G. Effects of sub-lethal exposure of rats to the herbicide glyphosate in drinking water: glutathione transferase enzyme activities, levels of reduced glutathione and lipid peroxidation in liver, kidneys and small intestine. *Environ. Toxicol. Pharmacol*. **2012**, *34*, 811–818.
45. Mañas, F.J.; Peralta, L.; Garca Ovando, H.; Weyers, A.; Ugnia, L.; Gorla, N. Genotoxicity of glyphosate and AMPA evaluated through comet assay in blood and hepatocytes of treated mice. *Biocell.* **2009**, *33*, A80.
46. Kim, Y.H.; Hong, J.R.; Gil, H.W.; Song, H.Y.; Hong, S.Y. Mixtures of glyphosate and surfactant TN20 accelerate cell death via mitochondrial damage-induced apoptosis and necrosis. *Toxicol. In Vitro* **2013**, *27*, 191–197.
47. Clair, E.; Linn, L.; Travert, C.; Amiel, C.; Séralini, G.E.; Panoff, J.M. Effects of Roundup and glyphosate on three food microorganisms: Geotrichum candidum, Lactococcus lactis subsp. cremoris and Lactobacillus delbrueckii subsp. bulgaricus. *Curr. Microbiol*. **2012**, *64*, 486–491.
48. Mariager, T.P.; Madsen, P.V.; Ebbehøj, N.E.; Schmidt, B.; Juhl, A. Severe adverse effects related to dermal exposure to a glyphosate-surfactant herbicide. *Clin. Toxicol. (Phila.).* **2013**, *51*, 111–113.
49. Deo. S.P.; Shetty, P. Accidental chemical burns of oral mucosa by herbicide. *JNMA J. Nepal Med. Assoc*. **2012**, *52*, 40–42.
50. Williams, B.L.; Hornig, M.; Buie, T.; Bauman, M.L.; Cho Paik, M.; Wick, I.; Bennettt, A.; Jabado, O.; Hirschberg, D.L.; Lipkin, W.I. Impaired carbohydrate digestion and transport and mucosal dysbiosis in the intestines of children with autism and gastrointestinal disturbances. *PLoS One* **2011**, *6*, e24585.
51. Horvath, K.; Perman, J.A. Autism and gastrointestinal symptoms. *Current Gastroenterology Reports* **2002**, *4*, 251–258.
52. Wang, L.; Christophersen, C.T.; Sorich, M.J.; Gerber, J.P.; Angley, M.T.; Conlon, M.A. Elevated fecal short chain fatty acid and ammonia concentrations in children with autism spectrum disorder. *Dig. Dis. Sci.* **2012**, *57*, 2096–2102.
53. MacFabe, D.F. Short-chain fatty acid fermentation products of the gut microbiome: implications in autism spectrum disorders. *Microb. Ecol. Health Di.* **2012**, *23*, 19260.
54. Song, Y.; Liu, C.; Finegold, S.M. Real-Time PCR quantitation of Clostridia in feces of autistic children. *Appl. Environ. Microbiol.* **2004**, *70*, 6459–6465.
55. Wakefield, A.J.; Puleston, J.M.; Montgomery, S.M.; Anthony, A.; O'Leary, J.J.; Murch, S.H. Review article: The concept of enterocolonic encephalopathy, autism and opioid receptor ligands. *Aliment. Pharmacol. Ther*. **2002**, *16*, 663–674.
56. Shawcross, D.; Jalan, R. The pathophysiologic basis of hepatic encephalopathy: central role for ammonia and inflammation. *Cell. Mol. Life Sci*. **2005**, *62*, 2295–2304.

57. Lemberg, A.; Fernández, A. Hepatic encephalopathy, ammonia, glutamate, glutamine and oxidative stress. *Ann. Hepatol*. **2009**, *8*, 95–102.
58. Romero-Gmez, M.; Jover, M.; Galn, J.J.; Ruiz, A. Gut ammonia production and its modulation. *Metab. Brain Dis.* **2009**, *24*, 147–157.
59. MacDonald, M.J. and DCunha, G.B. A modern view of phenylalanine ammonia lyase. *Biochem. Cell. Biol*. **2007**, *85*, 273–282.
60. Clayton, T.A. Metabolic differences underlying two distinct rat urinary phenotypes, a suggested role for gut microbial metabolism of phenylalanine and a possible connection to autism. *FEBS Lett.* **2012**, *586*, 956–961.
61. Hartzell, S.; Seneff, S. Impaired sulfate metabolism and epigenetics: Is there a link in autism? *Entropy* **2012**, *14*, 1953–1977.
62. Kern, J.K.; Grannemann, B.D.; Trivedi, M.H.; Waring, R.H.; Ramsden, D.B.; Garver, C.R. Abnormal sulfation chemistry in autism. In *Trends in Autism Research*; Ryaskin, O.T., Ed.; Nova Publishers: Hauppauge, NY, USA, 2004; Chapter XI.
63. Sivsammye, G.; Sims, H.V. Presumptive identification of Clostridium difficile by detection of p-cresol in prepared peptone yeast glucose broth supplemented with p-hydroxyphenylacetic acid. *J. Clin. Microbiol*. **1990**, *28*, 1851–1853.
64. D'Ari, L.; H.A. Barker, H.A. p-Cresol formation by cell free extracts of Clostridium difficile, *Arch. Microbiol*. **1985**, *143*, 311–312.
65. Kelly, C.P.; Pothoulakis, C.; LaMont, J.T. Clostridium difficile colitis. *N. Engl. J. Med*. **1994**, *330*, 257–262.
66. Issa, M.; Vijayapal, A.; Graham, M.B.; Beaulieu, D.B.; Otterson, M.F.; Lundeen, S.; Skaros, S.; Weber, L.R.; Komorowski, R.A.; Knox, J.F.; Emmons, J.; Bajaj, J.S.; Binion, D.G. Impact of Clostridium difficile on inflammatory bowel disease. *Clin. Gastroenterol. Hepatol*. **2007**, *5*, 345–351.
67. Clayton, T.A.; Baker, D.; Lindon, J.C.; Everett, J.R.; Nicholson, J.K. Phar-macometabonomic identification of a significant hostmicrobiome metabolic interaction affecting human drug metabolism. *Proc. Natl. Am. Sci.* **2009**, *106*, 14728–14733.
68. Altieri, L.; Neri, C.; Sacco, R.; Curatolo, P.; Benvenuto, A.; Muratori, F.; Santocchi, E.; Bravaccio, C.; Lenti, C.; Saccani, M. *et al.* Urinary p-cresol is elevated in small children with severe autism spectrum disorder. *Biomarkers* **2011**, *16*, 252–260.
69. Buckman, N.G.; Hill, J.O.; Magee, R.J.; McCormick, M.J. Separation of substituted phenols, including eleven priority pollutants using high performance liquid chromatography, *J. Chromatogr*. **1984**, *284*, 441–446.
70. Azad, M.B.; Konya, T.; Maughan, H.; Guttman, D.S.; Field, C.J.; Chari, R.S.; Sears, M.R.; Becker, A.B.; Scott, J.A.; Ozyrskyj, A.L. Gut microbiota of healthy Canadian infants: Profiles by mode of delivery and infant diet at 4 months. *Can. Med. Assoc. J.* **2013** *185*, 385–394.
71. Schultz, S.T.; Klonoff-Cohen, H.S.; Wingard, D.L.; Akshoomoff, N.A.; Macera, C.A.; Ji, M.; Bacher, C. Breastfeeding, infant formula supplementation, and autistic disorder: The results of a parent survey. *Int. Breastfeed. J.* **2006**, *1*, 16.
72. van der Heiden, C.; Wauters, E.A.K.; Ketting, D.; Duran, M.; Wadman, S.K. Gas chromatographic analysis of urinary tyrosine and phenylalanine metabolites in patients with gastrointestinal disorders. *Clin. Chim. Acta.* **1971**, *34*, 289–296.

73. Shaw, W. Increased urinary excretion of a 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPHPA), an abnormal phenylalanine metabolite of Clostridia spp. in the gastrointestinal tract, in urine samples from patients with autism and schizophrenia. *Nutr. Neurosci*. **2010**, *13*, 135–143.
74. Yap, I.K.; Angley, M.; Veselkov, K.A.; Holmes, E.; Lindon, J.C.; Nicholson, J.K. Urinary metabolic phenotyping differentiates children with autism from their unaffected siblings and age-matched controls. *J. Proteome Res*. **2010**, *9*, 2996–3004.
75. Gatley, S.J.; Sherratt, H.S. The synthesis of hippurate from benzoate and glycine by rat liver mitochondria. Submitochondrial localization and kinetics. *Biochem. J*. **1977**, *166*, 39–47.
76. Ashwood, P.; Anthony, A.; Pellicer, A.A.; Torrente, F.; Walker-Smith, J.A.; Wakefield, A.J. Intestinal lymphocyte populations in children with regressive autism: Evidence for extensive mucosal immunopathology. *J. Clin. Immunol*. **2003**, *23*, 504–517.
77. O'Reilly, B.A.; Waring, R.H. Enzyme and sulphur oxidation deficiencies in autistic children with known food/chemical intolerances. *Xenobiotica*. **1990**, *20*, 117–122.
78. Baldwin, R.L. How Hofmeister ion interactions affect protein stability. *Biophys. J*. **1996**, *71*, 2056–2063.
79. Hofmeister, F. Naunyn-Schmiedebergs Zur Lehre von der Wirkung der Salze (Article in German). *Arch. Pharmacol*. **1888**, *24*, 247–260.
80. Zouaoui, K.; Dulaurent, S.; Gaulier, J.M.; Moesch, C.; Lachâtre, G. Determination of glyphosate and AMPA in blood and urine from humans: About 13 cases of acute intoxication. *Forensic Sci. Int*. **2013**, *226*, e20–e25.
81. Xia, F.; Nagrath, D.; Garde, S.; Cramer, S.M. Evaluation of selectivity changes in HIC systems using a preferential interaction based analysis. *Biotech. Bioengineer*. **2004**, *87*, 354–363.
82. Falany, C.N. Molecular enzymology of human liver cytosolic sulfotransferases. *Trends Pharmacol. Sci*. **1991**, *12*, 255–259.
83. Berg, N.B.; Young, R.W. Sulfate metabolism in pancreatic acinar cells. *J. Cell. Biol*. **1971**, *50*, 469–483.
84. Goldman, R.; Claycamp, G.H.; Sweetland, M.A.; Sedlov, A.V.; Tyurin, V.A.; Kisin, E.R.; Tyurina, Y.Y.; Ritov, V.B.; Wenger, S.L.; Grant, S.G.; Kagan, V.E. Myeloperoxidase-catalyzed redox-cycling of phenol promotes lipid peroxidation and thiol oxidation in HL-60 cells. *Free Radic. Biol. Med*. **1999**, *27*, 1050–1063.
85. Prior, R.L.; Wu, X.; Gu, L. Flavonoid metabolism and challenges to understanding mechanisms of health effects. *J. Sci. Food Agric*. **2006**, *86*, 2487–2491.
86. Walle, T.; Hsieh, F.; DeLegge, M.H.; Oatis, J.E., Jr.; Walle, U.K. High absorption but very low bioavailability of oral resveratrol in humans. *Drug Metab. Dispos*. **2004**, *32*, 1377–1382.
87. Tang, L.; Zhou, J.; Yang, C.H.; Xia, B.J.; Hu, M.; Liu, Z.Q. Systematic studies of sulfation and glucuronidation of 12 flavonoids in the mouse liver S9 fraction reveal both unique and shared positional preferences. *J. Agric. Food Chem*. **2012**, *28*, 60, 3223–3233.
88. El-Demerdash, F.M.; Yousef, M.I.; Elagamy, E.I. Influence of paraquat, glyphosate, and cadmium on the activity of some serum enzymes and protein electrophoretic behavior (*in vitro*). *J. Environ. Sci. Health B* **2001**, *36*, 29–42.

89. Qiu, H.; Geng, J.; Ren, H.; Xia, X.; Wang, X.; Yu, Y. Physiological and biochemical responses of Microcystis aeruginosa to glyphosate and its Roundup® formulation. *J. Hazard. Mater.* **2012**, 172–176.
90. Adams, J.B.; George, F.; Audhya, T.J. Abnormally high plasma levels of vitamin B6 in children with autism not taking supplements compared to controls not taking supplements. *J. Altern. Complement. Med*. **2006**, *12*, 59–63.
91. Martineau, J.; Barthelemy, C.; Garreau, B.; Lelord, G. Vitamin B6, magnesium, and combined B6-Mg: Therapeutic effects in childhood autism. *Biol. Psych*. **1985**, *20*, 467–478.
92. Lelord, G.; Muh, J.P.; Barthelemy, C.; Martineau, J.; Garreau, B. Effects of pyridoxine and magnesium on autistic symptoms—Initial observations. *J. Autism Devel. Disord*. **1981**, *11*, 219–230.
93. Cohen, B.I. The significance of ammonia/gamma-aminobutyric acid (GABA) ratio for normality and liver disorders. *Med. Hypotheses* **2002**, *59*, 757–758.
94. Sweeten, T.L.; Posey, D.J.; Shankar, S.; McDougle, C.J. High nitric oxide production in autistic disorder: a possible role for interferon-gamma. *Biol. Psychiatry* **2004**, *55*, 434–437.
95. Sögüt,S.S.; Zoroglu, S.S.; Özyurt,H.; Ylmaz,H.R.; Ozugurlu,F.; Sivasli,E.; Yetkin,O.; Yanik,M.; Tutkun, H.; Savas, H.A.; *et al.* Changes in nitric oxide levels and antioxidant enzyme activities may have a role in the pathophysiological mechanisms involved in autism. *Clin. Chim. Acta.* **2003**, *331*, 111–117.
96. Zoroğlu, S.S. Yürekli, M.; Meram, I.; Söğüt, S.; Tutkun, H.; Yetkin, O.; Sivasli, E.; Savaş, H.A.; Yanik, M.; Herken, H.;l Akyol, O. Pathophysiological role of nitric oxide and adrenomedullin in autism. *Cell. Biochem. Funct*. **2003**, *21*, 55–60.
97. Launay, J.M.; Ferrari, P.; Haimart, M.; Bursztejn, C.; Tabuteau, F.; Braconnier, A.; Pasques-Bondoux, D.; Luong, C. Serotonin Metabolism and other biochemical parameters in infantile autism: A controlled study of 22 autistic children. *Neuropsychobiology.* **1988**, *20*, 1–11.
98. Al-Yafee, Y.A.; Al-Ayadhi, L.Y.; Haq, S.H.; El-Ansary, A.K. Novel metabolic biomarkers related to sulfur-dependent detoxification pathways in autistic patients of Saudi Arabia. *BMC Neurol*. **2011**, *11*, 139.
99. Alberti, A.; Pirrone, P.; Elia, M.; Waring, R.H.; Romano, C. Sulphation deficit in "low-functioning" autistic children: A pilot study. *Biolog. Psychiat.* **1999**, *46*, 420–424.
100. Waring, R.H.; Kovrza, L.V. Sulphur metabolism in autism. *J. Nutr. Environ. Med.* **2000**, *10*, 25–32.
101. Finegold, S.M. Therapy and epidemiology of autism–clostridial spores as key elements. *Med. Hypotheses* **2008**, *70*, 508–511.
102. Murch, S.H.; MacDonald, T.T.; Walker-Smith, J.A.; Levin. M.; Lionetti, P.; Klein, N.J., Disruption of sulphated glycosaminoglycans in intestinal inflammation. *Lancet* **1993**, *341*, 711–714.
103. Finegold, S.M. Desulfovibrio species are potentially important in regressive autism. *Med. Hypotheses* **2011**, *77*, 270–274
104. Evans, W.C. Anaerobic degradation of aromatic compounds. *Ann. Rev. Microbiol.* **1988**, *42*, 289–317.
105. Coates, J.D.; Anderson, R.T.; Lovley, D.R. Oxidation of polycyclic aromatic hydrocarbons under sulfate-reducing conditions. *Appl. Environ. Microbiol*. **1996**, *62*, 1099–1101.

106. Rueter, P.; Rabus, R.; Wilkest, H.; Aeckersberg, F.; Rainey, F.A.; Jannasch, H.W.; Widdel, F. Anaerobic oxidation of hydrocarbons in crude oil by new types of sulphate-reducing bacteria. *Nature* **1994**, *372*, 455–458.
107. Londry, K.L.; Suflita, J.M.; Tanner, R.S. Cresol metabolism by the sulfate-reducing bacterium Desulfotomaculum sp. strain Groll. *Can. J. Microbiol.* **1999**, *45*, 458–463.
108. Shangari, N.; Chan, T.S.; O'Brien, P.J. Sulfation and glucuronidation of phenols: Implications in coenyzme Q metabolism. *Methods Enzymol.* **2005**, *400*, 342–359.
109. Gasnier, C.; Dumont, C.; Benachour, N.; Clair, E.; Chagnon, M.C.; Séralini, G.E. Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. *Toxicology* **2009**, *262*, 184–191.
110. Richard, S.; Moslemi, S.; Sipahutar, H.; Benachour, N.; S´eralini, G.-E. Differential effects of glyphosate and roundup on human placental cells and aromatase. *Environ. Health Perspect.* **2005**, *113*, 716–720.
111. Mottier, A.; Kientz-Bouchart, V.; Serpentini, A.; Lebel, J.M.; Jha, A.N.; Costil, K. Effects of glyphosate-based herbicides on embryo-larval development and metamorphosis in the Pacific oyster, *Crassostrea gigas*. *Aquat. Toxicol*. **2013**, *128–129*, 67–78.
112. Aulehla, A.; Pourqui, O. Signaling gradients during paraxial mesoderm development. *Cold Spring Harb. Perspect. Biol*. **2010**, *2*, a000869.
113. Paganelli, A.; Gnazzo, V.; Acosta, H.; Lpez, S.L.; Carrasco, A.E. Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling. *Chem. Res. Toxicol*. **2010**, *23*, 1586–1595.
114. William J. Ray, W.J.; Gerard Bain, G.; Min Yao, M.; and David I. Gottlieb, D.I. CYP26, a novel mammalian cytochrome P450, is induced by retinoic acid and defines a new family. *J. Biol. Chem.* **1997**, *272*, 18702–18708.
115. Fujii, H.; Sato, T.; Kaneko, S.; Gotoh, O.; Fujii-Kuriyama, Y.; Osawa, K.; Kato, S.; Hamada, H. Metabolic inactivation of retinoic acid by a novel P450 differentially expressed in developing mouse embryos. *EMBO J*. **1997**, *16*, 4163–4173.
116. Lamb, D.C.; Kelly, D.E.; Hanley, S.Z.; Mehmood, Z.; Kelly, S.L. Glyphosate is an inhibitor of plant cytochrome P450: Functional expression of thlaspi arvensae cytochrome P45071b1/reductase fusion protein in Escherichia coli. *Biochem. Biophys. Res. Comm*. **1998**, *244*, 110–114.
117. Hietanen, E.; Linnainmaa, K.; Vainio, H. Effects of phenoxyherbicides and glyphosate on the hepatic and intestinal biotransformation activities in the rat. *Acta. Pharmacol. Toxicol*. **1983**, *53*, 103–112.
118. Khan, S.U.; Young, J.C. N-Nitrosamine formation in soil from the herbicide glyphosate. *J. Agric. Food Chem*. **1977**, *25*, 1430–1432.
119. Su, K. N-nitrosamine formation in soil from the herbicide glyphosate and its uptake by plants. *ACS Symposium Series*. **1981**, *174*, 275–287.
120. Buchmann, A.; Kuhlmann, W.D.; Schwarz, M.; Kunz, W.; Wolf, C.R; Moll, E.; Friedberg, T.; Oesch, F. Regulation and expression of four cytochromes P-450 isoenzymes, NADPH-cytochrome P-450 reductase, the glutathione transferases B and C and microsomal epoxide hydrolase in preneoplastic and neoplastic lesions in rat liver. *Carcinogenesis* **1985**, *6*, 513–521.

121. Abass, K.; Turpeinen, M.; Pelkonen, O. An evaluation of the cytochrome P450 inhibition potential of selected pesticides in human hepatic microsomes. *J. Environ. Sci. Health B.* **2009**, *44*, 553–563.
122. Abass, K.; Lämsä, V.; Reponen, P.; Küblbeck Honkakoski, P.; Mattila, S.; Pelkonen, O. Hakkola, J. Characterization of human cytochrome P450 induction by pesticides. *Toxicology* **2012**, *294*, 17–26.
123. Rendic, S.; di Carlo Herd, F.J. Human cytochrome P450 enzymes: A status report summarlzlng thelr reactions, substrates, inducers, and inhibitors. *Drug Metab. Rev.* **1997**, *29*, 413–580.
124. Schacker, M. A *Spring Without Bees: How Colony Collapse Disorder Has Endangered Our Food Supply*; Globe Pequot: Guilford, CT. USA, 2008.
125. Mao, W.; Schuler, M.A.; Berenbaum, M.R. CYP9Q-mediated detoxification of acaricides in the honey bee (*Apis mellifera). Proc. Natl. Am. Soi.* **2011**, *108*, 12657–12662.
126. Morandin, L.A.; Winston, M.L. Wild bee abundance and seed production in conventional organic, and genetically modified canola. *Ecol. Appl.* **2005**, *15*, 871–881.
127. Foulk, K.E.; Reeves, C. Identifying the role of glyphosate-containing herbicides on honeybee mortality rates and colony collapse disorder. In Proceedings of Junior Science, Engineering, and Humanities Symposium, Camdenton, MO, USA, 2009; 2–23.
128. Ratnieks, F.L.W.; Carreck, N.L. Clarity on honey bee collapse? *Science* **2010**, *327*, 152–153.
129. Mohamed, F.; Gawarammana, I.; Robertson, T.A.; Roberts, M.S.; Palangasinghe, C.; Zawahir, S.; Jayamanne, S.; Jegenathen, K.; Eddleston, M; Buckley, N.; *et al.* Acute Human self-poisoning with Imidacloprid compound: A neonicotinoid insecticide. *Plos One* **2009**, *4*, e5127.
130. Baillie-Hamilton, P.F.. Chemical toxins: A hypothesis to explain the global obesity epidemic. *J. Altern. Complem. Med.* **2002**, *8*, 185–192.
131. Zimmermann, R.C.; McDougle, C.J.; Schumacher, M.; Olcese, J.; Mason, J.W.; Heninger, G.R.; Price, L.H. Effects of acute tryptophan depletion on nocturnal melatonin secretion in humans. *J. Clin. Endocr. MeTable* **1993**, *76*, 1160–1164.
132. Breisch, S.T.; Zemlan, F.P.; Hoebel, B.G. Hyperphagia and obesity following serotonin depletion by intraventricular p-chlorphenylalanine. *Science* **1976**, *192*, 382–385.
133. Moffett, J.R.; and MA ARYAN Namboodiri, M.A. Tryptophan and the immune response. *Immunol. Cell. Biol.* **2003**, *81*, 247–265.
134. Moffett, J.R.; Espey, M.G.; Namboodiri, M.A. Antibodies to quinolinic acid and the determination of its cellular distribution within the rat immune system. *Cell. Tissue. Res.* **1994**, *278*, 461–469.
135. Werner-Felmayer, G.; Werner, E.R.; Fuchs, D.; Hausen, A.; Reibnegger, G.; Wachter, H. Induction of indoleamine 2,3-dioxygenase in human cells *in vitro*. *Adv. Exp. Med. Biol.* **1991**, *294*, 505–509.
136. Yoshida, R.; Nukiwa, T.; Watanabe, Y.; Fujiwara, M.; Hirata, F.; Hayaishi, O. Regulation of indoleamine 2,3-dioxygenase activity in the small intestine and the epididymis of mice. *Arch. Biochem. Biophys*. **1980**, *203*, 343–351.
137. Yoshida, R. Hayaishi, O. Induction of pulmonary indoleamine 2,3-dioxygenase by in-traperitoneal injection of bacterial lipo-polysaccharide. *Proc. Natl. Acad. Sci. USA* **1978**, *75*, 3998–4000.
138. Carson, D.A.; Seto, S.; Wasson, D.B.; Carrera, C.J. DNA strand breaks, NAD metabolism, and programmed cell death. *Exp. Cell. Res*. **1986**, *164*, 273–281.

139. Hageman, G.J.; Stierum, R.H. Niacin, poly (ADP-ribose) polymerase-1 and genomic stability. *Mutat. Res.* **2001**, *475*, 45–56.
140. Satoh, M.S.; Poirier, G.G.; Lindahl, T. Dual function for poly (ADP-ribose) synthesis in response to DNA strand breakage. *Biochemistry* **1994**, *33*, 7099–7106.
141. Hayaishi, O. Utilization of superoxide anion by indoleamine oxygenase-catalyzed tryptophan and indoleamine oxidation. *Adv. Exp. Med. Biol.* **1996**, *398*, 285–289.
142. Caballero, B.; Finer, N.; Wurtman, R.J. Plasma amino acids and insulin levels in obesity: response to carbohydrate intake and tryptophan supplements. *Metabolism* **1988**, *37*, 672–676.
143. Breum, L.; Rasmussen, M.H.; Hilsted, J.; Fernstrom, J.D. Twenty-four hour plasma tryptophan concentrations and ratios are below normal in obese subjects and are not normalized by substantial weight reduction. *Am. J. Clin. Nutr*. **2003**, *77*, 1112–1118.
144. Fei, N.; Liping Zhao, L. An opportunistic pathogen isolated from the gut of an obese human causes obesity in germfree mice. *ISME J*. **2013**, *7*, 880–884.
145. Woods, S.C.; Seeley, R.J.; Rushing, P.A.; DAlessio, D.; Tso, P. A controlled high-fat diet induces an obese syndrome in rats. *J. Nutr*. **2003**, *133*, 1081–1087.
146. Johnson, R.J.; Segal, M.S.; Sautin, Y.; Nakagawa, T.; Feig, D.I.; Kang, D.-H.; Gersch, M.S.; Benner, S.; Sanchez-Lozada, L.G. Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. *Am. J. Clin. Nutr*. **2007**, *86*, 899–906.
147. Deckelbaum, R.J.; Williams, C.L. Childhood obesity: The health issue. *Obes. Res.* **2001**, *9*, 239S–243S.
148. Rolls, B.J. The supersizing of America: Portion size and the obesity epidemic. *Nutrition Today* **2003**, *38*, 42–53.
149. Popkins, B.M.; Doak, C.M. The obesity epidemic is a worldwide phenomenon. *Nutr. Rev*. **1998**, *56*, 106–114.
150. Puoane, T.; Steyn, K.; Bradshaw, D.; Laubscher, R.; Fourie, J.; Lambert, V.; Mbananga, N. Obesity in South Africa: the South African demographic and health survey. *Obes. Res.* **2002**, *10*, 1038–1048.
151. Friedberg, S.; Horowitz, L. Converging Networks and Clashing Stories: South Africa's Agricultural Biotechnology Debate. *Africa Today* **2004**, *51*, 325.
152. Scoones, I. Mobilizing Against GM Crops in India, South Africa and Brazil. *J. Agrar. Change* **2008**, *8*, 315–344.
153. WHO Global Infobase, Available online: https://apps.who.int/infobase/Indicators.aspx/ (accessed on 18 February 2013).
154. Hidaka, H.; Nagatsu, T.; Takeya, K.; Matsumoto, S; Yagi, K. Inactivation of serotonin by sulfotransferase system. *J. Pharmacol. Exp.Ther.* **1969**, *166*, 272–275.
155. Strott, C.A.; Higashi, Y. Cholesterol sulfate in human physiology: What's it all about? *J. Lipid Res*. **2003**, *44*, 1268–1278.
156. Croonenberghs, J.; Spaas, K.; Wauters, A.; Verkerk, R.; Scharpe, S.; Deboutte, D.; Maes, M. Faulty serotonin--DHEA interactions in autism: Results of the 5-hydroxytryptophan challenge test. *Neuro. Endocrinol. Lett*. **2008**, *29*, 385–390.

157. Hernández-Morante, J.J.; Pérez-de-Heredia, F.; Luján, J.A.; Zamora, S.; Garaulet, M.Role of DHEA-S on body fat distribution: Gender- and depot-specific stimulation of adipose tissue lipolysis. *Steroids* **2008**, *73*, 209–215.
158. Gómez-Santos, C.; Hernández-Morante, J.J.;Tébar, F.J.; Granero, E.; Garaulet, M. Differential effect of oral dehydroepiandrosterone-sulphate on metabolic syndrome features in pre- and postmenopausal obese women. *Clin. Endocrinol.* **2012**, *77*, 548–554.
159. Szymczak, J.; Milewicz, A.; Thijssen, J.H.H.; Blankenstein, M.A.; Daroszewski, J. Concentration of sex steroids in adipose tissue after menopause. *Steroids* **1998**, *63*, 319–321.
160. Loftus, E.V. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. *Gastroenterology* **2004**, *126*, 1504–1517.
161. Nebert, D.W.; Russell, D.W. Clinical importance of the cytochromes P450. *The Lancet* **2002**, *360*, 1155–1162.
162. Anzenbacher, P.; Anzenbacherova, E. Cytochromes p450 and metabolism of xenobiotics. *Cell. Mol. Life Sci.* **2001**, *58*, 737–747.
163. Stiles, A.R.; McDonald, J.G.; Bauman, D.R.; Russell, D.W. CYP7B1: One cytochrome P450, two human genetic diseases, and multiple physiological functions. *J. Biol. Chem.* **2009**, *284*, 28485–28489.
164. Wikvall, K. Cytochrome P450 enzymes in the bioactivation of vitamin D to its hormonal form (review). *Int. J. Mol. Med*. **2001**, *7*, 201–209.
165. Schuster, I. Cytochromes P450 are essential players in the vitamin D signaling system. *Biochim. Biophys. Acta* **2011**, *1814*, 186–199.
166. Ginde, A.A.; Liu, M.C.; A. Camargo, C.A.; Demographic Differences and Trends of Vitamin D Insufficiency in the US Population, 1988–2004. *JAMA Internal Medicine* **2009**, *169*, 626–632.
167. Miller, W.L. P450 oxidoreductase deficiency: a disorder of steroidogenesis with multiple clinical manifestations. *Sci. Signal*. **2012**, *5*,11.
168. Sarachana, T.; Xu, M.; Wu, R.C.; Hu, V.W. Sex hormones in autism: Androgens and estrogens differentially and reciprocally regulate RORA, a novel candidate gene for autism. *Plos One* **2011**, *6*, e17116.
169. Baron-Cohen, S. The extreme male brain theory of autism. *Trends Cog. Sci.* **2002**, *6*, 248–254.
170. Andreola, F.; Fernandez-Salguero, P.M.; Chiantore, M.V.; Petkovich, M.P.; Gonzalez, F.J.; De Luca, L.M. Aryl hydrocarbon receptor Ahr(−/−) knockout mice exhibit liver retinoid accumulation and reduced retinoic acid metabolism. *Cancer Res*. **1997**, *57*, 2835–2838.
171. Jetten, A.M.; George, M.A.; Pettit, G.R.; Herald, C.L.; Rearick, J.I. Action of phorbol esters, bryostatins, and retinoic acid on cholesterol sulfate synthesis: Relation to the multistep process of differentiation in human epidermal keratinocytes. *J. Invest. Dermatol*. **1989**, *93*, 108–115.
172. Lorbek, G.; Lewinska, M.; Rozman, D. Cytochrome P450s in the synthesis of cholesterol and bile acids–from mouse models to human diseases. *FEBS J.* **2012**, *279*, 1516–1533.
173. Sibbing, D.; Stegherr, J.; Latz, W.; Koch, W.; Mehilli, J.; Dörrler, K.; Morath, T.; Schömig, A.; Kastrati, A.; von Beckerath, N. Cytochrome P450 2C19 loss-of-function polymorphism and stent thrombosis following percutaneous coronary intervention. *Eur. Heart J.* **2009**, *30*, 916–922.

174. Luo, Y.; Zhao, Y.-T.; Verdo, A.; Qi, W.-G.; Zhang, D.-F.; Hu, B. Relationship between cytochrome P450 2C19*2 polymorphism and stent thrombosis following percutaneous coronary intervention in Chinese patients receiving clopidogrel. *The J. Int. Med. Res.* **2011**, *39*, 2012–2019.
175. Slofstra, S.H.; Spek, C.A.; ten Cate, H. Disseminated intravascular coagulation. *Hematol. J.* **2003**, *4*, 295–302.
176. Gorren, A.C.; Mayer, B. Nitric-oxide synthase: A cytochrome P450 family foster child. *Biochim. Biophys. Acta.* **2007**, *1770*, 432–445.
177. Seneff, S.; Lauritzen, A.; Davidson, R.; Lentz-Marino, L. Is endothelial nitric oxide synthase a moonlighting protein whose day job is cholesterol sulfate synthesis? Implications for cholesterol transport, diabetes and cardiovascular disease. *Entropy* **2012**, *14*, 2492–2530.
178. Cryle, M.J.; De Voss, J.J. Is the ferric hydroperoxy species responsible for sulfur oxidation in cytochrome P450s? *Angew. Chem. Int. Ed.* **2006**, *45*, 8221–8223.
179. Engelberg, H. Endogenous heparin activity deficiency: The missing link in atherogenesis? *Atherosclerosis* **2001**, *159*, 253–260.
180. Khalili, H.; Huang, E.S.; Ananthakrishnan, A.N.; Higuchi, L.; Richter, J.M.; Fuchs, C.S.; Chan, A.T. Geographical variation and incidence of inflammatory bowel disease among US women. *Gut* **2012,** *61*, 1686–1692
181. Thum, T.; Fraccarollo, D.; Schultheiss, M.; Froese, S.; Galuppo, P.; Widder, J.D.; Tsikas, D.; Ertl, G.; Bauersachs, J. Endothelial nitric oxide synthase uncoupling impairs endothelial progenitor cell mobilization and function in diabetes. *Diabetes* **2007**, *56*, 666–674.
182. Valstar, M.J.; Ruijter, G.J.G.; van Diggelen, O.P. Sanfilippo syndrome: A minireview. *J. Inherit. Metab. Dis*. **2008**, *31*, 240252.
183. Friedman, L.G.; Lachenmayer, M.L.; Wang, J.; He, L.; Poulose, S.M.; Komatsu, M.; Holstein, G.R.; Yue, Z. Disrupted autophagy leads to dopaminergic axon and dendrite degeneration and promotes presynaptic accumulation of -Synuclein and LRRK2 in the brain. *J. Neurosci*. **2012**, *32*, 7585–7593.
184. Terman, A.; Kurz, T.; Gustafsson, B.; Brunk, U.T. The involvement of lysosomes in myocardial aging and disease. *Curr. Cardiol. Rev*. **2008**, *4*, 107–115.
185. Takemura, G.; Miyata, S.; Kawase, Y.; Okada, H.; Maruyama, R.; Fujiwara, H.; Autophagic Degeneration and Death of Cardiomyocytes in Heart Failure. *Autophagy* **2006**, *2*, 212–214.
186. Terman, A.; Gustafsson, B.; Brunk, U.T. The lysosomal-mitochondrial axis theory of postmitotic aging and cell death. *Chem. Biol. Interact*. **2006**, *163*, 29–37.
187. Kumar, S.; Sun, X.; Sharma, S.; Aggarwal, S.; Ravi, K.; Fineman, J.R.; Black, S.M. GTP cyclohydrolase I expression is regulated by nitric oxide: role of cyclic AMP. *Am. J. Physiol. Lung Cell. Mol. Physiol*. **2009**, *297*, L309–L317.
188. Landmesser, U.; Dikalov, S.; Price, R.; McCann, L.; Fukai, T.; Holland, S.M.; Mitch, W.E.; Harrison, D.G. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *J. Clin. Invest*. **2003**, *111*, 1201–1209.
189. Werner, E.R.; Werner-Felmayer, G.; Fuchs, D.; Hausen, A.; Reibnegger, G.; Wachter, H. Parallel induction of tetrahydrobiopterin biosynthesis and indoleamine 2,3-dioxygenase activity in human cells and cell lines by interferon-gamma. *Biochem. J.* **1989**, *262*, 861–866.
190. McCully, K.S. Chemical pathology of homocysteine V: Thioretinamide, thioretinaco, and cystathionine synthase function in degenerative diseases. *Ann. Clin. Lab. Sci*. **2011**, *41*, 300313.

191. McCully, K.S. Homocysteine, vitamins, and vascular disease prevention. *Am. J. Clin. Nutr*. **2007**, *86*, 1563S–1568S.
192. Vasan, R.S.; Beiser, A.; D'Agostino, R.B.; Levy, D.; Selhub, J.; Jacques, P.E.; Rosenberg, I.H.; Wilson, P.W.F. Plasma homocysteine and risk for congestive heart failure in adults without prior myocardial infarction. *J. Am. Med. Assoc.* **2003**, *289*, 1251–1257.
193. Seshadri, S.; Beiser, A.; Selhub, J.; Jacques, P.F.; Rosenberg, I.H.; D'Agostino, R.B.; Wilson, P.W.F.; Wolf, P.A. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N. Engl. J. Med.* **2002**, *346*, 476–483.
194. van Guldener, C.; Stam, F.; Stehouwer, C.D. Homocysteine metabolism in renal failure. *Kidney Int. Suppl*. **2001**, 78, S234-S237.
195. van Guldener, C. Why is homocysteine elevated in renal failure and what can be expected from homocysteine-lowering? *Nephrol. Dial. Transplant.* **2006**, *21*, 1161–1166.
196. Libby, P.; Ridker, P.M.; Maseri, A. Inflammation and atherosclerosis. *Circulation* **2002**, *105*, 1135–1143.
197. Cowen, P.J. Serotonin and depression: pathophysiological mechanism or marketing myth? *Trends Pharmacol. Sci*. **2008**, *29*, 433–436.
198. McDougle, C.J.; Naylor, S.T.; Cohen, D.J.; Aghajanian, G.K.; Heninger, G.R.; Price, L.H. Effects of tryptophan depletion in drug-free adults with autistic disorder. *Arch. Gen. Psychiatry* **1996**, *53*, 993–1000.
199. Geldenhuys, W.J.; van der Schyf, C.J. Role of serotonin in Alzheimer's disease: A new therapeutic target? *CNS Drugs* **2011**, *25*, 765–781.
200. Meltzer, C.C.; Smith, G.; DeKosky, S.T.; Pollock, B.G.; Mathis, C.A.; Moore, R.Y.; Kupfer, D.J.; Reynolds, C.F., III. Serotonin in aging, late-life depression, and Alzheimer's disease: The emerging role of functional imaging. *Neuropsychopharmacology* **1998**, *18*, 407–430.
201. Lansdowne, A.T.G.; Provost S.C. Vitamin D3 enhances mood in healthy subjects during winter *Psychopharmacology* **1998**, *135*, 319–323.
202. Maes, M.; Kubera, M.; Leunis, J.-C. The gut-brain barrier in major depression: Intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression. *Neuroendocrin. Lett.* **2008**, *29*, 117–124.
203. Maes, M.; Yirmyia, R.; Noraberg, J.;Brene, S.; Hibbeln, J.; Perini, G.; Kubera, M.; Bob, P.; Lerer, B.; Maj, M. The inflammatory and neurodegenerative (I&ND) hypothesis of depression: Leads for future research and new drug developments in depression. *Metab. Brain Dis.* **2009**, *24*, 27–53.
204. Song, C.; Lin, A.; Bonaccorso, S.; Heide, C.; Verkerk, R.; Kenis, G.; Bosmans, E.; Scharpe, S.; Whelan, A.;Cosyns, P.; de Jongh, R.; Maes, M. The inflammatory response system and the availability of plasma tryptophan in patients with primary sleep disorders and major depression. *J. Affect. Disord*. **1998**, *49*, 211–219.
205. Hallikainen, T.; Saito, T.; Lachman, H.M.; Volavka, J.; Pohjalainen, T.; Ryynnen, O.P.; Kauhanen, J.; Syvlahti, E.; Hietala, J.; Tiihonen, J. Association between low activity serotonin transporter promoter genotype and early onset alcoholism with habitual impulsive violent behavior. *Mol. Psychiatr.* **1999**, *4*, 385–388.

206. Anderson, M.; Kaufman, J.; Simon, T.R.; Barrios, L.; Paulozzi, L.; Ryan, G.; Hammond, R.; Modzeleski, W.; Feucht, T.; Potter, L.; School-associated violent deaths in the United States, 1994–1999. *J. Am. Medical Assoc.* **2001**, *286*, 2695–2702.
207. Retz, W.; Retz-Junginger, P.; Supprian, T.; Thome, J.; Rösler, M. Association of serotonin transporter promoter gene polymorphism with violence: relation with personality disorders, impulsivity, and childhood ADHD psychopathology. *Behav. Sci. Law* **2004**, *22*, 415–425.
208. Shiva, V.; Jafri, A.H.; Emani, A.; Pande, M. *Seeds of Suicide: the Ecological and Human Costs of Globalisation of Agriculture*; Zed Books: London, UK, 2005.
209. Roy, A.; Linnoila, M. Suicidal behavior, impulsiveness and serotonin. *Acta Psychiatr. Scand*. **1988**, *78*, 529–535.
210. Sutcliffe, J.S.; Delahanty, R.J.; Prasad, H.C.; McCauley, J.L.; Han, Q.; Jiang, L.; Chun Li, C.; Folstein, S.E.; Blakely, R.D. Allelic heterogeneity at the serotonin transporter locus (SLC6A4) confers susceptibility to autism and rigid-compulsive behaviors. *Am. J. Hum. Genet*. **2005**, *77*, 265–279.
211. D'Eufemia, P.; Finocchiaro, R.; Celli, M.; Viozzi, L.; Monteleone, D.; Giardini, O. Low serum tryptophan to large neutral amino acids ratio in idiopathic infantile autism. *Biomed. Pharmacother*. **1995**, *49*, 288–292.
212. Veenstra-VanderWeele, J.; Muller, C.L.; Iwamoto, H.; Sauer, J.E.; Owens, W.A.; Shah, C.R.; Cohen, J.; Mannangatti, P.; Jessen, T.; J. Thompson, B.J.; *et al.* Autism gene variant causes hyperserotonemia, serotonin receptor hypersensitivity, social impairment and repetitive behavior. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 5469–5474.
213. Pandi-Perumal, S.R.; BaHammam, A.S.; Brown, G.M.; Spence, D.W.; Bharti, V.K.; Kaur, C.; Hardeland, R.; Cardinali, D.P. Melatonin antioxidative defense: Therapeutical implications for aging and neurodegenerative processes. *Neurotox. Res.* **2013**, *23*, 267–300.
214. Ortiz, G.G.; Bentez-King, G.A.; Rosales-Corral, S.A.; Pacheco-Moiss, F.P.; Velzquez-Brizuela, I.E. Cellular and biochemical actions of melatonin which protect against free radicals: Role in neurodegenerative disorders. *Curr. Neuropharmacol.* **2008**, *6*, 203–214.
215. Anderson, K.N.; Jamieson, S.; Graham, A.J.; Shneerson, J.M. REM sleep behaviour disorder treated with melatonin in a patient with Alzheimers disease. *Clin. Neurol. Neurosurg*. **2008**, *110*, 492–495.
216. Asayama, K.; Yamadera, H.; Ito, T.; Suzuki, H.; Kudo, Y.; Endo, S. Double blind study of melatonin effects on the sleep-wake rhythm, cognitive and non-cognitive functions in Alzheimer type dementia. *J. Nippon Med. Sch*. **2003**, *70*, 334–341.
217. Antolin, I.; Mayo, J.C.; Sainz, R.M.; del Brio, M.L.; Herrera, F.; Martin, V.; Rodríıguez, M.V.; Protective effect of melatonin in a chronic experimental model of Parkinsons disease. *Brain Res.* **2002**, *943*, 163–173.
218. Borah, A.; Mohanakumar, K.P. Melatonin inhibits 6-hydroxydopamine production in the brain to protect against experimental parkinsonism in rodents. *J. Pineal. Res.* **2009**, *47*, 293–300.
219. Wakefield, A.J. The Gut-Brain Axis in Childhood Developmental Disorders. *JPGN* **2002**, *34*, S14–S17.
220. Basile, A.S.; Jones, E.A. Ammonia and GABA-ergic neurotransmission: Interrelated factors in the pathogenesis of hepatic encephalopathy. *Hepatology.* **1997**, *25*, 1303–1305.

221. Seiler, N. Ammonia and Alzheimer's disease. *Neurochem. Int*. **2002**, *41*, 189–207.
222. Caulfield, L.E.; Black, R.E. Zinc deficiency. *Comparative Quantification of Health Risks: Global and Regional Burden of Disease Attributable to Selected Major Risk Factors*; Ezzati, M., Lopez, A.D., Rodgers, A.A., Murray, C.J.L., Eds.; World Health Organization: Geneva, Swiss, 2004; Chapter 5.
223. Famularo, G.; de Simone, C.; Pandey, V.; Sahu, A.R.; Minisola, G. Probiotic lactobacilli: an innovative tool to correct the malabsorption syndrome of vegetarians? *Med. Hypotheses* **2005**, *65*, 1132–1135.
224. Watt, N.T.; Whitehouse, I.J.; Hooper, N.M. The role of zinc in Alzheimers disease. *Int. J. Alz. Dis.* **2011**, *2011*, 971021.
225. Yasuda, H.; Yoshida, K.; Yasuda, Y.; Tsutsui, T. Infantile zinc deficiency: Association with autism spectrum disorders. *Scientific Reports* **2011**, *1*, 129.
226. Akhondzadeh, S.; Mohammadi, M.R.; Khademi, M. Zinc sulfate as an adjunct to methylphenidate for the treatment of attention deficit hyperactivity disorder in children: a double blind and randomised trial. *BMC Psychiatr.***2004**, *4*, 9.
227. Arnold, L.E.; Bozzolo, H.; Hollway, J.; Cook, A.; DiSilvestro, R.A.; Bozzolo, D.R.; Crowl, L.; Ramadan, Y.; Williams, C. Serum zinc correlates with parent- and teacher-rated inattention in children with attention-deficit/hyperactivity disorder. *J. Child. Adolesc. Psychopharmacol*. **2005**, *15*, 628–636.
228. Adlard, P.A.; Parncutt, J.M.; Finkelstein, D.I.; Bush, A.I. Cognitive loss in zinc transporter-3 knock-out mice: a phenocopy for the synaptic and memory deficits of Alzheimer's disease? *J. Neurosci*. **2010**, *30*, 1631–1736.
229. Brewer, G.J. Copper excess, zinc deficiency, and cognition loss in Alzheimer's disease. *Biofactors.* **2012**, *38*, 107–113.
230. Potocnik, F.C.; van Rensburg, S.J.; Hon, D.; Emsley, R.A.; Moodie, I.M.; Erasmus, R.T. Oral zinc augmentation with vitamins A and D increases plasma zinc concentration: Implications for burden of disease. *Metab. Brain Dis.* **2006**, *21*, 139–147.
231. James, J.; Cutler, P.; Melnyk, S.; Jernigan, S.; Janak, L.; Gaylor, D.W.; Neubrander, J.A. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. *Am. J. Clin. Nutr*. **2004**, *80*, 1611–1617.
232. Morrison, L.D.; Smith, D.D.; Kish, S.J. Brain s-adenosylmethionine levels are severely decreased in Alzheimer's disease. *J. Neurochem.* **1996**, *67*, 1328–1331.
233. Ejim, L.J.; D'Costa, V.M. ; Elowe, N.H.; Concepcíon Loredo-Osti, J.; Malo, D.; Wright, G.D. Cystathionine-Lyase is important for virulence of salmonella enterica serovar typhimurium. *Infect. Immun*. **2004**, *72*, 3310–3314.
234. Alkhawajah, M. M.; Caminero, A. B.; Freeman, H. J.; Oger, J. J. Multiple sclerosis and inflammatory bowel diseases: What we know and what we would need to know! *Mult. Scler.* **2013**, *19*, 259–265.
235. Westall, F.C. Molecular Mimicry Revisited: Gut Bacteria and Multiple Sclerosis. *J. Clin. Microbiol*. **2006**, *44*, 2099–2104.
236. Noonan, C.W.; Kathman, S.J.; White, M.C. Prevalence estimates for MS in the United States and evidence of an increasing trend for women. *Neurology* **2002**, *58*, 136–138.

237. Montgomery, A.J.; McTavish, S.F.B.; Cowen, P.J.; Grasby, P.M. Reduction of brain dopamine concentration with dietary tyrosine plus phenylalanine depletion: An [11C] Raclopride PET study. *Am. J. Psychiatry* **2003**, *160*, 1887–1889.
238. Costello, S.; Cockburn, M.; Bronstein, J.; Zhang, X.; Ritz. B. Parkinson's disease and residential exposure to maneb and paraquat from agricultural applications in the central valley of California. *Am. J. Epidemiol.* **2009**, *169*, 919–926.
239. Negga, R.; Rudd, D.A.; Davis, N.S.; Justice, A.N; Hatfield, H.E; Valente, A.L.; Fields, A.S. Fitsanakis, V.A. Exposure to Mn/Zn ethylene-bis-dithiocarbamate and glyphosate pesticides leads to neurodegeneration in Caenorhabditis elegans. *Neurotoxicology* **2011**, *32*, 331–341.
240. Heafield, M.T.; Fearn, S.; Steventon, G.B.; Waring, R.H.; Williams, A.C.; Sturman, S.G. Plasma cysteine and sulphate levels in patients with Motor neurone, Parkinsons and Alzheimers disease. *Neurosci. Lett*. **1990**, *110*, 216–220.
241. Carter-Kent, C.; Zein, N.N.; Feldstein, A.E. Cytokines in the pathogenesis of fatty liver and disease progression to steatohepatitis: implications for treatment. *Am. J. Gastroenterol*. **2008**, *103*, 1036–1042.
242. Peraldi, P.; Hotamisligil, G.S.; Buurman, W.A.; White, M.F.; Spiegelman, B.M. Tumor necrosis factor (TNF)-alpha inhibits insulin signaling through stimulation of the p55 TNF receptor and activation of sphingomyelinase. *J. Biol. Chem.* **1996**, *271*, 13018–13022.
243. Plomgaard, P.; Bouzakri, K.; Krogh-Madsen, R.; Mittendorfer, B.; Zierath, J.R.; Pedersen, B.K. Tumor necrosis factor-alpha induces skeletal muscle insulin resistance in healthy human subjects via inhibition of Akt substrate 160 phosphorylation. *Diabetes* **2005**, *54*, 2939–2945.
244. Xu, H.; Uysal, K.T.; Becherer, J.D.; Arner, P.; Hotamisligil, G.S. Altered tumor necrosis factor-alpha (TNF-alpha) processing in adipocytes and increased expression of transmembrane TNF-alpha in obesity. *Diabetes* **2002**, *51*, 1876–1883.
245. Langlais, J.; Zollinger, M.; Plante, L.; Chapdelaine, A.; Bleau, G.; Roberts K.D. Localization of cholesterol sulfate in human spermatozoa in support of a hypothesis for the mechanism of capacitation. *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 7266–7270.
246. Hidiroglou, M.; Knipfel, J.E. Zinc in mammalian sperm: a review. *J. Dairy Sci*. **1984**, *67*, 1147–1156.
247. Mose, T.; Kjaerstad, M.B.; Mathiesen, L.; Nielsen, J.B.; Edelfors, S.; Knudsen, L.E. Placental passage of benzoic acid, caffeine, and glyphosate in an *ex vivo* human perfusion system. *J. Toxicol. Environ. Health A* **2008**, *71*, 984–991.
248. Seneff, S.; Davidson, R.M.; Liu, J. Is cholesterol sulfate deficiency a common factor in preeclampsia, autism, and pernicious anemia? *Entropy* **2012**, *14*, 2265–2290.
249. Robin, M.-M. In *Argentina: The Soybeans of Hunger. Chapter 13 in The World According to Monsanto. English Translation, Translated from French by George Holoch*; The New Press: New York, NY, USA, 2010.
250. Cerdeira, A.L.; Gazziero, D.L.; Duke, S.O.; Matallo, M.B.; Spadotto, C.A. Review of potential environmental impacts of transgenic glyphosate-resistant soybean in Brazil. *J. Environ. Sci. Health B* 2007, *42*, 539–549.
251. Silveira, M.F.; Santos, I.S.; Barros, A.J.D.; Matijasevich, A.; Barros, F.C.; Victora, C.G. Increase in preterm births in Brazil: Review of population-based studies. *Rev. Saúde. Pública.* **2008**, *42*, 1–7.

252. Arbuckle, T.E.; Lin, Z.; Mery, L.S. An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population. *Environ. Health Persp.* **2001**, *109*, 851–857.
253. Hamilton, B.E.; Martin, J.A.; Ventura, S.J. Births: Preliminary data for 2011. In *National Vital Statistics Reports*; National Center for Health Statistics: Hyattsville, MD, USA, 2012; Volume 61.
254. Clair, E.; Mesnage, R.; Travert, C.; Séralini, G.E. A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells *in vitro*, and testosterone decrease at lower levels. *Toxicol. In Vitro* **2012**, *26*, 269–279.
255. Walsh, L.P.; McCormick, C.; Martin, C.; Stocco, D.M. Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression. *Environ. Health Persp.* **2000**, *108*, 769–776.
256. Motoyama, N.; Naka, K. DNA damage tumor suppressor genes and genomic instability. *Curr. Opin. Genet. Dev*. **2004**, *14*, 11–16.
257. Marc, J.; Mulner-Lorillon, O.; Boulben, S.; Hureau, D.; Durand, G.; Bellé, R. Pesticide roundup provokes cell division dysfunction at the level of CDK1/cyclin B activation. *Chem. Res. Toxicol*. **2002**, *15*, 326–331.
258. Marc, J.; Bellé, R.; Morales, J.; Cormier, P.; Mulner-Lorillon, O. Formulated glyphosate activates the DNA-response checkpoint of the cell cycle leading to the prevention of G2/M transition. *Toxicol. Sci.* **2004**, *82*, 436–442.
259. de Roos, A.J.; Blair, A.; Rusiecki, J.A.; Hoppin, J.A.; Svec, M.; Dosemeci, M.; Sandler, D.P.; Alavanja, M.C. Cancer incidence among glyphosate-exposed pesticide applicators in the agricultural health study. *Environ. Health Persp.* **2005**, *113*, 49–54.
260. Walters, D.K.; Wu, X.; Tschumper, R.C.; Arendt, B.K.; Huddleston, P.M.; Henderson, K.J.; Dispenzieri, A.; Jelinek, D.F. Evidence for ongoing DNA damage in multiple myeloma cells as revealed by constitutive phosphorylation of H2AX. *Leukemia* **2011**, *25*, 1344–1353.
261. Alexander, D.D.; Mink, P.J.; Adami, H.-O.; Cole, P.; 5, Mandel, J.S.; Oken, M.M.; Trichopoulos, D.; Multiple myeloma: A review of the epidemiologic literature. *Int. J. Cancer* **2007**, *120*, 4061.
262. Troussard, X.; Avet-Loiseau, H.; Macro, M.; Mellerin, M.P.; Malet, M.; Roussel, M.; Sola, B. Cyclin D1 expression in patients with multiple myeloma. *Hematol. J.* **2000**, *1*, 181–185.
263. Yong, M.; Schwartz, S.M.; Atkinson, C.; Makar, K.W.; Thomas, S.S.; Newton, K.M.; Bowles, E.J.A.; Holt, V.L.; Leisenring, W.M.; Lampe, J.W. Associations between polymorphisms in glucuronidation and sulfation enzymes and mammographic breast density in premenopausal women in the United States. *Cancer Epidemiol. Biomarkers Prev*. **2010** 19, 537–546.
264. McCormack, V.A.; dos Santos Silva, I. Breast density and parenchymal patterns as markers of breast cancer risk: A meta-analysis. *Cancer Epidemiol. Biomarkers Prev.* **2006**, *15*, 1159–1169.
265. Hong, C.-C.; Tang, B.-K.; Hammond, G.L.; Tritchler, D.; Yaffe, M.; Boyd, N.F. Cytochrome P450 1A2 (CYP1A2) activity and risk factors for breast cancer: A cross-sectional study. *Breast Cancer Res*. **2004**, *6*, R352-R365.
266. Morimoto, L.M.; White, E.; Chen, Z.; Chlebowski, R.T.; Hays, J.; Kuller, L.; Lopez, A.M.; Manson, J.; Margolis, K.L.; Muti, P.C. *et al.* Obesity, body size, and risk of postmenopausal breast cancer: the Women's Health Initiative (United States). *Cancer Cause Control.* **2002**, *13*, 741–751.

267. Hakkak, R.; Holley, A.W.; MacLeod, S.L.; Simpson, P.M.; Fuchs, G.J.; Jo, C.H.; Kieber-Emmons, T.; Korourian, S. Obesity promotes 7,12-dimethylbenz(a)anthracene-induced mammary tumor development in female zucker rats. *Breast Cancer Res*. **2005**, *7*, R627-R633.
268. Subbaramaiah, K.; Howe, L.R.; Bhardwaj, P.; Du, B.; Gravaghi, C.; Yantiss, R.K.; Zhou, X.K.; Blaho, V.A.; Hla, T.; Yang, P.; Kopelovich, L.; Hudis, C.A.; Dannenberg, A.J. Obesity is associated with inflammation and elevated aromatase expression in the mouse mammary gland. *Cancer Prev. Res. (Phila.)* **2011**, *4*, 329–346.
269. Cleary, M.P.; Grossmann, M.E. Minireview: Obesity and breast cancer: The estrogen connection. *Endocrinology* **2009**, *150*, 2537–2542.
270. Jagoe, R.T.; Goldberg, A.L. What do we really know about the ubiquitin-proteasome pathway in muscle atrophy? *Curr. Opin. Clin. Nutr. Metab. Care* **2001**, *4*, 183–190.
271. Li, Y.-P.; Chen, Y.; John, J.; Moylan, J.; Jin, B.; Mann, D.L.; Reid, M.B. TNF-α acts via p38 MAPK to stimulate expression of the ubiquitin ligase atrogin1/MAFbx in skeletal muscle. *FASEB J.* **2005**, *19*, 362–370.
272. Gruber, A.; Donaldsson, D.; Kiely, T.; Wu, L. *Pesticides Industry Sales and Usage: 2006 and 2007 Market Estimates.* U.S. Environmental Protection Agency: Washington, DC, USA, 2011.
273. Johnson, R.J.; Perez-Pozo, S.E.; Sautin, Y.Y.; Manitius, J.; Sanchez-Lozada, L.G.; Feig, D.I.; Shafiu, M.; Segal, M.; Glassock, R.J.; Shimada, M.; Roncal, C.; Nakagawa, T. Hypothesis: could excessive fructose intake and uric acid cause type 2 diabetes? *Endocr. Rev*. **2009**, *30*, 96–116.
274. Vivancos, P.D.; Driscoll, S.P.; Bulman, C.A.; Ying, L.; Emami, K.; Treumann, A.; Mauve, C.; Noctor, G.; Foyer, C.H. Perturbations of amino acid metabolism associated with glyphosate-dependent inhibition of shikimic acid metabolism affect cellular redox homeostasis and alter the abundance of proteins involved in photosynthesis and photorespiration. *Plant Physiol.* **2011**, *157*, 256–268.
275. MacDonald, J.; McBride, W. The transformation of U.S. livestock agriculture: Scale, efficiency, and risks; *Economic Information Bulletin No. (EIB-43)*; USDA Economic Research Service: Washington, DC, USA, 2009.
276. European Food Safety Authority (EFSA). Modification of the existing MRL for glyphosate in lentils. *EFSA J.* **2012**, *10*, 2550–2575.
277. Seneff, S.; Lauritzen, A.; Davidson, R.M.; Lentz-Marino, L. Is encephalopathy a mechanism to renew sulfate in autism? *Entropy* **2013**, *15*, 372–406.
278. Dietert, R.R.; Dietert, J.M. Early-life immune insult and developmental immunotoxicity (DIT)-associated diseases: Potential of herbal- and fungal-derived medicinals. *Curr. Med. Chem*. **2007**, *14*, 1075–1085.
279. Dietert, R.R. Role of developmental immunotoxicity and immune dysfunction in chronic disease and cancer. *Reprod. Toxicol*. **2011**, *31*, 319–326.
280. Leifer, C.A.; Dietert, R.R. Early life environment and developmental immunotoxicity in inflammatory dysfunction and disease. *Toxicol. Environ. Chem*. **2011**, *93*, 1463–1485.
281. Seneff, S.; Liu, J.; Davidson, R. Empirical data confirm autism symptoms related to aluminum and acetaminophen exposure. *Entropy* **2012**, *14*, 2227–2253.

282. Chen, M.X.; Cao, Z.Y.; Jiang, Y.; Zhu, Z.W. Direct determination of glyphosate and its major metabolite, aminomethylphosphonic acid, in fruits and vegetables by mixed-mode hydrophilic interaction/weak anion-exchange liquid chromatography coupled with electrospray tandem mass spectrometry. *J. Chromatogr. A.* **2013**, *1272*, 90–99.
283. Arul, S.A.; Sreenivasa, M.A.; Manonmani, H.K. Enzyme-linked immunoassay for the detection of glyphosate in food samples using avian antibodies. *Food Agri. Immunol*. **2011**, *22*, 217–228.
284. Sun, Y.; Wang, C.; Wen, Q.; Wang, G.; Wang, H.; Qu, Q.; Hu, X. Determination of glyphosate and aminomethylphosphonic acid in water by LC using a new labeling reagent, 4-methoxybenzenesulfonyl fluoride. *Chromatographia.* **2010**, *72*, 679–686.
285. Sullivan, T.P.; Sullivan, D.S. The effects of glyphosate herbicide on food preference and consumption in black-tailed deer. *Can. J. Zool.* **1979**, *57*, 1406–1412.
286. Pesticide residues in food. In *FAO/WHO. Evaluations Part I: Residues*. 1st ed.; Volume 78, In Proceeedings of the Joint Meeting of the FAO Panel of Experts Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues, Rome, Italy, 29 September–8 October, 1986; Food and Agriculture Organization of the United Nations: Rome, Italy, 1986; FAO Plant Production and Protection Paper.

Interdiscip Toxicol. 2013; **Vol. 6**(4): 159–184.
doi: 10.2478/intox-2013-0026
Published online in:
www.intertox.sav.sk & www.versita.com/it

REVIEW ARTICLE

Glyphosate, pathways to modern diseases II: Celiac sprue and gluten intolerance

Anthony SAMSEL [1] and Stephanie SENEFF [2]

[1] *Independent Scientist and Consultant, Deerfield, NH 03037, USA*
[2] *Computer Science and Artificial Intelligence Laboratory, MIT, Cambridge, MA, USA*

ITX060413R01 • Received: 24 September 2013 • Revised: 10 November 2013 • Accepted: 12 November 2013

ABSTRACT

Celiac disease, and, more generally, gluten intolerance, is a growing problem worldwide, but especially in North America and Europe, where an estimated 5% of the population now suffers from it. Symptoms include nausea, diarrhea, skin rashes, macrocytic anemia and depression. It is a multifactorial disease associated with numerous nutritional deficiencies as well as reproductive issues and increased risk to thyroid disease, kidney failure and cancer. Here, we propose that glyphosate, the active ingredient in the herbicide, Roundup®, is the most important causal factor in this epidemic. Fish exposed to glyphosate develop digestive problems that are reminiscent of celiac disease. Celiac disease is associated with imbalances in gut bacteria that can be fully explained by the known effects of glyphosate on gut bacteria. Characteristics of celiac disease point to impairment in many cytochrome P450 enzymes, which are involved with detoxifying environmental toxins, activating vitamin D3, catabolizing vitamin A, and maintaining bile acid production and sulfate supplies to the gut. Glyphosate is known to inhibit cytochrome P450 enzymes. Deficiencies in iron, cobalt, molybdenum, copper and other rare metals associated with celiac disease can be attributed to glyphosate's strong ability to chelate these elements. Deficiencies in tryptophan, tyrosine, methionine and selenomethionine associated with celiac disease match glyphosate's known depletion of these amino acids. Celiac disease patients have an increased risk to non-Hodgkin's lymphoma, which has also been implicated in glyphosate exposure. Reproductive issues associated with celiac disease, such as infertility, miscarriages, and birth defects, can also be explained by glyphosate. Glyphosate residues in wheat and other crops are likely increasing recently due to the growing practice of crop desiccation just prior to the harvest. We argue that the practice of "ripening" sugar cane with glyphosate may explain the recent surge in kidney failure among agricultural workers in Central America. We conclude with a plea to governments to reconsider policies regarding the safety of glyphosate residues in foods.

KEY WORDS: celiac disease; gluten; glyphosate; food; cytochrome P450; deficiency

1 Introduction

Gluten intolerance is a growing epidemic in the U.S. and, increasingly, worldwide. Celiac sprue is a more specific disorder, characterized by gluten intolerance along with autoantibodies to the protein, transglutaminase, which builds crosslinks in undigested fragments of gliadin, a major constituent of gluten (Green & Cellier, 2007). The autoantibodies are produced as an immune response to undegraded fragments of proteins in gluten. A remarkable set of symptoms develop over time in association with celiac disease, including weight loss, diarrhea, chronic fatigue, neurological disorders, anemia, nausea, skin rashes, depression, and nutrient deficiencies. Usually, but not always, a strict gluten-free diet can alleviate many of the symptoms. A key associated pathology is an inflammatory response in the upper small intestine, leading to villous atrophy, a flattening of the microvilli which impairs their ability to function in their important role in absorbing nutrients.

Some have suggested that the recent surge in celiac disease is simply due to better diagnostic tools. However, a recent study tested frozen sera obtained between 1948 and 1954 for antibodies to gluten, and compared the results with sera obtained from a matched sample from people living today (Rubio-Topia *et al.*, 2009). They identified a four-fold increase in the incidence of celiac disease in the newer cohort compared to the older one. They also determined that undiagnosed celiac disease is associated with a 4-fold increased risk of death, mostly due to

Correspondence address:
Stephanie Seneff, PhD.
Computer Science and Artificial Intelligence Laboratory,
Massachusetts Institute of Technology,
Rm G-438 MIT Stata Center, 32 Vassar Street, Cambridge, MA 02139, USA
TEL.: +1-617-253-0451 • FAX: +1-617-258-8642
E-MAIL: seneff@csail.mit.edu

increased cancer risk. They concluded that the prevalence of undiagnosed celiac disease has increased dramatically in the United States during the past 50 years.

Transglutaminases play many important roles in the body, as they form covalent crosslinks in complex proteins in connection with blood coagulation, skin-barrier formation, extracellular matrix assembly, and fertilization, endowing the substrate with protection from degradation by proteases (Lorand & Graham, 2003). They also form crosslinks in undigested fragments of gliadin derived from wheat, and sensitivity to certain of these fragments leads to the development of autoantibodies to tissue transglutaminase (Esposito *et al.*, 2002) that inhibit its activity.

Glyphosate is the active ingredient in the herbicide Roundup. It is a broad-spectrum herbicide, considered to be nearly nontoxic to humans (Williams *et al.*, 2000). However, a recent paper (Samsel & Seneff, 2013), argued that glyphosate may be a key contributor to the obesity epidemic and the autism epidemic in the United States, as well as to several other diseases and conditions, such as Alzheimer's disease, Parkinson's disease, infertility, depression, and cancer. Glyphosate suppresses 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSP synthase), the rate-limiting step in the synthesis of the aromatic amino acids, tryptophan, tyrosine, and phenylalanine, in the shikimate pathway of bacteria, archaea and plants (de María *et al.*, 1996). In plants, aromatic amino acids collectively represent up to 35% of the plant dry mass (Franz, 1997). This mode of action is unique to glyphosate among all emergent herbicides. Humans do not possess this pathway, and therefore we depend upon our ingested food and our gut microbes to provide these essential nutrients. Glyphosate, patented as an antimicrobial (Monsanto Technology LLC, 2010), has been shown to disrupt gut bacteria in animals, preferentially killing beneficial forms and causing an overgrowth of pathogens. Two other properties of glyphosate also negatively impact human health – chelation of minerals such as iron and cobalt, and interference with cytochrome P450 (CYP) enzymes, which play many important roles in the body. We will have much more to say about these aspects in later sections of this paper.

A recent study on glyphosate exposure in carnivorous fish revealed remarkable adverse effects throughout the digestive system (Senapati *et al.*, 2009). The activity of protease, lipase, and amylase were all decreased in the esophagus, stomach, and intestine of these fish following exposure to glyphosate. The authors also observed "disruption of mucosal folds and disarray of microvilli structure" in the intestinal wall, along with an exaggerated secretion of mucin throughout the alimentary tract. These features are highly reminiscent of celiac disease. Gluten peptides in wheat are hydrophobic and therefore resistant to degradation by gastric, pancreatic and intestinal proteases (Hershko & Patz, 2008). Thus, the evidence from this effect on fish suggests that glyphosate may interfere with the breakdown of complex proteins in the human stomach, leaving larger fragments of wheat in the human gut that will then trigger an autoimmune

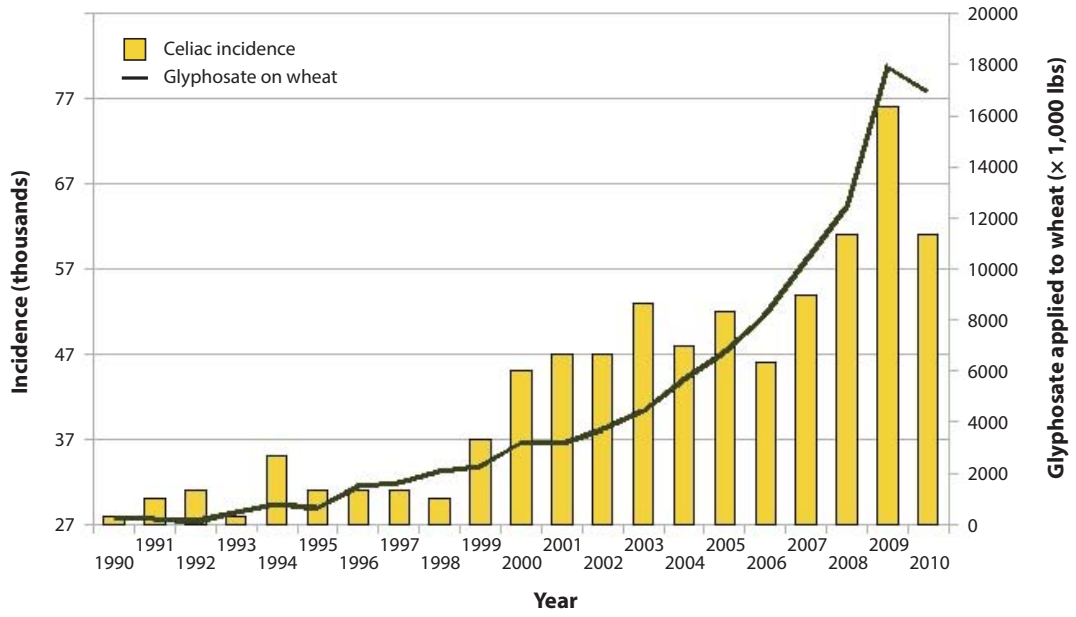


Figure 1. Hospital discharge diagnosis (any) of celiac disease ICD-9 579 and glyphosate applications to wheat (R=0.9759, $p \leq 1.862e\text{-}06$). Sources: USDA:NASS; CDC. (Figure courtesy of Nancy Swanson).

ISSN: 1337-6853 *(print version)* | 1337-9569 *(electronic version)*

Interdisciplinary Toxicology. 2013; **Vol. 6**(4): 159–184
Also available online on **PubMed Central**

response, leading to the defects in the lining of the small intestine that are characteristic of these fish exposed to glyphosate and of celiac patients. As illustrated in Figure 1, the usage of glyphosate on wheat in the U.S. has risen sharply in the last decade, in step with the sharp rise in the incidence of Celiac disease. We explain the reasons for increased application of glyphosate to wheat in Section 13.

In the remainder of this paper, we will first show that gut dysbiosis, brought on by exposure to glyphosate, plays a crucial role in the development of celiac disease. Many CYP enzymes are impaired in association with celiac disease, and we show that glyphosate's known suppression of CYP enzyme activity in plants and animals plausibly explains this effect in humans. In Section 4, we describe the role of excess retinoic acid in celiac disease, and show how this ties also to reproductive problems. We link this to the known effects of glyphosate on retinoic acid, mediated by its suppression of CYP enzymes. Section 5 addresses cobalamin deficiency, a known pathology associated with celiac disease that leads to macrocytic anemia. We argue that this follows as a direct consequence of glyphosate's ability to chelate cobalt. Section 6 discusses in more depth the role of anemia in celiac disease, a consequence of both cobalamin and iron deficiency. Section 7 discusses molybdenum deficiency and its link to microcephaly, which is associated with celiac disease. Section 8 discusses the link between selenium deficiency and autoimmune thyroid disease. Section 9 discusses kidney disease in connection with celiac disease and glyphosate. Section 10 discusses various nutritional deficiencies associated with celiac disease, and shows how these can directly be explained by glyphosate. Section 11 discusses the link between celiac disease and certain rare cancers that have also been linked to glyphosate. Section 12 goes into an in-depth discussion of how glyphosate might promote autoantibodies to transglutaminase. Following a section which presents compelling evidence that glyphosate residues in wheat, sugar and other crops are likely increasing in recent decades, and a section discussing the increased risk to kidney failure in agricultural workers exposed to excess glyphosate occupationally, we close with a discussion section that summarizes our findings, and a conclusion which implores governments to pay more attention to the damaging consequences of the escalation in chemical warfare on weeds that characterizes current agricultural practices.

2 Gut bacteria

In this section, we first discuss the role of pathogens in inducing the breakdown of tight junctions in enterocytes lining the small intestinal wall. We then show that glyphosate is associated with an overgrowth of pathogens along with an inflammatory bowel disease in animal models. A parallel exists with celiac disease where the bacteria that are positively and negatively affected by glyphosate are overgrown or underrepresented respectively in association with celiac disease in humans. We also discuss how the beneficial bacteria that are negatively impacted by glyphosate can protect from celiac disease through their enzymatic activities on gluten, and point to several articles recommending treatment plans based on probiotics.

Pathogens, through their activation of a potent signaling molecule called zonulin, induce a breakdown of the tight junctions in cells lining the gut, leading to "leaky gut" syndrome (Fasano, 2011). Concentrations of zonulin were sharply elevated ($p<0.000001$) in subjects with celiac disease during the acute phase (Fasano *et al.*, 2000). As many as 30% of celiac patients continue to experience GI symptoms after adopting a gluten-free diet, despite optimal adherence, a condition that was attributed to bacterial overgrowth in the small intestine (Tursi *et al.*, 2003). Figure 2 shows that there is a correlation between glyphosate application to wheat and the incidence of intestinal infections.

Evidence of disruption of gut bacteria by glyphosate is available for poultry (Shehata *et al.*, 2013), cattle (Krüger *et al.*, 2013), and swine (Carman *et al.*, 2013). Glyphosate disrupts the balance of gut bacteria in poultry (Shehata *et al.*, 2013), increasing the ratio of pathogenic bacteria to other commensal microbes. Salmonella and Clostridium are highly resistant to glyphosate, whereas Enterococcus, Bifidobacteria, and Lactobacillus are especially susceptible. Glyphosate was proposed as a possible factor in the increased risk to Clostridium botulinum infection in cattle in Germany over the past ten to fifteen years (Krüger *et al.*, 2013b). Pigs fed GMO corn and soy developed widespread intestinal inflammation that may have been due in part to glyphosate exposure (Carman *et al.*, 2013).

Celiac disease is associated with reduced levels of Enterococcus, Bifidobacteria and Lactobacillus in the gut and an overgrowth of pathogenic gram negative bacteria (Sanz *et al.*, 2011; Di Cagno *et al.*, 2011; Collado *et al.*, 2007). In (Di Cagno *et al.*, 2011), Lactobacillus, Enterococcus and Bifidobacteria were found to be significantly lower in fecal samples of children with celiac disease compared to controls, while levels of the pathogens, Bacteroides, Staphylococcus, Salmonella, a Shighella were elevated. In (Collado *et al.*, 2007), another study comparing the fecal material of celiac infants to healthy controls, Bacteroides, Clostridium and Staphylococcus were all found to be significantly higher ($p<0.05$). Sulfate-reducing bacterial counts were also elevated ($p<0.05$) (Nadal *et al.*, 2007; Collado *et al.*, 2007), an interesting observation which we will return to later in this paper. A significant reduction in Bifidobacteria was also found in (Nadal *et al.*, 2007). An increased excretion of the bacterial metabolites p-Cresol and phenol has also been recognized in association with celiac disease (Tamm, 1984). p-Cresol is produced via anaerobic metabolism of tyrosine by pathogenic bacteria such as Clostridium difficile (D'Ari and Barker, 1985). It is a highly toxic carcinogen, which also causes adverse effects on the central nervous system, the cardiovascular system, lungs, kidney and liver (Kelly *et al.*, 1994).

Probiotic treatments are recommended to aid in digestive healing in celiac disease. The proteolytic activity of

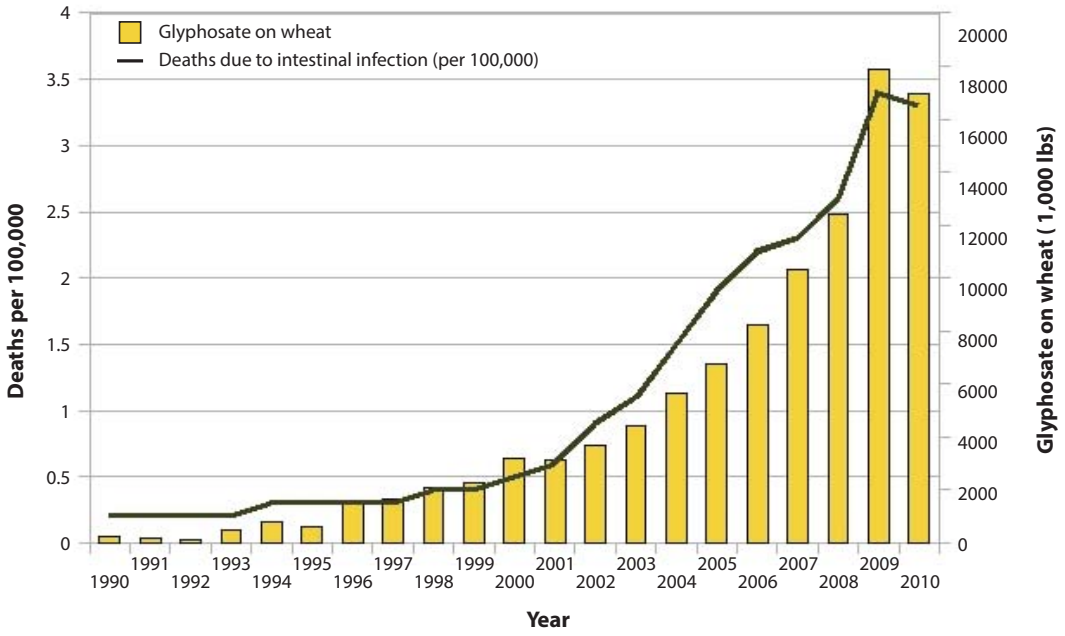


Figure 2. Deaths due to intestinal infections ICD A04, A09; 008, 009 with glyphosate applications to wheat (R=0.9834, $p \leq 3.975e\text{-}09$). Sources: USDA:NASS; CDC. (Figure courtesy of Nancy Swanson).

Lactobacilli aids the breakdown of wheat into less allergenic forms. Ongoing research aims to produce gluten-containing sourdough breads fermented by Lactobacilli that can then serve as probiotics to help ameliorate the symptoms of celiac disease and allow celiac patients to consume wheat (Gobbetti *et al.*, 2007). Probiotic Lactobacilli produce the enzyme phytase which breaks down phytates that would otherwise deplete important minerals and other cations through chelation (Famularo *et al.*, 2005). Their activities would therefore improve absorption of these micronutrients, a known problem in celiac patients (Cavallaro *et al.*, 2004). Glyphosate itself also chelates rare minerals, a subject we will address in the section on nutritional deficiencies.

Probiotic treatment with Bifidobacteria has been shown to alleviate symptoms associated with celiac disease (Smecuol *et al.*, 2013; Whorwell *et al.*, 2006). Bifidobacteria suppress the pro-inflammatory milieu triggered by the microbiota of celiac patients (Medina *et al.*, 2008). Live cultures of Bifidobacterium lactis would promote healing of the gut if offered as treatment in conjunction with the gluten-free diet, or might even allow the celiac patient to consume modest amounts of gluten without damaging effects (Lindfors *et al.*, 2008). In this in vitro study, it was demonstrated that B. lactis reduced epithelial permeability and improved the integrity of the tight junctions in human colon cells.

In summary, celiac disease is associated with a reduced presence in the gut of commensal bacteria such as Lactobacilli and Bifidobacteria, which are known to be preferentially killed by glyphosate, and with an overabundance of C. difficile, which is known to be promoted by glyphosate exposure. Bifidobacteria and Lactobacilli are both capable of modifying gluten in such a way as to make it less allergenic, a feature that is being exploited in recent efforts to develop gluten-containing foods that may be safe for consumption by celiac patients. Probiotics containing live forms of these bacteria are also being actively marketed today.

3 CYP Enzyme impairment and sulfate depletion

As mentioned previously, glyphosate has been shown to suppress CYP enzymes in plants (Lamb *et al.*, 1998) and animals (Hietanen *et al.*, 1983). A study on rats demonstrated that glyphosate decreased the levels of CYP enzymes and monooxygenase activities in the liver and the intestinal activity of aryl hydrocarbon hydroxylase (Hietanen *et al.*, 1983).

CYP enzymes are essential for detoxification of many compounds in the liver (Lindros, 1997). Intraperitoneal exposure of rats to Roundup in acute doses over a short time interval induced irreversible damage to hepatocytes and elevated urinary markers of kidney disease. This was associated with lipid peroxidation and elevated levels of the inflammatory cytokine tumor necrosis factor (TNF-α) (El-Shenawy, 2009). CYP3A is constitutively expressed in human intestinal villi and plays an important role in

ISSN: 1337-6853 *(print version)* | 1337-9569 *(electronic version)*

Interdisciplinary Toxicology. 2013; **Vol. 6**(4): 159–184
Also available online on **PubMed Central**

drug metabolism (Cupp & Tracy, 1998). Celiac disease is associated with a decrease in the intestinal CYP3A (Lang *et al.*, 1996). This defect is restored by a gluten free diet.

Impaired gallbladder bile acid production (Colombato *et al.*, 1977) and biliary cirrhosis, an inflammatory liver disease characterized by obstruction of the bile duct (Dickey *et al.*, 1997), have been shown to co-occur with celiac disease. CYP enzymes are crucial in the production of bile acids (Lorbek *et al.*, 2012). An obligatory CYP enzyme in bile acid synthesis, CYP27A, has been identified as being identical to the mitochondrial vitamin D3 activating enzyme (Wikvall, 2001). In (Kemppainen *et al.*, 1999), 64% of men and 71% of women with celiac disease were found to be vitamin D3 deficient, manifested as low spinal bone mineral density. Celiac disease is associated with impaired gall bladder function and decreased pancreatic secretions (Brown *et al.*, 1987; Benini *et al.*, 2012) along with recurrent pancreatitis (Patel *et al.*, 1999). Abnormalities in bile acid secretion have been found in children suffering from celiac disease (Ejderhamn *et al.*, 1992). Celiac patients exhibit abnormally low synthesis of cholecystokinin (Deprez *et al.*, 2002), but it has also become apparent that the gall bladder is less responsive to stimulation of contraction by cholecystokinin (Brown *et al.*, 1987). A reversible defect of gallbladder emptying and cholecystokinin release has been identified in association with celiac disease (Maton *et al.*, 1985). These pathologies may be related to impaired CYP enzyme activity induced by glyphosate.

While it is clear that CYP enzymes play an important role in bile acid synthesis and in cholesterol homeostasis, the details have not yet been worked out (Lorbek *et al.*, 2012). However, some mouse knockout experiments produce embryonically lethal effects, pointing to the importance of these enzymes to biological systems. Disruption of Cyp7A1, involved in bile acid synthesis in mice, induces elevated serum cholesterol and early death.

A link has been established between celiac disease and non-alcoholic fatty liver, which is likely due to the liver's inability to export cholesterol sulfate through the bile acids due to impaired CYP enzymes (Lorbek *et al.*, 2012). This requires a private store of fats to house the excess cholesterol that cannot be exported in bile. This would also likely lead to insufficient sulfate supplies to the small intestine, and could result in impaired heparan sulfate synthesis in the glycosaminoglycans and subsequent pathologies. Heparan sulfate populating the glycosaminoglycans (GAGs) surrounding enterocytes is essential for the proper functioning of the small intestines. Leakage of both albumin and water in both the vasculature and tissues results when the negative charge is reduced due to insufficient sulfation of the polysaccharide units (Sunergren *et al.*, 1987). Vascular leakage may be a consequence of degradation of sulfated GAGs due to inflammatory agents (Klein *et al.*, 1992). A similar problem may occur in the kidneys leading to albumin loss into urine during nephrosis (Vernier *et al.*, 1983). Intestinal protein loss in inflammatory enteropathy associated with celiac disease may also be due to a deficiency in the sulfated GAGs (Murch *et al.*, 1993; Murch, 1995). A case study of three infants with congenital absence of enterocyte heparan sulfate demonstrated profound enteric protein loss with secretory diarrhoea and absorption failure, even though their intestines were not inflamed (Murch *et al.*, 1996).

In (Samsel and Seneff, 2013), a hypothesis was developed that glyphosate disrupts the transport of sulfate from the gut to the liver and pancreas, due to its competition as a similarly kosmotropic solute that also increases blood viscosity. (Kosmotropes are ions that induce "structure ordering" and "salting out" of suspended particles in colloids). Insufficient sulfate supply to the liver is a simple explanation for reduced bile acid production. The problem is compounded by impaired CYP enzymatic action and impaired cycling of bile acids through defective enterocytes in the upper small intestine. The catastrophic effect of loss of bile acids to the feces due to impaired reuptake compels the liver to adopt a conservative approach of significantly reduced bile acid synthesis, which, in turn, leads to gall bladder disease.

The protein, Nuclear factor κ-lightchain-enhancer of activated B cells (NF-κB) controls DNA transcription of hundreds of genes and is a key regulator of the immune response to infection (Tieri *et al.*, 2012). Light chains are polypeptide subunits of immunoglobulins. NF-κB responds to stimulation from bacterial and viral antigens, inflammatory cytokines like TNF-α, free radicals, oxidized LDL, DNA damage and UV light. The incidence of acute pancreatitis has been increasing in recent years (Bhatia, 2012), and it often follows billiary disease. A local inflammatory reaction at the site of injury coincides with an increase in the synthesis of hydrogen sulfide (H_2S) gas. H_2S regulates the inflammatory response by exciting the extracellular signal regulated (ERK) pathway, leading to production of NF-κB (Bhatia, 2012). We hypothesize that H_2S, while toxic, is a source of both energy and sulfate for the pancreas, derived from sulfur-containing amino acids such as cysteine and homocysteine. Dehydroepiandrosterone (DHEA) sulfate, but not DHEA, inhibits NF-κB synthesis, suggesting that sulfate deficiency is a driver of inflammation (Iwasaki *et al.*, 2004).

While H_2S is well known as a toxic gas through its inhibition of aerobic respiration, a recent paradigm shift in the research surrounding H_2S has been inspired by the realization that it is an important signaling gas in the vasculature, on par with nitric oxide (Li *et al.*, 2011). H_2S can serve as an inorganic source of energy to mammalian cells (Módis *et al.*, 2013). 3-mercaptopyruvate sulfurtransferae (3MST) is expressed in the vascular endothelium, and it produces H_2S from mercaptopyruvate, an intermediary in the breakdown of cysteine (Kimura, 2011). Endogenously produced H_2S derived from 3-mercaptopyruvate stimulates additional mitochondrial H_2S production, which then is oxidized to thiosulfate via at least three different pathways (Ingenbleek and Kimura, 2013; Hildebrandt and Grieshaber, 2008; Goubern *et al.*, 2007), producing ATP. The inflammatory agent superoxide can act as substrate for the oxidation of H_2S to sulfite and subsequently sulfate

and the activated form, PAPS (Seneff *et al.*, 2012), but will likely induce oxidative damage in the pancreas, particularly, as we will see in section 7, if molybdenum deficiency impairs sulfite-to-sulfate synthesis.

Pancreatic beta cells express extraordinarily high levels of heparan sulfate, which is essential for their survival (Ziolkowski *et al.*, 2012), since it protects them from ROS-induced cell death. Because sulfate transport via the hepatic portal vein is likely disrupted by glyphosate, H_2S, whether derived from sulfur-containing amino acids or supplied via diffusion following its production by sulfur-reducing bacteria in the gut, can become an important source of sulfur for subsequent sulfate production locally in the pancreatic cells. Pancreatic elastase is a serine protease that is needed to assist in protein degradation, but an overabundance can lead to autolysis of tissues (Ito *et al.*, 1998). Cholesterol sulfate inhibits pancreatic elastase (Ito *et al.*, 1998), so a deficiency in cholesterol sulfate supply due to impaired sulfate supply to the liver and impaired CYP function should increase the risk of tissue digestion by pancreatic enzymes, contributing to the loss of villi in the upper small intestine observed in celiac disease.

In the early 1990's a newly recognized disease began to appear, characterized by eosinophil infiltration into the esophagus, which manifested as dysphagia in adults and refractory reflux symptoms in children (Lucendo & Sánchez-Cazalilla, 2012). This disease, termed eosinophilic esophagitis (EOE), is associated with a Th2 immune profile and synthesis of the cytokine IL-13, which has direct cytotoxic effects on epithelial cells. A dose-dependent induction of eosinophilia by intratracheal delivery of IL-13 confirms its association with EOE (Mishra and Rothenberg, 2003). An association has been found between EOE and celiac disease (Leslie *et al.*, 2010). Patients with refractory celiac disease that is not corrected by dietary gluten restriction show an increased production of IL-13 in the gut (Gross *et al.*, 2013). The incidence of EOE has increased at alarming rates in Western countries in the last three decades (Furuta *et al.*, 2007; Liacouras *et al.*, 2011; Prasad *et al.*, 2009).

Glyphosate is highly corrosive to the esophageal epidermal lining, with upper GI tract injury observed in 94% of patients following glyphosate ingestion (Chang *et al.*, 1999). In (Zouaoui *et al.*, 2013), the most common symptoms in an acute response from glyphosate poisoning were oropharyngeal ulceration, nausea and vomiting. We hypothesize that glyphosate induces EOE via a systemic response as well as through direct contact. The pathogenesis of EOE is related to food sensitivities, but airborne exposure to chemicals in the lungs can also induce it, so it does not require physical contact to the allergen (Blanchard & Rothenberg, 2008). It is conceivable that glyphosate is responsible for the emergence of EOE.

The cytochrome P450 reductase (CPR) and cytochrome P450 (CP) enzyme system is essential for inducing nitric oxide release from organic nitrates (Li, 2006). The nitrate moiety is reduced while simultaneously oxidizing NADPH to NADP+. This system is invoked in organic nitrate drug treatment for cardiovascular therapy. The reaction depends on anaerobic, acidic conditions, a feature of venous rather than arterial blood. Since L-arginine is substrate for NO synthesis by endothelial nitric oxide synthase (eNOS) under oxidative conditions (Förstermann and Münze, 2006), it is likely that CPR and CP play an important role mainly in stimulating *venous* smooth muscle relaxation. Impaired venous relaxation would likely contribute to venous thrombosis, which is a well-established complication of celiac disease (Zenjari *et al.*, 1995; Marteau *et al.*, 1994, Grigg, 1999, Halfdanarson *et al.*, 2007).

In summary, celiac disease is associated with multiple pathologies in the digestive system, including impaired gall bladder function, fatty liver, pancreatitis, and EOE. We have argued here that many of these problems can be traced to impaired CYP function in the liver due to glyphosate exposure, leading to insufficient flow of bile acids through the circular pathway between the liver and the gut. This results in a system-wide depletion in sulfate, which induces inflammation in multiple organs to produce sulfate locally. A potential sulfur source for sulfate synthesis could be hydrogen sulfide gas, provided in part by the local breakdown of sulfur-containing amino acids like cysteine and homocysteine and in part by diffusion of the gas produced from inorganic dietary sources by sulfur-reducing bacteria in the large intestine. Impaired CYP enzyme function may also contribute to venous thrombosis, for which celiac disease is an established risk factor.

4 Retinoic acid, celiac disease and reproductive issues

In this section, we first establish that excess retinoic acid (RA) is a risk factor for celiac disease. We then show that excess RA leads to complications in pregnancy and teratogenic effects in offspring. Glyphosate has been shown to exhibit teratogenic effects in line with known consequences of excess RA exposure to the embryo, and we propose that the mechanism for this effect may be glyphosate's known disruption of CYP enzymes (Samsel & Seneff, 2013), which are involved in RA catabolism. This then links glyphosate to increased risk to celiac disease via its direct effects on RA. And it identifies a possibly important factor in the association of celiac disease with reproductive issues. We also discuss other adverse effects of excess retinoic acid and a possible relationship to impaired sulfate supply to the gut.

In celiac disease, T cells develop antibody responses against dietary gluten, a protein present in wheat (Jabri & Sollid, 2009). RA, a metabolite of vitamin A, has been shown to play a critical role in the induction of intestinal regulatory responses (Mora *et al.*, 2008; Coombes *et al.*, 2007; Mucida *et al.*, 2007). The peptide in gluten, A-gliadin p31-43, induces interleukin 15 (IL-15), a key cytokine promoting T-cell activation (Hershko & Patz, 2008). RA synergizes with high levels of IL-15 to promote JNK phosphorylation (Nanda, 2011; DePaolo *et al.*, 2011), which potentiates cellular apoptosis (Putcha *et al.*, 2003).

ISSN: 1337-6853 *(print version)* | 1337-9569 *(electronic version)*

Interdisciplinary Toxicology. 2013; **Vol. 6**(4): 159–184
Also available online on **PubMed Central**

IL-15 is a causative factor driving the differentiation of precursor cells into anti-gluten CD4+ and CD8+ Th1 cells in the intestinal mucosa. Furthermore, in (DePaolo *et al.*, 2011), it was discovered that RA exhibits an unanticipated co-adjuvant property to induce Th1 immunity to antigens during infection of the intestinal mucosa with pathogens. Retinoic acid has also been shown to directly suppress transglutaminase activity, another way in which it would negatively impact celiac disease (Thacher *et al.*, 1985). Thus, it is becoming clear that excess exposure to RA would increase risk to celiac disease, and warnings have been issued regarding potential adverse effects of RA supplements on celiac disease.

It is well established that high RA levels leads to teratogenic effects both in human and experimental models. Brain abnormalities such as microcephaly, impairment of hindbrain development, mandibular and midfacial underdevelopment, and cleft palate are all implicated (Sulik *et al.*, 1988; Clotman *et al.*, 1998). Women with celiac disease are known to have higher rates of infertility, miscarriages, and birth defects in their offspring (Freeman, 2010; Martinelli *et al.*, 2000; Dickey *et al.*, 1996; Collin *et al.*, 1996). Excess RA could be a significant factor in these complications.

A possible mechanism by which glyphosate might induce excess RA is via its interference with the CYP enzymes that metabolize RA. There are at least three known CYPs (CYP26A1, CYP26B1 and CYP26C1) that catabolize RA, and they are active in both the embryo and the adult (Taimi *et al.*, 2004). A 1/5000 dilution of glyphosate was sufficient to induce reproducible malformations characteristic of RA exposure in frog embryos (Paganelli *et al.*, 2010). Pathologies included shortening of the trunk, reduction in the size of the head, abnormally small eyes or the presence of only one eye (cyclopia), and other craniofacial malformations in the tadpole. Glyphosate's toxicity to tadpoles has been well demonstrated, as it killed nearly 100% of larval amphibians exposed in experimental outdoor pond mesocosms (Relyea, 2005).

According to official records, there has been a recent 4-fold increase in developmental malformations in the province of Chaco, Argentina, where glyphosate is used massively on GMO monocrops of soybeans (Carrasco, 2013). In Paraguay, 52 cases of malformations were reported in the offspring of women exposed during pregnancy to agrochemicals, including anencephaly, microcephaly, facial defects, cleft palate, ear malformations, polydactily, and syndactily (Benítez-Leite *et al.*, 2009). In in vitro studies on human cell lines, DNA strand breaks, plasma membrane damage and apoptosis were observed following exposure to glyphosate-based herbicides (Gasnier *et al.*, 2009). Another factor in teratogenetic effects of glyphosate may be the suppression of the activity of androgen-to-estrogen conversion by aromatase, a CYP enzyme (Gasnier *et al.*, 2009).

Ingested vitamin A, a fat-soluble vitamin, is delivered to the blood via the lymph system in chylomicrons, and excess vitamin A is taken up by the liver as retinoic acid for catabolism by CYP enzymes (Russell, 2000). Any remaining retinoic acid that is not catabolized is exported inside LDL particles, and it lingers much longer as retinyl esters in the vasculature in this form (Krasinski *et al.*, 1990). Excess retinoic acid is more readily stored in this way in LDL particles in the elderly. Vitamin A toxicity can lead to fatty liver and liver fibrosis (Russell, 2000) as well as hypertriglyceridemia (Ellis *et al.*, 1986). Vitamin A has a negative effect on cholesterol sulfate synthesis (Jetten *et al.*, 1989), which might negatively impact the liver's ability to maintain adequate supplies of cholesterol sulfate for the bile acids, and therefore also interfere with the supply of cholesterol sulfate to the gastrointestinal tract.

In summary, glyphosate's disruption of the CYP enzymes responsible for RA catabolism could lead to an excess bioavailability of RA that could contribute adversely to celiac disease, as well as damaging the liver and leading to teratogenic effects in offspring of exposed individuals.

In addition to higher risk to birth defects, individuals with celiac disease have increased risk to infertility (Meloni *et al.*, 1999; Farthing *et al.*, 1982). Increased incidence of hypogonadism, infertility and impotence was observed in a study of 28 males with celiac disease (Farthing *et al.*, 1982). Marked abnormalities of sperm morphology and motility were noted, and endocrine dysfunction was suggested as a probable cause. In studies conducted on Sertoli cells in prepubertal rat testis, exposure to Roundup induced oxidative stress leading to cell death (de Liz Oliveira Cavalli *et al.*, 2013). Roundup induced the opening of L-type voltage dependent calcium channels as well as ryanodine receptors, initiating ER stress and leading to calcium overload and subsequent necrosis. Glutathione was depleted due to upregulation of several glutathione-metabolizing enzymes. This suggests that Roundup would interfere with spermatogenesis, which would impair male fertility.

5 Cobalamin deficiency

Untreated celiac disease patients often have elevated levels of homocysteine, associated with folate and/or cobalamin deficiency (Saibeni *et al.*, 2005; Dickey *et al.*, 2008). Species of Lactobacillus and Bifidobacterium have the capability to biosynthesize folate (Rossi *et al.*, 2011), so their disruption by glyphosate could contribute to folate deficiency. Malabsorption in the proximal small intestine could also lead to iron and folate deficiencies. Cobalamin was originally thought to be relatively spared in celiac disease because its absorption is mostly through the terminal ileum, which is unaffected by celiac disease. However, a recent study found that cobalamin deficiency is prevalent in celiac patients. 41% of the patients studied were found to be deficient in cobalamin (<220 ng/L), and 31% of these cobalamin-deficient patients also had folate deficiency (Dahele & Ghosh, 2001). Either cobalamin or folate deficiency leads directly to impaired methionine synthesis from homocysteine, because these two vitamins are both required for the reaction to take place. This induces

hyperhomocysteinemia (Refsum *et al.*, 2001), an established risk factor in association with celiac disease (Hadithi *et al.*, 2009). Long-term cobalamin deficiency also leads to neurodegenerative diseases (Herrmann & Obeid, 2012).

Because a deficiency in cobalamin can generate a large pool of methyl-tetrahydrofolate that is unable to undergo reactions, cobalamin deficiency will often mimic folate deficiency. Cobalamin requires cobalt, centered within its corrin ring, to function. We depend upon our gut bacteria to produce cobalamin, and impaired cobalt supply would obviously lead to reduced synthesis of this critical molecule. Glyphosate is known to chelate +2 cations such as cobalt. Glyphosate complexes with cobalt as a dimer [Co(glyphosate)2]3 in fifteen different stereoisomeric configurations, and it is facile at switching among the different stereoisomers, an unusual kinetic property compared to most Co(III) systems (Cusiel, 2005).

In fact, studies have revealed that glyphosate inhibits other cytosolic enzymes besides EPSP synthase in plants and microbes that also activate steps in the shikimate pathway (Ganson and Jensen, 1988; Bode *et al.*, 1984). Glyphosate potently inhibits three enzymes in the shikimate pathway in yeast (Bode *et al.*, 1984). It has been confirmed that these other enzymes depend upon cobalt as a catalyst, and glyphosate inhibition works through competitive cobalt binding and interference with cobalt supply (Ganson and Jensen, 1988). It has also been proposed that chelation by glyphosate of both cobalt and magnesium contributes to impaired synthesis of aromatic amino acids in Escherichia coli bacteria (Hoagland and Duke, 1982). Thus, it is plausible that glyphosate similarly impairs cobalamin function in humans by chelating cobalt.

6 Anemia and iron

Anemia is one of the most common manifestations of celiac disease outside of the intestinal malabsorption issues (Halfdanarson *et al.*, 2007; Bottaro *et al.*, 1999), and is present in up to half of diagnosed celiac patients. Celiac patients often have both cobalamin and folate deficiency, which can cause anemia, but iron deficiency may be the most important factor (Hershko & Patz, 2008). Celiac patients often don't respond well to iron treatment.

Glyphosate's chelating action can have profound effects on iron in plants (Eker *et al.*, 2006; Bellaloui *et al.*, 2009). Glyphosate interferes with iron assimilation in both glyphosate-resistant and glyphosate-sensitive soybean crops (Bellaloui *et al.*, 2009). It is therefore conceivable that glyphosate's chelation of iron is responsible for the refractory iron deficiency present in celiac disease.

Erythropoietin (EPO), also called hematopoietin, is a cytokine produced by interstitial fibroblasts in the kidney that regulates red blood cell production. Low EPO levels, leading to a low turnover rate of red blood cells, is a feature of celiac disease (Bergamaschi *et al.*, 2008; Hershko & Patz, 2008). This can lead to megaloblastic anemia, where red blood cells are large (macrocytic) and reduced in number due to impaired DNA synthesis. A recent hematological study on mice exposed to Roundup at subacute levels for just 15 days revealed an anemic syndrome in both male and female mice, with a significant reduction in the number of erythrocytes and in hemoglobin, reduced hematocrit and increased mean corpuscular volume, indicative of macrocytic anemia (Jasper *et al.*, 2012).

7 Molybdenum deficiency

Molybdenum deficiency is rarely considered in diagnoses, as it is only needed in trace amounts. However, molybdenum is essential for at least two very important enzymes: sulfite oxidase and xanthine oxidase. Sulfite oxidase converts sulfite, a highly reactive anion, to sulfate, which is much more stable. Sulfite is often present in foods such as wine and dried fruits as a preservative. Sulfate plays an essential role in the sulfated proteoglycans that populate the extracellular matrices of nearly all cell types (Turnbull *et al.*, 2001; Murch *et al.*, 1993; Murch, 1995). So, impaired sulfite oxidase activity leads to both oxidative damage and impaired sulfate supplies to the tissues, such as the enterocytes in the small intestine. The excess presence of sulfur-reducing bacteria such as Desulfovibrio in the gut in association with celiac disease (Collado *et al.*, 2007; Nadal *et al.*, 2007) could be protective, because these bacteria can reduce dietary sulfite to hydrogen sulfide, a highly diffusable gas that can migrate through tissues to provide a source of sulfur for sulfate regeneration at a distant site, as previously discussed. These distal sites could reoxidize the H_2S through an alternative pathway that does not require molybdenum for sulfur oxidation (Ingenbleek and Kimura, 2013).

Xanthine oxidase (XO) produces uric acid from xanthine and hypoxanthine, which are derived from purines. It is activated by iron, which, as we have seen, is often intractably deficient in association with celiac disease. Impaired XO activity would be expected to drive purines towards other degradation pathways. Adenosine deaminase (ADA), a cytoplasmic enzyme that is involved in the catabolism of purine bases, is elevated in celiac disease, and is therefore a useful diagnostic marker (Cakal *et al.*, 2010). In fact, elevation of ADA is correlated with an increase in several inflammatory conditions. Impaired purine synthesis is expected in the context of cobalamin deficiency as well, because methyl melonlyl CoA mutase depends on catalytic action by cobalamin (Allen *et al.*, 1993). Decreased purine synthesis results in impaired DNA synthesis, which then leads to megaloblastic anemia (Boss, 1985), due to slowed renewal of RBC's from multipotent progenitors, a problem that is compounded by suppressed EPO activity (Bergamaschi *et al.*, 2008), a feature of celiac disease.

A remarkable recent case of a three-month old infant suffering from molybdenum deficiency links several aspects of glyphosate toxicity together, although glyphosate exposure was not considered as a possible cause in this case (Boles *et al.*, 1993). This child presented with microcephaly, developmental delay, severe irritability,

ISSN: 1337-6853 *(print version)* | 1337-9569 *(electronic version)*

Interdisciplinary Toxicology. 2013; **Vol. 6**(4): 159–184
Also available online on **PubMed Central**

and lactic acidosis. Lactic acidosis is a striking feature of intentional glyphosate poisoning induced by drinking Roundup (Zouaoui *et al.*, 2013; Beswick & Millo, 2011), and it suggests impaired oxidative respiration, as is seen in E. coli exposed to glyphosate (Lu *et al.*, 2013). In vitro studies of glyphosate in the formulation Roundup have demonstrated an ability to disrupt oxidative respiration by inducing mitochondrial swelling and inhibiting mitochondrial complexes II and III (Peixoto, 2005). This would explain a massive build-up of lactic acid following ingestion of Roundup, due to a switch to anaerobic metabolism. Glyphosate has also been shown to uncouple mitochondrial phosphorylation in plants (Haderly *et al.*, 1977; Ali & Fletcher, 1977).

As has been stated previously, microcephaly is a feature of excess RA, which could be induced by glyphosate due to its inhibitory action on CYP enzymes. In the case study on molybdenum deficiency (Boles *et al.*, 1993), urinary sulfite levels were high, indicative of defective sulfite oxidase activity. Serum hypouricemia was also present, indicative of impaired XO activity. So, the induction of excess RA, depletion of molybdenum, and lactic acidosis by glyphosate provide a plausible environmental factor in this case.

One final aspect of molybdenum deficiency involves nitrate metabolism. As a source of nitric oxide, inorganic nitrite regulates tissue responses to ischemia. While nitrate reductase activity has been known to be a capability of microbes for many years, it has only recently been realized that mammals also possess a functioning nitrate reductase capability, utilizing a molybdenum-dependent enzyme to produce nitrite from nitrate (Jansson *et al.*, 2008). Molybdenum deficiency would impair this capability, likely contributing to the higher risk to venous thrombosis observed in celiac disease (Zenjari *et al.*, 1995; Marteau *et al.*, 1994, Grigg, 1999). This could also explain the excess nitrates in the urine observed in association with celiac disease (Högberg *et al.*, 2011).

8 Selenium and thyroid disorders

Autoimmune thyroid disease is associated with celiac disease (Collin *et al.*, 2002; Valentino *et al.*, 2002). In (Valentino *et al.*, 2002), up to 43% of patients with Hashimoto's thyroiditis showed signs of mucosal T-cell activation typical of celiac disease. Selenium, whose deficiency is associated with celiac disease (Hinks *et al.*, 1984), plays a significant role in thyroid hormone synthesis, secretion and metabolism, and selenium deficiency is therefore a significant factor in thyroid diseases (Sher, 2000; Chanoine *et al.*, 2001; Khrle, 2013).

Selenium is required for the biosynthesis of the "twenty first amino acid," selenocysteine. Twenty five specific selenoproteins are derived from this amino acid. Selenium deficiency can lead to an impairment in immune function and spermatogenesis in addition to thyroid function (Papp *et al.*, 2007). One very important selenoprotein is glutathione peroxidase, which protects cell membranes and cellular components against oxidative damage by both hydrogen peroxide and peroxynitrite ($ONOO^-$) (Prabhakar *et al.*, 2006).

Wheat can be a good source of selenoproteins. However, the content of selenium in wheat can range from sufficient to very low, depending upon soil physical conditions. Soil compaction, which results from modern practices of "no till" agriculture (Huggins & Reganold, 2008), can lead to both reduced selenium content and a significant increase in arsenic content in the wheat (Zhao *et al.*, 2007). Since glyphosate has been shown to deplete sulfur in plants (Saes Zobiole *et al.*, 2010), and selenium is in the same column of the periodic table as sulfur, it is likely that glyphosate also disrupts selenium uptake in plants. A gluten-free diet will guarantee, however, that no selenium is available from wheat, inducing further depletion of selenoproteins, and therefore increasing the risk to immune system, thyroid and infertility problems in treated celiac patients.

The gut bacterium Lactobacillus, which is negatively impacted by glyphosate (Shehata *et al.*, 2013) and depleted in association with celiac disease (Di Cagno *et al.*, 2011), is able to fix inorganic selenium into more bioavailable organic forms like selenocysteine and selenomethionine (Pessione, 2012). Selenocysteine is present in the catalytic center of enzymes that protect the thyroid from free radical damage (Triggiani *et al.*, 2009). Free radical damage would lead to apoptosis and an autoimmune response (Tsatsoulis, 2002). Glyphosate's disruption of these bacteria would lead to a depletion in the supply of selenomethionine and selenocysteine. Methionine depletion by glyphosate (Nafziger *et al.*, 1984) would further compound this problem.

Thus, there are a variety of ways in which glyphosate would be expected to interfere with the supply of selenoproteins to the body, including its effects on Lactobacillus, its depletion of methionine, the no-till farming methods that are possible because weeds are killed chemically, and the likely interference with plant uptake of inorganic selenium. This aligns well with the observed higher risk of thyroid problems in association with celiac disease, in addition to infertility problems and immune issues, which are discussed elsewhere in this paper. Further support for an association between glyphosate and thyroid disease comes from plots over time of the usage of glyphosate in the U.S. on corn and soy time-aligned with plots of the incidence rate of thyroid cancer in the U.S., as shown in Figure 3.

9 Indole and kidney disease

The prevalence of kidney disease and resulting dialysis is increasing worldwide, and kidney disease is often associated with increased levels of celiac disease autoantibodies. Kidney disease and thyroid dysfunction are intimately connected (Iglesias & Díez, 2009). A population-based study in Sweden involving nearly 30,000 people with diagnosed celiac disease determined that there was nearly a three-fold increased risk for kidney failure in this population group (Welander *et al.*, 2012).

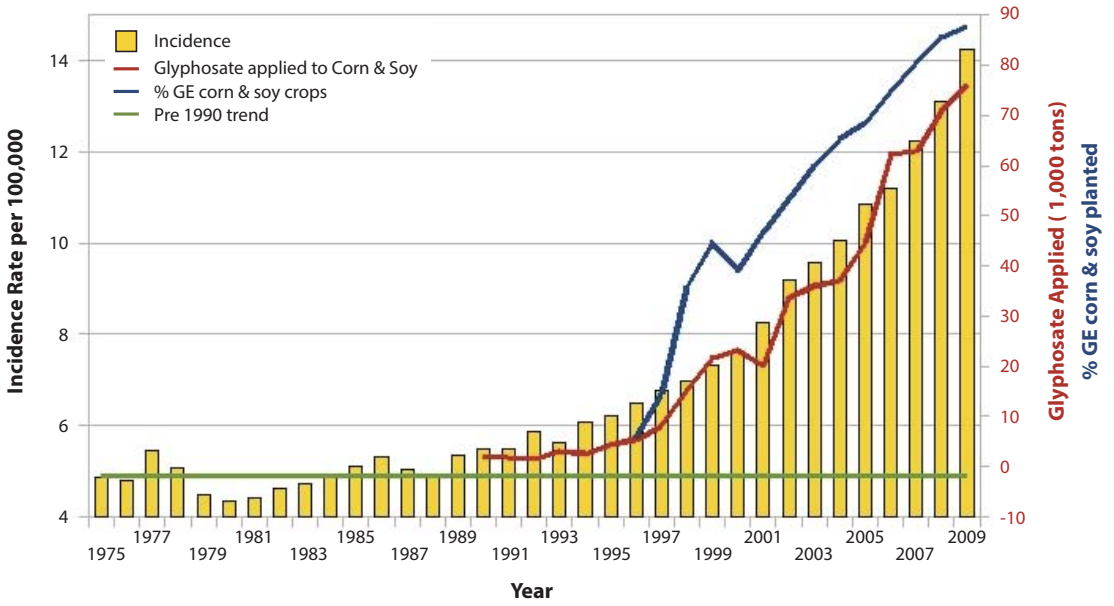


Figure 3. Thyroid cancer incidence rate plotted against glyphosate applied to U.S. corn & soy crops (R=0.988, $p \leq 7.612e\text{-}09$) along with %GE corn & soy crops (R=0.9377, $p \leq 2.152e\text{-}05$). Sources: USDA:NASS; SEER. (Figure courtesy of Nancy Swanson).

Inflammation plays a crucial role in kidney disease progression (Tonelli *et al.*, 2005; Bash *et al.*, 2009; Rodriguez-Iturbe *et al.*, 2010). Chronic kidney disease develops as a consequence of assaults on the kidney from inflammatory agents, brought on by the induction of pro-inflammatory cytokines and chemokines in the kidney. The toxic phenol p-Cresol sulfate, as well as indoxyl sulfate, a molecule that is chemically similar to p-Cresol, have been shown to induce activation of many of these cytokines and chemokines (Sun *et al.*, 2012). p-Cresol and indoxyl sulfate both decrease endothelial proliferation and interfere with wound repair (Dou *et al.*, 2004). p-Cresol is produced by the pathogenic bacterium C. difficile, and indoxyl sulfate, derived from indole through sulfation in the liver (Banoglu & King, 2002), accumulates at high levels in association with chronic kidney disease (Niwa, 2010).

The aromatic amino acid tryptophan contains an indole ring, and therefore disruption of tryptophan synthesis might be expected to generate indole as a by-product. Indeed, glyphosate has been shown to induce a significant increase in the production of indole-3-acetic acid in yellow nutsedge plants (Caal *et al.*, 1987). Indole is produced by coliform microorganisms such as E. coli under anaerobic conditions. Glyphosate induces a switch in E. coli from aerobic to anaerobic metabolism due to impaired mitochondrial ATP synthesis (Lu *et al.*, 2013; Samsel & Seneff, 2013), which would likely result in excess production of indole. Besides, E. coli, many other pathogenic bacteria can produce indole, including Bacillus, Shigella, Enterococcus, and V. cholerae (Lee & Lee, 2010). At least 85 different species of both Gram-positive and Gram-negative bacteria produce indole, and its breakdown by certain bacterial species depends on CYP enyzmes (Lee & Lee, 2010). Feeding indole to rats deprived of sulfur metabolites leads to macrocytic anemia (Roe, 1971). Indole is an important biological signaling molecule among microbes (Lee & Lee, 2010). Indole acetic acid inhibits the growth of cobalamin-dependent microorganisms, which then causes macrocytic (pernicious) anemia in the host due to cobalamin deficiency (Drexler, 1958).

Experiments on exposure of mouse fetuses to indole-3-acetic acid have shown that it dramatically induces microcephaly in developing fetuses exposed at critical times in development (Furukawa *et al.*, 2007). A case study found celiac disease associated with microcephaly and developmental delay in a 15-month-old girl (Bostwick *et al.*, 2001; Lapunzina, 2002). A gluten-free diet restored head growth. The authors suggested that poor head growth might precede other manifestations of celiac disease in infants. A study on plants demonstrated a concentration gradient of indole-3-acetic acid in the plant embryo, similar to the gradient in retinoic acid that controls fetal development in mammals (Uggla *et al.*, 1996). This alternative may be another way in which glyphosate would promote microcephaly.

Thus, solely through its effect on indole production and indole catabolism in gut bacteria, chronic glyphosate exposure would be expected to lead to cobalamin deficiency, pernicious anemia, microcephaly in a fetus during pregnancy, and kidney failure. p-Cresol supply by overgrown pathogens like C. difficile would likely contribute in a similar way as indole, due to its similar biochemical and biophysical properties.

ISSN: 1337-6853 *(print version)* | 1337-9569 *(electronic version)*

Interdisciplinary Toxicology. 2013; **Vol. 6**(4): 159–184
Also available online on **PubMed Central**

10 Nutritional deficiencies

The damaged villi associated with celiac disease are impaired in their ability to absorb a number of important nutrients, including vitamins B6, B12 (cobalamin) and folate, as well as iron, calcium and vitamins D and K (Hallert *et al.*, 2002). Thus, long-term celiac disease leads to major deficiencies in these micronutrients. Cobalamin deficiency has been well addressed previously. We have also already mentioned the chelation of trace minerals by phytates and by glyphosate. However, other factors may be at play as well, as discussed here.

Glyphosate disrupts the synthesis of tryptophan and tyrosine in plants and in gut bacteria, due to its interference with the shikimate pathway (Lu *et al.*, 2013; María *et al.*, 1996), which is its main source of toxicity to plants. Glyphosate also depletes methionine in plants and microbes. A study on serum tryptophan levels in children with celiac disease revealed that untreated children had significantly lower ratios of tryptophan to large neutral amino acids in the blood, and treated children also had lower levels, but the imbalance was less severe (Hernanz & Polanco, 1991). The authors suggested a metabolic disturbance in tryptophan synthesis rather than impaired absorption, as other similar amino acids were not deficient in the serum. It was proposed that this could lead to decreased synthesis of the monoamine neurotransmitter, serotonin, in the brain associated with behavior disorders in children with celiac disease, such as depression (Koyama & Melzter, 1986). Deficiencies in tyrosine and methionine were also noted (Hernanz & Polanco, 1991). "Functional dyspepsia" is an increasing and mainly intractable problem in the Western world, which is estimated to affect 15% of the U.S. population (Saad & Chey, 2006). Dyspepsia, a clinical symptom of celiac disease, is likely mediated by excess serotonin synthesis following ingested tryptophan-containing foods (Manocha *et al.*, 2012).

Serotonin (5-hydroxytryptamine or 5-HT) is produced by enterochromaffin (EC) cells in the gut and is an important signaling molecule for the enteric mucosa (Kim *et al.*, 2001). EC cells are the most numerous neuroendocrine cell type in the intestinal lumen, and they regulate gut secretion, motility, pain and nausea by activating primary afferent pathways in the nervous system (Chin *et al.*, 2012). Serotonin plays an important role in activating the immune response and inflammation in the gut, and also induces nausea and diarrhea when it is overexpressed. Anaerobic bacteria in the colon convert sugars into short-chain fatty acids, which can stimulate 5-HT release from EC cells (Fukumoto *et al.*, 2003; Grider & Piland, 2007). This is likely an important source of fats to the body in the case of a low-fat diet induced by impaired fatty acid metabolism due to insufficient bile acids.

The number of 5-HT expressing EC cells in the small intestine is increased in association with celiac disease, along with crypt hyperplasia (Wheeler & Challacombe, 1984; Challacombe *et al.*, 1977), and, as a consequence, serotonin uptake from dietary sources of tryptophan is greatly increased in celiac patients (Erspamer, 1986). Postprandial dyspepsia is associated in celiac disease with increased release of 5-HT, and this may account for the digestive symptoms experienced by celiac patients (Coleman *et al.*, 2006). An explanation for these observations is that a chronic tryptophan insufficiency due to the impaired ability of gut bacteria to produce tryptophan induces aggressive uptake whenever dietary tryptophan is available.

Glyphosate forms strong complexes with transition metals, through its carboxylic, phosphonic, and amino moieties, each of which can coordinate to metal ions, and it can also therefore form complexes involving two or three atoms of the targeted transition metal (Madsen *et al.*, 1978; Motekaitis & Martell, 1985; Undabeytia *et al.*, 2002). This means that it is a metal chelator par excellence. One can expect, therefore, deficiencies in multiple transition (trace) metals, such as iron, copper, cobalt, molybdenum, zinc and magnesium in the presence of glyphosate. Glyphosate has been shown to reduce levels of iron, magnesium, manganese and calcium in non-GMO soybean plants (Cakmak *et al.*, 2009). We have already discussed iron, selenium, cobalt and molybdenum deficiencies in association with celiac disease.

Zinc deficiency seems to be a factor in celiac disease, as a recent study of 30 children with celiac disease demonstrated a significantly reduced serum level of zinc (0.64 vs 0.94 μg/mL in controls) (Singhal *et al.*, 2008). Copper deficiency is a feature of celiac disease (Halfdanarson *et al.*, 2009), and copper is one of the transition metals that glyphosate binds to and chelates (Madsen, 1978; Undabeytia, 2002). Confirmed magnesium deficiency in celiac disease has been shown to be due to significant loss through the feces (Goldman *et al.*, 1962). This would be expected through binding to phytates and/or glyphosate. A study of 23 patients with gluten-sensitive enteropathy to assess magnesium status revealed that only one had *serum* magnesium levels below the normal range, whereas magnesium levels in *erythrocytes* and *lymphocytes* was markedly below normal, and this was associated with evidence of osteoporosis due to malabsorption (Rude and Olerich, 1996). Daily treatment with $MgCl_2$ or Mg lactate led to a significant increase in bone mineral density, and was correlated with a rise in RBC Mg^{2+}.

A recent study investigated the status of 25(OH) vitamin D3 in adults and children with celiac disease (Lerner *et al.*, 2012). It was determined that vitamin D3 deficiency was much more prevalent in the adults than in the children, suggesting a deterioration in vitamin D3 serum levels with age. This could be explained by a chronic accumulation of glyphosate, leading to increasingly impaired vitamin D3 activation in the liver. The liver converts 1,25(OH) vitamin D3 to the active form, 25(OH) vitamin D3, using CYP27A (Ponchon *et al.*, 1969; Sakaki *et al.*, 2005), which might be disrupted by glyphosate exposure, given its known interference with CYP function in mice (Hietanen *et al.*, 1983). On a broader level, this might also explain the recent epidemic in the U.S. in vitamin D3 deficiency (Holick, 2005).

Another issue to consider is whether the food being consumed by celiac patients is itself depleted in nutrients.

This is likely the case for the transgenic Roundup-Ready crops that increasingly supply the processed food industry. A recent study on the effects of glyphosate on Roundup-Ready soy revealed a significant effect on growth, as well as an interference with the uptake of both macronutrients and micronutrients (Saes Zobiole *et al.*, 2010). Transgenic soybeans exposed to glyphosate are often affected by a "yellow flashing" or yellowing of the upper leaves, and an increased sensitivity to water stress. An inverse linear relationship was observed between glyphosate dosage and levels of the macronutrients, sodium, calcium, sulfur, phosphorus, potassium, magnesium, and nitrogen, as well as the micronutrients, iron, zinc, manganese, copper, cobalt, molybdenum, and boron. Glyphosate's ability to form insoluble metal complexes likely mediates these depletions (Glass, 1984). Glyphosate also interferes with photosynthesis, as reflected in several measures of photosynthesis rate (Saes *et al.*, 2010) and reductions in chlorophyll (Ali & Fletcher, 1977; Kitchen *et al.*, 1981). This could be due to depletion of zinc and manganese, since chloroplasts require these micronutrients to function well (Homann, 1967; Thompson & Weier, 1962).

11 Cancer

Chronic inflammation, such as occurs in celiac disease, is a major source of oxidative stress, and is estimated to account for 1/3 of all cancer cases worldwide (Ames *et al.*, 1993; Coussens & Werb, 2002). Oxidative stress leads to DNA damage and increased risk to genetic mutation. Several population-based studies have confirmed that patients with celiac disease suffer from increased mortality, mainly due to malignancy (Nielsen *et al.*, 1985; Logan *et al.*, 1989; Pricolo *et al.*, 1998; Cottone *et al.*, 1999; Corrao *et al.*, 2001; Green *et al.*, 2003). These include increased risk to non-Hodgkin's lymphoma, adenocarcinoma of the small intestine, and squamous cell carcinomas of the esophagus, mouth, and pharynx, as well as melanoma. The non-Hodgkin's lymphoma was not restricted to gastrointestinal sites, and the increased risk remained following a gluten-free diet (Green *et al.*, 2003).

Celiac disease is associated with a lifelong risk of any malignancy between 8.1 and 13.3%, with the risk for non-Hodgkin's lymphoma alone being 4.3 to 9.6% (Matheus-Vliezen *et al.*, 1994; Egan *et al.*, 1995). This risk is 19-fold higher than the risk in the general population. Selenium deficiency in association with celiac disease may be a significant factor in the increased cancer risk. Selenium deficiency is associated with increased risk to several cancers, and selenium supplements are beneficial in reducing the incidence of liver cancer and decreasing mortality in colorectal, lung and prostate cancer (Nelson *et al.*, 1999; Björnstedt *et al.*, 2010).

Children with celiac disease, whether or not they are on a gluten-free diet, exhibit elevated urinary biomarkers of DNA damage (Zaflarska-Popawska *et al.*, 2010). Human colon carcinoma cells exposed to peptides extracted from wheat responded with a sharp increase in the GSSG/GSH ratio (ratio of oxidized to reduced glutathione), a well-established indicator of oxidative stress (Rivabene, 1999). The authors did not provide information as to whether the wheat plants were exposed to glyphosate, but they did suggest that this effect could explain the increased risk to intestinal cancer associated with celiac. Intriguingly, studies on pea plants have shown that *glyphosate* induces a sharp increase in the GSSG/GSH ratio in plants (Miteva *et al.*, 2003), which suggests that glyphosate contamination could explain the results observed in (Rivabene, 1999).

Interestingly, it was noted in 1996 that the incidence of both non-Hodgkin's lymphoma and melanoma had been rising sharply worldwide in recent decades, and so it was decided to investigate whether there might be a link between the two cancers associated with sunlight exposure. Surprisingly, the authors found an *inverse* relationship between non-Hodgkin's lymphoma and UV exposure. More recently, such UV protection has been reaffirmed in a review of epidemiologic studies on the subject (Negri, 2010). This suggests that vitamin D3 is protective, so vitamin D3 deficiency due to impaired CYP function in the liver could be contributory to increased risk in celiac disease.

The incidence of non-Hodgkins lymphoma has increased rapidly in most Western countries over the last few decades. Statistics from the American Cancer Society show an 80% increase since the early 1970's, when glyphosate was first introduced on the market.

While there have been only a few studies of lymphoma and glyphosate, nearly all have indicated a potential relationship (Vigfusson & Vyse, 1980; Pavkov & Turnier, 1986; Hardell & Eriksson, 1999; McDuffie *et al.*, 2001; De Roos *et al.*, 2003). A dose-response relationship for non-Hodgkin's lymphoma was demonstrated in a cross-Canada study of occupational exposure to glyphosate in men (McDuffie *et al.*, 2001), and a larger study in the U.S. noted a similar result (De Roos *et al.*, 2003). A population-based study in Sweden showed an increased risk to non-Hodgkins lymphoma upon prior exposure to herbicides and fungicides but not insecticides (Hardell & Eriksson, 1999). Glyphosate exposure resulted in an odds ratio of 2.3, although the number of samples was small, and the authors suggested that further study is necessary. A study on mice showed increases in carcinoma, leukemia and lymphoma (Pavkov & Turnier, 1986) and an in vitro mutagenic test on human lymphocytes revealed increased sister-chromatid exchanges (Vigfusson & Vyse, 1980) upon exposure to glyphosate.

12 Proposed transglutaminase-glyphosate interactions

Establishing the mechanism by which glyphosate might promote autoantibodies to transglutaminase is a challenging task, not because this possibility seems unlikely but rather because multiple disruptions are plausible. In this section, we present evidence from the research literature that supports various hypotheses for the interaction

ISSN: 1337-6853 *(print version)* | 1337-9569 *(electronic version)*

Interdisciplinary Toxicology. 2013; **Vol. 6**(4): 159–184
Also available online on **PubMed Central**

of glyphosate with the transglutaminase enzymatic pathways. The definitive studies that clarify which of these hypotheses is correct have yet to be conducted.

Celiac disease is thought to be primarily caused by ingestion of wheat gluten proteins, particularly gliadin, due to a high concentration of proline- and glutamine-rich sequences, which imparts resistance to degradation by proteases. Transglutaminase autoimmunity arises when specific epitopes of wheat gliadin activate sensitized T-cells which then stimulate B-cell synthesis of IgA or IgM autoantibodies to transglutaminase. Transglutaminase bound to gliadin can induce false recognition by a T-cell.

Transglutaminase acts on gluten in wheat to form crosslinks between glutamine residues and lysine residues, producing ammonia as a by-product. Ammonia is known to induce greater sensitivity to glyphosate in plants, and it is common practice to apply ammonium sulfate simultaneously with glyphosate for this reason (Nalewaja & Matysiak, 1993). This enhanced effect is due to ammonium binding to glyphosate at three sites – one on the carbonyl group and two on the phosphonyl group, which displaces cations such as calcium and endows glyphosate with enhanced reactivity.

Transglutaminase sometimes only achieves half of its intended reaction product, by converting a glutamine residue to glutamate, and leaving lysine intact, thus not producing the desired crosslink. It has been established that gluten fragments containing "deamidated glutamine" residues instead of the crosslinks are much more highly allergenic than those that contain the crosslinks (Dørum *et al.*, 2010; Qiao *et al.*, 2005). These have been referred to as "celiac disease T-Cell epitopes." T-cells of celiac patients preferentially recognize epitopes that are augmented with negatively charged deamidated glutamine residues – the product of the reaction when the lysine linkage does not occur. Thus, if there is a mechanism by which glyphosate interferes with crosslink formation, this would explain its ability to enhance gluten sensitivity.

A clue can be found from the research literature on glyphosate sensitivity in plants, where it has been determined that the substitution of a lysine residue in a critical locale in EPSP synthase greatly increases sensitivity to glyphosate (Selvapandiyan *et al.*, 1995). Lysine's NH3+ group is highly reactive with negatively charged ions, and this makes it a common constituent of DNA binding proteins due to its ability to bind to phosphates in the DNA backbone. Glyphosate contains a phosphonyl group that binds easily to ammonia and behaves as a phosphate mimetic. It also contains a carboxyl group that substitutes well for the carboxyl group of glutamate, the intended reaction partner.

Thus, it seems possible that glyphosate would be drawn to the ammonia released when the glutamine residue is deamidated by transglutaminase, and then the ammonium glyphosate would react with the lysine residue, releasing the ammonia and resulting in the binding of glyphosate to the lysine residue. This would yield a gluten fragment bound to glyphosate that is likely highly allergenic. An analogous EPSP synthase-EPSP-glyphosate ternary complex has been identified in numerous studies on the physiology of glyphosate in plants (Sammons *et al.*, 1995).

Research in the food industry has concerned producing breads that, while not gluten free, may contain forms of gluten to which celiac patients are less sensitive. Such research has revealed that enzymatic modification to promote methionine binding to glutamine reduces IgA immunoreactivity (Cabrera-Chávez *et al.*, 2010). Whether methionine binding to glutamine residues in wheat takes place in vivo is not known, but it is established that glyphosate depletes methionine by 50 to 65 percent in plants, as well as the aromatic amino acids (Nafziger *et al.*, 1984; Haderlie *et al.*, 1977). As we have already discussed, glyphosate interferes with cobalt bioavailability for cobalamin synthesis, and cobalamin is an essential catalyst for the conversion of cysteine to methionine.

Transglutaminase also cross-links proteins in the extracellular matrix, and therefore is important for wound healing, tissue remodeling, and stabilization of the extracellular matrix. Thus, autoimmunity to transglutaminase leads to destabilization of the microvilli lining the small intestines. Transglutaminase has 18 free cysteine residues which are targets for S-nitrosylation. A cysteine residue is also involved in the catalytic active site. A unique Ca^{2+} dependent mechanism regulates nitrosylation by NO, mediated by CysNO (S-nitrosocysteine). It was shown experimentally that up to 15 cysteines of transglutaminase were nitrosylated by CysNO in the presence of Ca^{2+}, and this inhibited its enzymatic activity (Lai *et al.*, 2001).

Thus, another plausible mechanism by which glyphosate might enhance the development of autoantibodies to transglutaminase is by nitrosylating its cysteines, acting similarly to CysNO. A precedent for this idea is set with research proposing nitrosylation as the means by which glyphosate interferes with the heme active site in CYP enzymes (Lamb *et al.*, 1998). It is conceivable that cysteine nitrosylation by glyphosate at the active site inactivates the molecule, in which case glyphosate is itself acting as an "antibody."

13 Evidence of glyphosate exposure in humans and animals

The US EPA has accepted Monsanto's claim that glyphosate is essentially harmless to humans. Due to this position, there have been virtually no studies undertaken in the US to assess glyphosate levels in human blood or urine. However, a recent study involving multiple countries in Europe provides disturbing confirmation that glyphosate residues are prevalent in the Western diet (Hoppe, 2013). This study involved exclusively city dwellers, who are unlikely to be exposed to glyphosate except through food sources. Despite Europe's more aggressive campaign against GMO foods than that in the Americas, 44% of the urine samples contained quantifiable amounts of glyphosate. Diet seems to be the main source of exposure. One can predict that, if a study were undertaken in the U.S., the percentage of the affected population would be much larger.

A recent study conducted on dairy cows in Denmark shows conclusively that the cows' health is being adversely affected by glyphosate (Krüger *et al.*, 2013a). All of the cows had detectable levels of glyphosate in their urine, and it was estimated that from 0.1 to 0.3 mg of glyphosate was excreted daily from each cow. More importantly, all of the cows had serum levels of cobalt and manganese that were far below the minimum reference level for nutrient sufficiency. Half of the cows had high serum urea, and there was a positive linear relationship between serum urea and glyphosate excretion. High serum urea is indicative of nephrotoxicity. Blood serum levels of enzymes indicative of cytotoxicity such as creatine kinase (CK) and alkaline phosphatase (ALP) were also elevated. CK is indicative of rhabdomyolysis or kidney failure. High levels of ALP indicate liver damage, and it is often used to detect blocked bile ducts (Kaplan *et al.*, 1983).

Thus, the low cobalt levels and the indicators of liver, kidney, and gall bladder stress are all consistent with our previous discussion. The results of this study were also consistent with results of a study on rats exposed experimentally to glyphosate (Beuret *et al.*, 2005) in which Roundup was shown to be even more toxic than its active ingredient, glyphosate.

Glyphosate-metal complexes serve to reduce glyphosate's toxicity in the soil to plants, but they also protect glyphosate from attack by microorganisms that could decompose it (Cusiel, 2005). The degree of reactivity of the complex depends on which metals glyphosate binds to, which in turn depends upon the particular soil conditions (Nomura & Hilton, 1977). Glyphosate usually degrades relatively quickly (Vencill, 2002); however, a half-life of up to 22 years has also been reported in conditions where pH is low and organic matter contents are high (Nomura & Hilton, 1977). Therefore, glyphosate may survive much longer in certain soils than has been claimed by the industry, and could be taken up by crops planted subsequent to glyphosate application to kill weeds.

A disturbing trend of crop desiccation by glyphosate pre-harvest (O'Keeffe, 1980; O'Keeffe, 1981; Stride *et al.*, 1985; Darwent *et al.*, 1994; Orson & Davies, 2007) may be a key factor in the increased incidence of celiac disease. According to Monsanto, glyphosate was used on some 13% of the wheat area pre-harvest in the UK in 2004. However, by 2006 and 2007, some 94% of UK growers used glyphosate on at least 40% of cereal and 80% of oilseed crops for weed control or harvest management (Monsanto International Sàrl, 2010).

An increasing number of farmers now consider the benefits of desiccating their wheat and sugar cane crops with glyphosate shortly before the harvest (Monsanto International Sàrl, 2010). The advantage is improved harvesting efficiency because the quantity of materials other than grain or cane is reduced by 17%, due to a shutdown of growth following glyphosate treatment. Treated sugar cane crops produce drier stalks which can be baled more easily. There is a shorter delay before the next season's crop can be planted, because the herbicide was applied pre-harvest rather than post-harvest. Several pests can be controlled due to the fact that glyphosate is a broad-spectrum herbicide. These include Black grass, Brome grasses, and Rye grasses, and the suggestion is that this would minimize the risk of these weeds developing resistance to other herbicides.

A complete list of the latest EPA residue levels for glyphosate as of September 18, 2013 are shown in Table 1. Tolerances are established on all crops for both human and animal consumption resulting from the application of glyphosate.

As glyphosate usage continues unabated, glyphosate resistance among weeds is becoming a growing problem (Waltz, 2010), necessitating a strategy that either involves an increase in the amount of glyphosate that is applied or a supplementation with other herbicides such as glufosinate, dicampa, 2-4D, or atrazine. Agrochemical companies are now actively developing crops with resistance to multiple herbicides (Culpepper, 2000), a disturbing trend, especially since glyphosate's disruption of CYP enzymes leads to an impaired ability to break down many other environmental chemicals in the liver.

14 Kidney disease in agricultural workers

Chronic kidney disease is a globally increasing problem (Ramirez-Rubio *et al.*, 2013), and glyphosate may be playing a role in this epidemic. A plot showing recent trends in hospitalization for acute kidney injury aligned with glyphosate usage rates on corn and soy shows strong correlation, as illustrated in Figure 4, and a similar correlation is seen for deaths due to end-stage renal disease in Figure 5. Recently, it has been noted that young men in Central America are succumbing in increasing numbers to chronic kidney disease (Trabanino *et al.*, 2002; Cerdas, 2005; Torres *et al.*, 2010; Peraza *et al.*, 2012; Ramirez-Rubio *et al.*, 2013; Sanoff *et al.*, 2010). The problem appears to be especially acute among agricultural workers, mainly in sugar cane fields (Cerdas, 2005; Torres *et al.*, 2010; Peraza *et al.*, 2012). Since we have shown in Section 8 how glyphosate can produce toxic effects on the kidneys through its disruption of gut bacteria, it is fruitful to consider whether glyphosate could be playing a role in the fate of Central American workers in the sugar cane fields.

In attempting to explain this phenomenon, physicians and pharmacists have proposed that it may be due to dehydration caused by over-exertion in high temperature conditions, combined with an acute reaction to commonly administered non-steroidal anti-inflammatory drugs (NSAIDs) to treat pain and/or antibiotics to treat infection (Ramirez-Rubio *et al.*, 2013). NSAIDs require CYP enzymes in the liver for detoxification (Agúndez *et al.*, 2009), so impaired CYP function by glyphosate would lead to a far more toxic effect of excessive NSAID administration. Kidney disease among agricultural workers tends to be associated with chronic glomerulonephritis and interstial nephritis, which was proposed in (Soderland *et al.*, 2010) to be

ISSN: 1337-6853 *(print version)* | 1337-9569 *(electronic version)*

Interdisciplinary Toxicology. 2013; **Vol. 6**(4): 159–184
Also available online on **PubMed Central**

Table 1. Complete list of glyphosate tolerances for residues in food crops in the U.S. as of September 18, 2013, as reported in: EPA: Title 40: Protection of Environment.

Commodity	PPM
Acerola	0.2
Alfalfa, seed	0.5
Almond, hulls	25
Aloe vera	0.5
Ambarella	0.2
Animal feed, nongrass, group 18	400
Artichoke, globe	0.2
Asparagus	0.5
Atemoya	0.2
Avocado	0.2
Bamboo, shoots	0.2
Banana	0.2
Barley, bran	30
Beet, sugar, dried pulp	25
Beet, sugar, roots	10
Beet, sugar, tops	10
Berry and small fruit, group 13-07	0.20
Betelnut	1.0
Biriba	0.2
Blimbe	0.2
Breadfruit	0.2
Cacao bean, bean	0.2
Cactus, fruit	0.5
Cactus, pads	0.5
Canistel	0.2
Canola. seed	20
Carrot	5.0
Chaya	1.0
Cherimoya	0.2
Citrus, dried pulp	1.0
Coconut	0.1
Coffee, bean, green	1.0
Corn, pop, grain	0.1
Corn, sweet, kernel plus cob with husk removed	3.5
Cotton, gin byproducts	210
Custard apple	0.2
Dried fruit	0.2
Dokudami	2.0
Durian	0.2
Epazote	1.3
Feijoa	0.2
Fig	0.2
Fish	0.25
Fruit, citrus, group 10-10	0.50
Fruit, pome, group 11-10	0.20
Fruit, stone, group 12	0.2
Galangal, roots	0.2
Ginger, white, flower	0.2
Gourd, buffalo, seed	0.1
Governor's plum	0.2
Gow kee, leaves	0.2
Grain, cereal, forage, fodder and straw, group 16, except field corn, forage and field corn, stover	100
Grain, cereal, group 15 except field corn, popcorn, rice, sweet corn, and wild rice	30
Grass, forage, fodder and hay, group 17	300
Guava	0.2
Herbs subgroup 19A	0.2
Hop, dried cones	7.0
Ilama	0.2
Imbe	0.2
Imbu	0.2
Jaboticaba	0.2
Jackfruit	0.2
Kava, roots	0.2
Kenaf, forage	200
Leucaena, forage	200
Longan	0.2
Lychee	0.2
Mamey apple	0.2
Mango	0.2
Mangosteen	0.2
Marmalade box	0.2
Mioga, flower	0.2
Noni	0.20
Nut, pine	1.0
Nut, tree, group 14	1.0
Oilseeds, group 20, except canola	40
Okra	0.5
Olive	0.2
Oregano, Mexican, leaves	2,0
Palm heart	0.2
Palm heart, leaves	0.2
Palm, oil	0.1
Papaya	0.2
Papaya, mountain	0.2
Passionfruit	0.2
Pawpaw	0.2
Pea, dry	8.0
Peanut	0.1
Peanut, hay	0.5
Pepper leaf, fresh leaves	0.2
Peppermint, tops	200
Perilla, tops	1.8
Persimmon	0.2
Pineapple	0.1
Pistachio	1.0
Pomegranate	0.2
Pulasan	0.2
Quinoa. grain	5.0
Rambutan	0.2
Rice, grain	0.1
Rice, wild, grain	0.1
Rose apple	0.2
S apod ilia	0.2
Sapote, black	0.2
Sapote, mamey	0.2
Sapote, white	0.2
Shellfish	3.0
Soursop	0.2
Spanish lime	0.2
Spearmint, tops	200
Spice subgroup 19B	7.0
Star apple	0.2
Starfruit	0.2
Stevia, dried leaves	1.0
Sugar apple	0.2
Sugarcane, cane	2.0
Sugarcane, molasses	30
Surinam cherry	0.2
Sweet potato	3.0
Tamarind	0.2
Tea. dried	1.0
Tea, instant	7.0
Teff, forage	100
TefF, grain	5.0
Teff, hay	100
Ti, leaves	0.2
Ti, roots	0.2
Ugli fruit	0.5
Vegetable, bulb, group 3-07	0.20
Vegetable, cucurbit, group 9	0.5
Vegetable, foliage of legume, subgroup 7A, except soybean	0.2
Vegetable, fruiting, group 8-10 (except okra)	0.10
Vegetable, leafy, brassica. group 5	0.2
Vegetable, leafy, except brassica, group 4	0.2
Vegetable, leaves of root and tuber, group 2, except sugar beet tops	0.2
Vegetable, legume, group 6 except soybean and dry pea	5.0
Vegetables, root and tuber, group 1, except carrot, sweet potato, and sugar beet	0.20
Wasabi. roots	0.2
Water spinach, tops	0.2
Watercress, upland	0.2
Wax jambu	0.2

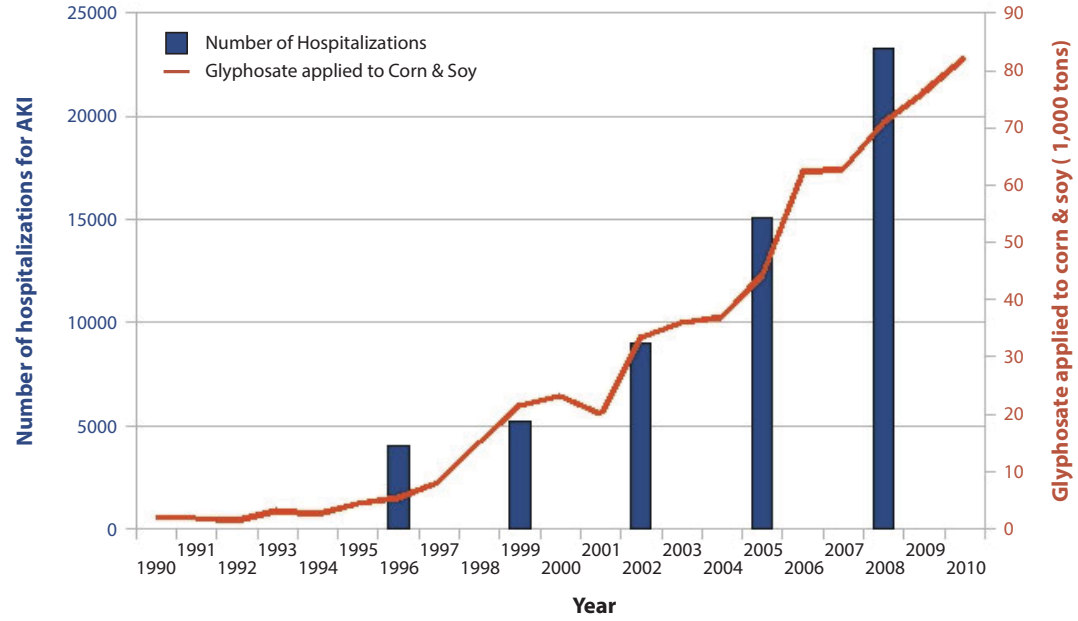


Figure 4. Number of hospitalizations for acute kidney injury plotted against glyphosate applied to com & soy (in 1000 tons). (Figure courtesy of Nancy Swanson).

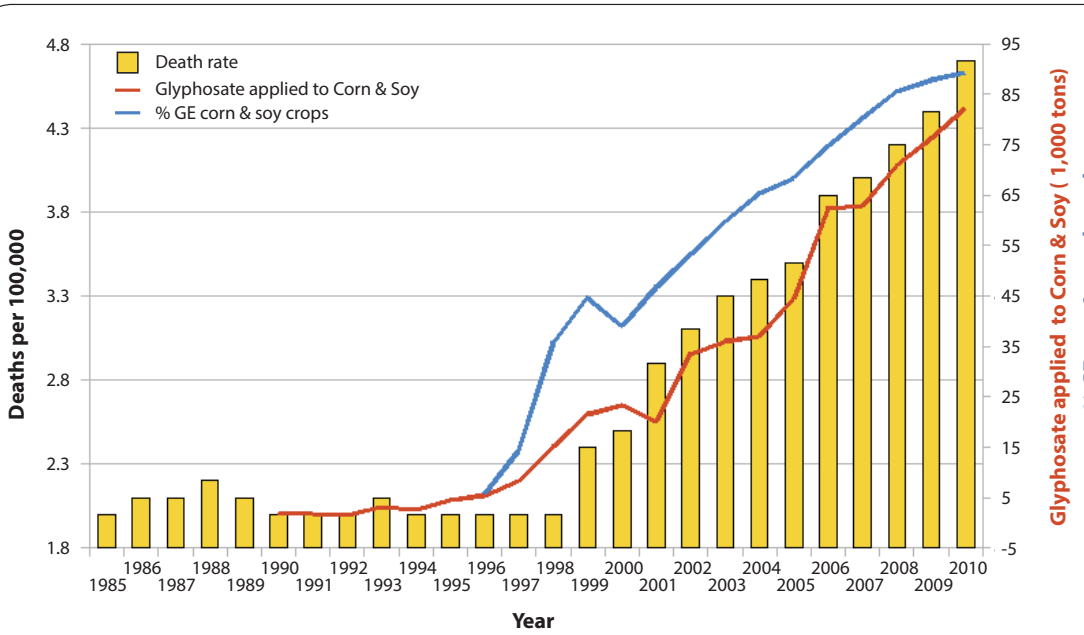


Figure 5. End stage renal disease deaths (ICD N18.0 & 585.6) plotted against %GE corn & soy planted (R=0.9585, p<4.03e-6) and glyphosate applied to corn & soy (R=0.9844, $p \leq 3.704e-09$). Sources: USDA:NASS; CDC. (Figure courtesy of Nancy Swanson).

due to environmental toxins such as heavy metals or toxic chemicals. Glomerulonephritis is also found in association with celiac disease (Katz *et al.*, 1979; Peters *et al.*, 2003). A Swedish study showed a five-fold increase in nephritis risk in celiac patients (Peters *et al.*, 2003).

A strong hint comes from epidemiological studies conducted in Costa Rica (Cerdas, 2005). The demographic features of those with chronic renal failure revealed a remarkably specific pattern of young men, between 20 and 40 years old, with chronic interstitial nephritis. All

ISSN: 1337-6853 *(print version)* | 1337-9569 *(electronic version)*

Interdisciplinary Toxicology. 2013; **Vol. 6**(4): 159–184
Also available online on **PubMed Central**

of them were sugar-cane workers. These authors wrote: "A specific study of their work environment is needed to determine what in their daily activities puts them at increased risk for chronic renal failure."

Agriculture is an important part of the economy of the state of Louisiana in the United States, and sugar cane is a significant agricultural product. Chemical methods to ripen sugar cane are commonly used, because they can substantially increase the sucrose content of the harvest (Richard & Dalley, 2009). Glyphosate, in particular, has been the primary ripener used in Louisiana since 1980 (Orgeron, 2012). As of 2001, Louisiana had the highest rate of kidney failure in the U.S. (State-Specific Trends in Chronic Kidney Failure – United States, 1990–2001). Louisiana's death rate per 100,000 from nephritis/kidney disease is 26.34 as compared to a U.S. rate of 14.55 (Network Coordinating Council, 2013). The number of patients on dialysis has risen sharply in the last few years.

By 2005, it is estimated that 62% of the total harvested hectares of sugar cane in Louisiana were ripened with glyphosate (Legendre *et al.*, 2005). A paper published in 1990 showed that glyphosate applied as a ripener on three different sugar cane varieties grown *in Costa Rica* produced up to a 15% increase in the sucrose content of the harvested sugar cane (Subiros, 1990). Glyphosate applied before the harvest is the *only* sugarcane ripener currently registered for use in the U.S.

A disturbing recent trend is the repeated application of glyphosate over the course of the season with the hope of further increasing yields (Richard & Dalley, 2009). Responses to the standard application rate (0.188 lb/acre) of glyphosate have been inconsistent, and so farmers are increasing both the amount and the frequency of application. In (Richard & Dalley, 2009), growers are encouraged not to apply glyphosate beyond mid-October, as results are counterproductive, and not to use higher rates in an attempt to improve yield. But it is doubtful that these recommendations are being followed. It is likely, although we have not been able to confirm this, that glyphosate usage has expanded in scope on the sugar cane fields in Central America since 2000, when the expiration of Monsanto's patent drove prices down, and that the practices of multiple applications of glyphosate in the U.S. are also being followed in Central America. Several other ripening agents exist, such as Ethephon, Trinexapacethyl, and Sulfometuron-methyl, but glyphosate is likely growing in popularity recently due to its more favorable pricing and perceived non-toxicity. Larger amounts are needed for effective ripening in regions that are hot and rainy, which matches the climate of Costa Rica and Nicaragua.

15 Discussion

In this paper, we have developed an argument that the alarming rise in the incidence of celiac disease in the United States and elsewhere in recent years is due to an increased burden of herbicides, particularly glyphosate exposure in the diet. We suggest that a principal factor is the use of glyphosate to desiccate wheat and other crops prior to the harvest, resulting in crop residue and increased exposure. Strong evidence for a link between glyphosate and celiac disease comes from a study on predatory fish, which showed remarkable effects in the gut that parallel the features of celiac disease (Shenapati *et al.*, 2009).

More generally, inflammatory bowel disease has been linked to several environmental factors, including a higher socioeconomic status, urban as opposed to rural dwelling, and a "Westernized" cultural context (Shapira *et al.*, 2010). Disease incidence is highest in North America and Europe, and is higher in northern latitudes than in southern latitudes within these regions, suggesting a beneficial role for sunlight. According to the most recent statistics from the U.S. Environmental Protection Agency (EPA) (Grube *et al.*, 2011), the U.S. currently represents 25% of the total world market on herbicide usage. Glyphosate has been the most popular herbicide in the U.S. since 2001, whereas it was the 17th most popular herbicide in 1987 (Kiely *et al.*, 2004). Since 2001, glyphosate usage has grown considerably, due to increased dosing of glyphosate-resistant weeds and in conjunction with the widespread adoption of "Roundup-Ready" genetically modified crops. Glyphosate is probably now the most popular herbicide in Europe as well (Kimmel *et al.*, 2013). Glyphosate has become the number one herbicide worldwide, due to its perceived lack of toxicity and its lower price after having become generic in 2000 (Duke & Powles, 2008).

A recent estimate suggests that one in twenty people in North America and Western Europe suffer from celiac disease (Koning, 2005; Fasano *et al.*, 2003). Outdoor occupational status is protective (Sonnenberg *et al.*, 1991). First generation immigrants into Europe or North America are generally less susceptible, although second generation non-Caucasian immigrants statistically become even more susceptible than native Caucasians (Shapira *et al.*, 2010). This may in part stem from the increased need for sunlight exposure given darker skin pigmentation.

Table 2 summarizes our findings relating glyphosate to celiac disease. All of the known biological effects of glyphosate – cytochrome P450 inhibition, disruption of synthesis of aromatic amino acids, chelation of transition metals, and antibacterial action – contribute to the pathology of celiac disease.

Celiac disease is associated with deficiencies in several essential micronutrients such as vitamin D3, cobalamin, iron, molybdenum, selenium and the amino acids, methionine and tryptophan, all of which can be explained by glyphosate. Glyphosate depletes multiple minerals in both genetically modified soybeans (Saes *et al.*, 2010) and conventional soybeans (Cakmak *et al.*, 2009), which would translate into nutritional deficiencies in foods derived from these crops. This, together with further chelation in the gut by any direct glyphosate exposure, could explain deficiencies in cobalt, molybdenum and iron. Glyphosate's effect on CYP enzymes should lead to inadequate vitamin D3 activation in the liver (Hietanen *et al.*, 1983; Ponchon *et al.*, 1969). Cobalamin depends

Table 2. Illustration of the myriad ways in which glyphosate can be linked to celiac disease or its associated pathologies.

(a) Disruption of gut bacteria

Glyphosate Effect	Dysfunction	Consequences
reduced Bifidobacteria	impaired gluten breakdown	transglutaminase antibodies
reduced Lactobacillus	impaired phytase breakdown reduced selenoproteins	metal chelation autoimmune thyroid disease
anaerobic E. coli	indole toxicity	kidney failure
C. diff overgrowth	p-Cresol toxicity	kidney failure
Desulfovibrio overgrowth	hydrogen sulfide gas	inflammation

(b) Transition metal chelation

Glyphosate Effect	Dysfunction	Consequences
cobalt deficiency	cobalamin deficiency reduced methionine elevated homocysteine	neurodegenerative diseases impaired protein synthesis heart disease
molybdenum deficiency	inhibited sulfite oxidase inhibited xanthine oxidase	impaired sulfate supply DNA damage/cancer teratogenesis megaloblastic anemia
iron deficiency		anemia

(c) CYP enzyme inhibition

Glyphosate Impairment	Dysfunction	Consequences
vitamin D3 inactivation	impaired calcium metabolism	osteoporosis; cancer risk
retinoic acid catabolism	suppressed transglutaminase	teratogenesis
bile acid synthesis	impaired fat metabolism impaired sulfate supply	gall bladder disease pancreatitis
xenobiotic detoxification	increased toxin sensitivity impaired indole breakdown	liver disease macrocytic anemia kidney failure
nitrate reductase	venous constriction	venous thrombosis

(d) Shikimate pathway suppression

Glyphosate Effect	Dysfunction	Consequences
tryptophan deficiency	impaired serotonin supply hypersensitive receptors	depression nausea, diarrhea

on cobalt, and cobalt-dependent enzymes in plants and microbes have been shown to be inhibited by glyphosate (Bode *et al.*, 1984; Ganson and Jensen, 1988). Glyphosate has been shown to severely impair methionine and tryptophan synthesis in plants (Nafziger *et al.*, 1984), which would reduce the bioavailability of these nutrients in derived foods.

There are multiple intriguing connections between celiac disease and microcephaly, all of which can be linked to glyphosate. Celiac disease is found in association with microcephaly in infants (Bostwick *et al.*, 2001; Lapunzina, 2002), and teratogenic effects are also observed in children born to celiac mothers (Dickey *et al.*, 1996; Martinelli *et al.*, 2000). Microcephaly in an infant where confirmed molybdenum deficiency was present (Boles *et al.*, 1993) suggests that molybdenum deficiency could be causal. However, elevated RA also induces microcephaly, as does indole-3-acetic acid, which has been dramatically linked to microcephaly in mice (Furukawa *et al.*, 2007). Elevated RA is predicted as a response to glyphosate due to its expected inhibition of CYP enzymes which catabolize RA in the liver (Lamb *et al.*, 1998; Hietanen *et al.*, 1983). Molybdenum deficiency is expected due to glyphosate's ability to chelate cationic minerals. Glyphosate has been shown to induce indole-3-acetic acid synthesis in plants (Caal *et al.*, 1987), and it induces a shift to anaerobic metabolism in E. coli (Lu *et al.*, 2013), which is associated with indole synthesis.

Celiac disease is associated with impaired serotonin metabolism and signaling in the gut, and this feature leads us to propose a novel role for serotonin in transporting sulfate to the tissues. It is a curious and little known fact that glucose and galactose, but not fructose or mannose, stimulate 5-HT synthesis by EC cells in the intestinal

ISSN: 1337-6853 *(print version)* | 1337-9569 *(electronic version)*

Interdisciplinary Toxicology. 2013; **Vol. 6**(4): 159–184
Also available online on **PubMed Central**

lumen (Kim *et al.*, 2001), suggesting a role for EC cells as "glucose sensors." Glucose and galactose are the two sugars that make up the heparan sulfate chains of the syndecans and glypicans that attach to the membrane-bound proteins in most cells, serving as the innermost constituency of the extracellular matrix (Bernfield *et al.*, 1999). In (Seneff *et al.*, 2012), it was proposed that part of the post-prandial glucose that is taken up by the tissues is temporarily stored in the extracellular matrix as heparan sulfate, and that a deficiency in sulfate supply impairs this process, which impedes glucose uptake in cells. These heparan sulfate units have a high turnover rate, as they are typically broken down within three hours of their initial placement (Turnbull *et al.*, 2001). This provides the cells with a convenient temporary buffer for glucose and galactose that can allow them to more efficiently remove these sugars from the serum. Insufficient sulfate supplies would impair this process and lead to insulin resistance.

As is the case for other monoamine neurotransmitters as well as most sterols, 5-HT is normally transported in the serum in a sulfated form. The sulfate moiety must be removed for the molecule to activate it. Therefore, 5-HT, as well as these other monoamine neurotransmitters and sterols, can be viewed as a sulfate "escort" in the plasma. In (Samsel & Seneff, 2013), it was argued that such carbon-ring-containing molecules are necessary for safe sulfate transport, especially in the face of co-present kosmotropes like glyphosate, in order to protect the blood from excess viscosity during transport. Support for the concept that glyphosate gels the blood comes from the observation that disseminated coagulation is a characteristic feature of glyphosate poisoning (Zouaoui *et al.*, 2013). Since glyphosate disrupts sterol sulfation and it disrupts monoamine neurotransmitter synthesis, in addition to its physical kosmotropic feature, it can be anticipated that a chronic exposure to even a small amount of glyphosate over the course of time will lead to a system-wide deficiency in the supply of sulfate to the tissues. We believe that this is the most important consequence of glyphosate's insidious slow erosion of health.

An interesting consideration regarding a known link between celiac disease and hypothyroidism (Collins *et al.*, 2012) emerges when one considers that iodide is one of the few chaotropic (structure breaking) anions available to biological systems: another important one being nitrate, which is elevated in the urine in association with celiac disease (Laurin *et al.*, 2003). It is intriguing that the conversion of T4 to T3 (the active form of thyroid hormone) involves selenium as an essential cofactor. Furthermore, iodide is released in the process, thus providing chaotropic buffering in the blood serum. Therefore, impaired conversion due to deficient selenium results in an inability to buffer this significant chaotrope in the blood, despite the fact that chaotropic buffering is likely desperately needed in the context of the kosmotropic effects of glyphosate. While speculative, it is possible that the autoimmune thyroid disease that develops in association with celiac disease is a direct consequence of the inability to activate thyroid hormone due to insufficient selenium. Indeed, celiac patients with concurrent hypothyroidism require an elevated dose of levothyroxine (T4) compared to non-celiac hypothyroid patients (Collins *et al.*, 2012), which could be due to impaired activation to T3.

The link between autoimmune (type 1) diabetes and autoimmune thyroiditis is likely tied to deficiencies in selenoproteins leading to apoptosis. Diabetic rats produce significantly less glomerular heparan sulfate in the kidneys than controls, and this is associated with increased albuminurea (Jaya *et al.*, 1993). However, children with type-1 diabetes and celiac disease excrete lower levels of albumin than type-1 diabetic children without celiac disease, suggesting a protective role for celiac disease (Gopee *et al.*, 2013). Wheat is a good source of tryptophan, so it is likely that tryptophan-derived serotonin induces the symptoms of diarrhea and nausea associated with wheat ingestion, but, at the same time, transports available sulfate through the vasculature, to help maintain adequate supplies of heparan sulfate to the glomerulus. Thus, the increased metabolism of dietary tryptophan to serotonin observed in association with celiac disease may help ameliorate the sulfate deficiency problem. Glyphosate's interference with CYP enzymes links to impaired bile-acid production in the liver, which in turn impairs *sterol*-based sulfate transport, placing a higher burden on serotonin for this task.

We have argued here that kidney failure, a known risk factor in celiac disease, is a consequence of depleted sulfate supplies to the kidneys. An alarming increase in kidney failure in young male agricultural workers in sugar cane fields in South America can be directly linked to the recent increase in the practice of using Roundup to "ripen" the crop just prior to the harvest. Furthermore, glyphosate's interference with selenoprotein supply would lead to thyroid dysfunction, which greatly increases risk to kidney disease. We propose here that glyphosate is the key environmental factor contributing to this epidemic, but further investigation is warranted.

While we have covered a broad range of pathologies related to celiac disease in this paper, and have shown how they can be explained by glyphosate exposure, there are likely still other aspects of the disease and the connection to glyphosate that we have omitted. For example, in a remarkable case study (Barbosa, 2001), a 54-year-old man who accidentally sprayed himself with glyphosate developed skin lesions six hours later. More significantly, one month later he exhibited symptoms of Parkinson's disease. Movement disorders such as Parkinsonism are associated with gluten intolerance (Baizabal-Carvallo, 2012). Figure 6 shows plots of glyphosate application to corn and soy alongside plots of deaths due to Parkinson's disease. These and other connections will be further explored in future research.

16 Conclusion

Celiac disease is a complex and multifactorial condition associated with gluten intolerance and a higher risk to thyroid disease, cancer and kidney disease, and there is also

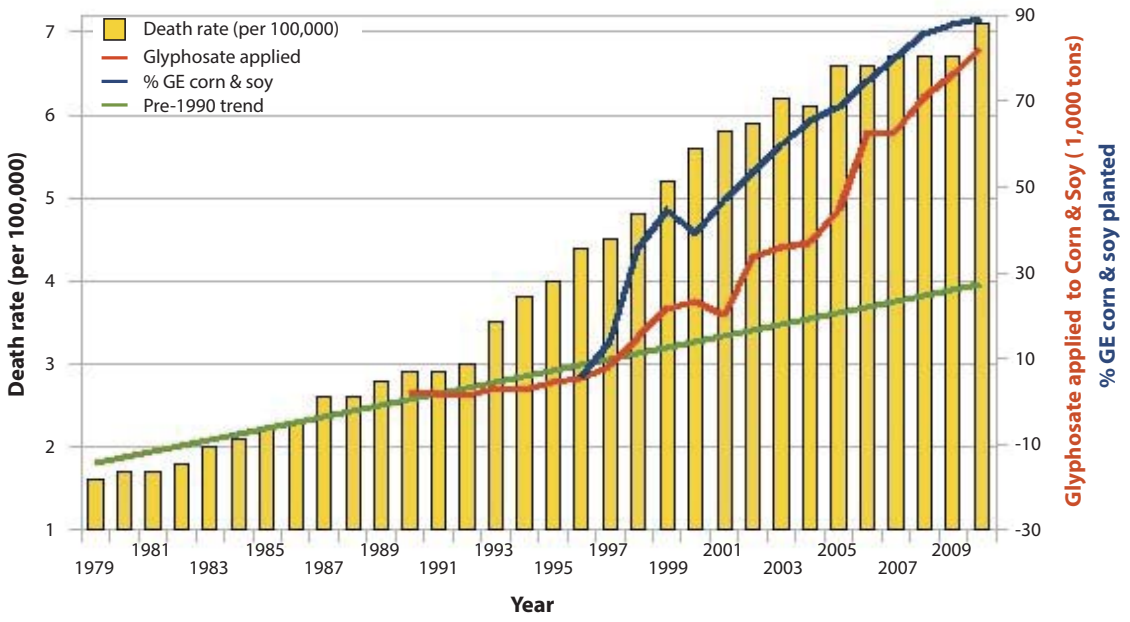


Figure 6. Deaths from Parkinson's disease (ICD G20 & 332.0) plotted against glyphosate use on corn & soy (R=0.9006, $p \leq 5.063e\text{-}07$) and %GE corn & soy planted (R=0.9676, $p \leq 2.714e\text{-}06$). Sources: USDA:NASS; CDC. (Figure courtesy of Nancy Swanson).

an increased risk to infertility and birth defects in children born to celiac mothers. While the principal diagnostic is autoantibodies to tissue transglutaminase, celiac disease is associated with a spectrum of other pathologies such as deficiencies in iron, vitamin D3, molybdenum, selenium, and cobalamin, an overgrowth of pathogens in the gut at the expense of beneficial biota, impaired serotonin signaling, and increased synthesis of toxic metabolites like p-Cresol and indole-3-acetic acid. In this paper, we have systematically shown how all of these features of celiac disease can be explained by glyphosate's known properties. These include (1) disrupting the shikimate pathway, (2) altering the balance between pathogens and beneficial biota in the gut, (3) chelating transition metals, as well as sulfur and selenium, and (4) inhibiting cytochrome P450 enzymes. We argue that a key system-wide pathology in celiac disease is impaired sulfate supply to the tissues, and that this is also a key component of glyphosate's toxicity to humans.

The monitoring of glyphosate levels in food and in human urine and blood has been inadequate. The common practice of desiccation and/or ripening with glyphosate right before the harvest ensures that glyphosate residues are present in our food supply. It is plausible that the recent sharp increase of kidney failure in agricultural workers is tied to glyphosate exposure. We urge governments globally to reexamine their policy towards glyphosate and to introduce new legislation that would restrict its usage.

Acknowledgements

The authors would like to thank Nancy Swanson for her gracious effort in the creation of the informative pictorial graphs included with the text. Her statistical research for glyphosate usage and disease over time is an invaluable contribution to our paper. Stephanie Seneff would also like to personally thank Jennifer Moeny for her most informative discussions concerning current associative prognosis, research and trends in Celiac disease and gluten intolerance. This work was funded in part by Quanta Computers, Taipei, Taiwan, under the auspices of the Qmulus Project.

Disclosures: *The authors have nothing to disclose.*

REFERENCES

Agúndez JA, García-Martín E, Martínez C. (2009). Genetically based impairment in CYP2C8- and CYP2C9-dependent NSAID metabolism as a risk factor for gastrointestinal bleeding: Is a combination of pharmacogenomics and metabolomics required to improve personalized medicine? *Expert Opin Drug Metab Toxicol* **5**(6): 607–620.

Ali A. and Fletcher R. A. (1977), Phytotoxic action of glyphosate and amitrole on corn seedlings. *Can J Bot* **56**: 2196–2202.

Allen RH, Stabler SP, Savage DG, and Lindenbaum J. Metabolic abnormalities in cobalamin (vitamin B12) and folate deficiency. (1993) *FASEB J* **7**: 1344–1353.

Ames BN, Shigenaga MK, Hagen TM. (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci U S A* **90**: 7915–22.

ISSN: 1337-6853 *(print version)* | 1337-9569 *(electronic version)*

Interdisciplinary Toxicology. 2013; **Vol. 6**(4): 159–184
Also available online on **PubMed Central**

Baizabal-Carvallo JF, Jankovic J. (2012). Movement disorders in autoimmune diseases. *Mov Disord* **27**(8): 935–46.

Banoglu E, King RS. (2002). Sulfation of indoxyl by human and rat aryl (phenol) sulfotransferases to form indoxyl sulfate. *Eur J Drug Metab Pharmacokinet* **27**(2): 135–140.

Barbosa ER, Leiros da Costa MD, Bacheschi LA, Scaff M, Leite CC. (2001). Parkinsonism after glycine-derivate exposure. *Mov Disord* **16**(3): 565–8.

Bash LD, Erlinger TP, Coresh J, Marsh-Manzi J, Folsom AR, Astor BC. (2009). Inflammation, hemostasis, and the risk of kidney function decline in the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Kidney Dis* **53**: 596–605.

Bellaloui N, Reddy KN, Zablotowicz RM, Abbas HK, Abel CA. (2009). Effects of glyphosate application on seed iron and root ferric (III) reductase in soybean cultivars. *J Agric Food Chem* **57**(20): 9569–74.

Benini F, Mora A, Turini D, Bertolazzi S, Lanzarotto F, Ricci C, Villanacci V, Barbara G, Stanghellini V, Lanzini A. (2012). Slow gallbladder emptying reverts to normal but small intestinal transit of a physiological meal remains slow in celiac patients during gluten-free diet. *Neurogastroenterol Motil* **24**(2): 100–7, e79–80.

Benítez-Leite S, Macchi ML, Acosta M (2009). Malformaciones congenítas asociadas a agrotóxicos. *Archivos de Pediatría del Uruguay* **80**: 237–247.

Bergamaschi G, Markopoulos K, Albertini R, Di Sabatino A, Biagi F, Ciccocioppo R, Arbustini E, Corazza GR. (2008). Anemia of chronic disease and defective erythropoietin production in patients with celiac disease. *Haematologica* **93**(12): 1785–91.

Bernfield M, Götte M, Park P-W, Reizes O, Fitzgerald ML, Lincecum J, Zako M. (1999). Functions of cell surface heparan sulfate proteoglycans. *Annu Rev Biochem* **68**: 729–777.

Beswick E, Millo J. (2011). Fatal poisoning with glyphosate-surfactant herbicide. *JICS* **12**(1): 37–39.

Beuret CJ, Zirulnik F, Gimenez MS (2005) Effect of the herbicide glyphosate on liver lipoperoxidation in pregnant rats and their fetuses. *Reprod Toxicol* **19**: 501–504.

Bhatia M. (2012). Role of hydrogen sulfide in the pathology of inflammation. **2012**: Article ID 159680.

Björnstedt M, & Aristi P. Fernandes AP. (2010). Selenium in the prevention of human cancers. *EPMA Journal* **1**: 389–395.

Blanchard C, Rothenberg ME. (2008). Basics pathogenesis of eosinophilic esophagitis. *Gastrointest Endosc Clin N Am* **18**(1): 133–143.

Bode R, Melo C, Birnbaum D. (1984). Mode of action of glyphosate in Candida maltosa. *Arch Microbiol* **140**(1): 83–5.

Boles RG, Ment LR, Meyn MS, Horwich AL, Kratz LE, Rinaldo P. (1993). Short-term response to dietary therapy in molybdenum cofactor deficiency. *Ann Neurol* **34**(5): 742–4.

Boss GR. (1985). Cobalamin inactivation decreases purine and methionine synthesis in cultured lymphoblasts. *J Clin Invest* **76**: 213–218.

Bostwick HE, Berezin SH, Halata MS, Jacobson R, Medow MS. (2001). Celiac disease presenting with microcephaly. *J Pediatr* **138**(4): 589–92.

Bottaro G, Cataldo F, Rotolo N, Spina M, Corazza GR. (1999). The clinical pattern of subclinical/silent celiac disease: an analysis on 1026 consecutive cases. *Am J Gastroenterol* **94**: 691–6.

Brown AM, Bradshaw MJ, Richardson R, Wheeler JG, Harvey RF. (1987). Pathogenesis of the impaired gall bladder contraction of coeliac disease. *Gut* **28**(11): 1426–1432.

Cabrera-Chávez F, Islas-Rubio AR, Rouzaud-Sández O, Sotelo-Cruz N, Calderón de la Barcaa AM. (2010). Modification of gluten by methionine binding to prepare wheat bread with reduced reactivity to serum IgA of celiac disease patients. *J Cereal Sci* **52**(2): 310–313.

Cakal B, Beyazit Y, Koklu S, Akbal E, Biyikoglu I, Yilmaz G. (2010). Elevated adenosine deaminase levels in celiac disease. *J Clin Lab Anal* **24**(5): 323–326.

Cakmak I, Yazici A, Tutus Y, Ozturk L (2009). Glyphosate reduced seed and leaf concentrations of calcium, manganese, magnesium, and iron in non-glyphosate resistant soybean. *Eur J Agron* **31**(3): 114–119.

Cañal MJ, Tamés RS, Fernández B, (1987). Glyphosate-increased levels of indole-3-acetic acid in yellow nutsedge leaves correlate with gentisic acid levels. *Physiol Plantar* **71**(3): 384–388.

Carman JA, Vlieger HR, Ver Steeg LJ, Sneller VE, Robinson GW, Clinch-Jones CA, Haynes JI, Edwards JW. (2013). A long-term toxicology study on pigs fed a combined genetically modified (GM) soy and GM maize diet. *J Organic Syst* **8**(1): 38–54.

Carrasco A. (2013). *Teratogenesis by glyphosate based herbicides and other pesticides: Relationship with the retinoic acid pathway*. In: Breckling B, Verhoeven R. GM-Crop Cultivation Ecological Effects on a Landscape Scale. Theorie in der kologie 17. Frankfurt, Peter Lang.

Cavallaro R, Iovino P, Castiglione F, Palumbo A, Marino M, Di Bella S, Sabbatini F, Labanca F, Tortora R, Mazzacca G, Ciacci C. (2004). Prevalence and clinical associations of prolonged prothrombin time in adult untreated coeliac disease. *Eur J Gastroenterol Hepatol* **16**(2): 219–223.

Cerdas M. (2005). Chronic kidney disease in Costa Rica. *Kidney Int Suppl* **97**: 31–33.

Challacombe DN, Dawkins PD, Baker P. (1977). Increased tissue concentrations of 5-hydroxy-tryptamine in the duodenal mucosa of patients with coeliac disease. *Gut* **18**: 882–886.

Chang CY, Peng YC, Hung DZ, Hu WH, Yang DY, Lin TJ. (1999). Clinical impact of upper gastrointestinal tract injuries in glyphosate-surfactant oral intoxication. *Hum Exp Toxicol* **18**: 475–78.

Chanoine JP, Neve J, Wu S, Vanderpas J, Bourdoux P. (2001). Selenium decreases thyroglobilin concentrations but does not affect the increased thyroxine-to-triiodothyronine ratio in chidren with congenital hypothyroidism. *J Clin Endocrinol Metab* **86**: 1160–1163.

Chin A, Svejda B, Gustafsson BI, Granlund AB, Sandvik AK, Timberlake A, Sumpio B, Pfragner R, Modlin IM, Kidd M. (2012). The role of mechanical forces and adenosine in the regulation of intestinal enterochromaffin cell serotonin secretion. *Am J Physiol Gastrointest Liver Physiol* **302**: G397–G405.

Clotman F, van Maele-Fabry G, Chu-Wu L, Picard JJ. (1998). Structural and gene expression abnormalities induced by retinoic acid in the forebrain. *Reprod Toxicol* **12**: 169–176.

Coleman NS, Foley S, Dunlop SP, Wheatcroft J, Blackshaw E, Perkins AC, Singh G, Marsden CA, Holmes GK, Spiller RC. (2006) Abnormalities of serotonin metabolism and their relation to symptoms in untreated celiac disease. *Clin Gastroenterol Hepatol* **4**: 874–881.

Collado MC, Calabuig M, Sanz Y. (2007). Differences between the fecal microbiota of coeliac infants and healthy controls. *Curr Issues Intest Microbiol* **8**(1): 9–14.

Collin P, Kaukinen K, Valimaki M, Salmi J. (2002). Endocrinological disorders and celiac disease. *Endocrine Rev* **23**(4): 464–483.

Collin P, Vilska S, Heinonen PK, Hällström O, Pikkarainen P. (1996). Infertility and coeliac disease. *Gut* **39**(3): 382–384.

Collins D, Wilcox R, Nathan M, Zubarik R. (2012). Celiac disease and hypothyroidism. *Am J Med* **125**(3): 278–82.

Colombato LO, Parodi H, Cantor D. (1977) Biliary function studies in patients with celiac sprue. *Am J Dig Dis* **22**(2): 96–8.

Coombes JL, Siddiqui KR, Arancibia-Cárcamo CV, Hall J, Sun CM, Belkaid Y, Powrie F.. (2007). A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J Exp Med* **204**(8): 1757–64.

Corrao G, Corazza GR, Bagnardi V, Brusco G, Ciacci C, Cottone M, Sategna Guidetti C, Usai P, Cesari P, Pelli MA, Loperfido S, Volta U, Calabr A, Certo M; Club del Tenue Study Group. (2001). Mortality in patients with coeliac disease and their relatives: A cohort study. *Lancet* **358**: 356–361.

Cottone M, Termini A, Oliva L, Magliocco A, Marrone C, Orlando A, Pinzone F, Di Mitri R, Rosselli M, Rizzo A, Pagliaro L. (1999). Mortality and causes of death in celiac disease in a Mediterranean area. *Dig Dis Sci* **44**: 2538–41.

Coussens LM, Werb Z. (2002). Inflammation and cancer. *Nature* **420**: 860–67.

Culpepper, AS, York, AC, Batts, RB, Jennings KM. (2000). Weed Management in Glufosinate- and Glyphosate-Resistant Soybean (Glycine max). *Weed Technology* **14**(1): 77–88.

Cupp MJ, Tracy TS. (1998). Cytochrome P450: New nomenclature and clinical implications. *Am Fam Physician* **57**(1): 107–16.

Cusiel AL. (2005). The Synthesis and Reactivity of Novel Co(L)(PMG).n+ Complexes. University of Canterbury, MS Thesis, April.

Dahele A, Ghosh S. (2001). Vitamin B12 deficiency in untreated celiac disease. *Am J Gastroenterol* **96**(3): 745–50.

D'Ari L, Barker HA. (1985). p-Cresol formation by cell free extracts of Clostridium difficile. *Arch Microbiol* **143**: 311–312.

Darwent AL, Kirkland KJ, Townley-Smith L, Harker KN, Cessna AJ, Lukow OM, Lefkovitch LP. (1994). Effect of preharvest applications of glyphosate on the drying, yield and quality of wheat. *Can J Plant Sci* **74**(2): 221–230.

de Liz Oliveira Cavalli VL, Cattani D, Heinz Rieg CE, Pierozan P, Zanatta L, Benedetti Parisotto E, Wilhelm Filho D, Mena Barreto Silva FR, Pessoa-Pureur R, Zamoner A. (2013). Roundup disrupts male reproductive functions by triggering calcium-mediated cell death in rat testis and Sertoli cells. *Free Radic Biol Med* **29**(65C): 335–346.

de María N, Becerril JM, Garca-Plazaola JI, Hernandez AH, de Felipe MR, Fernández-Pascual M. (1996). New insights on glyphosate mode of action in nodular metabolism: Role of shikimate accumulation. *J Agric Food Chem* **54**: 2621–2628.

DePaolo RW, Abadie V, Tang F, Fehlner-Peach H, Hall JA, Wang W, Marietta EV, Kasarda DD, Waldmann TA, Murray JA, Semrad C, Kupfer S, Belkaid Guandalini YS, Jabri1 B. (2011). Co-adjuvant effects of retinoic acid and IL-15 induce inflammatory immunity to dietary antigens. *Nature* **471**(7337): 220–224.

Deprez P, Sempoux C, Van Beers BE, Jouret A, Robert A, Rahier J, Geubel A, Pauwels S, Mainguet P. (2002). Persistent decreased plasma cholecystokinin levels in celiac patients under gluten-free diet: respective roles of histological changes and nutrient hydrolysis. *Regul Pept* **110**(1): 55–63.

De Roos AJ, Zahm SH, Cantor KP, Weisemburger DD, Holmes FF, Burmeister LF, Blair A. (2003). Integrative assessment of multiple pesticides as risk factors for non-Hodgkins lymphoma among men. *Occup Environ Med* **60**(9): e11.

Di Cagno R, De Angelis M, De Pasquale I, Ndagijimana M, Vernocchi P, Ricciuti P, Gagliardi F, Laghi L, Crecchio C, Guerzoni ME, Gobbetti M, Francavilla R. (2011). Duodenal and faecal microbiota of celiac children: Molecular, phenotype and metabolome characterization. *BMC Microbiol* **11**: 219.

Dickey W, McMillan SA, Callender ME. (1997). High prevalence of celiac sprue among patients with primary biliary cirrhosis. *J Clin Gastroenterol* **25**(1): 328–9.

Dickey W, Ward M, Whittle CR, Kelly MT, Pentieva K, Horigan G, Patton S, McNulty H. (2008). Homocysteine and related B-vitamin status in coeliac disease: Effects of gluten exclusion and histological recovery. *Scand J Gastroenterol* **43**: 682688.

Dickey W, Stewart F, Nelson J, McBreen G, McMillan SA, Porter KG. (1996). Screening for coeliac disease as a possible maternal risk factor for neural tube defect. *Clin Genet* **49**(2): 107–8.

Dørum S, Arntzen MØ, Qiao S-W, Holm A, Koehler CJ, Thiede B, Sollid LM, Fleckenstein B. (2010). The preferred substrates for transglutaminase 2 in a complex wheat gluten digest are peptide fragments harboring celiac disease T-cell epitopes. *PLoS ONE* **5**(11): e14056.

Dou L, Bertrand E, Cerini C, Faure V, Sampol J, Vanholder R, Berland Y, Brunet P. (2004). The uremic solutes p-cresol and indoxyl sulfate inhibit endothelial proliferation and wound repair. *Kidney Int* **65**: 442–451.

Drexler J. (1958). Effect of indole compounds on vitamin B12 utilization. *Blood* **13**(3): 239–44.

Duke, S.O.; Powles, S.B. (2008). Glyphosate: A once-in-a-century herbicide. *Pest Manag Sci* **64**: 319–325.

Egan LJ, Walsh SV, Stevens FM, Connolly CE, Egan EL, McCarthy CF. (1995). Celiac associated lymphoma: A single institution experience of 30 cases in the combination chemotherapy era. *J Clin Gastroenterol* **21**(2): 123–9.

Ejderhamn J, Samuelson K, Strandvik B. (1992) Serum primary bile acids in the course of celiac disease in children. *J Pediatr Gastroenterol Nutr* **14**(4): 443–9.

Eker S, Ozturk L, Yazici A, Erenoglu B, Romheld V, Cakmak I. (2006). Foliar-applied glyphosate substantially reduced uptake and transport of iron and manganese in sunflower (Helianthus annuus L.) plants. *J Agric Food Chem* **54**(26): 10019–25.

El-Shenawy N. (2009). Oxidative stress responses of rats exposed to Roundup and its active ingredient glyphosate. *Environ Toxicol Pharmacol* **28**(3): 379–385.

Ellis JK, Russell RM, Makrauer FL, Schaefer EJ. (1986). Increased risk of vitamin A toxicity in severe hypertriglyceridemia. *Ann Intern Med* **105**: 877–9.

Erspamer V. (1986). Historical introduction: The Italian contribution to the discovery of 5-hydroxytryptamine (enteramine, serotonin). *J Hypertens Suppl* **4**(1): S3–S5.

Esposito C, Paparo F, Caputo I, Rossi M, Maglio M, Sblattero D, Not T, Porta R, Auricchio S, Marzari R, Troncone R. (2002). Anti-tissue transglutaminase antibodies from coeliac patients inhibit transglutaminase activity both in vitro and in situ. *Gut* **51**(2): 177–181.

Famularo G, De Simone C, Pandey V, Sahu AR, Minisola G (2005). Probiotic lactobacilli: an innovative tool to correct the malabsorption syndrome of vegetarians? *Med Hypotheses* **65** (6): 11325.

Farthing MJG, Edwards CRW, Rees LH, Dawson AM (1982). Male gonadal function in coeliac disease: 1. Sexual dysfunction, infertility, and semen quality. *Gut* **23**: 608–614.

Fasano A. (2011). Zonulin and its regulation of intestinal barrier function: The biological door to inflammation, autoimmunity, and cancer. *Physiol Rev* **91**: 151–175.

Fasano A, Berti I, Gerarduzzi T, Not T, Colletti RB, *et al.* (2003). Prevalence of celiac disease in at-risk and not-at-risk groups in the United States a large multicenter study. *Arch Intern Med* **163**: 286–292.

Fasano A, Not T, Wang W, Uzzau S, Berti I, Tommasini A, Goldblum SE. (2000). Zonulin, a newly discovered modulator of intestinal permeability, its expression in coeliac disease. *Lancet* **358**: 1518–1519.

Förstermann U, Münzel T. (2006). Endothelial nitric oxide synthase in vascular disease: From marvel to menace. *Circulation* **113**: 1708–14.

Fountoulakis S, Tsatsoulis A. (2004). On the pathogensis of autoimmune thyroid disease: A unifying hypothesis. *Clin Endocrinol* **60**: 397–409.

Franz JE, Mao MK, Sikorski JA. (1997). *Glyphosate: A unique global herbicide*. American Chemical Society, Publisher.

Freeman HJ. (2010). Reproductive changes associated with celiac disease. *World J Gastroenterol* **16**(46): 5810–5814.

Fukumoto S, Tatewaki M, Yamada T, Fujimiya M, Mantyh C, Voss M, Eubanks S, Harris M, Pappas TN, Takahashi T. (2003). Short-chain fatty acids stimulate colonic transit via intraluminal 5-HT release in rats. *Am J Physiol Regul Integr Comp Physiol* **284**: R1269–R1276.

Furukawa S, Usuda K, Abe M, Hayashi S, Ogawa I. (2007). Indole-3-acetic acid induces microencephaly in mouse fetuses. *Exp Toxicol Pathol* **59**(1): 43–52.

Furuta GT, Liacouras CA, Collins MH, Gupta SK, Justinich C, Putnam PE, Bonis P, Hassall E, Straumann A, Rothenberg ME; First International Gastrointestinal Eosinophil Research Symposium (FIGERS) Subcommittees. (2007). Eosinophilic esophagitis in children and adults: A systematic review and consensus recommendations for diagnosis and treatment. *Gastroenterology* **133**: 1342–1363.

Ganson RJ, Jensen RA. (1988). The essential role of cobalt in the inhibition of the cytosolic lsozyme of 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase from Nicotiana silvestris by glyphosate. *Arch Biochem Biophys* **260**(1): 85–73.

Gasnier C, Dumont C, Benachour N, Clair E, Chagnon M-C, Seralini G-E. (2009). Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. *Toxicology* **262**: 184–191.

Glass RL. (1984). Metal complex formation by glyphosate. *Journal of Agricultural and Food Chemistry* **32**: 1249–1253.

Gobbetti M, Giuseppe Rizzello C, Di Cagno R, De Angelis M.(2007). Sourdough lactobacilli and celiac disease. *Food Microbiol* **24**(2): 187–96.

Goldman AS, Van Fossan DD, Baird EE. (1962). Magnesium deficiency in celiac disease. *Pediatrics* **29**(6): 948–952.

Gopee E, van den Oever EL, Cameron F, Thomas MC. (2013). Coeliac disease, gluten-free diet and the development and progression of albuminuria in children with type 1 diabetes. *Pediatr Diabetes* **14**(6): 455–8.

Goubern M, Andriamihaja M, Nubel T, Blachier F, Bouillaud F. (2007). Sulfide, the first inorganic substrate for human cells. *FASEB J* **21**(8): 1699–1706.

Green PHR, Cellier C. (2007). Celiac Disease. *N Engl J Med* **357**: 1731–1743.

Green PH, Fleischauer AT, Bhagat G, Goyal R, Jabri B, Neugut AI. (2003). Risk of malignancy in patients with celiac disease. *Am J Med* **115**(3): 191–5.

Grider JR, Piland BE. (2007). The peristaltic reflex induced by short-chain fatty acids is mediated by sequential release of 5-HT and neuronal CGRP but not BDNF. *Am J Physiol Gastrointest Liver Physiol* **292**: G429–G437.

Grigg AP. (1999). Deep venous thrombosis as the presenting feature in a patient with coeliac disease and homocysteinaemia. *Aust N Z J Med* **29**: 566–567.

Gross S, van Wanrooij RL, Nijeboer P, Gelderman KA, Cillessen SAGM, Meijer GA, Mulder CJJ, Bouma G, von Blomberg BME, Bontkes HJ. (2013). Differential IL-13 production by small intestinal leukocytes in active coeliac disease versus refractory coeliac disease. *Mediators of Inflammation* **2013**: Article ID 939047.

Grube A, Donaldson D, Kiely T, Wu L. (2011). *Pesticide industry sales and usage: 2006 and 2007 market estimates*. U.S. Environmental Protection Agency: Washington, DC, USA.

Haderlie LC, Widholm JM, Slife FW. (1977). Effect of glyphosate on carrot and tobacco cells. *Plant Physiol* **60**: 40–43.

ISSN: 1337-6853 *(print version)* | 1337-9569 *(electronic version)*

Interdisciplinary Toxicology. 2013; **Vol. 6**(4): 159–184
Also available online on **PubMed Central**

Hadithi M, Mulder CJJ, Stam F, Azizi J, Crusius JBA, Peña AS, Stehouwer CDA, Smulders YM. (2009). Effect of B vitamin supplementation on plasma homocysteine levels in celiac disease. *World J Gastroenterol* **15**(8): 955–960.

Halfdanarson TR, Kumar N, Hogan WJ, Murray JA. (2009). Copper deficiency in celiac disease. *J Clin Gastroenterol* **43**(2): 162–4.

Halfdanarson TR, Litzow MR, Murray JA. (2007). Hematologic manifestations of celiac disease. *Blood* **109**: 412–21.

Hallert C, Grant C, Grehn S, Granno C, Hultén S, Midhagen, G, Ström M, Svensson H. Valdimarsson T. (2002). Evidence of poor vitamin status in celiac patients on a gluten-free diet for 10 years. *Alimentary Pharmacology & Therapeutics* **16**: 1333–1339.

Hardell L, Eriksson M. (1999). A casecontrol study of non-Hodgkin lymphoma and exposure to pesticides. *Cancer* **85**(6): 1353–1360.

Hernanz, A, Polanco I. (1991). Plasma precursor amino acids of central nervous system monoamines in children with coeliac disease. *Gut* **32**: 1478–1481.

Herrmann W, Obeid R. (2012). Cobalamin deficiency. *Subcell Biochem* **56**: 301–22.

Hershko C, Patz J. (2008). Ironing out the mechanism of anemia in celiac disease. *Haematologica* **93**(12): 1761–1765.

Hietanen E, Linnainmaa K, Vainio H. (1983). Effects of phenoxyherbicides and glyphosate on the hepatic and intestinal biotransformation activities in the rat. *Acta Pharmacol Toxicol (Copenh).* **53**(2): 103–12.

Hildebrandt TM, Grieshaber MK. (2008). Three enzymatic activities catalyze the oxidation of sulfide to thiosulfate in mammalian and invertebrate mitochondria. *FEBS J* **275**(13): 3352–3361.

Hinks LJ, Inwards KD, Lloyd B, Clayton BE. (1984). Body content of selenium in coeliac disease. *Br Med J* **288**: 1862–1863.

Hoagland, RE, Duke SE. (1982). *Biochemical effects of glyphosate. In Biochemical Responses Induced by Herbicides*; Moreland, D. E., St. John, J. B., Hess, F. D., Eds.; ACS Symposium Series 181; American Chemical Society: Washington, DC, pp 175–205.

Högberg L, Webb C, Fälth-Magnusson K, Forslund T, Magnusson KE, Danielsson L, Ivarsson A, Sandström O, Sundqvist T. (2011). Children with screening-detected coeliac disease show increased levels of nitric oxide products in urine. *Acta Paediatr* **100**(7): 1023–7.

Holick MF. (2005). The Vitamin D Epidemic and its Health Consequences. *J Nutr* **135**(11): 2739S–48S.

Homann PE. (1967). Studies on the manganese of the chloroplast. *Plant Physiology* **42**: 997–1007.

Hoppe H-W. (2013). *Determination of Glyphosate residues in human urine samples from 18 European countries.* Report Glyphosate MLHB-2013-06-06. Medical Laboratory Bremen, Haferwende 12, 28357 Bremen, Germany, March.

Huggins DR, Reganold JP. (2008). No till: the quiet revolution. *Sci Am* **99**(1): 70–7.

Iglesias P, Díez JJ. (2009). Thyroid dysfunction and kidney disease. *European J Endocrinol* **160**: 503–515.

Ingenbleek Y, Kimura H. (2013). Nutritional essentiality of sulfur in health and disease. *Nutr Rev* **71**(7): 413–32.

Ito N, Iwamori Y, Hanaoka K, Iwamori M. (1998). Inhibition of pancreatic elastase by sulfated lipids in the intestinal mucosa. *J Biochem* **123**: 107–114.

Iwasaki Y, Asai M, Yoshida M, Nigawara T, Kambayashi M, Nakashima N. (2004). Dehydroepiandrosterone-sulfate inhibits nuclear factor-κB-dependent transcription in hepatocytes, possibly through antioxidant effect. *J Clin Endocrinol Metab* **89**(7): 3449– 3454.

Jabri B, Sollid LM. (2009). Tissue-mediated control of immunopathology in coeliac disease. *Nat Rev Immunol* **9**(12): 858–870.

Jansson EA, Huang L, Malkey R, Govoni M, Nihlén C, Olsson A, Stensdotter M, Petersson J, Holm L, Weitzberg E, Lundberg JO. (2008). A mammalian functional nitrate reductase that regulates nitrite and nitric oxide homeostasis. *Nat Chem Biol* **4**(7): 411–7.

Jasper R, Locatelli GO, Pilati C, Locatelli C. (2012). Evaluation of biochemical, hematological and oxidative parameters in mice exposed to the herbicide glyphosate- Roundup. *Interdiscip Toxicol* **5**(3): 133–140.

Jaya B, Hu L, Bauman JW, Fu SC, Reddi AS. (1993). Effect of galactose regimen on glomerular heparan sulfate synthesis and albumin excretion in diabetic rats. *Res Commun Chem Pathol Pharmacol* **80**(2): 143–52.

Jetten AM, George MA, Pettit GR, Herald CL, Rearick JI. (1989). Action of phorbol esters, bryostatins, and retinoic acid on cholesterol sulfate synthesis: Relation to the multistep process of differentiation in human epidermal keratinocytes. *Journal of Investigative Dermatology* **93**: 108–115.

Kaplan MM, Ohkubo A, Quaroni EG, Sze-Tu D. (1983). Increased synthesis of rat liver alkaline phosphatase by bile duct ligation. *Hepatology* **3**(3): 368–76.

Katz A, Dyck RF, Bear RA. (1979). Celiac disease associated with immune complex glomerulonephritis. *Clin Nephrol* **11**(1): 39–44.

Kelly CP, Pothoulakis C, LaMont JT. (1994). Clostridium difficile colitis. *N Engl J Med* **330**: 257–262.

Kemppainen T, Kröger H, Janatuinen E, Arnala I, Kosma VM, Pikkarainen P, Julkunen R, Jurvelin J, Alhava E, Uusitupa M. (1999). Osteoporosis in adult patients with celiac disease. *Bone* **24**(3): 249–255.

Kiely T, Donaldson D, Grube A. (2004). *Pesticides industry sales and usage – 2000 and 2001 market estimates*. U.S. Environmental Protection Agency; Washington DC, USA. Ref Type: Report.

Kim M, Cooke HJ, Javed NH, Carey HV, Christofi F, Raybould HE. (2001). D-glucose releases 5-hydroxytryptamine from human BON cells as a model of enterochromaffin cells. *Gastroenterology* **121**: 1400–1406.

Kimmel GL, Kimmel CA, Williams AL, DeSesso JM. (2013). Evaluation of developmental toxicity studies of glyphosate with attention to cardiovascular development. *Crit Rev Toxicol* **43**(2): 79–95.

Kimura H (2011). Hydrogen sulfide: its production and functions. *Experimental Physiology* **96**: 833–835.

Kitchen LM, Witt WW, Rieck CE. (1981). Inhibition of chlorophyll accumulation by glyphosate. *Weed Science* **29**: 513–516.

Klein NJ, Shennan GI, Heyderman RS, Levin M. (1992). Alteration in glycosaminoglycan metabolism and surface charge on humanumbilical vein endothelial cells induced by cytokines, endotoxin and neutrophils. *J Cell Sci* **102**: 821–32.

Köhrle J. (2013). Selenium and the thyroid. *Curr Opin Endocrinol Diabetes Obes* **20**(5): 441–8.

Koning F. (2005). Celiac disease: caught between a rock and a hard place. *Gastroenterology* **129**(4): 1294–1301.

Koyama, T, Melzter HY. (1986). *A biochemical and neuroendocrine study of the serotonergic system in depression.* In: Hippius H, Klerman GL, Matussek N, Eds. New results in depression research. New York: Springer-Verlag, 1986: 164–88.

Krasinski SD, Cohn JS, Schaefer EJ, Russell RM. (1990). Postprandial plasma retinyl ester response is greater in older subjects compared with younger subjects. *J Clin Invest* **85**: 883–92.

Krüger M, Schrödl W, Neuhaus J, Shehata AA. (2013a). Field investigations of glyphosate in urine of Danish dairy cows. *J Environ Anal Toxicol* **3**(5): 100186.

Krüger M, Shehata AA, Schrödl W, Rodloff A. (2013b). Glyphosate suppresses the antagonistic effect of Enterococcus spp. on Clostridium botulinum. *Anaerobe* **20**: 74–78.

Lai TS, Hausladen A, Slaughter TF, Eu JP, Stamler JS, Greenberg CS. (2001). Calcium regulates S-nitrosylation, denitrosylation, and activity of tissue transglutaminase. *Biochemistry* **40**(16): 4904–10.

Lamb DC, Kelly DE, Hanley SZ, Mehmood Z, Kelly SL. (1998). Glyphosate is an inhibitor of plant cytochrome P450: Functional expression of Thlaspi arvensae cytochrome P45071B1/reductase fusion protein in Escherichia coli. *Biochem Biophys Res Commun* **244**: 110–114.

Lang CC, Brown RM, Kinirons MT, Deathridge MA, Guengerich FP, Kelleher D, O'Briain DS, Ghishan FK, Wood AJ. (1996). Decreased intestinal CYP3A in celiac disease: Reversal after successful gluten-free diet: a potential source of interindividual variability in first-pass drug metabolism. *Clin Pharmacol Ther* **59**(1): 41–6.

Lapunzina P. (2002). Celiac disease and microcephaly. *J Pediatr* **140**(1): 141–142.

Laurin P, Fälth-Magnusson K, Sundqvist T. (2003). Increase in nitric oxide urinary products during gluten challenge in children with coeliac disease. *Scand J Gastroenterology* **38**(1): 55–60.

Lee J-H, Lee J.(2010). Indole as an intercellular signal in microbial communities. *FEMS Microbiol Rev* **34**: 426–444.

Legendre BL, Gravois KA, Bischoff KP, Griffin JL. (2005). Timing of glyphosate applications, alternatives to the use of glyphosate and response of new varieties to glyphosate in maximizing the yield of sugar per acre of Louisiana sugarcane in 2005. *LSU AgCenter Sugarcane Ann Rep* 182–191.

Lerner A, Shapira Y, Agmon-Levin N, Pacht A, Ben-Ami Shor D, López HM, Sanchez-Castanon M, Shoenfeld Y. (2012). The clinical significance of 25OH-vitamin D status in celiac disease. *Clin Rev Allergy Immunol* **42**(3): 322–30.

Leslie C, Mews C, Charles A, Ravikumara M. (2010). Celiac disease and eosinophilic esophagitis: a true association. *J Pediatr Gastroenterol Nutr* **50**(4): 397–9.

Li H, Liu X, Cui H, Chen Y-R, Cardounel AJ, Zweier JL. (2006). Characterization of the mechanism of cytochrome P450 reductase-cytochrome P450-mediated nitric oxide and nitrosothiol generation from organic nitrates. *JBC* **281**(18): 12546–12554.

Li L, Rose P, Moore PK. (2011). Hydrogen sulfide and cell signaling. *Annu Rev Pharmacol Toxicol* **51**: 169–87.

Liacouras CA, Furuta GT, Hirano I, Atkins D, Attwood SE, Bonis PA, Burks AW, Chehade M, Collins MH, Dellon ES, Dohil R, Falk GW, Gonsalves N, Gupta SK, Katzka DA, Lucendo AJ, Markowitz JE, Noel RJ, Odze RD, Putnam PE, Richter JE, Romero Y, Ruchelli E, Sampson HA, Schoepfer A, Shaheen NJ, Sicherer SH, Spechler S, Spergel JM, Straumann A, Wershil BK, Rothenberg ME, Aceves SS. (2011). Eosinophilic esophagitis: Updated consensus recommendations for children and adults. *J Allergy Clin Immunol* **128**: 3–20.

Lindfors K, Blomqvist T, Juuti-Uusitalo K, Stenman S, Venalainen J, Maki M, Kaukinen K. (2008). Live probiotic Bifidobacterium lactis bacteria inhibit the toxic effects induced by wheat gliadin in epithelial cell culture. *Clin Exp Immunol* **152**(3): 552–558.

Lindros KO. (1997). Zonation of cytochrome P450 expression, drug metabolism and toxicity in liver. *Gen Pharmacol* **28**(2): 191–196.

Logan RF, Rifkind EA, Turner ID. (1989). Ferguson A. Mortality in celiac disease. *Gastroenterol*ogy **97**: 265–271.

Lorand L, Graham RM. (2003). Transglutaminases: Crosslinking enzymes with pleiotropic functions. *Nat Rev Mol Cell Biol* **4**: 140–156.

Lorbek G, Lewinska M, Rozman D. (2012). Cytochrome P450s in the synthesis of cholesterol and bile acids–from mouse models to human diseases. *FEBS J* **279**(9): 1516–33.

Lu W, Li L, Chen M, Zhou Z, Zhang W, Ping S, Yan Y, Wang J, Lin M. (2013). Genome-wide transcriptional responses of Escherichia coli to glyphosate, a potent in- hibitor of the shikimate pathway enzyme 5-enolpyruvylshikimate-3-phosphate synthase. *Mol Biosyst* **9**: 522–530.

Lucendo AJ, Sánchez-Cazalilla M. (2012). Adult versus pediatric eosinophilic esophagitis: Important differences and similarities for the clinician to understand. *Expert Rev Clin Immunol* **8**(8): 733–45.

Madsen HEL, Christensen HH, Gottlieb-Petersen C. (1978). Stability constants of copper(II), zinc, manganese(II), calcium, and magnesium complexes of N-(phosphonomethyl)glycine (glyphosate). *Acta Chem Scand* **32**: 79–83.

Manocha M, Khan WI. (2012). Serotonin and GI disorders: An update on clinical and experimental studies. *Clin Transl Gastroenterol* **3**: e13.

Marteau P, Cadranel JF, Messing B, Gargot D, Valla D, Rambaud JC. (1994). Association of hepatic vein obstruction and coeliac disease in North African subjects. *J Hepatol* **20**: 650–653.

Martinelli P, Troncone R, Paparo F, Torre P, Trapanese E, Fasano C, Lamberti A, Budillon G, Nardone G, Greco L. (2000). Coeliac disease and unfavourable outcome of pregnancy. *Gut* **46**(3): 332–335.

Matheus-Vliezen EME, Van Halteran H, Tylgut GNJ. (1994). Malignant lymphoma in coeliac disease: various manifestations with distinct symptomatology and prognosis? *J Intern Med* **236**(1): 43–9.

Maton PN, Selden AC, Fitzpatrick ML, Chadwick VS. (1985). Defective gallbladder emptying and cholecystokinin release in celiac disease. Reversal by gluten-free diet. *Gastroenterol*ogy **88**(2): 391–6.

McDuffie HH, Pahwa P, McLaughlin JR, Spinelli JJ, Fincham S, Dosman JA, Robson D, Skinnider LF, Choi NW. (2001). Non-Hodgkins lymphoma and specific pesticide exposures in men: Cross-Canada study of pesticides and health. *Cancer Epidemiol Biomarkers Prev* **10**(11): 1155–1163.

Medina M, De Palma G, Ribes-Koninckx C, Calabuig M, Sanz Y. (2008). Bifidobacterium strains suppress in vitro the pro-inflammatory milieu triggered by the large intestinal microbiota of coeliac patients. *J Inflamm (Lond)* **5**: 19.

Meloni GF, Dessole S, Vargiu N, Tomasi PA, Musumeci S (1999). The prevalence of coeliac disease in infertility. *Hum Reprod* **14**(11): 2759–2761.

Mishra A, Rothenberg ME. (2003). Intratracheal IL-13 induces eosinophilic esophagitis by an IL-5, eotaxin-1, and STAT6 dependent mechanism. *Gastroenterol*ogy **125**: 1419–1427.

Miteva L, Ivanov S, Alexieva V, Karanov E. (2003). Effect of herbicide glyphosate on glutathione levels, glutathione-S-transferase and glutathione reductase activities in two plant species. *Comptes Rendus de l'Academie Bulgare des Sciences* **56**: 79–84.

Módis K, Coletta C, Erdélyi K, Papapetropoulos A, Szabo C. (2013). Intramitochondrial hydrogen sulfide production by 3-mercaptopyruvate sulfurtransferase maintains mitochondrial electron flow and supports cellular bioenergetics. *FASEB J* **27**(2): 601–11.

Monsanto International Sàrl. (2010). *The agronomic benefits of glyphosate in Europe. Review of the benefits of glyphosate per market use.* Monsanto Europe SA. www.monsanto.com/products/Documents/glyphosate-background-materials/Agronomic%20benefits%20of%20glyphosate%20in%20Europe.pdf [Last accessed Sep. 4, 2013].

Monsanto Technology LLC, Missouri. (2010). *Glyphosate formulations and their use for the inhibition of 5-enolpyruvylshikimate-3-phosphate synthase.* US Patent number 7771736 B2. https: //www.google.com/patents/US7771736. [Last accessed Sep. 1, 2013]

Motekaitis RJ, Martell AE. (1985). Metal chelate formation by N-phosphonomethylglycine and related ligands. *J Coord Chem* **14**: 139–149.

Mora JR, Iwata M, von Andrian UH. (2008). Vitamin effects on the immune system: Vitamins A and D take centre stage. *Nat Rev Immunol* **8**(9): 685–98.

Mucida D, Park Y, Kim G, Turovskaya O, Scott I, Kronenberg M, Cheroutre H. (2007). Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science* **317**(5835): 256–260.

Murch SH. (1995). Sulphation of proteoglycans and intestinal function. *J Gastroenterol Hepatol* **10**: 210–12.

Murch SH, MacDonald TT, Walker-Smith JA, Levin M, Lionetti P, Klein NJ. (1993). Disruption of sulphated glycosaminoglycans in intestinal inflammation. *Lancet* **341**: 711–14.

Murch SH, Winyard PJ, Koletzko S, Wehner B, Cheema HA, Risdon RA, Phillips AD, Meadows N, Klein NJ, Walker-Smith JA. (1996). Congenital enterocyte heparan sulphate deficiency with massive albumin loss, secretory diarrhoea, and malnutrition. *Lancet* **347**(9011): 1299–301.

Nadal I, Donat E, Ribes-Koninckx C, Calabuig M, Sanz Y. (2007). Imbalance in the composition of the duodenal microbiota of children with coeliac disease. *J Med Microbiol* **56**: 1669–74.

Nafziger ED, Widholm JM, Steinrücken HC, Killmer JL. (1984). Selection and characterization of a carrot cell line tolerant to glyphosate. *Plant Physiol* **76**(3): 571–4.

Nalewaja JD, Matysiak R. (1993). Influence of diammonium sulfate and other salts on glyphosate phytotoxicity. *Pesticide Science* **38**: 77–84.

Nanda S. (2011). Celiac disease: Retinoic acid and IL-15 jointly implicated in reversal of oral tolerance. *Nat Rev Gastroenterol Hepatol* **8**: 181.

Negri E. (2010). Sun exposure, vitamin D, and risk of Hodgkin and non-Hodgkin lymphoma. *Nutr Cancer* **62**(7): 878–82.

Nelson MA, Porterfield BW, Jacobs ET, Clark LC. (1999). Selenium and prostate cancer prevention. *Semin Urol Oncol* **17**(2): 91–6.

Network Coordinating Council. (May, 2013). *2012 Annual Report. End Stage Renal Disease Network* 13. http: //www.network13.org/PDFs/NW13 Annual Report 2012 Final.pdf [Last accessed Sept 3, 2013]

Nielsen OH, Jacobsen O, Pedersen ER, Rasmussen SN, Petri M, Laulund S, Jarnum S. (1985). Non-tropical sprue. Malignant diseases and mortality rate. *Scand J Gastroenterol* **20**: 13–18.

Niwa T. (2010). Indoxyl sulfate is a nephro-vascular toxin. *J Ren Nutr* **20**(5 Suppl): S2–6.

Nomura NS, Hilton HW. (1977). The adsorption and degradation of glyphosate in five Hawaiian sugarcane soils. *Weed Res* **17**: 113–121.

O'Keeffe MG. (1980). *The control of Agropyron repens and broad-leaved weeds pre-harvest of wheat and barley with the isopropylamine salt of glyphosate.* Proceedings of British Crop Protection Conference - Weeds, 53–60.

O'Keeffe MG.(1981). *The control of perennial grasses by pre-harvest applications of glyphosate.* Proceedings of the Conference on Grass Weeds in Cereals in the United Kingdom. Association of Applied Biologists, Warwick, UK, 137–144.

Orgeron AJ. (2012). *Sugarcane growth, sucrose content, and yield response to the ripeners glyphosate and trinexapacethyl.* PhD Dissertation, School of Plant, Environmental, and Soil Sciences, Louisiana State University.

Orson JH, Davies DKH. (2007). *Pre-harvest glyphosate for weed control and as a harvest aid in cereals.* Research Review No. 65. HGCA.

ISSN: 1337-6853 *(print version)* | 1337-9569 *(electronic version)*

Interdisciplinary Toxicology. 2013; **Vol. 6**(4): 159–184
Also available online on **PubMed Central**

Paganelli A, Gnazzo V, Acosta H, López SL, Carrasco AE. (2010). Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid Signaling. *Chem Res Toxicol* **23**: 1586–1595.

Papp LV, Lu J, Holmgren A, Khanna KK. (2007). From selenium to selenoproteins: Synthesis, identity, and their role in human health. *Antiox Redox Signal* **9**: 775–806.

Patel RS, Johlin FC Jr, Murray JA. (1999). Celiac disease and recurrent pancreatitis. *Gastrointest Endosc* **50**(6): 823–827.

Pavkov KL, Turnier JC. (1986). *2-Year chronic toxicity and oncogenicity dietary study with SCm-0224 in mice.* T-11813. Farmington: Stauffer Chemical Company.

Peixoto F. (2005). Comparative effects of the Roundup and glyphosate on mitochondrial oxidative phosphorylation. *Chemosphere* **61**(8): 1115–1122.

Peraza S, Wesseling C, Aragon A, Leiva R, Garca-Trabanino RA, Torres C, Jakobsson K, Elinder C, Hogstedt C. (2012). Decreased kidney function among agriculture workers in El Salvador. *Am J Kidney Dis* **59**: 531–540.

Pessione E. (2012). Lactic acid bacteria contribution to gut microbiota complexity: Lights and shadows. *Front Cell Infect Microbiol* **2**: 86.

Peters U, Askling J, Gridley G, Ekbom A, Linet M. (2003). Causes of death in patients with celiac disease in a population-based Swedish cohort. *Arch Intern Med* **163**(13): 1566–1572.

Ponchon G, Kennan AL, DeLuca HF. (1969). Activation of vitamin D by the liver. *J Clin Invest* **48**(11): 2032–2037.

Prabhakar R, Morokuma K, Musaev DG. (2006). Peroxynitrite reductase activity of selenoprotein glutathione peroxidase: A computational study. *Biochemistry* **45**: 6967–6977.

Prasad GA, Alexander JA, Schleck CD, Zinsmeister AR, Smyrk TC, Elias RM, Locke GR 3rd, Talley NJ. (2009). Epidemiology of eosinophilic esophagitis over three decades in Olmsted County, Minnesota. *Clin Gastroenterol Hepatol* **7**: 1055–1061.

Pricolo VE, Mangi AA, Aswad B, Bland KI. (1998). Gastrointestinal malignancies in patients with celiac sprue. *Am J Surg* **176**: 344–7.

Putcha GV, Le S, Frank S, Besirli CG, Clark K, Chu B, Alix S, Youle RJ, LaMarche A, Maroney AC, Johnson EM Jr. (2003). JNK-mediated BIM phosphorylation potentiates BAX-dependent apoptosis. *Neuron* **38**(6): 899–914.

Qiao S-W, Bergseng E, Molberg, Ø, Jung G, Fleckenstein B, Solli LM. (2005). Refining the Rules of Gliadin T Cell Epitope Binding to the Disease-Associated DQ2 Molecule in Celiac Disease: Importance of Proline Spacing and Glutamine Deamidation. *J Immunol* **175**(1): 254–261.

Ramirez-Rubio O, Brooks DR, Amador JJ, Kaufman JS, Weiner DE, Scammell MK. (2013). Chronic kidney disease in Nicaragua: A qualitative analysis of semi-structured interviews with physicians and pharmacists. *MC Public Health* **13**: 350.

Refsum H, Yajnik CS, Gadkari M, Schneede J, Vollset SE, Orning L, Guttormsen AB, Joglekar A, Sayyad MG, Ulvik A, Ueland PM. (2001). Hyperhomocysteinemia and elevated methylmalonic acid indicate a high prevalence of cobalamin deficiency in Asian Indians. *Am J Clin Nutr* **74**: 233–41.

Relyea RA. (2005). The lethal impact of Roundup on aquatic and terrestrial amphibians. *Ecol Appl* **15**: 1118–1124.

Richard EP Jr, Dalley CD. (2009). Effects of glyphosate ripener timing and rate on cane and sugar yields. *J Am Soc Sug Cane Technol* **29**: 81–82.

Rivabene R, Mancini E, Vincenzi M. (1999). In vitro cytotoxic e¡ect of wheat gliadin-derived peptides on the Caco-2 intestinal cell line is associated with intracellular oxidative imbalance: implications for coeliac disease. *Biochi Biophys Acta* **1453**: 152–160.

Rodríguez-Iturbe B, Garca Garca G. (2010). The role of tubulointerstitial inflammation in the progression of chronic renal failure. *Nephron Clin Pract* **116**: c81–c88

Roe DA. (1971). Effects of methionine and inorganic sulfate on indole toxicity and indican excretion in rats. *J Nutr* **101**(5): 645–53.

Rossi M, Amaretti A, Raimondi S. (2011). Folate production by probiotic bacteria. *Nutrients* **3**(1): 118–34.

Rubio-Tapia A, Kyle RA, Kaplan EL, Johnson DR, Page W, Erdtmann F, Brantner TL, Kim WR, Phelps TK, Lahr BD, Zinsmeister AR, Melton LJ 3rd, Murray JA. (2009). Increased prevalence and mortality in undiagnosed celiac disease. *Gastroenterology* **137**(1): 88–93.

Rude RK, Olerich M. (1996). Magnesium deficiency: possible role in osteoporosis associated with gluten-sensitive enteropathy. *Osteoporos Int* **6**(6): 453–61.

Russell RM. (2000). The vitamin A spectrum: From deficiency to toxicity. *Am J Clin Nutr* **71**: 878–84.

Saad RJ, Chey WD. (2006). Review article: Current and emerging therapies for functional dyspepsia. *Aliment Pharmacol Ther* **24**(3): 475–492.

Saes Zobiole LH, de Oliveira RS Jr, Kremer RJ, Muniz AS, de Oliveira, A Jr. (2010). Nutrient accumulation and photosynthesis in glyphosate-resistant soybeans is reduced under glyphosate use. *J Plant Nutr* **33**: 1860–1873.

Saibeni S, Lecchi A, Meucci G, Cattaneo M, Tagliabue L, Rondonotti E, Formenti S, De Franchis R, Vecchi M. (2005). Prevalence of hyperhomocysteinemia in adult gluten-sensitive enteropathy at diagnosis: Role of B12, folate, and genetics. *Clin Gastroenterol Hepatol* **3**: 574–580.

Sakaki T, Kagawa N, Yamamoto K, Inouye K. (2005). Metabolism of vitamin D3 by cytochromes P450. *Front Biosci* **10**: 119–34.

Sammons RD, Gruys KJ, Anderson KS, Johnson KA, Sikorski JA. (1995). Reevaluating glyphosate as a transition-state inhibitor of EPSP synthase: Identification of an EPSP synthase.EPSP.glyphosate ternary complex. *Biochemistry* **34**(19): 6433–40.

Samsel A, Seneff S. (2013). Glyphosate's suppression of cytochrome P450 enzymes and amino acid biosynthesis by the gut microbiome: Pathways to modern diseases. *Entropy* **15**: 1416–1463.

Sanoff SL, Callejas L, Alonso CD, Hu Y, Colindres RE, Chin H, Morgan DR, Hogan SL. (2010). Positive association of renal insufficiency with agriculture employment and unregulated alcohol consumption in Nicaragua. *Ren Fail* **32**: 766–777.

Sanz Y, De Palma G, Laparra M. (2011). Unraveling the ties between celiac disease and intestinal microbiota. *International Reviews of Immunology* **30**(4): 207–218.

Selvapandiyan A, Majumder K, Fattah FA, Ahmad S, Arora N, Bhatnagar RK. (1995). Point mutation of a conserved arginine (104) to lysine introduces hypersensitivity to inhibition by glyphosate in the 5-enolpyruvylshikimate-3-phosphate synthase of Bacillus subtilis. *FEBS Lett* **374**(2): 253–6.

Senapati T, Mukerjee AK, Ghosh AR. (2009). Observations on the effect of glyphosate based herbicide on ultra structure (SEM) and enzymatic activity in different regions of alimentary canal and gill of Channa punctatus (Bloch). *Journal of Crop and Weed* **5**(1): 236–245.

Seneff S, Lauritzen A, Davidson R, Lentz-Marino L. (2012). Is endothelial nitric oxide synthase a moonlighting protein whose day job is cholesterol sulfate synthesis? Implications for cholesterol transport, diabetes and cardiovascular disease. *Entropy* **14**: 2492–2530.

Shapira Y, Agmon-Levina N, Shoenfeld Y. (2010). Defining and analyzing geoepidemiology and human autoimmunity. *Journal of Autoimmunity* **34**: J168–J177.

Shehata AA, Schrödl W, Aldin AA, Hafez HM, Krüger M. (2013). The effect of glyphosate on potential pathogens and beneficial members of poultry microbiota in vitro. *Curr Microbiol* **66**: 350–358.

Sher L. (2000). Selenium and human health. *Lancet* **356**: 233–241.

Singhal N, Alam S, Sherwani R, Musarrat J. (2008). Serum zinc levels in celiac disease. *Indian Pediatr* **45**(4): 319–21.

Smecuol E, Hwang HJ, Sugai E, Corso L, Cheravsky AC, Bellavite FP, González A, Vodánovich F, Moreno ML, Vázquez H, Lozano G, Niveloni S, Mazure R, Meddings J, Maurio E, Bai JC. (2013). Exploratory, randomized, double-blind, placebo-controlled study on the effects of Bifidobacterium infantis natren life start strain super strain in active celiac disease. *J Clin Gastroenterol* **47**(2): 139–47.

Soderland P, Lovekar S, Weiner DE, Brooks DR, Kaufman JS. (2010). Chronic kidney disease associated with environmental toxins and exposures. *Adv Chronic Kidney Dis* **17**(3): 254–64.

Sonnenberg A, McCarty DJ, Jacobsen SJ. (1991). Geographic variation of inflammatory bowel disease within the United States. *Gastroenterology* **100**: 143e9.

State-Specific Trends in Chronic Kidney Failure —United States, 1990–2001. http: //www.cdc.gov/mmwr/preview/mmwrhtml/mm5339a3.htm. [Last accessed Sept. 3, 2013]

Stride CD, Edwards RV and Seddon JC. (1985). *Sward destruction by application of glyphosate before cutting or grazing.* British Crop Protection Conference – Weeds 7B–6, 771–778.

Subiros JF. (1990). The effect of applying glyphosate as ripener in three varieties. *Turrialba* **40**(4): 527–534.

Sulik KK, Cook CS, Webster WS. (1988) Teratogens and craniofacial malformations: relationships to cell death. *Development* **103** Suppl: 213–231.

Sun C-Y, Hsu H-H, Wu M-S. (2012). p-Cresol sulfate and indoxyl sulfate induce similar cellular inflammatory gene expressions in cultured proximal renal tubular cells. *Nephrol Dial Transplant* **28**(1): 70–8.

Sunergren KP, Fairman RP, deBlois GG, Glauser FL. (1987). Effects of protamine, heparinase and hyaluronidase on endothelial permeability and surface charge. *J Appl Physiol* **63**: 1987–92.

Taimi M, Helvig C, Wisniewski J, Ramshaw H, White J, Amad M, Korczak B, Petkovich M. (2004). A novel human cytochrome P450, CYP26C1, involved in metabolism of 9-cis and all-trans isomers of retinoic acid. *J Biol Chem* **279**: 77–85.

Tamm AO. (1984). Biochemical activity of intestinal microflora in adult coeliac disease. *Nahrung* **28**(6–7): 711–5.

Thacher SM, Coe EL, Rice RH. (1985). Retinoid suppression of transglutaminase activity and envelope competence in cultured human epidermal carcinoma cells: Hydrocortisone is a potent antagonist of retinyl acetate but not retinoic acid. *Differentiation* **29**(1): 82–87.

Thompson WW, Weier TE. (1962). The fine structure of chloroplasts from mineral-deficient leaves of Phaseolus vulgaris. *Am J Bot* **49**: 1047–1056.

Tieri P, Termanini A, Bellavista E, Salvioli S, Capri M, Franceschi C. (2012). Charting the NF-κB pathway interactome map. *PLoS One* **7**(3): e32678.

Tonelli M, Sacks F, Pfeffer M, Jhangri GS, Curhan G; Cholesterol and Recurrent Events (CARE) Trial Investigators. (2005). Biomarkers of inflammation and progression of chronic kidney disease. *Kidney Int* **68**: 237–245.

Torres C, Aragon A, Gonzalez M, Lopez I, Jakobsson K, Elinder CG, Lundberg I, Wesseling C. (2010). Decreased kidney function of unknown cause in Nicaragua: a community-based survey. *Am J Kidney Dis* **55**: 485–496.

Trabanino RG, Aguilar R, Silva CR, Mercado MO, Merino RL. (2002). End-stage renal disease among patients in a referral hospital in El Salvador. *Rev Panam Salud Publica* **12**: 202–206. [article in Spanish]

Triggiani V, Tafaro E, Giagulli VA, Sabbà C, Resta F, Licchelli B, Guastamacchia E. (2009). Role of iodine, selenium and other micronutrients in thyroid function and disorders. *Endocr Metab Immune Disord Drug Targets* **9**(3): 277–94.

Turnbull J, Powell A, Guimond S. (2001). Heparan sulfate: Decoding a dynamic multifunctional cell regulator. *Trends Cell Biol* **11**: 75–82.

Tursi A, Brandimarte G, Giorgetti G. (2003). High prevalence of small intestinal bacterial overgrowth in celiac patients with persistence of gastrointestinal symptoms after gluten withdrawal. *Am J Gastroenterol* **98**: 839–843.

Uggla C, Moritz T, Sandberg G, Sundberg B. (1996). Auxin as a positional signal in pattern formation in plants. *Proc Natl Acad Sci U S A* **93**(17): 9282–86.

Undabeytia TS, Morillo E, Maqueda C. (2002). FTIR study of glyphosate-copper complexes. *J Agric Food Chem* **50**: 1918–1921.

Valentino R, Savastano S, Maglio M, Paparo F, Ferrara F, Dorato M, Lombardi G, Troncone R. (2002). Markers of potential coeliac disease in patients with Hashimoto's thyroiditis. *Eur J Endocrinol* **146**: 479–483.

Vencill WK (ed) (2002). *Herbicide handbook*. 8th ed. Weed Science Society of America, Lawrence, KS. USA.

Vernier RL, Klein DJ, Sisson SP, Mahan JD, Oegema TR, Brown DM. (1983). Heparan sulphate-rich anionic sites in the human glomerular basement membrane: decreased concentration in congential nephrotic syndrome. *N Engl J Med* **309**: 1001–9.

Vigfusson NV, Vyse ER. (1980).The effect of the pesticides, Dexon, Captan and Roundup, on sister-chromatid exchanges in human lymphocytes in vitro. *Mutat Res* **79**: 53–7.

Waltz E. (2010). Glyphosate resistance threatens Roundup hegemony. *Nat Biotechnol* **28**: 537–538.

Welander A, Prütz KG, Fored M, Ludvigsson JF. (2012). Increased risk of end-stage renal disease in individuals with coeliac disease. *Gut* **61**(1): 64–8.

Wheeler EE, Challacombe DN. (1984). Quantification of enterochromaffin cells with serotonin immunoreactivity in the duodenal mucosa in coeliac disease. *Arch Dis Child* **59**: 523–527.

Whorwell PJ, Altringer L, Morel J, Bond Y, Charbonneau D, O'Mahony L, Kiely B, Shanahan F, Quigley EM. (2006). Efficacy of an encapsulated probiotic Bifidobacterium infantis 35624 in women with irritable bowel syndrome. *Am J Gastroenterol* **101**(7): 1581–90.

Wikvall K. (2001). Cytochrome P450 enzymes in the bioactivation of vitamin D to its hormonal form (review). *Int J Mol Med* **7**(2): 201–9.

Williams GM, Kroes R, Munro IC. (2000). Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. *Regul Toxicol Pharmacol* **31**(2 Pt 1): 117–165.

Zaflarska-Popławska A, Siomek A, Czerwionka-Szaflarska M, Gackowski D, Rozalski R, Guz J, Szpila A, Zarakowska E, Olinski R. (2010). Oxidatively damaged DNA/oxidative stress in children with celiac disease. *Cancer Epidemiol Biomarkers Prev* **19**(8): 1960–1965.

Zenjari T, Boruchowicz A, Desreumaux P, Laberenne E, Cortot A, Colombel JF. (1995). Association of coeliac disease and portal venousthrombosis. *Gastroenterol Clin Biol.* **19**: 953–954.

Zhao F-J, Lopez-Bellido FJ, Gray CW, Whalley WR, Clark LJ, McGrath SP. (2007). *Science of The Total Environment* **372**(2–3): 433–439.

Ziolkowski AF, Popp SK, Freeman C, Parish CR, Simeonovic CJ. (2012). Heparan sulfate and heparanase play key roles in mouse cell survival and autoimmune diabetes. *J Clin Invest* **122**(1): 132–141.

Zouaoui K, Dulaurent S, Gaulier JM, Moesch C, Lachâtre G. (2013). Determination of glyphosate and AMPA in blood and urine from humans: About 13 cases of acute intoxication. *Forensic Sci Int* **226**(1–3): e20–5.

ISSN: 1337-6853 *(print version)* | 1337-9569 *(electronic version)*

Surgical Neurology International

OPEN ACCESS

For entire Editorial Board visit : http://www.surgicalneurologyint.com

Editor: **James I. Ausman,** MD, PhD University of California, Los Angeles, CA, USA

Original Article

Glyphosate, pathways to modern diseases III: Manganese, neurological diseases, and associated pathologies

Anthony Samsel, Stephanie Seneff[1]

Research Scientist and Consultant, Deerfield, NH 03037, [1]Spoken Language Systems Group, Computer Science and Artificial Intelligence Laboratory, MIT, Cambridge MA 02139, USA

E-mail: Anthony Samsel - anthonysamsel@acoustictracks.net; *Stephanie Seneff - seneff@csail.mit.edu
*Corresponding author

Received: 22 September 14 Accepted: 21 January 15 Published: 24 March 15

This article may be cited as:
Samsel A, Seneff S. Glyphosate, pathways to modern diseases III: Manganese, neurological diseases, and associated pathologies. Surg Neurol Int 2015;6:45.
Available FREE in open access from: http://www.surgicalneurologyint.com/text.asp?2015/6/1/45/153876

Abstract

Manganese (Mn) is an often overlooked but important nutrient, required in small amounts for multiple essential functions in the body. A recent study on cows fed genetically modified Roundup®-Ready feed revealed a severe depletion of serum Mn. Glyphosate, the active ingredient in Roundup®, has also been shown to severely deplete Mn levels in plants. Here, we investigate the impact of Mn on physiology, and its association with gut dysbiosis as well as neuropathologies such as autism, Alzheimer's disease (AD), depression, anxiety syndrome, Parkinson's disease (PD), and prion diseases. Glutamate overexpression in the brain in association with autism, AD, and other neurological diseases can be explained by Mn deficiency. Mn superoxide dismutase protects mitochondria from oxidative damage, and mitochondrial dysfunction is a key feature of autism and Alzheimer's. Chondroitin sulfate synthesis depends on Mn, and its deficiency leads to osteoporosis and osteomalacia. Lactobacillus, depleted in autism, depend critically on Mn for antioxidant protection. Lactobacillus probiotics can treat anxiety, which is a comorbidity of autism and chronic fatigue syndrome. Reduced gut Lactobacillus leads to overgrowth of the pathogen, Salmonella, which is resistant to glyphosate toxicity, and Mn plays a role here as well. Sperm motility depends on Mn, and this may partially explain increased rates of infertility and birth defects. We further reason that, under conditions of adequate Mn in the diet, glyphosate, through its disruption of bile acid homeostasis, ironically promotes toxic accumulation of Mn in the brainstem, leading to conditions such as PD and prion diseases.

Key Words: Autism, cholestasis, glyphosate, manganese, Parkinson's disease

Access this article online

Website: www.surgicalneurologyint.com

DOI: 10.4103/2152-7806.153876

Quick Response Code:

INTRODUCTION

Glyphosate is the active ingredient in Roundup®, the most widely used herbicide on the planet.[314] Glyphosate enjoys widespread usage on core food crops, in large part because of its perceived nontoxicity to humans. The adoption of genetically engineered "Roundup®-Ready" corn, soy, canola, cotton, alfalfa, and sugar beets has made it relatively easy to control weeds without killing the crop plant, but this means that glyphosate will be present as a residue in derived foods. Unfortunately, weeds among GM Roundup®-Ready crops are developing ever-increasing resistance to Roundup®,[107,221] which requires an increased rate of herbicide application.[26]

In 1987, glyphosate was the 17th most commonly used herbicide in the United States, but, in large part due to the introduction of glyphosate-resistant core crops, it became the number one herbicide by 2001.[146] Its usage has increased steadily since then, in step with the rise in autism rates. Glyphosate's perceived nontoxicity is predicated on the assumption that our cells do not possess the shikimate pathway, the biological pathway in plants, which is disrupted by glyphosate, and whose disruption is believed to be the most important factor in its toxicity.

It may seem implausible that glyphosate could be toxic to humans, given the fact that government regulators appear nonchalant about steadily increasing residue limits, and that the levels in food and water are rarely monitored by government agencies, presumably due to lack of concern. However, a paper by Antoniou *et al*.[12] provided a scathing indictment of the European regulatory process regarding glyphosate's toxicity, focusing on potential teratogenic effects. They identified several key factors leading to a tendency to overlook potential toxic effects. These include using animal studies that are too short or have too few animals to achieve statistical significance, disregarding *in vitro* studies or studies with exposures that are higher than what is expected to be realistically present in food, and discarding studies that examine the effects of glyphosate formulations rather than pure glyphosate, even though formulations are a more realistic model of the natural setting and are often orders of magnitude more toxic than the active ingredient in pesticides.[189] Regulators also seemed unaware that chemicals that act as endocrine disruptors (such as glyphosate[108]) often have an inverted dose–response relationship, wherein very low doses can have more acute effects than higher doses. Teratogenic effects have been demonstrated in human cell lines.[212] An *in vitro* study showed that glyphosate in parts per trillion can induce human breast cancer cell proliferation.[289]

Adjuvants in pesticides are synergistically toxic with the active ingredient. Mesnage *et al*.[189] showed that Roundup® was 125 times more toxic than glyphosate by itself. These authors wrote: "Despite its relatively benign reputation, Roundup® was among the most toxic herbicides and insecticides tested."[189]

The industry dictates that 3 months is a sufficiently long time to test for toxicity in rodent studies, and as a consequence none of the industry studies have run for longer than 3 months. The only study we are aware of that was a realistic assessment of the long-term effects of GM Roundup®-Ready corn and soy feed on mammals was the study by Séralini *et al*. that examined the effects on rats fed these foods for their entire life span.[261] This study showed increased risk to mammary tumors in females, as well as kidney and liver damage in the males, and a shortened lifespan in both females and males. These effects occurred both in response to Roundup and to the GM food alone. These effects only began to be apparent after 4 months.

There are multiple pathways by which glyphosate could lead to pathology.[248] A major consideration is that our gut bacteria *do* have the shikimate pathway, and that we depend upon this pathway in our gut bacteria as well as in plants to supply us with the essential aromatic amino acids, tryptophan, tyrosine, and phenylalanine. Methionine, an essential sulfur-containing amino acid, and glycine, are also negatively impacted by glyphosate. Furthermore, many other biologically active molecules, including serotonin, melatonin, melanin, epinephrine, dopamine, thyroid hormone, folate, coenzyme Q10, vitamin K, and vitamin E, depend on the shikimate pathway metabolites as precursors. Gut bacteria and plants use exclusively the shikimate pathway to produce these amino acids. In part because of shikimate pathway disruption, our gut bacteria are harmed by glyphosate, as evidenced by the fact that it has been patented as an antimicrobial agent.[298]

Metal chelation and inactivation of cytochrome P450 (CYP) enzymes (which contain heme) play important roles in the adverse effects of glyphosate on humans. A recent study on rats showed that both males and females exposed to Roundup® had 50% reduction in hepatic CYP enzyme levels compared with controls.[156] CYP enzyme dysfunction impairs the liver's ability to detoxify xenobiotics. A large number of chemicals have been identified as being porphyrinogenic.[77] Rossignol *et al*.[242] have reviewed the evidence for environmental toxicant exposure as a causative factor in autism, and they referenced several studies showing that urinary excretion of porphyrin precursors to heme is found in association with autism, suggesting impaired heme synthesis. Impaired biliary excretion leads to increased excretion of heme precursors in the urine, a biomarker of multiple chemical sensitivity syndrome.[77] We later discuss the ability of glyphosate to disrupt bile homeostasis, which we believe is a major source of its toxic effects on humans.

Glyphosate is a likely cause of the recent epidemic in celiac disease.[249] Glyphosate residues are found in wheat due to the increasingly widespread practice of staging and desiccation of wheat right before harvest. Many of the pathologies associated with celiac disease can be explained by disruption of CYP enzymes.[156] Celiac patients have a shortened life span, mainly due to an increased risk to cancer, most especially non-Hodgkin's lymphoma, which has also been linked to glyphosate.[85,253] Celiac disease trends over time match well with the increase in glyphosate usage on wheat crops.

Glyphosate is also neurotoxic.[59] Its mammalian metabolism yields two products: Aminomethylphosphonic acid (AMPA) and glyoxylate, with AMPA being at least as

toxic as glyphosate. Glyoxylate is a highly reactive glycating agent, which will disrupt the function of multiple proteins in cells that are exposed.[90] Glycation has been directly implicated in Parkinson's disease (PD).[57] Glyphosate has been detected in the brains of malformed piglets.[155] In a report produced by the Environmental Protection Agency (EPA), over 36% of 271 incidences involving acute glyphosate poisoning involved neurological symptoms, indicative of glyphosate toxicity in the brain and nervous system.[122]

In the remainder of this paper, we first introduce the link between glyphosate and manganese (Mn) dysbiosis, and briefly describe the main biological roles of Mn. We then describe how glyphosate's disruption of gut bacteria may be a major player in the recent epidemic in antibiotic resistance. We then explain how glyphosate can influence the uptake of arsenic and aluminum, and propose similar mechanisms at work with Mn. In the next section, we describe how Mn deficiency can lead to a reduction in Lactobacillus in the gut, and we link this to anxiety disorder. We follow with a discussion on mitochondrial dysfunction associated with suppressed Mn superoxide dismutase (Mn-SOD), and then a section on implications of Mn deficiency for oxalate metabolism. The following section explains how Mn deficiency can lead to the overexpression of ammonia and glutamate in many neurological diseases. The next two sections show how Mn accumulation in the liver is linked to cholestasis and high serum low density lipoprotein (LDL), and how this can also induce increased susceptibility to Salmonella poisoning. We then identify a role for Mn in chondroitin sulfate synthesis, and the implications for osteomalacia. The next two sections explain how glyphosate exposure can lead to Mn toxicity in the brain, and discuss two neurological diseases that are associated with excess Mn, PD and prion diseases. After a section on the link between male infertility and Mn deficiency in the testes, we discuss evidence of exposure to glyphosate and end with a short summary of our findings.

SUPPORTIVE EVIDENCE OF MANGANESE DYSBIOSIS DUE TO GLYPHOSATE

Glyphosate's disruption of the shikimate pathway is due in part to its chelation of Mn, which is a catalyst for enolpyruvylshikimate phosphate synthase (EPSPS), a critical early enzyme in the pathway.[63] A recent study on Danish dairy cattle investigated mineral composition in serum of cattle fed Roundup®-Ready feed.[154] The study identified a marked deficiency in two minerals: Serum cobalt and serum Mn. All of the cattle on eight different farms had severe Mn deficiency, along with measurable amounts of glyphosate in their urine. In Australia, following two seasons of high levels of stillbirths in cattle, it was found that all dead calves were Mn deficient.[184] Furthermore, 63% of newborns with birth defects were found to be deficient in Mn.

Mn, named after the Greek word for "magic," is one of 14 essential trace elements. Mn plays essential roles in antioxidant protection, glutamine synthesis, bone development, and sperm motility, among other things. Although Mn is essential, it is only required in trace amounts. And an excess of Mn can be neurotoxic.

Remarkably, Mn deficiency can explain many of the pathologies associated with autism and Alzheimer's disease (AD). The incidence of both of these conditions has been increasing at an alarming rate in the past two decades, in step with the increased usage of glyphosate on corn and soy crops in the United States, as shown in [Figures 1 and 2]. Although correlation does not necessarily mean causation, from 1995 to 2010, the autism rates in first grade in the public school correlates almost perfectly ($P = 0.997$) with total glyphosate application on corn and soy crops over the previous 4 years (from age 2 to 6 for each child) [Figure 1]. Such remarkable correlation necessitates further experimental investigation. These neurological disorders are associated with mitochondrial impairment[197,241,243,281,316] and with excess glutamate and ammonia in the brain,[2,109,265] leading to a chronic low-grade encephalopathy.[256,260] As we will show later, Mn deficiency is critically associated with these pathologies.

Thyroid dysfunction can be predicted as well, and low maternal thyroid function predicts autism in the fetus.[238] Furthermore, increases in bone fractures in both children and the elderly can also be explained by Mn deficiency, due to its critical role in bone development.[276] Osteoporosis, which is a serious problem

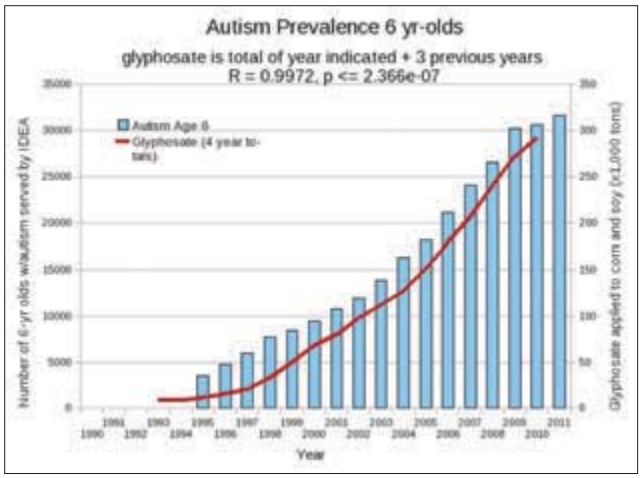


Figure 1: Plots of amount of glyphosate applied to corn and soy crops in the US over the previous 4 years (red), provided by the US Department of Agriculture, compared with number of children enrolled in the first grade in the public school system under the autism category according to the Individuals with Disabilities Education Act (IDEA) (blue bars). (Figure courtesy of Dr. Nancy Swanson)

among the elderly today, is also likely promoted by Mn deficiency,[247] and osteoporosis leads to increased risk to fractures.[98,139,140]

Sprague-Dawley rats fed a Mn-deficient diet had significantly reduced concentrations of Mn in liver, kidney, heart, and pancreas, compared with controls.[18] Furthermore, pancreatic insulin content was only 63% of control levels, and insulin release following glucose administration was also reduced. Mn deficiency not only impairs insulin secretion in Sprague-Dawley rats, but it also causes reduced glucose uptake in adipose tissue,[19] so Mn deficiency could contribute to impaired glucose metabolism in both type 1 and type 2 diabetes, which are a growing problem worldwide.[199] Type 1 diabetes in children is associated with a decrease in Lactobacillus and Bifidobacterium, and an increase in Clostridium, in the gut.[195] These same pathologies are also found in gut bacteria from poultry fed Roundup®-Ready feed.[263] The increased incidence of diabetes in the US is strongly correlated with glyphosate usage on corn and soy, as shown in [Figure 3].

Much remains elusive about Mn's roles in cellular metabolism, but it is clear that it is very important. For instance, Target of Rapamycin Complex 1 (TORC1) accelerates the aging process in cells from yeast to mammals,[231] and Mn inhibits TORC1, but only if it is present in the Golgi.[86] Zinc (Zn) is essential for DNA and RNA replication and cell division. Zn deficiency leads to greatly enhanced Mn uptake by cells, and this induces modifications to messenger RNA such that the ratio of guanine and cytosine nucleotides (C + G) to adenine and thymine (A + T) is sharply increased.[100] Clearly, more research is needed to explain the significance of these phenomena.

We infer, paradoxically, that both Mn deficiency and Mn toxicity, attributable to glyphosate, can occur simultaneously. Because of glyphosate's disruption of CYP enzymes, the liver becomes impaired in its ability to dispose of Mn via the bile acids, and instead it transports the Mn via the vagus nerve to brainstem nuclei, where excess Mn leads to PD. Recently, PD has also increased dramatically, in step with glyphosate usage on corn and soy [Figure 4].

Ironically, while the brainstem suffers from excess Mn, the rest of the brain incurs Mn deficiency due to the depressed serum levels of Mn. Mn is particularly important in the hippocampus, and deficiency there can lead to seizures. A high incidence of seizures is found in children with autism.[302] Seizures are also associated with reduced serum Mn,[54,88,269] and this is consistent with the liver's inability to distribute Mn to the body via the bile acids. Antibiotics have been found to induce seizures.[132]

Mn uptake in the brain is normally enhanced during the neonatal period in rats, and proper development

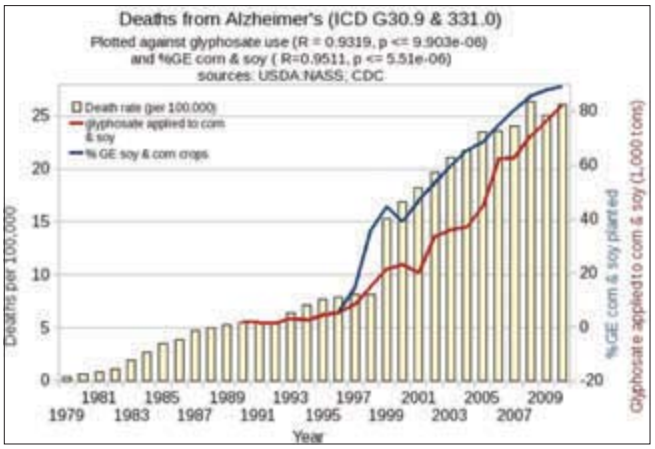


Figure 2: Plots of amount of glyphosate applied to corn and soy crops in the US over time, compared to the rate of death from AD. (Figure courtesy of Dr. Nancy Swanson)

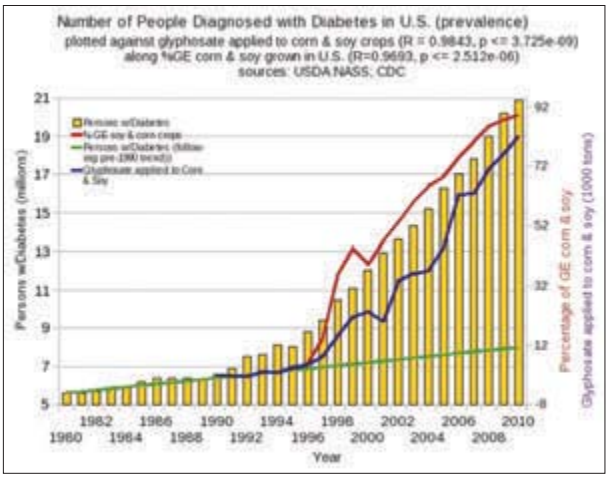


Figure 3: Plots of glyphosate usage on corn and soy crops (blue), percent of corn and soy that is genetically engineered to be "Roundup Ready" (red), and prevalence of diabetes (yellow bars) in the US. (Figure courtesy of Dr. Nancy Swanson)

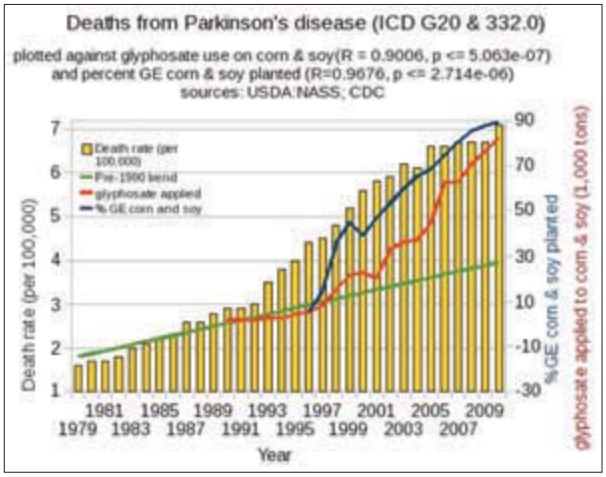


Figure 4: Plots of glyphosate usage on corn and soy crops (blue), percent of corn and soy that is genetically engineered to be "Roundup Ready," (red), and deaths from PD (yellow bars) in the US. (Figure courtesy of Dr. Nancy Swanson)

of the hippocampus depends on Mn.[284] Soy formula increases the risk of seizures in autism,[310] hardly surprising when one considers that soybean crops are now 90% Roundup®-Ready. A recent paper has confirmed that alarmingly high glyphosate residues appear in Roundup®-Ready soy.[35] The US Department of Agriculture analyzed glyphosate residues in soy in 2011, and reported that 91% of the 300 samples tested were positive for glyphosate, with 96% being positive for AMPA, an equally toxic by-product of glyphosate breakdown.[297] Our own analysis confirms that glyphosate is present in infant formula. Out of several soy-based baby formulas we tested, only one contained glyphosate residues. We found levels of 170 ppb in Enfamil ProSobee liquid concentrate. Further testing is underway. Soybean product sourcing and residue testing should be required prior to product manufacturing and is necessary to prevent inadvertent infant exposure.

Another mechanism by which glyphosate in soy formula could cause seizures is through bilirubin production. Serum concentrations of bilirubin were elevated in catfish exposed to sublethal doses of Roundup®, in a dose-dependent relationship.[208] Neonates, due to an immature digestive system, are unable to metabolize bilirubin in the gut, and it can therefore build up in the blood and even penetrate their immature blood–brain barrier to cause seizures.[308]

GLYPHOSATE AND MICROBIAL ANTIBIOTIC INTOLERANCE

Microbial antibiotic tolerance and resistance are a growing problem worldwide, likely fueled by horizontal gene transfer among different bacterial species.[106,121] Multiple-drug resistant commensal bacteria in the guts of both animals and humans form a reservoir of resistance genes that can spread to pathogenic species. Methicillin-resistant *Staphylococcus aureus* (MRSA),[119] *Clostridium difficile*,[183] and *Pseudomonas aeruginosa*[169] are all becoming major threats, especially in the hospital environment. A generic mechanism of upregulated efflux through membrane pores offers broad-domain resistance to multiple antibiotics.[169] Exposure to antibiotics early in life can even lead to obesity as a direct consequence of the resulting imbalance in gut bacteria.[73]

Studies have shown that increased mutation rates due to chronic low level exposure to one antibiotic can induce an accelerated rate of development of resistance to diverse other antibiotics.[151] Glyphosate, patented as an antimicrobial agent,[298] is present in steadily increasing amounts in the GM Roundup-Ready corn and soy feed of cows, pigs, chickens, farmed shrimp, and fish, and it is ubiquitous in the Western diet of humans. *Pseudomonas aeruginosa* can use glyphosate as a sole source of phosphorus,[192] and it is one of a small number of resistant bacterial species with the ability to metabolize glyphosate, a feature that might be exploited for soil remediation.[1] However, DNA mutations due to exposure would enhance tolerance to glyphosate and other antibiotics, perhaps explaining the current epidemic in multiple antibiotic resistant *P. aeruginosa* infections, which have a 20% mortality rate.[190] Antibiotic resistance sequences engineered into GM crops may also play a role in the current crisis concerning antibiotic resistant pathogens.

Glyphosate has also been demonstrated as a remarkable antimicrobial synergist. It greatly increases the cidal effects of other antimicrobials, particularly when combined as salts of glyphosate. A concentration dependent synergy index (SI) ranging from 0.34 to 5.13 has been recorded for the Zn salt of glyphosate.[299] This has serious implications for glyphosate ingested with pharmaceuticals or residues of other widely used agricultural chemicals, such as the herbicides Diquat, Paraquat, 2,4 D and Glufosinate, the fungicide Chlorothalonil and the systemic neonicotinoid insecticides Acetamiprid, Imidacloprid, Thiacloprid, Thiamethoxam, and Clothianidin.

Glyphosate acts as a catalyst for the development of antibiotic resistance genes in pathogens. Since both poultry and cow manure are used as natural fertilizers in crops, it can be expected that a vector for microbial resistance to multiple drugs is through contamination of fruits and vegetables. Indeed, multiple resistance genes have been identified from diverse phyla found in cow manure, including Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria, that is, in phylogenetically diverse organisms.[311]

One of the ways in which glyphosate is toxic to plants is through disruption of chlorophyll synthesis, due to suppression of the activity of the first enzyme in pyrrole synthesis.[61,69,143,319] Pyrrole is the core building block of both chlorophyll and the porphyrin rings, including corrin in cobalamin and heme in hemoglobin and cytochrome enzymes. Several cofactors containing a structurally complex tetrapyrrole-derived framework chelating a metal ion (cobalt (Co), magnesium (Mg), iron (Fe), or nickel (Ni)) are synthesized by gut bacteria and supplied to the host organism, including heme and corrin.[236]

Thus, glyphosate can be expected to disrupt synthesis of these biologically essential molecules. Pseudomonas normally thrives in the small bowel and produces abundant cobalamin that may be a significant source for the human host.[6] *P. aeruginosa*'s successful colonization may be due in part to its ability to produce cobalamin despite the presence of glyphosate. Only recently has it also been recognized that a Mn–porphyrin complex can protect from mitochondrial overproduction of hydrogen peroxide (H_2O_2) in response to ionizing radiation.[274] It can be predicted that homeostasis of all of these minerals

in the gut (Co, Mn, Fe, Ni, and Mg) is impaired in the presence of glyphosate, and this will have serious consequences not only to the gut bacteria but also to the impaired regulation of these minerals. The implications of impaired heme and cobalamin synthesis will be further addressed in a future paper.

A key component of glyphosate's action is its ability to chelate minerals, particularly transition metals such as Mn. Glyphosate forms strong complexes with the transition metals via the amino, the carboxylic, and the phosphonic moieties in the molecule. Each of these can coordinate separately to metal ions or in combination as bidentate or tridentate ligands.[194,296]

ANALOGY WITH ARSENIC AND ALUMINUM

Chronic kidney disease is clearly associated with multiple environmental toxicants.[268] There has been an epidemic in recent years in kidney failure among young agricultural workers in Central America, India, and Sri Lanka, particularly those working in the sugar cane fields.[249] A recent paper reached the unmistakable conclusion that glyphosate plays a critical role in this epidemic.[133] A growing practice of spraying sugar cane with glyphosate as a ripener and desiccant right before the harvest has led to much greater exposure to the workers in the fields. The authors, who focused their studies on affected workers in rice paddies in Sri Lanka, identified a synergistic effect of arsenic, which contaminated the soil in the affected regions. This paper is highly significant, because it proposes a mechanism whereby glyphosate greatly increases the toxicity of arsenic through chelation, which promotes uptake by the gut. Glyphosate also depletes glutathione (GSH)[60,128] and glutathione S transferase (GST) is a critical enzyme for liver detoxification of arsenic.[295] As a consequence, excess arsenic in the kidney causes acute kidney failure, without evidence of other symptoms such as diabetes usually preceding kidney failure.

Arsenic is normally disposed of by the liver through biliary excretion. In rats exposed to arsenic, large amounts of GSH appeared in the bile simultaneously with biliary excretion of arsenic.[113] It was first hypothesized, and later confirmed, that arsenic is transported in bile acids in the form of unstable GSH complexes (monomethylarsonous acid), which release GSH upon decomposing. Since glyphosate disrupts CYP enzymes necessary for bile acid formation,[248,249] as well as depleting GSH,[60,128] it can be expected that glyphosate would disrupt the process of biliary excretion of arsenic, thus forcing arsenic to be redirected toward urinary excretion, leading ultimately to kidney failure.

Glyphosate also chelates aluminum,[230] and it has been reasoned that this enables aluminum to get past the gut barrier more readily through direct analogy with the situation with arsenic, which is also a 3+ cation.[193] However, it has been demonstrated through experimentation that glyphosate prefers divalent cations. Thus, aluminum would enter the bloodstream via the digestive tract's portal vein to the organs traveling with albumin, which is known to attach and transport many xenobiotics. It is well established that citrate also binds aluminum and promotes its uptake past the gut barrier through a mechanism that parallels glyphosate's binding to aluminum.[68,148] Both are small molecules that easily pass through a leaky gut barrier.

Considering these observations regarding aluminum and arsenic, it is reasonable to expect that something similar might happen with Mn. Unlike these other two, however, Mn plays many essential roles in the body, and so its chelation by glyphosate would interfere with its bioavailability in the general circulation. Just as for arsenic, bile acids play a critical role in Mn homeostasis. Bile is the major excretory route of injected Mn.[17] Malecki *et al*. wrote: "Biliary excretion may be a major homeostatic mechanism for preventing both deficiency and toxicity of Mn."[179, p. 489]

Glyphosate, a dipolar zwitterion, is toxic in part due to its bio-transformative properties as pH varies. We postulate that Mn, which is transported in the blood stream bound to glyphosate, is oxidized to Mn^{3+} following its release in the localized acidic environment of sulfated glycosaminoglycans (GAGs) in the glycocalyx lining the capillary wall.[234] As we will later explain, Mn uniquely is able to travel along axons and across synapses, and this results in a novel path via the vagus nerve for brain toxicity following excess Mn accumulation in the liver.

Mn is a transition metal, and therefore it can catalyze oxidative reactions in neurons via the Fenton reaction.[305] While Mn^{2+} is the form of Mn that catalyzes enzyme reactions, Mn^{3+}, similar to Al^{3+}, is directly toxic to neuronal membranes.[13] *In vitro* studies have shown that Mn^{3+} complexes auto-oxidize catecholamines, and therefore exposure to excess Mn leads to a decrease in the bioavailability of dopamine, serotonin, and noradrenaline in the striatum.[227]

It has been shown that glyphosate enhances the oxidation of Mn from a 2^+ oxidation state to 3^+, both in solution and on an inert surface.[21] It can be inferred, therefore, that Mn^{2+} oxidation to the toxic form, Mn^{3+}, might occur in the artery wall following exposure to superoxide in the presence of glyphosate. Mn^{3+} also enhances glyphosate degradation to AMPA,[21] which would produce the highly glycating by-product, glyoxylate. Glyoxylate and Mn^{3+} would both cause significant arterial damage in association with the inflammatory response.

We suggest that another route for Mn transport is the vagus nerve, which delivers Mn from the liver to the brainstem nuclei. When bile acid synthesis is impaired,

the brainstem nuclei can acquire neurotoxic levels of Mn, while serum levels are simultaneously depressed.

GUT BACTERIA DYSBIOSIS AND ANXIETY

Anxiety disorder is a comorbidity of autism,[110] AD,[288] and PD,[235] and, in this section, we argue that disruption of Lactobacillus due to impaired Mn bioavailability is a likely cause. Anxiety disorder is also correlated with glyphosate usage on corn and soy, as illustrated in [Figure 5].

Glyphosate has been shown to severely deplete Mn uptake by plants, both by the roots and by the shoots.[127] Experiments on plants demonstrated that Mn applied as fertilizer antagonizes glyphosate's effectiveness in weed control,[29] and this implied that Mn chelation was an important part of glyphosate's toxicity to plants. Electron paramagnetic resonance (EPR) spectroscopy analyses conducted by these authors demonstrated that glyphosate's binding to Mn increased with pH as pH rose from 2.8 to 7.5. The pH of plant symplast is typically 7.5, a level at which glyphosate would be an effective chelator of Mn.

Certain species of gut bacteria, such as members of the Lactobacillus family, utilize Mn in novel ways for protection from oxidation damage, and, as a consequence, their requirements for Mn are much higher than those of other species.[13,14] Thus, Mn chelation by glyphosate would lead to reduced numbers of these essential bacteria in the gut. This leads directly to neurological symptoms such as anxiety, due to the influence of the gut–brain axis.[70,75,106] In the small intestine, the pH increases gradually from pH 6 to pH 7.4 in the terminal ileum.[101] At pH 7.4, Mn bioavailability can be expected to be reduced by 50% due to glyphosate chelation.[173]

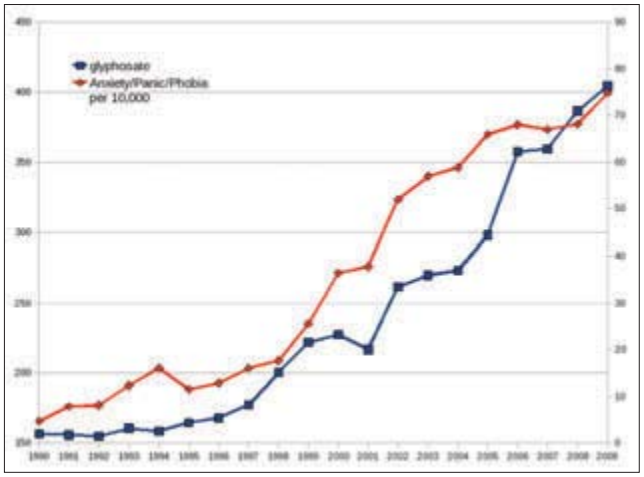


Figure 5: Plots of glyphosate usage on corn and soy crops (blue), provided by the US Department of Agriculture, and rates per 10,000 of phobia, anxiety disorder, and panic disorder (red) in the US, provided by the Centers for Disease Control. (Figure courtesy of Dr. Nancy Swanson)

The liver regulates the amount of Mn in the general vascular circulation, by incorporating any excess into bile acids, which gives the gut bacteria repeated chances to take it up. However, production of bile acids depends upon CYP enzymes, which are disrupted by glyphosate.[248,249] Hence, glyphosate can be expected to lead to severe impairment of Mn bioavailability to the gut bacteria, while at the same time allowing too much Mn to accumulate in the liver.

Lactobacillus tends to reside in the foregut.[286] The pH of the foregut is higher than that of the cecum,[100] so Mn chelation by glyphosate is a bigger issue there, since glyphosate's chelation effects increase with increasing pH. Mn-SOD is an important enzyme in mitochondria for protection from oxidative damage. Most Lactobacillus species lack Mn-SOD, but they have devised a way to protect themselves from oxidation damage due to the superoxide radical by using active transport of Mn in the +2 oxidation state. Many Lactobacilli normally have high intracellular concentrations of Mn.[14] For example, *Lactobacillus plantarum* accumulates over 30 mM of intracellular Mn (II).[13]

A recent study demonstrated that Roundup® in concentrations lower than those recommended in agriculture inhibited microbial growth of three microorganisms that are widely used as starters in fermentation of milk products,[66] including a species of Lactobacillus. Research into genetically engineering an Mn-SOD-encoding gene derived from *Streptococcus thermophilus* into various Lactobacillus species has shown that they can produce Mn-SOD from these heterologous genes and use it to improve their resistance to oxidative stress.[48]

Bruno-Bárcena *et al.*[48] proposed that such genetically engineered Lactobacilli might provide benefit as probiotics to people suffering from colitis or peptic ulcers. Colitis is associated with increased inflammation in the gut, which may be due to impaired function of Mn-SOD. An experiment on a mouse model of colitis demonstrated that *Lactobacilus gasseri* treatment alleviated inflammation in the colon of Il-10 deficient mice.[55] Genetically modified forms of *L. gasseri* as described above, which overproduce Mn-SOD, showed enhanced therapeutic effects.

Several members of the Lactobacillus family are capable of producing the inhibitory neurotransmitter γ-aminobutyric acid (GABA) via the enzyme glutamate decarboxylase, and this may be a reason for their ability to improve symptoms of anxiety. Experiments with Lactobacillus probiotics in mice demonstrated neurochemical and behavioral effects related to changes in GABAergic expression in regions of the brain that control mood.[165] These effects were absent in vagotomized mice, pointing to the vagus nerve as the bacterial communication pathway between gut and brain.

Lactobacillus have been successfully cultivated to produce fermented food containing high levels of GABA, proposed to be a health benefit in probiotics.[40]

Patients suffering from chronic fatigue syndrome (CFS) often have imbalances in microbial flora,[177] along with anxiety as a frequent comorbidity.[104] A pilot placebo-controlled study involved daily administration of *Lactobacillus casei* probiotics over a 2-month period to CFS patients.[232] The outcome was a significant rise in both Lactobacillus and Bifidobacteria in the gut, along with a significant decrease in anxiety symptoms ($P = 0.01$). This study reinforces the gut–brain connection, specifically implicating Lactobacillus in the etiology of anxiety disorder.

MN-SUPEROXIDE DISMUTASE AND MITOCHONDRIAL DYSFUNCTION

Mitochondrial dysfunction, particularly for the neutrophils, which perform important immune system functions, is implicated in CFS,[196] and this could be due to impaired Mn supply to Mn-SOD. Mn-SOD is dramatically upregulated in association with the inflammatory markers tumor necrosis factor α (TNF-α), lipopolysaccharide (LPS), and interleukin-1,[301] presumably to protect mitochondria from oxidative damage. SOD plays an important role in antioxidant defenses, by converting superoxide into H_2O_2, which can then be further detoxified by other enzymes such as catalase.[182] There are three major classes of SOD, which are distinguished by the metal catalyst, which can be copper (Cu), Zn, Mn, iron, or nickel. Eukaryotes rely on a distinct form in the mitochondria, which depends on Mn, whereas Cu/Zn SOD is present in the cytoplasm and extracellularly. Many bacterial species including *Escherichia coli* use Fe-SOD as well as Mn-SOD. The adjuvants in Roundup® may play an important role in enabling glyphosate to penetrate the mitochondrial membrane,[215] where it can interfere with the activities of Mn-SOD via chelation of Mn.

Recent experiments on goldfish involved exposing them for 96 h to Roundup® at concentrations ranging from 2.5 to 20 mg/L.[174] Several metabolites were measured from the liver, kidney, and brain. Remarkably, Roundup® inhibited SOD activity in all three organs examined, by 51–68% in the brain, 58–67% in the liver, and 33–53% in the kidney, and this was the most striking effect that was observed. Unfortunately, they did not specify whether the cytoplasmic Cu/Zn SOD or the mitochondrial Mn-SOD was most affected. Regardless, a plausible explanation of this effect is the chelation of Mn, Cu, and Zn, which are essential cofactors for the two SODs.

A recent study on the fish species *Anguilla anguilla* exposed to environmentally realistic levels of glyphosate over a short time period revealed DNA strand breaks in both liver and gills, along with a suppression of SOD activity in the liver.[116] A plausible explanation is that the breakdown product of glyphosate, glyoxylate, which is a potent glycating agent, would cause DNA damage by attacking Cu, Zn-SOD. Experiments have shown that released Cu in combination with H_2O_2 produced by glycated Cu, Zn-SOD triggers a Fenton reaction, resulting in nuclear DNA cleavage.[138]

Aconitase, an enzyme that converts citrate to isocitrate, is a crucial participant in Complex I of the citric acid cycle in the mitochondria. Many neurodegenerative diseases have been linked to decreased aconitase activity due to oxidative stress, including Friedreich ataxia,[245,249] Huntington's disease,[282] progressive supranuclear palsy,[213] autism,[239] and epilepsy.[300] The presence of a single unligated iron atom in the iron–sulfur cluster of aconitase makes it uniquely sensitive to oxidative inactivation by superoxide.[105] Aconitase inactivation has a cascade effect, because the released iron results in ferrous iron toxicity, further promoting cell death.[52] The pervasive herbicide Paraquat has been implicated in oxidative inactivation of aconitase, and this likely explains its known role in PD.[52]

A postmortem study of brains of autistic individuals showed a striking decrease in aconitase activity in the cerebellum associated with a similar decrease in glutathione redox antioxidant capacity (GSH/GSSG), with the plot producing a near 100% separation between cases and controls.[239] Inadequate clearance of superoxide due to Mn-SOD inactivation can easily account for this observation. Aconitase is a crucial participant in the citric acid cycle in the mitochondria, so this effect has catastrophic consequences on the renewal of adenosine triphosphate (ATP) as an energy source for the neurons.

In another study, cells from children with autism exhibited higher oxidative stress than control cells, including a 1.6-fold increase in reactive oxygen species (ROS) production, 1.5-fold increase in mitochondrial DNA copy number per cell, and more deletions.[198] Furthermore, oxidative phosphorylation capacity of granulocytes from children with autism was 3-fold lower than in controls. These are all indicators of oxidative stress, which could be due to SOD inhibition by glyphosate, mediated by Mn deficiency.

OXALATE

Monsanto's Roundup herbicide, WeatherMax® with Transorb II Technology, is now a preferred formulation and uses a potassium salt of glyphosate (48.8%). The Transorb Technology, which utilizes a dual surfactant system and adjuvants, was first introduced in 1996 as Roundup ULTRA. In 1998, the formula was altered and released as WeatherMAX, then further improved and released in 2005 as the current product.

The inert ingredients have undergone several changes over the years to include formula variations, which included the use of dual surfactants of siloxane copolymers and Polyoxyethylated tallow amine (POEA). As noted by Monsanto, "Promotion of stomatal infiltration of glyphosate by an organosilicone surfactant reduces the critical rainfall period," hence the rain-fastness of Roundup WeatherMax® with Transorb® 2 Technology. In 1995, US Patent #5,464,806 was issued which reflected another product formulation improvement and move from the use of Silwet-77 siloxane surfactant due to phase separation problems, to the use of an acetylenic diol.[142] The new formula provides protection from herbicide loss due to rain within 30 min of application. Additional adjuvants, well known in the paper-making industry, were used to quickly break down cell walls and collapse the plant. These chemicals originally included sodium sulfite with a later change to oxalic acid (oxalate) as patented in 2006.[315] In this patent, it was noted that "it has been discovered that the addition of oxalic acid or salts thereof to glyphosate compositions increases the cell membrane permeability of plant cells or suppresses oxidative burst to increase cellular uptake of glyphosate."

This modification may be related to the recent increase in health issues concerning excess serum oxalate in the United States and elsewhere, linked to both autism and kidney stones. A study comparing children with autism with controls found a 3-fold increase in serum oxalate levels in the children with autism,[152] and it was suggested that this might be due to excess absorption through the gut barrier, and that oxalate crystals in the brain could potentially disrupt brain function. Calcium oxalate crystals are responsible for up to 80% of kidney stones, and there has been an increased incidence of kidney stones recently in the US.[250] In a study on prisoners in Illinois who complained of gastrointestinal distress following a change to a high-soy diet, it was proposed that the unusually high levels of oxalate in the processed soy protein might be responsible for the observed symptoms.[175]

Both the high oxalate content of the soy and the high serum oxalate in humans could be due to impaired oxalate metabolism. Oxalate metabolism by oxalate oxidase in plants and by oxalate decarboxylases in fungi and a few bacteria, such as *Bacillus subtilis*, are both dependent on Mn as a cofactor.[280] Bifidobacteria, which are highly sensitive to glyphosate,[263] possess an oxalate-metabolizing enzyme that depends on magnesium rather than Mn as a cofactor.[102] However, glyphosate decreases the content of both magnesium and Mn in plants.[50] Furthermore, gamma-glutamyl carboxylase, a liver enzyme that metabolizes oxalate, is catalyzed by vitamin K, which depends on the shikimate pathway.[51] It has been shown that patients with calcium oxalate urolithiasis have significantly reduced activity of this enzyme in the liver.[65]

The sulfate ion transporter, Sat-1, plays an important role not only in sulfate transport but also in oxalate transport,[273] as evidenced by the fact that mice with a disrupted Sat-1 gene develop urolithiasis.[252] Glyoxylate is not only a substrate of Sat-1 but it is also a key regulator.[252] The upregulation of SAT-1 by glyoxylate in hepatic cells likely serves to flush oxalate and glyoxylate from the liver, to avoid hepatotoxicity. However, this can lead to nephrotoxicity due to glyoxylate glycation damage and the formation of kidney stones. Due to competition between oxalate and sulfate for transport via Sat-1, glyoxylate, and oxalate, likely, also disrupt sulfate homeostasis in the liver. Sulfate is critical for bile acid formation and for detoxification of xenobiotics such as acetaminophen.

The conversion of glyoxylate to oxalate by the enzyme lactate dehydrogenase is inhibited by oxalate.[87] Hence gloxylate, derived from glyphosate breakdown, would accumulate in the presence of excess oxalate.

Aside from the obvious damaging effects of oxalate crystals on tissues, the oxalate, whose metabolism is impaired due to Mn deficiency, will also interfere with the metabolism of glyphosate, likely greatly increasing both its effectiveness as an herbicide and its toxicity to mammals. Under oxalate stress conditions, both superoxide and the hydroxyl radical are produced in excess amounts.[258] Obviously, the ineffectiveness of Mn-SOD due to Mn deficiency would further enhance the damage due to excess oxalate. SOD activity has been shown to be reduced in association with the urolithic kidney, and methionine supplementation can alleviate this problem.[257] As mentioned previously, methionine is depleted by glyphosate.

AMMONIA, GLUTAMATE, AND NEUROTOXICITY

In this section, we will show that both glutamate and ammonia are implicated as neurotoxins in connection with autism and other neurological diseases, and we will offer the simple explanation that Mn deficiency leads to impaired activity of glutamine synthase and arginase, both of which utilize Mn as a cofactor. Mn deficiency can also explain the increased risk to epilepsy found in autism, due to the fact that Mn decreases T2 relaxation time. Mn-deprived rats are more susceptible to convulsions.[129]

Blaylock and Strunecká[32] have proposed that immune-glutamatergic dysfunction may be the central mechanism of autism spectrum disorders. Ghanizadeh[109] reported that glutamate and homocysteine are elevated in the serum in association with autism, and that glutamine and tryptophan are depleted. Tryptophan, which depends

upon the shikimate pathway in plants and microbes, is the precursor to serotonin and melatonin. An increase in glutamate and a corresponding decrease in glutamine can be entirely explained by an inactive glutamine synthase enzyme. Another extensive study on children with autism compared with controls found low serum tryptophan, high serum glutamate and homocysteine, and significantly reduced free sulfate, as well as high levels of oxidative stress markers,[1] all of which are consistent with these assertions. High serum homocysteine is one associated consequence of folate deficiency:[76] Folate is produced by Lactobacillus and Bifidobacteria from products of the shikimate pathway.[240]

The neurotransmitter glutamate has been implicated as an excitatory neurotoxin in the brain not only in autism but also in association with multiple neurological diseases, including AD, PD, amyotrophic lateral sclerosis (ALS), and multiple sclerosis.[93] Ordinarily, following glutamate release into the synaptic cleft, microglia in the brain take up excess glutamate and convert it to glutamine, using the enzyme glutamine synthase.[204] Glutamine is then released into the extracellular space, taken up by neurons, and converted in the cytoplasm to glutamate to be held within internal vesicles in anticipation of future activity. Conversion to glutamine for the transport stage from microglia to neurons renders the molecule inactive as a neurotransmitter, and therefore as a neurotoxin, when it is out of service.

TNF-α, secreted by activated microglia in the brain, is a major cytokine leading to neurotoxicity in association with multiple neurological diseases. A major component of the damaging effect of TNF-α is the autocrine induction of the release of glutamate from microglia.[285] Experiments exposing immature rats to Roundup®, whether via exposure to the dam during pregnancy and lactation or via acute exposure to the pup for 30 min, demonstrated lipid peroxidation and NMDA receptor activation in the hippocampus, indicative of oxidative stress and glutamate excitotoxicity.[59] Acute exposure increased the release of glutamate into the synaptic cleft, and depleted GSH.

Glutamine synthase depends upon Mn as a cofactor, so depleted Mn supplies would lead to a build-up of glutamate that cannot be returned to the neurons using normal channels. Multiple sclerosis is associated with both depleted Mn in the cerebrospinal fluid[185] and depleted GSH synthase in the white matter lesions.[309] Cerebellar brain samples taken postmortem from 10 individuals with autism demonstrated an anomalous increase in mRNA expression of excitatory amino acid transporter 1 and glutamate receptor AMPA 1, both involved in the glutamate system.[226] Glutamate receptor binding proteins were also abnormally expressed, and AMPA-type glutamate receptor density was low. These effects could be explained as a response to the excess bioavailability of glutamate due to an inability to convert it to the inactive form, glutamine.

Further confirmation of glutamate dysbiosis in autism comes from a study on levels of 25 amino acids in the platelet-poor plasma of high-functioning autistic children compared with normal controls, which revealed that only glutamate and glutamine were abnormally expressed in the children with autism, with a highly significant ($P < 0.002$) excess of glutamate and a highly significant ($P < 0.004$) decrease in glutamine.[264] They linked these findings to glutamatergic abnormalities reported by others.

It is intriguing to us that Mn deficiency leads to a pair of complementary pathologies – excess glutamate along with aconitase deficiency, which together allow for the cells to generate ATP by metabolizing glutamate instead of glucose. Glutamate enters the citric acid cycle beyond Complex I, thus bypassing the step that is impaired by aconitase deficiency. This is a rather elegant regulatory system that provides energy even in the face of Complex I impairment.

The prevalence of epilepsy in the US is similar to that of diabetes, making it a common disorder affecting 2 million Americans.[47] Epilepsy is associated with increased T2 relaxation time in nagnetic resonance imaging (MRI) signaling analysis of the hippocampus, both in the ipsilateral sclerotic hippocampus as well as the contralateral hippocampus and anterior temporal lobe.[42] Following intracerebral injection of Mn, a large amount of Mn accumulates in the hippocampal fissure, which results in a reduction in T2 relaxation time.[78] Epilepsy may therefore be a consequence of insufficient Mn in the hippocampus, which could easily account for the associated increase in T2. Autism is associated with a high risk of epilepsy,[293,162] and the hippocampus has been the focus of many studies on the neurological pathology of autism.[84,95]

Ammonia is a well-established neurotoxin, which accumulates when the urea cycle is unable to keep up with ammonia released from protein breakdown.[290] Ammonia can induce tremor, ataxia, seizures, coma, and death.[49] Ammonia is a highly diffusible gas that readily crosses the blood–brain barrier, and its detoxification depends upon the conversion of glutamate to glutamine, which is catalyzed by glutamine synthase, the enzyme in microglia that relies upon Mn as a cofactor.[71] Thus, impaired function of glutamine synthase leads to the accumulation of both glutamate and ammonia in the brain, both of which are established neurotoxins.

Excess ammonia due to impaired ability to detoxify excess nitrogen via the urea cycle can lead to impaired memory, shortened attention span, sleep–wake cycle disruption, brain edema, intracranial hypertension, seizures, ataxia,

and coma.[37] Arginase, the final enzyme of the urea cycle, is ubiquitous in living systems, and depends upon Mn as a cofactor. Therefore, Mn deficiency due to glyphosate's chelation of the metal would be expected to lead directly to impaired arginase function. The excess accumulation of ammonia due to inactive glutamine synthase combined with the decreased ability to metabolize ammonia to urea constitute a double-hit leading to ammonia toxicity in the brain.

A case study of a 4-year-old girl showed that a genetic defect in arginase could present as pervasive developmental delay not otherwise specified (PDD-NOS) with many similarities to autism.[112] Its manifestations include brain edema and signs of epilepsy, as would be expected with ammonia toxicity in the brain. A Mn-deficient diet administered to rats induced a reduction in hepatic arginase activity ($P < 0.01$), along with a significant rise in plasma ammonia ($P < 0.01$).[43]

Seneff *et al.*[260] put forward the idea that excess ammonia due to disrupted gut bacteria could lead to a chronic low-grade encephalopathy that could explain much of the pathology associated with autism. Furthermore, glyphosate is known to induce excess ammonia production in plants, due to overactivity of the enzyme phenylalanine ammonia lyase (PAL).[86,117,125] This enzyme may also be overactive in gut bacteria exposed to glyphosate, further compounding the problem.

CHOLESTASIS AND BILIRUBINEMIA

Mn stimulates cholesterol synthesis in the liver, presumably because bile acids, derived from cholesterol, are needed to bind to Mn and carry it back to the gut for recycling.[5] However, CYP enzyme inhibition will impair the liver's ability to oxidize cholesterol, since the formation of oxysterols in the liver for incorporation into bile acids depends on several CYP enzymes (members of the CYP3, CYP7, CYP8, CYP27, CYP39, and CYP46 families).[200,217] As a result, it can be anticipated that, when Mn supplies are plentiful, both the Mn and the cholesterol will accumulate to toxic levels in the liver, unless another method can be found for their redistribution. In the case of cholesterol, this may lead to a necessary increase in the synthesis and release of LDL particles. People with a defective CYP7A1 gene have significantly elevated total and LDL cholesterol levels, as well as substantial accumulation of cholesterol in the liver and a markedly decreased rate of bile acid excretion.[225] Neonatal cholestasis and hypercholesterolemia (elevated LDL) were produced in mice with a defective CYP7A1 gene fed a normal chow diet.[96] The increased serum levels of LDL associated with heart disease risk may therefore be a consequence of the production of cholesterol that cannot be exported through the biliary ducts.

Studies with rats have shown that *Lactobacillus plantarum* probiotic supplements lower serum LDL levels.[166] Lactobacillus levels were reduced in chickens exposed to antimicrobial drugs, which resulted in reduced bile salt deconjugation in the gut.[114] Impaired bile salt deconjugation by gut bacteria results in significant weight gain along with elevated plasma cholesterol and liver triglycerides in mice.[136] Thus, glyphosate acting as an antibiotic that preferentially kills Lactobacillus can be expected to lead to elevated serum cholesterol and triglycerides through a similar mechanism.

Cholestasis is a blockage of bile acid flow, which often occurs as a side effect of various pharmaceutical drugs.[211] Glyphosate administration to rats over a period of 13 weeks induced increases in serum bile acids, indicative of cholestasis.[64] Severe cholestasis can induce bilirubinemia,[28] and glyphosate also independently induces bilirubinemia in catfish.[208] At least two other studies have shown bile stagnation in fish exposed to glyphosate.[80,135]

Inflammatory bowel disease (colitis and Crohn's disease) has been increasing in frequency in the US over the past 20 years, in step with glyphosate usage on corn and soy crops, as shown in [Figure 6]. According to analyses by Cappello *et al.* in a hospital-based survey,[53] cholestasis is a common feature of inflammatory bowel disease. They observed a cholestatic pattern in 60% of patients studied, mainly related to older age, longer duration of disease, and hypertension. Gallstones were commonly found, often in association with abnormal bile salt absorption, especially in Crohn's disease patients.

Ironically, while Mn-SOD depends upon Mn as a cofactor, *excess* exposure to Mn can inhibit SOD expression. Experiments exposing rats to excess Mn revealed several

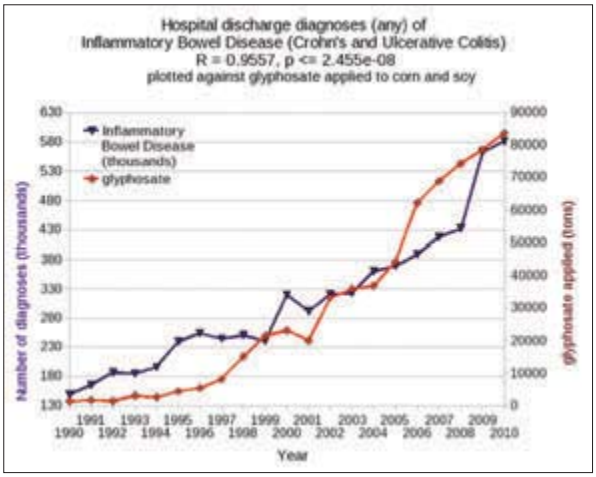


Figure 6: Plots of glyphosate usage on corn and soy crops (blue), and hospital discharge diagnoses of inflammatory bowel disease (Crohn's andulcerative colitis) in the US, over time. (Figure courtesy of Dr. Nancy Swanson)

pathological effects on the liver, including inhibition of SOD and GSH peroxidase, as well as decreased levels of GSH and reduced levels of sodium-potassium ATPase activity.[126] It is striking that glyphosate has also been shown to have these very same effects in animal experiments,[60,174,89] and it is conceivable that these effects may be in part mediated by excess Mn accumulation in the liver.

Bilirubinemia in neonates is a risk factor for autism, particularly when it is unbound and unconjugated.[10] Glucose 6-phosphate dehydrogenase (G6PD) deficiency can induce bilirubin toxicity in neonates,[176] due to the fact that G6PD enables conjugation of bilirubin.[141] Glyphosate was shown to induce a 2.67-fold reduction in G6PD expression in *E. coli*.[171] Studies on goldfish demonstrated that glyphosate significantly decreased G6PD activity in liver, kidney, and brain.[174]

G6PD is the main enzyme responsible for regeneration of NADPH, an essential requirement for GSH reductase activity.[174] Cholestasis is associated with a reduction in the supply of reduced GSH.[317] Furthermore, glyphosate has been shown to reduce the activity of GSH reductase in the liver.[174] Preeclampsia, affecting 4% of pregnancies, is associated with G6PD deficiency in red blood cells along with a reduction in reduced GSH.[3] The ratio of oxidized to reduced GSH is consistently high in association with autism, in plasma, immune cells, and the brain.[239]

A mouse model of cholestasis can be induced by exposing mice to Mn, followed shortly by exposure to bilirubin.[82] Mn induces cholesterol synthesis in the liver, and bilirubin disrupts 7-α oxidation of cholesterol, a crucial step in bile acid formation.[5] Cholesterol 7-α hydroxylase is the CYP enzyme CYP7A1 and is likely disrupted independently by glyphosate. Therefore, excess Mn in the liver works synergistically with glyphosate to induce cholestasis.

The incorporation of cholesterol products into exported bile acids is crucial for regulating cholesterol homeostasis.[205] Bile acid transport depends on ATP,[203] so mitochondrial disruption due to oxidation damage consequential to excess Mn could contribute to disturbed bile acid export, leading again to cholestasis. *In vitro* experiments exposing rat H9c2 cells to glyphosate plus the surfactant TN-20, which is a polyoxyethylene tallow amine commonly used in glyphosate herbicides, demonstrated that the surfactant in conjunction with glyphosate causes collapse of the mitochondrial membrane potential, leading to necrosis and apoptosis.[147] Even in the absence of a catastrophic death cascade, a drop in mitochondrial membrane potential would obviously negatively impact ATP production. The effect could be due in part to polyoxyethylenealkylamine (POEA), which includes polyethoxylated tallow amine surfactants that enable glyphosate to penetrate the mitochondrial membrane.[188] But, in addition, excess Mn, which would accumulate due to the lack of bile flow, itself induces a collapse in mitochondrial membrane potential.[137]

Intracellular accumulation of bile acids, associated with cholestasis, is known to be toxic to hepatocytes. The accumulation of bile acids in the cholestatic liver induces oxidative stress and apoptosis, resulting in damage to the liver parenchyma, and, ultimately, extrahepatic tissues as well.[180] The lipophilic bile acids are much more damaging than the hydrophilic ones,[15] and they can induce proton leakage and the permeability transition pore (PTP), resulting in programmed cellular death.[237] Their toxic effect is directly due to their role as surfactants.[180] Therefore, they enhance the effects of the surfactants in Roundup®, acting in a cascade reaction.

On the other hand, the hydrophilic bile acid, ursodeoxycholic acid (UDCA), is protective, and its protective effects have been proposed to be due to its ability to induce the expression of CYP3A4, a bile-metabolizing enzyme, in hepatocytes.[11] Hydroxylation, which depends on CYP enzymes, converts lipophilic compounds into hydrophilic products. So one can expect that, in the context of the CYP-enzyme suppressing effects of glyphosate, [248,249] lipophilic bile acids would accumulate in hepatocytes, and their export would be impeded by the loss of ATP subsequent to mitochondrial damage, in a positive feedback loop.

Another enzyme class that was discovered to be downregulated in the liver by glyphosate in the goldfish study is GST, an important class of enzymes. The main function of the GST enzymes is the detoxification of electrophilic xenobiotics by GSH conjugation.[275] Beyond their important role in the detoxification of xenobiotics, gene variants where the enzyme is inactive are associated with increased risk to basal cell carcinoma, and a gain-of-function mutation leads to decreased risk to asthma.[275] Arsenic, whose toxicity is synergistically enhanced by glyphosate[132] is a risk factor for basal cell carcinoma.[62] Asthma is reaching epidemic proportions today[91] and is associated with autism.[24,25] GST is increasingly recognized as an important enzyme in gut bacteria, which allows them to assist in the detoxification of xenobiotics.[7]

One final factor in cholestasis is vitamin K deficiency, which is associated with cholestatic liver disease.[278] Chorismate, the intermediary in the shikimate pathway whose synthesis is blocked by glyphosate, is a precursor not only for the three aromatic amino acids but also for tetrahydrofolate and phylloquinone (vitamin K1) in plants.[294] Similarly, menaquinone (vitamin K2) is synthesized by bacteria from chorismate.[27] Thus, disruption of the shikimate pathway contributes to vitamin K deficiency, which can lead to cholestasis.

SALMONELLA INFECTION

Multiple drug-resistant strains of Salmonella are becoming an increasing problem both in the industrialized[191] and in the developing world.[246] This may be due in part to the fact that Salmonella is more resistant to glyphosate than other species. *Salmonella typhimurium* is among a small set of microbes that possess phosphonatase genes that allow it to use the commonly occurring natural phosphonate, 2-aminoethyl-phosphonate (AEPn), as a sole source of phosphorus,[134] which likely contributes to its growth advantage in the presence of the phosphonate, glyphosate. Furthermore, Lactobacillus, which are especially vulnerable to glyphosate, produce antimicrobial compounds that can defend against Salmonella.[81] Salmonella infections often originate from contamination of plant-based foods exposed to manure of chickens and pigs. A study on poultry showed that *Salmonella entritidis*, *Salmonella galliarum*, and *Salmonella typhimurium* were all highly resistant to glyphosate compared with other more benign species.[263]

Our research into the pathology of Salmonella has uncovered a complex interplay of many factors that may be responsible for the epidemic, which includes an important role for Mn, as well as a role for industrial processing of lecithin from soy, and bile acid disruption by glyphosate. Commercial lecithin is a mixture of phospholipids and various metabolites, often derived from soy. In food processing, phospholipase A (PLA) is applied enzymatically to hydrolyze phospholipids in lecithin into lysophospholipids and fatty acids, in order to improve its emulsification, surfactant, and lubricant properties.[97] This may be a factor in inducing both increased virulence and an inflammatory response to Salmonella in the gut, contributing to inflammatory bowel disease.

Salmonella depend on cyclic adenosine monophosphate (cAMP) for flagella formation, and therefore for motility.[318] Salmonella possess a lysophospholipid sensing mechanism that triggers the synthesis of flagellin, mediated by cAMP, and this activates toll-like receptor 5 (TLR5) in macrophages, inducing an inflammatory response.[279] Experiments have shown, as might be expected, that flagella, produced from flagellin, not only enhance mobility but also protect *S. typhimurium* from internal death in macrophages and enhance their ability to multiply within an infected cell.[306]

Salmonella are remarkably resilient in an inflammatory environment, and, in a novel strategy for survival in a hostile environment, they take advantage of tetrathionate produced from oxidation of thiosulfate by ROS as a terminal electron acceptor in processing ethanolamine derived from host lysophospholipids as a source of energy not available to other microbes.[8,313] They can also uniquely use a glycated L-asparagine (fructose-asparagine, F-asn) as a source of both carbon and nitrogen.[8] Concentrations of F-asn, an Amadori product, are surprisingly high in heated fruits and vegetables.[92]

It has only recently been appreciated that Mn plays an important role in the virulence of Salmonella.[144] Salmonella depend on Mn to resist the oxidative attack of macrophages via Mn-SOD.[292] Since glyphosate's chelation of Mn makes it unavailable to gut bacteria, a mystery arises as to how Salmonella might acquire adequate Mn for defense against oxidative damage. Salmonella possess a Mn uptake system based on a protein that is homologous to eukaryotic Nramp transporters. This protein, MntH, is a proton-dependent metal transporter that is highly selective for Mn^{2+} over Fe^{2+},[144] or any other cation. Intracellular Mn^{2+} can accumulate in millimolar amounts even when environmental Mn^{2+} is scarce.

A feature unique to Salmonella is that they are especially adept at binding to cholesterol in the gall bladder, particularly in association with gallstones, and they also possess adaptive responses that allow them to survive the harsh environment of the bile acid milieu, as well as the highly acidic environment of the stomach.[9] In fact, studies have shown that they can survive a lower pH environment than either Shigella flexneri or *E. coli*.[167]

Several different species of Salmonella can form biofilms on human gallstones, which is dependent upon the presence of bile.[222,74] Since bile is an excellent source of Mn, and glyphosate's chelation of Mn is dependent on a basic environment, they could accumulate Mn while resident in the bile acids present in the gallstones. Bile acids offer antibacterial defenses, but Salmonella have developed resistance genes to protect them from bile acids.[123] In association with gallstones, *S. typhi* colonize the human gallbladder and persist in an asymptomatic carrier state.[9,187] Thus, impaired bile acid flow due to glyphosate would promote both gallstones and microbial growth.

Fibrates are hypolipidemic agents that are known to suppress bile acid synthesis via suppression of peroxisome proliferator-activated receptor γ (PPAR-γ).[220] Studies on humans exposed to fibrates have shown reduced activity of cholesterol 7α-hydroxylase (CYP7A1), leading to reduced bile acid production, and concurrent increased risk of gallstones.[271,31] Thus, it is plausible that Salmonella are able as well to gain a foothold on the gallstones caused by suppression of bile-acid production by glyphosate due to CYP enzyme inactivation, and they are able to take up Mn in the immobilized bile acids and utilize it for Mn-SOD production, protecting them from oxidative attack by macrophages. The pathogen responsible for Lyme disease, *Borrelia burgdorferi*, is also uniquely dependent on Mn,[4] and the disruption of Mn homeostasis by glyphosate may therefore play a role in its emergence.

CHONDROITIN SULFATE, OSTEOMALACIA AND ARTHRITIS

Vitamin D deficiency has reached epidemic proportions in the US and increasingly around the world in recent years.[124] In a large population study in the US, Bodnar *et al*.[34] found deficient levels of vitamin D in 83% of Black women and 92% of their newborns, as well as in 47% of White women and 66% of their newborns, despite the fact that over 90% of the women were on prenatal vitamins. This deficiency is associated with an increased risk to bone fractures, likely due to impaired calcium homeostasis.[145] It is even likely that care-takers are being falsely accused ("Shaken Baby Syndrome") of abusing children in their care who suffer from bone fractures.[255] These children are highly vulnerable to bone fractures due to impaired bone development. Bone fractures in the elderly due to osteoporosis have also risen sharply recently in the industrialized world.[139] The cause of a surging incidence of hip fractures across multiple age groups remains a mystery to medical personnel.[140]

Samsel and Seneff[248,249] proposed that the current vitamin D deficiency epidemic is caused by glyphosate, due to glyphosate's interference with CYP enzymes. The metabolite that is usually measured, 25-hydroxy vitamin D, is the product of activation in the liver by a CYP enzyme that is also critical in bile acid formation. However, there is a larger problem with bone development due to impaired Mn homeostasis. Bone mineralization depends critically on Mn, due to its essential role in chondroitin sulfate synthesis.[36,158] Several enzymes in the osteoblasts needed for this crucial step in bone development require Mn as a cofactor, including the polymerase, which polymerizes uridine diphosphate N-acetyle-galactosamine (UDP-N-acetyl-galactosamine) and UDP-glucuronic acid to form the polysaccharide, and galactotransferase, which incorporates galactose from UDP-galactose into the trisaccharide that links the polysaccharide to the glycosylated protein.[157,158] Chondroitin sulfate, together with osteocalcin, forms the ground substance to which collagen adheres to maintain healthy bone, ligaments and cartilage. Sulfate uptake by GAGs in chicks fed a Mn deficient diet was only 50% of that in control chicks, and the deficient chicks suffered from growth retardation and skeletal abnormalities.[36]

Osteoarthritis is another pathology likely related to Mn deficiency, impaired chondroitin sulfate synthesis and impaired vitamin D activation. Vitamin D deficiency is associated with rheumatoid arthritis.[207] Mn is necessary for the synthesis of GAGs or mucopolysaccharides,[156,254] which provide lubrication and protection for the joints. Rheumatoid arthritis is associated with Mn accumulation in the liver,[72] which is consistent with impaired bile flow. Glucosamine sulfate has been demonstrated to be effective in treating osteoarthritis, and it may even delay disease progression.[259] A combination therapy of glucosamine, chondroitin sulfate, and Mn ascorbate was shown to be effective in treating knee osteoarthritis in a placebo-controlled study conducted on US Navy specialists.[160]

A mysterious epidemic of a new disease, called "sea star wasting syndrome," is currently sweeping across the Pacific coast of North America, affecting at least 12 different species of sea stars.[307] We highly suspect that glyphosate plays an important role in this disease, and that it does so by chelating Mn, and therefore disrupting chondroitin sulfate synthesis. A likely source is Roundup® applied to oyster beds to kill the invading sea grass, since oysters are a common food for sea stars.[118] The state of Massachusetts was forced to close oyster beds in Edgartown on Cape Cod recently, due to infection with a pathogen, *Vibrio parahaemolyticus*.[214] This species synthesizes an N-acetyl transferase (NAT) protein, which is capable of detoxifying glyphosate by acetylating the nitrogen moiety,[58] and this could explain its overgrowth as being linked to glyphosate contamination. An analysis of the GAGs isolated from brittle stars showed exceptionally high proportions of di- and trisulfated chondroitin sulfate/dermatan sulfate disaccharides,[233] whose synthesis would be severely impaired by Mn deficiency due to chelation by glyphosate.

A protective layer of mucopolysaccharides called mucus is secreted by corals, and it has been characterized as containing sulfated glycoproteins similar to chondroitin sulfate,[44] which play an important role in controlling pH and the transepithelial movement of electrolytes and water, just as is the case in vertebrate mucosa. Mucus pathology is implicated in coral disease leading to mortality, particularly in the Caribbean.[219] Thus, an interesting hypothesis that should be considered is that glyphosate chelation of Mn is a crucial factor in the worldwide coral die-off.

PARKINSON'S DISEASE

It might be anticipated that a simple Mn mineral supplement could correct for the problem of glyphosate's chelation of Mn, but this assumption is almost certainly false. We suggest that glyphosate's disruption of Mn homeostasis leads to extreme sensitivity to Mn bioavailability, making it easy to err in the direction of either too little or too much.[103] Our investigations into the body's mechanisms for transporting Mn has revealed a likely pathway from the liver to the brain that would induce Mn toxicity in the brainstem nuclei whenever Mn is plentiful but glyphosate is also present. A strong clue comes from the condition, "manganism," closely resembling PD, which develops following

chronic occupational exposure to airborne nanoparticles containing Mn.[163,172,244] In addition to evidence from direct occupational exposure, geographical studies in the US have shown a higher incidence of PD in urban areas with higher industrial release of Mn.[312]

The fact that death from PD has increased in the past two decades in step with glyphosate usage on corn and soy suggests that there may be a role for glyphosate to play in Parkinson's pathology [Figure 4]. A case of accidental acute exposure to glyphosate through skin contact showed a remarkable development of Parkinsonian symptoms beginning just 1 month following exposure.[20] T2-weighted images revealed a hyperintense signal in the globus pallidus and substantia nigra. Another case study involved a 44-year-old female who developed PD after a 3-year period of job-related exposure at a chemical plant.[304] Glyphosate has also been shown to induce Parkinsonian-like effects in the worm, *Caenorhabditis elegans*, characterized by damage to both GABAergic and dopaminergic neurons.[201] The mechanism behind this effect remains obscure. However, a Mn-containing fungicide, MANCOZEB, ethylene bisdithio-carbamate, also induced similar damage.

An experiment where rats were exposed to Mn via intranasal instillation demonstrated that the Mn could enter the brain through the olfactory bulbs by following major neuronal pathways.[291] Mn migrated via both secondary and tertiary olfactory pathways and beyond to ultimately reach most parts of the brain and the spinal cord. By contrast, cadmium was unable to pass through synaptic junctions and was therefore limited in its penetration. These authors concluded that the olfactory nerve is the likely pathway by which Mn gains access to the brain in manganism, thus circumventing the blood–brain barrier. Autoradiographic studies tracking the distribution of radiolabeled Mn injected into rat brain verified that Mn is subject to widespread axonal transport in neuronal circuits.[283]

Elder *et al*.[94] reinforced this idea, in a study involving exposure of rats to ultrafine particles (UFPs) of inhaled Mn oxide. Their conclusion sums up the situation quite well: "We conclude from our studies that the olfactory neuronal pathway represents a significant exposure route of central nervous system (CNS) tissue to inhaled solid Mn oxide UFPs. In rats, which are obligatory nose breathers, translocation of inhaled nanosized particles along neurons seems to be a more efficient pathway to the CNS than via the blood circulation across the blood–brain barrier. Given that this neuronal translocation pathway was also demonstrated in nonhuman primates, it is likely to be operative in humans as well." [94, p. 1178]

Manganism is distinct from PD mainly in that it is the locus coeruleus that is preferentially damaged rather than the substantia nigra.[216] In the rat, at least 40% of all locus coeruleus neurons project to the olfactory bulb.[266] Since glyphosate likely interferes with the normal recycling of Mn via the bile acids, the liver will need to find an alternative route to dispose of excess Mn. Following the example of nerve-fiber migration from the olfactory bulb,[291] and the study on injected Mn,[283] a likely path is the vagus nerve, which has extensive innervation in the liver, with 10 times as many afferent nerves as efferent nerves, and particularly concentrated on the outer surface of the bile ducts.[30] MRI abnormalities indicative of Mn toxicity in the globi pallidi and substantia nigra were noted in three cases of patients with liver disease.[120] PD is associated with nonmotor symptoms that often precede the movement impairment aspects.[320] These include depression and gastrointestinal disturbances.

Berthoud *et al*.[30] proposed that the onset of PD may be associated with impairment of the vagus nerve, and subsequent functional inhibition of the dopamine system. Clinical trials have revealed pathological alteration of the vagus nerve in PD patients.[218] The dorsal motor nucleus of the vagus is an early site of pathology.[38] Liver failure can lead to excessive build-up of Mn in the brainstem, particularly the globus pallidus in the basal ganglia, which regulates voluntary movement. This is due primarily to decreased billiary excretion from the liver,[99,131] as bile acids recycle Mn back to the gut, where it is taken up by gut bacteria or disposed of. Liver cirrhosis is associated with excess Mn in the brain and associated Parkinsonian symptoms.[99] Mn neurotoxicity in the basal ganglia causing Parkinsonian symptoms has also been identified in association with chronic liver failure.[150] Despite the fact that Mn is an essential cofactor for glutamine synthase, excess Mn actually downregulates expression and activity of the enzyme, causing neuropathology. Its toxicity is linked to disruption of the cycling between astrocytes and neurons of glutamine, glutamate, and GABA.[267] Mn inhibits ATP-dependent calcium signaling in astrocytes, which likely contributes to the toxic effects of excess Mn on neurons.[277] Decreased bile flow associated with the birth defect, biliary atresia, leads to Mn accumulation in the liver[23] and in the globus pallidus.[130] As discussed previously, bile acid synthesis and export are disrupted by glyphosate and by surfactants, which is also a common effect of many toxic chemicals whose detoxification would be impaired by glyphosate's disruption of CYP enzymes.[248]

A study using MRI technology to detect Mn distribution in the brain in marmosets and rats following Mn injections revealed an accumulation in the basal ganglia as well as other parts of the brain that were situated near the ventricles, suggesting redistribution via the cerebrospinal fluid.[33] There was also considerable liver damage, especially in the marmosets, including hemosiderosis, congestion, and hepatic necrosis. Marmosets were far more susceptible than rats to Mn toxicity.

Both the substantia nigra and the locus coeruleus are characterized by high concentrations of neuromelanin. While it is unclear what role neuromelanin plays, one hypothesis is that it carries a protective action through its unique ability to accumulate and retain various amines and metallic cations, especially Mn.[168] The neuromelanin produced by the substantia nigra is closely related to dopamine, and therefore derived from tyrosine. The locus coeruleus' melanin is related to noradrenaline, and therefore derived from tryptophan. Both tyrosine and tryptophan are products of the shikimate pathway, and are therefore likely to be deficient in the context of glyphosate exposure to plants and microbes. This would result in a reduced ability to temporarily house excess Mn in the brainstem nuclei until it can be disposed of.

Glyphosate itself likely also contributes directly to PD. PD is caused by degeneration of dopaminergic neurons in the substantia nigra, attributed to mitochondrial dysfunction, oxidative stress, and protein aggregation.[79,251] PC12 cells are a popular model cell line for investigating neurological disease, and they produce dopamine in vesicles, as is appropriate for cells from the substantia nigra. A study on PC12 cells exposed to glyphosate demonstrated that glyphosate induced cell death via autophagy pathways as well as apoptotic pathways.[115]

A study on serum Mn levels in infants and their association with neurodevelopment revealed a U-shaped curve, with both too little and too much Mn leading to impaired development.[67] This study was conducted on children born in Mexico City between 1997 and 2000. We hypothesize that extreme sensitivity to Mn levels can be expected in the context of glyphosate, because it would prevent the liver from disposing of Mn via the bile acids, and therefore cause a flooding of the brainstem nuclei with excess Mn delivered via the vagus nerve. At the same time, Mn uptake into the blood stream from the gut is suppressed, both because of glyphosate's chelation of Mn at higher pH and the impaired recycling to the gut from the liver. This can lead to a paradoxical situation in which the brainstem nuclei are overwhelmed with Mn while the precortex and cortex are deprived because of the low bioavailability from the blood stream. One might postulate that seizures play a role in enhancing Mn redistribution from the brainstem to the cortex by mobilizing Mn transport along axons, and this effect might therefore explain the benefit of electroconvulsive therapy (ECT) to depression.[16]

Dopamine suppresses thyroid stimulating hormone, and therefore dopamine insufficiency can lead to overactive thyroid and potential burnout of the thyroid gland.[270] This problem is compounded by the fact that thyroid hormone itself is derived from tyrosine, one of the three aromatic amino acids that are negatively impacted by glyphosate through disruption of the shikimate pathway. The thyroid gland also depends critically on selenoproteins as antioxidants.[249] Glyphosate's depletion of both selenium and methionine will lead to reduced bioavailability of selenoproteins. It is conceivable that all of these factors working together can explain the strong correlation of glyphosate application to corn and soy with thyroid cancer [Figure 7], as well as the association between maternal thyroid disease and autism.[238]

PRION DISEASES AND MANGANESE

Prions are a special class of proteins that acquire alternative conformations that can become self-propagating. There is a class of neurological diseases called transmissible spongiform encephalopathies that are believed to be caused by misfolding of prion proteins, predominantly accumulating in the gray matter in the brain.[223] These include Creutzfeldt–Jakob disease (CJD) in humans, scrapie in sheep, chronic wasting disease of deer, and bovine spongiform encephalopathy or Madcow disease.

Prion proteins have been shown to bind Cu *in vivo*,[46] and this is probably an important factor in their normal functioning. Cu protects against conversion of prions to the pathogenic form, and studies have shown that gene variants with extra octarepeat inserts exhibit decreased Cu binding in association with markedly increased disease risk.[272] A theory first proposed by Purdey[228,229] is that the prion protein misfolds following binding to Mn instead of Cu. The pathology is then explained by a high Mn to Cu ratio in the diet. Brown *et al*.[45] investigated metal binding properties of prion proteins, and demonstrated that prions only bind to Cu and Mn. Furthermore, Mn binding induces a resistance to protein degradation by

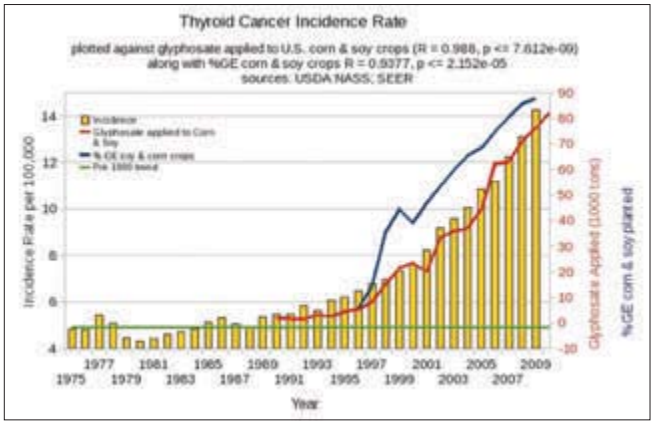


Figure 7: Plots of glyphosate usage on corn and soy crops (blue), percent of corn and soy that is genetically engineered to be "Roundup Ready" (red), and incidence of thyroid cancer (yellow bars) in the US. (Figure courtesy of Dr. Nancy Swanson)

protease, a characteristic feature of prion diseases. Aging of the Mn-bound version of prion proteins leads to loss of function. A later experiment demonstrated that Mn promotes prion protein aggregation.[161] Thus, Mn is causal in the formation of fibrils characteristic of the scrapie isoform of the protein in prion diseases.

The possible link between excess Mn and prion diseases was also supported in a later study by Masánová *et al*.,[181] who compared different regions of the Slovak republic and found a correlation between an elevated Mn/Cu ratio in core food items such as potato and bread, as well as in the soil, and a higher incidence of CJD.

Mn-SOD-/- mice die between days 3 and 13 following birth. They exhibit a marked dilated cardiomyopathy, neurodegeneration and fatty liver disease.[159,164] Mn 5, 10, 15, 20-tetrakis (4-benzoic acid) porphyrin (MnTBAP) rescues mice from this pathology and dramatically improves their lifespan. It operates at 10% of the efficiency of Mn-SOD to oxidize superoxide to H_2O_2. However, these supplemented mice develop a spongiform encephalopathy very similar to CJD.[186] This is most likely caused by excess delivery of Mn to the brain by the MnTBAP.

Purdey[227] has noted that madcow disease epidemiology aligned with the regulatory requirement to apply the organo phthalimido-phosphorus insecticide, phosmet, on the backs of cattle, for the control of warble fly during the 1980s. He maintained that phosmet chelated Cu in the CNS, but also caused oxidation of Mn to Mn^{3+}, leading to its toxicity. Like glyphosate, phosmet also disrupts CYP enzyme function,[262] which would lead to a similar disabling of bile acids.

New experimental data support the idea that the class of prion diseases should be expanded to include AD, PD, and related tauopathies.[224] Indeed, it has been proposed that Cu deficiency may be a factor in AD.[149] Glyphosate chelates Cu down to much lower pH values than those at which it chelates Mn [173,296] [Figure 8], and it has also been shown to oxidize Mn to the +3 oxidation state.[21] Thus, one can surmise that glyphosate might behave similarly to both phosmet and MnTBAP to be causal in prion diseases. In the pH 4 environment adjacent to the sulfates in the glycocalyx, Mn, but not Cu, would be released by glyphosate. Abundant bioavailability of Mn^{3+} alongside Cu deficiency further aggravated by glyphosate could set up a situation whereby excess Mn supplied to the neurons in the cortex, bound to glyphosate, over-competes with Cu in binding to prion protein. One can predict that glyphosate's tenacious binding to Cu will render Cu systemically unavailable, which argues for a role for glyphosate in prion diseases through Cu binding.[45,228,229]

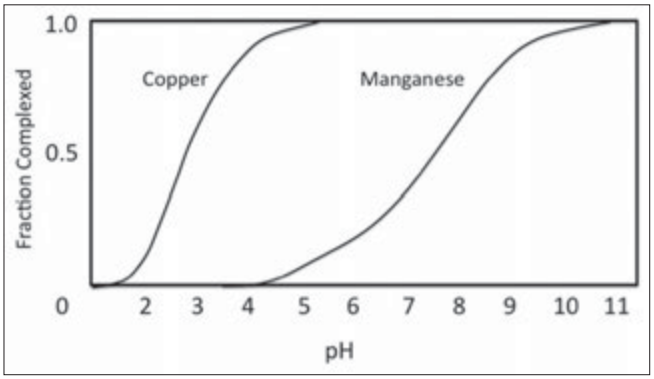


Figure 8: Fractions of metal complexed with glyphosate as a function of pH, for copper and manganese. Figure adapted from Lundager Madsen *et al*[170]

MN AND INFERTILITY

Multiple papers on rodent studies have indicated disruption of the male reproductive system by glyphosate. Acute treatment of 60-day-old male Sprague-Dawley rats to Roundup® caused a marked increase in aromatase mRNA in testicular tissue,[56] likely reflecting an increase in production due to suppression of the activity of aromatase (CYP 19), accompanied by abnormal sperm morphology. Aromatase participates in both hormone synthesis and metabolism. It is an important enzyme in testes that converts testosterone to estrogen, thus regulating the balance of sex hormones.

In vitro studies on Sertoli cells from mouse testis demonstrated that glyphosate opens L-type voltage-dependent calcium channels, leading to calcium-overload cell death.[83] However, glyphosate's disruption of the supply of Mn to sperm may be a more important factor leading to infertility, due to immobilization of the sperm. Sperm are critically dependent on Mn for their motility. Mammalian sperm contain a distinctive form of Mn-dependent adenylate cyclase, which first appears during development in seminiferous tubules simultaneously with the appearance of spermatid cells.[39] It is expressed at the highest levels following sexual maturity. Adenylate cyclase catalyzes the synthesis of cAMP. Bacteria such as *E. coli* and *Salmonella typhimurium* depend on cAMP for flagella formation, and therefore for motility.[318] cAMP-dependent phosphorylation has been linked to activation of motility in sperm flagella from sea squirts[209] and from dogs.[287] Human sperm also depend on cAMP for increased flagellar motility.[210,303] A study on zebrafish demonstrated that glyphosate exposure at concentrations of 5 and 10 mg/L over a 24-h time period reduced sperm motility.[170] Mn stimulated the progressive motility of human sperm in a time- and dose-dependent manner, and this was linked to adenyl cyclase activity.[178] The decrease in male fertility levels today in the industrialized world[111] may therefore be explained by Mn deficiency.

EVIDENCE OF EXPOSURE

Glyphosate-containing herbicides are applied to crops several times each season both for killing weeds and for desiccation just prior to the harvest in non-Roundup®-Ready crops, such as wheat and sugar cane. It accumulates in the leaves, grains, and fruit, and thus cannot be removed by washing and, furthermore, is not broken down by cooking.[41] Nowell *et al*.[206] identified pesticides as a leading cause for declines and deformities among amphibians and pollinators in the United States. A total of 100% of all water surfaces and 96% of all examined fish contained detectable levels of pesticides. In a recent US-based study by Battaglin *et al*.,[22] glyphosate and AMPA were detected frequently in soils and sediment, ditches and drains, rainwater, rivers, and streams.

A recently published study by Shehata *et al*.[264] showed that glyphosate residues can be found in the organs and muscles of chickens that consume glyphosate in their feed, including the liver, spleen, lung, intestine, heart, and kidney. All animals fed a diet contaminated by glyphosate would be subject to such bioaccumulation due to the molecule's ability to act as an attaching ligand. Feed supplementation with humic acid was able to significantly reduce the glyphosate burden in tissues.

A recent paper by Nevison[202] investigated temporal trends in autism since 1988 to assess to what degree the recent observed rate increases are due to increased diagnosis versus increased incidence. She concluded that increased incidence accounts for 75–80% of the tracked increase. She also investigated trend lines for a variety of environmental toxicants potentially implicated in autism. She wrote in the abstract: "Among the suspected toxins surveyed, polybrominated diphenyl ethers, aluminum adjuvants, and the herbicide glyphosate have increasing trends that correlate positively to the rise in autism." As we have stated earlier, glyphosate and aluminum are synergistically toxic.

There has been very little testing of glyphosate levels in either humans or other mammals. Glyphosate is passed in both the urine and the feces. Recent research from Europe has shown that glyphosate is consistently present in significant amounts in the urine of cows consuming Roundup®-Ready feed, as well as in the organs and meat of cattle.[153] Furthermore, they also detected glyphosate in the urine of humans, and the generally healthy population had significantly lower levels than the sick population. Those consuming a predominantly organic diet also had a significantly lower glyphosate burden. Another study found detectable levels of glyphosate in lungs, liver, kidney, *brain*, gut wall, and heart of malformed euthanized 1-day-old Danish piglets, and the authors proposed that glyphosate could be the cause of the deformities.[155]

CONCLUSION

Glyphosate is the most widely used herbicide on the planet, in part because of its perceived low toxicity to humans. In this paper, we propose that glyphosate's chelation of Mn, working together with other known effects of glyphosate such as CYP enzyme suppression and depletion of derivatives of the shikimate pathway in microorganisms, may explain the recent increase in incidence of multiple neurological diseases and other pathologies. We have shown that glyphosate's disruption of Mn homeostasis can lead to extreme sensitivity to variations in Mn bioavailability: While Mn deficiency in the blood leads to impairment of several Mn dependent enzymes, in contrast, excess Mn readily accumulates in the liver and in the brainstem due to the liver's impaired ability to export it in the bile acids. This pathology can lead to liver damage and PD. Mn depletion in the gut due to chelation by glyphosate selectively affects Lactobacillus, leading to increased anxiety via the gut–brain access. Both low Lactobacillus levels in the gut and anxiety syndrome are known features of autism, and Lactobacillus probiotic treatments have been shown to alleviate anxiety. Increased incidence of Salmonella poisoning can also be attributed to glyphosate, through its impairment of bile acid synthesis. Low Mn bioavailability from the blood supply to the brain leads to impaired function of glutamine synthase and a build-up of glutamate and ammonia in the brain, both of which are neurotoxic. Excess brain glutamate and ammonia are associated with many neurological diseases. At the same time, impaired function of Mn-SOD in the mitochondria results in mitochondrial damage, also a hallmark of many neurological diseases. Mn deficiency can account for poor sperm motility and therefore low fertilization rates, as well as poor bone development leading to osteoporosis and osteomalacia. Sea star wasting syndrome and the collapse of coral reefs may in fact be an ecological consequence of the environmental pervasiveness of the herbicide. Many diseases and conditions are currently on the rise in step with glyphosate usage in agriculture, particularly on GM crops of corn and soy. These include autism, AD, PD, anxiety disorder, osteoporosis, inflammatory bowel disease, renal lithiasis, osteomalacia, cholestasis, thyroid dysfunction, and infertility. All of these conditions can be substantially explained by the dysregulation of Mn utilization in the body due to glyphosate.

ACKNOWLEDGMENTS

This work was funded in part by Quanta Computers, Taipei, Taiwan, under the auspices of the Qmulus Project. The authors thank Dr. Nancy Swanson for providing the plots showing correlations over time of multiple diseases and conditions with glyphosate usage on corn and soy in the US.

REFERENCES

1. Abdel-Megeed A, Sadik MW, Al-Shahrani HO, Ali HM. Phyto-microbial degradation of glyphosate in Riyadh area. Int J Microbiol Res 2013;5:458.
2. Adams JB, Audhya T, McDonough-Means S, Rubin RA, Quig D, Geis E, *et al*. Nutritional and metabolic status of children with autism vs. neurotypical children, and the association with autism severity. Nutr Metab 2011;8:34.
3. Afzal-Ahmed I, Mann GE, Shennan AH, Poston L, Naftalin RJ. Preeclampsia inactivates glucose-6-phosphate dehydrogenase and impairs the redox status of erythrocytes and fetal endothelial cells. Free Radic Biol Med 2007;42:1781-90.
4. Aguirre JD, Clark HM, McIlvin M, Vazquez C, Palmere SL, Grab DJ, *et al*. A manganese-rich environment supports superoxide dismutase activity in a Lyme disease pathogen, Borrelia burgdorferi. J Biol Chem 2013;288:8468-78.
5. Akoume MY, Perwaiz S, Yousef IM, Plaa GL. Synergistic role of 3-hydroxy-3-methylglutaryl coenzyme A reductase and cholesterol 7α-hydroxylase in the pathogenesis of manganese-bilirubininduced cholestasis in rats. Toxicol Sci 2003;73:378-85.
6. Albert MJ, Mathan VI, Baker SJ. Vitamin B12 synthesis by human small intestinal bacteria. Nature 1980;283:781-2.
7. Allocati N, Federici L, Masulli M, Di Ilio C. Glutathione transferases in bacteria. FEBS J 2009;276:58-75.
8. Ali MM, Newsom DL, González JF, Sabag-Daigle A, Stahl C, Steidley B, *et al*. Fructose-asparagine is a primary nutrient during growth of Salmonella in the inflamed intestine. PLOS Pathogens 2014;10:e1004209.
9. Álvarez-Ordóñez A, Begley M, Prieto M, Messens W, López M, Bernardo A, *et al*. Salmonella spp. survival strategies within the host gastrointestinal tract. Microbiology 2011;157:3268-81.
10. Amin SB, Smith T, Wang H. Is neonatal jaundice associated with autism spectrum disorders: A systematic review. J Autism Dev Disord 2011;41:1455-63.
11. Ananthanarayanan M, Balasubramanian N, Makishima M, Mangelsdorf DJ, Suchy FJ. Human bile salt export pump promoter is transactivated by the farnesoid X receptor/bile acid receptor. J Biol Chem 2001;276:28857-65.
12. Antoniou M, Habib ME, Howard CV, Jennings RC, Leifert C, Nodari RO, *et al*. Teratogenic effects of glyphosate-based herbicides: Divergence of regulatory decisions from scientific evidence. J Environ Anal Toxicol 2012;S4:006.
13. Archibald FS, Duong MN. Manganese acquisition by Lactobacillus plantarum. J Bacteriol 1984;158:1-8.
14. Archibald FS, Fridovich I. Manganese, superoxide dismutase, and oxygen tolerance in some lactic acid bacteria. J Bacteriol 1981;146:928-36.
15. Attili AF, Angelico M, Cantafora A, Alvaro D, Capocaccia L. Bile acid- induced liver toxicity: Relation to the hydrophobic-hydrophilic balance of bile acids. Med Hypotheses 1986;19:57-69.
16. Avery D, Winokur G. The efficacy of electroconvulsive therapy and antidepressants in depression. Biol Psychiatry 1977;12:507-23.
17. Ballatori N, Miles E, Clarkson TW. Homeostatic control of manganese excretion in the neonatal rat. Am J Physiol 1987;252:R842-7.
18. Baly DL, Curry DL, Keen CL, Hurley LS. Effect of manganese deficiency on insulin secretion and carbohydrate homeostasis in rats. J Nutr 1984;114:1438-46.
19. Baly DL, Schneiderman JS, Garcia-Welsh AL. Effect of manganese deficiency on insulin binding, glucose transport and metabolism in rat adipocytes. J Nutr 1990;120:1075-9.
20. Barbosa ER, Leiros da Costa MD, Bacheschi LA, Scaff M, Leite CC. Parkinsonism after glycine-derivate exposure. Mov Disord 2001;16:565-8.
21. Barrett KA, McBride MB. Oxidative degradation of glyphosate and aminomethyl-phosphonate by manganese oxide. Environ Sci Technol 2005;39:9223-8.
22. Battaglin WA, Meyer MT, Kuivila KM, Dietze JE. Glyphosate and its degradation product AMPA occur frequently and widely in U.S. soils, surface water, groundwater, and precipitation. J Am Water Resour Assoc 2014;50:275-90.
23. Bayliss EA, Hambidge KM, Sokol RJ, Stewart B, Lilly JR. Hepatic concentrations of zinc, copper and manganese in infants with extrahepatic biliary atresia. J Trace Elem Med Biol 1995;9:40-3.
24. Becker KG, Schultz ST. Similarities in features of autism and asthma and a possible link to acetaminophen use. Med Hypotheses 2010;74:7-11.
25. Becker KG. Autism, asthma, inflammation, and the hygiene hypothesis. Medl Hypotheses 2007;69:731-40.
26. Benbrook CM. Impacts of genetically engineered crops on pesticide use in the U.S. – the first sixteen years. Environ Sci Europe 2012;24:24.
27. Bentley R, Meganathan R. Biosynthesis of Vitamin K (Menaquinone) in Bacteria. Microbiol Rev 1982;46:241-80.
28. Berk PD, Javitt NB. Hyperbilirubinemia and cholestasis. Am J Med 1978;64:311-26.
29. Bernards ML, Thelen KD, Penner D, Muthukumaran RB, McCracken JL. Glyphosate interaction with manganese in tank mixtures and its effect on glyphosate absorption and translocation. Weed Sci 2005;53:787-94.
30. Berthoud HR, Kressel M, Neuhuber WL. An anterograde tracing study of the vagal innervation of rat liver, portal vein and biliary system. Anat Embryol 1992;186:431-42.
31. Bertolotti M, Concari M, Loria P, Abate N, Pinetti A, Guicciardi ME, *et al*. Effects of different phenotypes of hyperlipoproteinemia and of treatment with fibric acid derivative on the rates of cholesterol 7alpha-hydroxylation in humans. Arterioscler Thromb Vasc Biol 1995;15:1064-9.
32. Blaylock RL, Strunecka A. Immune-glutamatergic dysfunction as a central mechanism of the autism spectrum disorders. Curr Med Chem 2009;16:157-70.
33. Bock NA, Paiva FF, Nascimento GC, Newman JD, Silva AC. Cerebrospinal fluid to brain transport of manganese in a non-human primate revealed by MRI. Brain Res 2008;1198:160-70.
34. Bodnar LM, Klebanoff MA, Gernand AD, Platt RW, Parks WT, Catov JM, *et al*. Maternal Vitamin D status and spontaneous preterm birth by placental histology in the US Collaborative Perinatal Project. Am J Epidemiol 2014;179:168-76.
35. Bøhn T, Cuhra M, Traavik T, Sanden M, Fagan J, Primicerio R. Compositional differences in soybeans on the market: Glyphosate accumulates in Roundup Ready GM soybeans. Food Chem 2014;153:207-15.
36. Bolze MS, Reeves RD, Lindbeck FE, Kemp SF, Elders MJ. Influence of manganese on growth, somatomedin and glycosaminoglycan metabolism. J Nutr 1985;115:352-8.
37. Bosoi CR, Rose CF. Identifying the direct effects of ammonia on the brain. Metab Brain Dis 2009;24:95-102.
38. Braak H, Del Tredici K, Rb U, De Vos RA, Ernst NH, Steur J, *et al*. Staging of brain pathology related to sporadic Parkinsons disease. Neurobiol Aging 2003;24:197-211.
39. Braun T, Dods RF. Development of a Mn^{2+}-sensitive, "soluble" adenylate cyclase in rat testis. Proc Nat Acad Sci USA 1975;72:1097-101.
40. Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, *et al*. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc Natl Acad Sci U S A 2011;108:16050-5.
41. Brewster DW, Warren J, Hopkins WE. Metabolism of glyphosate in SpragueDawley rats: Tissue distribution, identification, and quantitation of glyphosate-derived materials following a single oral dose. Fundam Appl Toxicol 1991;17:43-51.
42. Briellmann RS, Syngeniotis A, Fleming S, Kalnins RM, Abbott DF, Jackson GD. Increased anterior temporal lobe T2 times in cases of hippocampal sclerosis: A multi-echo T2 relaxometry study at 3 T. AJNR Am J Neuroradiol 2004;25:389-94.
43. Brock AA, Chapman SA, Ulman EA, Wu G. Dietary manganese deficiency decreases rat hepatic arginase activity. J Nutr 1994;124:340-4.
44. Brown BE, Bythell JC. Perspectives on mucus secretion in reef corals. Mar Ecol Prog Ser 2005;296:291-309.
45. Brown DR, Hafiz F, Glasssmith LL, Wong BS, Jones IM, Clive C, *et al*. Consequences of manganese replacement of copper for prion protein function and proteinase resistance. EMBO J 2000;19:1180-6.
46. Brown DR, Qin K, Herms JW, Madlung A, Manson J. Strome R, *et al*. The cellular prion protein binds copper *in vivo*. Nature 1997;390:684-7.
47. Browne T, Holmes G. Epilepsy. N Engl J Med 2001;344:1145-51.
48. Bruno-Bárcena JM, Andrus JM, Libby SL, Klaenhammer TR, Hassan HM. Expression of a heterologous manganese superoxide dismutase gene in intestinal lactobacilli provides protection against hydrogen peroxide toxicity. Appl Environ Microbiol 2004;70:4702-10.
49. Cagnon L, Braissant O. Hyperammonemia-induced toxicity for the developing central nervous system. Brain Res Rev 2007;56:183-97.

50. Cakmak I, Yazici A, Tutus Y, Ozturk L. Glyphosate reduced seed and leaf concentrations of calcium, manganese, magnesium, and iron in non-glyphosate resistant soybean. Eur J Agron 2009;31:114-9.
51. Campbell IM, Robins DJ, Kelsey M, Bentley R. Biosynthesis of Bacterial Menaquinones (Vitamins K)$_2$). Biochemistry 1971;10:3069-78.
52. Cantu D, Fulton RE, Drechsel DA, Patel M. Mitochondrial aconitase knockdown attenuates paraquat-induced dopaminergic cell death via decreased cellular metabolism and release of iron and H_2O_2. J Neurochem 2011;118:79-92.
53. Cappello M, Randazzo C, Bravatà I, Licata A, Peralta S, Craxì A, *et al*. Liver function test abnormalities in patients with inflammatory bowel diseases: A hospital-based survey. Clin Med Insights Gastroenterol 2014;7:25-31.
54. Carl GF, Keen CL, Gallagher BB, Clegg MS, Littleton WH, Flannery DB, *et al*. Association of low blood manganese concentrations with epilepsy. Neurology 1986;36:1584-7.
55. Carroll IM, Andrus JM, Bruno-Brcena JM, Klaenhammer TR, Hassan HM, Threadgill DS. Anti-inflammatory properties of Lactobacillus gasseri expressing manganese superoxide dismutase using the interleukin 10-deficient mouse model of colitis. Physiol Gastrointest Liver Physiol 2007;293:G729-38.
56. Cassault-Meyer E, Gress S, Séralini GE, Galeraud-Denis I. An acute exposure to glyphosate-based herbicide alters aromatase levels in testis and sperm nuclear quality. Environ Toxicol Pharmacol 2014;38:131-40.
57. Castellani R, Smith MA, Richey GL, Perry G. Glycoxidation and oxidative stress in Parkinson disease and diffuse Lewy body disease. Brain Res 1996;737:195-200.
58. Castle LA, Siehl DL, Gorton R, Patten PA, Chen YH, Bertain S, *et al*. Discovery and directed evolution of a glyphosate tolerance gene. Science 2004;304:1151-4.
59. Cattani D, de Liz Oliveira Cavalli VL, Heinz Rieg CE, Domingues JT, Dal-Cim T, Tasca CI, *et al*. Mechanisms underlying the neurotoxicity induced by glyphosate-based herbicide in immature rat hippocampus: Involvement of glutamate excitotoxicity. Toxicology 2014;320C: 34-45.
60. Cavuşoğlu K, Yapar K, Oruç E, Yalçın E. Protective effect of Ginkgo biloba L. leaf extract against glyphosate toxicity in Swiss albino mice. J Med Food 2011;14:1263-72.
61. Cebeci O, Budak H. Global expression patterns of three Festuca species exposed to different doses of glyphosate using the affymetrix genechip wheat genome array. Comp Funct Genomics 2009;2009:505701.
62. Centeno JA, Mullick FG, Martinez L, Page NP, Gibb H, Longfellow D, *et al*. Pathology related to chronic arsenic exposure. Environ Health Perspect 2002;110(Suppl 5):S883-6.
63. Cerdeira AL, Duke SO. The current status and environmental impacts of glyphosate-resistant crops: A review. J Environ Qual 2006;35:1633-58.
64. Chan P, Mahler J. NTP technical report on the toxicity studies of glyphosate (CAS No. 1071-83-6) administered in dosed feed to F344/N rats and B6C3F1 mice. Toxic Rep Ser 1992;16:1-D3.
65. Chen J, Liu J, Zhang Y, Ye Z, Wang S. Decreased renal vitamin K-dependent gamma-glutamyl carboxylase activity in calcium oxalate calculi patients. Chin Med J (Engl) 2003;116:569-72.
66. Clair E, Linn L, Travert C, Amiel C, Sralini GE, Panoff JM. Effects of Roundup() and glyphosate on three food microorganisms: Geotrichum candidum, Lactococcus lactis subsp. cremoris and Lactobacillus delbrueckii subsp. bulgaricus. Curr Microbiol 2012;64:486-91.
67. Claus Henn B, Ettinger AS, Schwartz J, Téllez-Rojo MM, Lamadrid-Figueroa H, Hernández-Avila M, *et al*. Early postnatal blood manganese levels and childrens' neurodevelopment. Epidemiology 2010;21:433-9.
68. Coburn JW, Mischel MG, Goodman WG, Salusky IB. Calcium citrate markedly enhances aluminum absorption from aluminum hydroxide. Am J Kidney Dis 1991;17:708-11.
69. Cole DJ. Mode of action of glyphosate - A literature analysis. In: Grossbard E, Atkinson D, editors. The herbicide glyphosate. London: Butterworths; 1985. p. 48-74.
70. Collins SM, Surette M, Bercik P. The interplay between the intestinal microbiota and the brain. Nat Rev Microbiol 2012;10:735-42.
71. Cooper AJ. 13N as a tracer for studying glutamate metabolism. Neurochem Int 2011;59:456-64.
72. Cotzias GC, Papavasiliou PS, Hughes ER, Tang L, Borg DC. Slow turnover of manganese in active rheumatoid arthritis accelerated by prednisone. J Clin Invest 1968;47:992-1001.
73. Cox LM, Yamanishi S, Sohn J, Alekseyenko AV, Leung JM, Cho I, *et al*. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. Cell 2014;158:705-21.
74. Crawford RW, Reeve, KE, Gunn JS. Flagellated but not hyperfimbriated Salmonella enterica serovar Typhimurium attaches to and forms biofilms on cholesterol-coated surfaces. J Bacteriol 2010;192:2981-90.
75. Cryan JF, Dinan TG. Mind-altering microorganisms: The impact of the gut microbiota on brain and behaviour. Nat Rev Neurosci 2012;13:701-12.
76. Curtis D, Sparrow R, Brennan L, Van der Weyden MB. Elevated serum homocysteine as a predictor for vitamin B12 or folate deficiency. Eur J Haematol 1994;52:227-32.
77. Daniell WE, Stockbridge HL, Labbe RF, Woods JS, Anderson KE, Bissell DM, *et al*. Environmental chemical exposures and disturbances of heme synthesis. Environ Health Perspect 1997;105 Suppl 1 :37-53.
78. Daoust A, Barbier EL, Bohic S. Manganese enhanced MRI in rat hippocampus: A correlative study with synchrotron X-ray microprobe. Neuroimage 2013;64:10-8.
79. Dauer W, Przedborski S. Parkinsons disease: Mechanisms and models. Neuron 2003;39:889-909.
80. de Carmo Langiano V, Martinez CB. Toxicity and effects of a glyphosate-based herbicide on the Neotropical fish Prochilodus lineatus. Comp Biochem Physiol Part C 2008;147:222-31.
81. De Keersmaecker SC, Verhoeven TL, Desair J, Marchal K, Vanderleyden J, Nagy I. Strong antimicrobial activity of Lactobacillus rhamnosus GG against Salmonella typhimurium is due to accumulation of lactic acid. FEMS Microbiol Lett 2006;259:89-96.
82. de Lamirande E, Tuchweber B, Plaa GL. Morphological aspects of manganese-bilirubin induced cholestasis. Liver 1982;2:22-7.
83. de Liz Oliveira Cavalli LV, Cattani D, Heinz Rieg CE, Pierozan P, Zanatta L, Benedetti Parisotto E, *et al*. Roundup disrupts male reproductive functions by triggering calcium-mediated cell death in rat testis and Sertoli cells. Free Radic Biol Med 2013;65:335-46.
84. DeLong GR. Autism, amnesia, hippocampus, and learning. Neurosci Biobehav Rev 1992;16:63-70.
85. De Roos AJ, Zahm SH, Cantor KP, Weisenburger DD, Holmes FF, Burmeister L, *et al*. Integrative assessment of multiple pesticides as risk factors for non-Hodgkins lymphoma among men. Occup Environ Med 2003;60:E11.
86. Devasahayam G, Burke DJ, Sturgill TW. Golgi manganese transport is required for rapamycin signaling in saccharomyces cerevisiae. Genetics 2007;177:231-8.
87. Duncan RJ, Tipton KF. The oxidation and reduction of glyoxylate by lactic dehydrogenase. Eur J Biochem 1969;11:58-61.
88. Dupont CL, Tanaka Y. Blood manganese levels in children with convulsive disorder. Biochem Med 1985;33:246-55.
89. Dutra BK, Fernandes FA, Failace DM, Oliveira GT. Effect of Roundup (glyphosate formulation) in the energy metabolism and reproductive traits of Hyalella castroi (Crustacea, Amphipoda, Dogielinotidae). Ecotoxicology 2011;20:255-63.
90. Dutta U, Cohenford MA, Guha M, Dain JA. Non-enzymatic interactions of glyoxylate with lysine, arginine, and glucosamine: A study of advanced non-enzymatic glycation like compounds. Bioorg Chem 2007;35:11-24.
91. Eder W, Ege MJ, von Mutius E. The Asthma Epidemic. N Engl J Med 2006;355:2226-35.
92. Eichner K, Reutter M, Wittmann R. Detection of Amadori compounds in heated foods. Thermally Generated Flavors (ACS Symposium Series 543). Parliament TH, Morello MJ, McGorrin RJ, editors. Chapter 5. Washington D.C.: American Chemical Society; 1994.
93. Eisen A, Calne D. Amyotrophic lateral sclerosis, Parkinson's disease and Alzheimer's disease: Phylogenetic disorders of the human neocortex sharing many characteristics. Can J Neurol Sci 1992;19 (1 Suppl):117-23.
94. Elder A, Gelein R, Silva V, Feikert T, Opanashuk L, Carter J, *et al*. Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. Environ Health Perspect 2006;114:1172-8.
95. Endo T, Shioiri T, Kitamura H, Kimura T, Endo S, Masuzawa N, *et al*. Altered chemical metabolites in the amygdala-hippocampus region contribute to autistic symptoms of autism spectrum disorders. Biol Psychiatry 2007;62:1030-7.
96. Erickson SK, Lear SR, Deane S, Dubrac S, Huling SL, Nguyen L, *et al*. Hypercholesterolemia and changes in lipid and bile acid metabolism in male and female cyp7A1-deficient mice. J Lipid Res 2003;44:1001-9.

97. Estiasih T, Kgs. Ahmadi, Ginting E, Priyanto AD. Modification of soy crude lecithin by partial enzymatic hydrolysis using phosholipase A1. Int Food Res J 2013;20:843-9.
98. Ettinger B, Black DM, Mitlak BH, Knickerbocker RK, Nickelsen T, Genant HK, *et al*. Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: Results from a 3-year randomized clinical trial. JAMA 1999;282:637-45.
99. Fabiani G, Rogacheski E, Wiederkehr JC, Khouri J, Cianfarano A. Liver transplantion in a patient with rapid onset Parkinsonism-dementia complex induced by manganism secondary to liver failure. Arq Neuropsiquiatr 2007;65:685-8.
100. Falchuk KH, Hardy C, Ulpino L, Vallee BL. RNA metabolism, manganese, and RNA polymerases of zinc-sufficient and zinc-deficient Euglena gracilis. Proc Natl Acad Sci 1978;75:4175-9.
101. Fallingborg J. Intraluminal pH of the human gastrointestinal tract. Dan Med Bull 1999;46:183-96.
102. Federici F, Vitali B, Gotti R, Pasca MR, Gobbi S, Peck AB, *et al*. Characterization and Heterologous Expression of the Oxalyl Coenzyme A Decarboxylase Gene from Bifidobacterium lactis. Appl Environ Microbiol 2004;70:5066-73.
103. Finley JW, Davis CD. Manganese deficiency and toxicity: Are high or low dietary amounts of manganese cause for concern? BioFactors 1999;10:15-24.
104. Fischler B, Cluydts R, De Gucht Y, Kaufman L, De Meirleir K. Generalized anxiety disorder in chronic fatigue syndrome. Acta Psychiatr Scand 1997;95:405-13.
105. Flint DH, Tuminello JF, Emptage MH. The inactivation of Fe-S cluster containing hydro-lyases by superoxide. J Biol Chem 1993;268:22369-76.
106. Foster JA, Neufeld KA. Gut-brain axis: How the microbiome influences anxiety and depression. Trends Neurosci 2013;36:305-12.
107. Fridman O, Goldberg A, Ronin I, Shoresh N, Balaban NQ. Optimization of lag time underlies antibiotic tolerance in evolved bacterial populations. Nature 2014;513:418-21.
108. Gasnier C, Dumont C, Benachour N, Clair E, Chagnon MC, Séralini GE. Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. Toxicology 2009;262:184-91.
109. Ghanizadeh A. Increased glutamate and homocysteine and decreased glutamine levels in autism: A review and strategies for future studies of amino acids in autism. Dis Markers 2013;35:281-6.
110. Gillott A. Anxiety in high-functioning children with autism. Autism 2001;5:277-86.
111. Giwercman A, Bonde JP. Declining male fertility and environmental factors. Endocrinol Metab Clin North Am 1998;27:807-30.
112. Görker I, Tüzün Ü. Autistic-like findings associated with a urea cycle disorder in a 4-year-old girl. Rev Psychiatr Neurosci 2005;30:133-5.
113. Gregus Z, Gyurasics A, Csanaky I. Biliary and urinary excretion of inorganic arsenic: Monomethylarsonous acid as a major biliary metabolite in rats. Toxicol Sci 2000;56:18-25.
114. Guban J, Korver DR, Allison GE, Tannock GW. Relationship of dietary antimicrobial drug administration with broiler performance, decreased population levels of Lactobacillus salivarius, and reduced bile salt deconjugation in the ileum of broiler chickens. Poult Sci 2006;85:2186-94.
115. Gui YX, Fan XN, Wang HM, Wang G, Chen SD. Glyphosate induced cell death through apoptotic and autophagic mechanisms. Neurotoxicol Teratol 2012;34:344-9.
116. Guilhermea S, Gaivãob I, Santosa MA, Pachecoa M. DNA damage in fish (Anguilla anguilla) exposed to a glyphosate-based herbicide – Elucidation of organ-specificity and the role of oxidative stress. Mutat Res 2012;743:1-9.
117. Guillet G, Poupart J, Basurco J, De Luca V. Expression of tryptophan decarboxylase and tyrosine decarboxylase genes in tobacco results in altered biochemical and physiological phenotypes. Plant Physiol 2000;122:933-43.
118. Hancock DA. The feeding behaviour of starfish on Essex oyster beds. J Mar Biol Assoc U.K. 1955;34:313-31.
119. Harris SR, Feil EJ, Holden MT, Quail MA, Nickerson EK, Chantratita N, *et al*. Evolution of MRSA during hospital transmission and intercontinental spread. Science 2010;327:469-74.
120. Hauser RA, Zesiewicz TA, Rosemurgy AS, Martinez C, Olanow CW. Manganese intoxication and chronic liver failure. Ann Neurol 1994;36:871-5.
121. Hawkey PM, Jones AM. The changing epidemiology of resistance. J Antimicrob Chemother 2009;64(Suppl 1):i3i10.
122. Hawkins M. Updated Review of Glyphosate (103601). Incident Reports. Memorandum, EPA Toxicology and Epidemiology Branch. February 26, 2009. Available from: http://www.epa.gov/pesticides/chemical/foia/cleared-reviews/reviews/103601/103601-2009-02-26a.pdf [Last accessed on 2014 Aug 28].
123. Hofmann AF, Eckmann L. How bile acids confer gut mucosal protection against bacteria. Proc Natl Acad Sci U S A 2006;103:4333-4.
124. Hollick MF, Chen TC. Vitamin D deficiency a worldwide problem with health consequences. Am J Clin Nutr 2008;87:10805-68.
125. Howles PA, Sewalt VJ, Paiva NL, Elkind Y, Bate NJ, Lamb C, *et al*. Overexpression of L-phenylalanine ammonia-lyase in transgenic tobacco plants reveals control points for flux into phenylpropanoid biosynthesis. Plant Physiol 1996;112:1617-24.
126. Huang P, Li G, Chen C, Wang H, Han Y, Zhang S, *et al*. Differential toxicity of Mn2+and Mn3+to rat liver tissues: Oxidative damage, membrane fluidity and histopathological changes. Exp Toxicol Pathol 2012;64:197-203.
127. Huber D. What about glyphosate-induced manganese deficiency? Fluid J 2007;20-22.
128. Hultberg M. Cysteine turnover in human cell lines is influenced by glyphosate. Environ Toxicol Pharmacol 2007;24:19-22.
129. Hurley ES, Woolley DE, Rosenthal F, Timiras PS. Influence of manganese on susceptibility of rats to convulsions. Am J Physiol 1963;204:493-6.
130. Ikeda S, Yamaguchi Y, Sera Y, Ohshiro H, Uchino S, Yamashita Y, *et al*. Manganese deposition in the globus pallidus in patients with biliary atresia. Transplantation 2000;69:2339-43.
131. Inoue E, Hori S, Narumi Y, Fujita M, Kuriyama K, Kadota T, *et al*. Portal-systemic encephalopathy: Presence of basal ganglia lesions with high signal intensity on MR images. Radiology 1991;179:551-5.
132. Ito Y, Ishige K, Aizawa M, Fukuda H. Characterization of quinolone antibacterial-induced convulsions and increases in nuclear AP-1 DNA- and CRE-binding activities in mouse brain. Neuropharmacology 1999;38:717-23.
133. Jayasumana C, Gunatilake S, Senanayake P. Glyphosate, hard water and nephrotoxic metals: Are they the culprits behind the epidemic of chronic kidney disease of unknown etiology ina Sri Lanka? Int J Environ Res Public Health 2014;11:2125-47.
134. Jiang W, Metcalf WW, Lee KS, Wanner BL. Molecular cloning, mapping, and regulation of Pho regulon genes for phosphonate breakdown by the phosphonatase pathway of Salmonella typhimurium LT2. J Bacteriol 1995;177:6411-21.
135. Jiraungkoorskul W, Upatham ES, Kruatrachue M, Sahaphong S, Vichasri-Grams S, Pokethitiyook P. Histopathological effects of Roundup, a glyphosate herbicide, on Nile tilapia (Oreochromis niloticus). Sci Asia 2002;28:121-7.
136. Joyce SA, MacSharry J, Casey PG, Kinsell M, Murphy EF, Shanahan F, *et al*. Regulation of host weight gain and lipid metabolism by bacterial bile acid modification in the gut. Proc Natl Assoc Sci U S A 2014;111:7421-6.
137. Kakulavarapu V, Rao R, Norenberg MD. Manganese induces the mitochondrial permeability transition in cultured astrocytes. J Biol Chem 2004;279:32333-8.
138. Kaneta H, Fujii J, Suzuki K, Kasai H, Kawamore R, Kamadaa T. DNA cleavage induced by glycation of Cu, Zn-superoxide dismutase. Biochem J 1994;304:219-25.
139. Kannus P, Palvanen M, Niemi S, Parkkari J, Järvinen M, Ilkka Vuori I. Osteoporotic fractures of the proximal humerus in elderly Finnish persons: Sharp increase in 1970-1998 and alarming projections for the new millennium. Acta Orthop Scand 2000;71:465-70.
140. Kannus P, Parkkari J, Sievnen H, Heinonen A, Vuori I, Järvinen M. Epidemiology of hip fractures. Bone 1996;18 Suppl 1:557-63.
141. Kaplan M, Rubaltelli FF, Hammerman C, Vilei MT, Leiter C, Abramov A, *et al*. Conjugated bilirubin in neonates with glucose-6-phosphate dehydrogenase deficiency. J Pediatr 1996;128:695-7.
142. Kassebaum JW, Dayawon MM, Sandbrink JJ. glyphosate-containing herbicidal compositions having enhanced effectiveness. Published Nov. 7, 1995. US Patent #5,464,806. Available from: http://www.google.com/patents/US5464806. [Last accessed on 2014 Sep 15].
143. Kearney PC, Kaufman DD, editor. Herbicides Chemistry: Degradation and Mode of Action. USA: CRC Press; 1988.
144. Kehres DG, Maguire ME. Emerging themes in manganese transport, biochemistry and pathogenesis in bacteria. FEMS Microbiol Rev 2003;27:263-90.
145. Keller KA, Barnes PD. Rickets vs abuse: A national and international epidemic.

Pediatr Radiol 2008;38:1210-6.
146. Kiely T, Donaldson D, Grube A. Pesticides industry sales and usage-2000 and 2001 market estimates. Washington, DC: U.S. Environmental Protection Agency; 2004.
147. Kim YH, Hong JR, Gil HW, Song HY, Hong SY. Mixtures of glyphosate and surfactant TN20 accelerate cell death via mitochondrial damage-induced apoptosis and necrosis. Toxicol *In Vitro* 2013;27:191-7.
148. Kirschbaum BB, Schoolwerth AC. Acute aluminum toxicity associated with oral citrate andaluminum-containing antacids. Am J Med Sci 1989;297:9-11.
149. Klevay LM. Alzheimer's disease as copper deficiency. Med Hypotheses 2008;70:802-7.
150. Klos KJ, Ahlskog JE, Josephs KA, Fealey RD, Cowl CT, Kumar N. Neurologic spectrum of chronic liver failure and basal ganglia T1 hyperintensity on magnetic resonance imaging: Probable manganese neurotoxicity. Arch Neurol 2005;62:1385-90.
151. Kohanski MA, DePristo MA, Collins JJ. Sublethal antibiotic treatment leads to multidrug resistance via radical-induced mutagenesis. Mol Cell 2010;37:311-20.
152. Konstantynowicz J, Porowski T, Zoch-Zwierz W, Wasilewska J, Kadziela-Olech H, Kulak W, *et al*. A potential pathogenic role of oxalate in autism. Eur J Paediatr Neurol 2012;16:485-91.
153. Krüger M, Schledorn P, Schrödl W, Hoppe HW, Lutz W, Shehata AA. Detection of glyphosate residues in animals and humans. J Environ Anal Toxicol 2014;4:2.
154. Krüger M, Schrödl W, Neuhaus J, Shehata AA. Field investigations of glyphosate in urine of Danish dairy cows. J Environ Anal Toxicol 2013;3:1-7.
155. Krüger M, Schrödl W, Pedersen I, Shehata AA. Detection of glyphosate in malformed piglets. J Environ Anal Toxicol 2014;4:5.
156. Larsen K, Najle R, Lifschitz A, Maté ML, Lanusse C, Virkel GL. Effects of sublethal exposure to a glyphosate-based herbicide formulation on metabolic activities of different xenobiotic-metabolizing enzymes in rats. Int J Toxicol 2014;33:307-18.
157. Leach RM Jr. Role of manganese in the synthesis of mucopolysaccharides. Fed Proc 1967;26:118-20.
158. Leach RM Jr, Muenster AM, Wien EM. Studies on the role of manganese in bone formation: II. Effect upon chondroitin sulfate synthesis in chick epiphyseal cartilage. Arch Biochem Biophys 1969;133:22-8.
159. Lebovitz RM, Zhang H, Vogel H, Cartwright J, Dionne L, Lu N, *et al*. Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. Proc Natl Acad Sci USA 1996;93:9782-7.
160. Leffler CT, Philippi AF, Leffler SG, Mosure JC, Kim PD. Glucosamine, chondroitin, and manganese ascorbate for degenerative joint disease of the knee or low back: A randomized, double-blind, placebo-controlled pilot study. Mil Med 1999;164:85-91.
161. Levin J, Bertsch U, Kretzschmar H, Giese A. Single particle analysis of manganese-induced prion protein aggregates. Biochem Biophys Res Commun 2005;329:1200-7.
162. Levisohn PM. The autism-epilepsy connection. Epilepsia 2007;48(Suppl 9):33-5.
163. Levy BS, Nassetta WJ. Neurologic effects of manganese in humans: A review. Int J Occup Environ Health 2003;9:153-63.
164. Li Y, Huang TT, Carlson EJ, Melov S, Ursell PC, Olson JL, *et al*. Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. Nat Genet 1995;11:376-81.
165. Lin Q. Submerged fermentation of Lactobacillus rhamnosus YS9 for γ-aminobutyric acid (GABA) production. Braz J Microbiol 2013;44:183-7.
166. Lia C, Nie SP, Ding Q, Zhu KX, Wang ZJ, Xiong T, *et al*. Cholesterol-lowering effect of Lactobacillus plantarum NCU116 in a hyperlipidaemic rat model. J Funct Foods 2014;8:340-7.
167. Lin J, Lee IS, Frey J, Slonczewski JL, Foster JW. Comparative analysis of extreme acid survival in Salmonella typhimurium, Shigella flexneri, and *Escherichia coli*. J Bacteriol 1995;177:4097-104.
168. Lindquist NG, Larsson BS, Lydén-Sokolowski A. Neuromelanin and its possible protective and destructive properties. Pigment Cell Res 1987;1:133-6.
169. Livermore DM. Multiple mechanisms of antimicrobial resistance in Pseudomonas aeruginosa: Our worst nightmare? Clin Infect Dis 2002;34:634-40.
170. Lopes FM, Varela Junior AS, Corcini CD, da Silva AC, Guazzelli VG, Tavares G, *et al*. Effect of glyphosate on the sperm quality of zebrafish Danio rerio. Aquat Toxicol 2014;155:322-6.
171. Lu W, Li L, Chen M, Zhou Z, Zhang W, Ping S, *et al*. Genome-wide transcriptional responses of *Escherichia coli* to glyphosate, a potent inhibitor of the shikimate pathway enzyme 5-enolpyruvylshikimate-3-phosphate synthase. Mol Biosyst 2013;9:522-30.
172. Lucchini RG, Martin CJ, Doney BC. From manganism to manganese-induced Parkinsonism: A conceptual model based on the evolution of exposure. Neuromol Med 2009;11:311-21.
173. Lundager Madsen HE, Christensen HH, Gottlieb-Petersen C, Andresen AF, Smidsrod O, Pontchour CO, *et al*. Stability Constants of Copper (II), Zinc, Manganese (II), calcium, and magnesium complexes of N-(phosphonomethyl) glycine (Glyphosate). Acta Chem Scand A 1978;32:79-83.
174. Lushchak OV, Kubrak OI, Storey JM, Storey KB, Lushchak VI. Low toxic herbicide Roundup induces mild oxidative stress in goldfish tissues. Chemosphere 2009;76:932-7.
175. Lyke K. A study on the effects of a high soy-content diet on urinary oxalate levels in humans. PhD Thesis, Hawthorn University Whitethorn, CA. January, 2013.
176. MacDonald MG. Hidden risks: Early discharge and bilirubin toxicity due to glucose 6-phosphate dehydrogenase deficiency. Pediatrics 1995;96:734-8.
177. Maes M, Mihaylova I, Leunis JC. Increased serum IgA and IgM against LPS of enterobacteria in chronic fatigue syndrome (CFS): Indication for the involvement of gram-negative enterobacteria in the etiology of CFS and for the presence of an increased gut intestinal permeability. J Affect Disord 2007;99:237-40.
178. Magnus Ø, Brekke I, Åbyholm T, Purvis K. Effects of manganese and other divalent cations on progressive motility of human sperm. Syst Biol Reprod Med 1990;25:159-66.
179. Malecki EA, Radzanowski GM, Radzahowski TJ, Gallaher DD, Greger JL. Biliary manganese excretion in conscious rats is affected by acute and chronic manganese intake but not by dietary fat. J Nutr 1996;126:489-98.
180. Marin JJ. Bile acids: Chemistry, physiology, and pathophysiology World J Gastroenterol 2009;15:804-16.
181. Masánová V, Mitrova E, Ursinyova M, Uhnakova I, Slivarichova D. Manganese and copper imbalance in the food chain constituents in relation to Creutzfeldt-Jakob disease. Int J Environ Health Res 2007;17:419-28.
182. Matés JM, Sánchez-Jiménez F. Antioxidant enzymes and their implications in pathophysiologic processes. Front Biosci 1999;4:d339-45.
183. McDonald LC, Killgore GE, Thompson A, Owens RC Jr, Kazakova SV, Sambol SP, *et al*. An epidemic, toxin genevariant strain of Clostridium difficile. N Engl J Med 2005;353:2433-41.
184. McLaren PJ, Cave JG, Parker EM, Slocombe RF. Chondrodysplastic calves in Northeast Victoria. Vet Pathol 2007;44:342-54.
185. Melø TM, Larsen C, White LR, Aasly J, Sjøbakk TE, Flaten TP, *et al*. Manganese, copper, and zinc in cerebrospinal fluid from patients with multiple sclerosis. Biol Trace Elem Res 2003;93:1-8.
186. Melov S, Schneider JA, Day BJ, Hinerfeld D, Coskun P, Mirra SS, *et al*. A novel neurological phenotype in mice lacking mitochondrial manganese superoxide dismutase. Nat Genet 1998;18:159-63.
187. Menendez A, Arena ET, Guttman JA, Thorson L, Vallance BA, Vogl W, *et al*. Salmonella infection of gallbladder epithelial cells drives local inflammation and injury in a model of acute typhoid fever. J Infect Dis 2009;200:1703-13.
188. Mesnage R, Bernay B, Séralini GE. Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. Toxicology 2013;313:122-8.
189. Mesnage R, Defarge N, de Vendômois JS, Séralini GE. Major pesticides are more toxic to human cells than their declared active principles. Biomed Res Int 2014;2014:179691.
190. Micek ST, Lloyd AE, Ritchie DJ, Reichley RM, Fraser VJ, Kollef MH. Pseudomonas aeruginosa bloodstream infection: Importance of appropriate initial antimicrobial treatment. Antimicrob Agents Chemother 2005;49:1306-11.
191. Molbak K, Baggesen DL, Aarestrup FM, Ebbesen JM, Engberg J, Frydendahl K, *et al*. An outbreak of multidrug-resistant, quinolone-resistant Salmonella enterica serotype typhimurium DT104. N Engl J Med 1999;341:1420-5.
192. Moore JK, Braymer HD, Larson AD. Isolation of a Pseudomonas sp. which utilizes the phosphonate herbicide glyphosate. Appl Environ Microbiol 1983;46:316-20.
193. Morley WA, Seneff S. Diminished brain resilience: A modern day neurological pathology of increased susceptibility to mild brain trauma, concussion and

downstream neurodegeneration. Surg Neurol Int 2014;5:97.
194. Motekaitis RJ, Martell AE. Metal chelate formation by N-Phosphono-methylglycine and related ligands. J Coord Chem 1985;14:139-49.
195. Murri M, Leiva I, Gamez-Zumaquero JM, Tinahones FJ, Cardona F, Soriguer F, *et al*. Gut microbiota in children with type 1 diabetes differs from that in healthy children: A case-control study. BMC Med 2013;11:46.
196. Myhill S, Booth NE, McLaren-Howard J. Chronic fatigue syndrome and mitochondrial dysfunction. Int J Clin Exp Med 2009;2:1-16.
197. Napoli E, Wong S, Giulivi C. Evidence of reactive oxygen species-mediated damage to mitochondrial DNA in children with typical autism. Mol Autism 2013;4:2.
198. Napoli E, Wong S, Hertz-Picciotto I, Giulivi C. Deficits in bioenergetics and impaired immune response in granulocytes from children with autism. Pediatrics 2014;133:e1405-10.
199. Narayan KM, Gregg EW, Fagot-Campagna A, Engelgau MM, Vinicor F. Diabetes a common, growing, serious, costly, and potentially preventable public health problem. Diabetes Res Clin Pract 2000;50:S77-84.
200. Nebert DW. Clinical importance of the cytochromes P450. Lancet 2002;360:1155-62.
201. Negga R, Stuart JA, Machen ML, Salva J, Lizek AJ, Richardson SJ, *et al*. Exposure to glyphosate- and/or Mn/Zn-ethylene-bisdithiocarbamate-containing pesticides leads to degeneration of γ-aminobutyric acid and dopamine neurons in Caenorhabditis elegans. Neurotox Res 2012;21:281-90.
202. Nevison CD. A comparison of temporal trends in United States autism prevalence to trends in suspected environmental factors. Environ Health 2014;13:73.
203. Nishida T, Gaitmatan Z, Che M, Arias IM. Rat liver canalicular membrane vesicles contain an ATP-dependent bile acid transport system. Proc Natl Acad Sci U S A 1991;88:6590-4.
204. Norenberg MD. The distribution of glutamine synthetase in the central nervous system. J Histochem Cytochem 1979;27:469-75.
205. Norlin M, Wikvall K. Enzymes in the conversion of cholesterol into bile acids. Curr Mol Med 2007;7:199-218.
206. Nowell LH, Capel PD, Dileanis PD. Pesticides in stream sediment and aquatic biota- distribution, trends, and governing factors. In: Pesticides in the Hydrologic System series, Vol. 4. Boca Raton, Florida: CRC Press; 1999. p. 1040.
207. Oelzner P, Müller A Deschner F, Hüller M, Abendroth K, Hein G, *et al*. Relationship between disease activity and serum levels of vitamin D metabolites and PTH in rheumatoid arthritis. Calcif Tissue Int 1998;62:193-8.
208. Okonkwo FO, Ejike CE, Anoka AN, Onwurah IN. Toxicological studies on the short term exposure of Clarias albopunctatus (Lamonte and Nichole 1927) to sub-lethal concentrations of Roundup. Pak J Biol Sci 2013;16:939-44.
209. Opresko LK, Brokaw CJ. cAMP-dependent phosphorylation associated with activation of motility of Ciona sperm flagella. Gamete Res 1983;8:201-18.
210. O'Rand MG, Widgren EE, Beyler S, Richardson RT. Inhibition of human sperm motility by contraceptive anti-eppin antibodies from infertile male monkeys: Effect on cyclic adenosine monophosphate. Biol Reprod 2009;80:279-85.
211. Padda MS, Sanchez M, Akhtar AJ, Boyer JL. Drug induced cholestasis. Hepatology 2011;53:1377-87.
212. Paganelli A, Gnazzo V, Acosta H, López SL, Carrasco AE. Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling. Chem Res Toxicol 2010;23:1586-95.
213. Park LC, Albers DS, Xu H, Lindsay JG, Beal MF, Gibson GE. Mitochondrial impairment in the cerebellum of the patients with progressive supranuclear palsy. J Neurosci Res 2001;66:1028-34.
214. Patrick D, Polanowicz J, Bartlett C. Department Of Public Health and Division of Marine Fisheries Announce Closure of Oyster Beds in Edgartown. Sep 9, 2013. Available from: http://www.mass.gov/eohhs/gov/newsroom/press-releases/dph/closure-of-oyster-beds-in-edgartown-announced.html [Last accessed on 2014 Sep 04].
215. Peixoto F. Comparative effects of the Roundup and glyphosate on mitochondrial oxidative phosphorylation. Chemosphere 2005;61:1115-22.
216. Perl, DP, Warren OC. The neuropathology of manganese-induced Parkinsonism. J Neuropathol Exp Neurol 2007;66:675-82.
217. Pikuleva IA. Cholesterol-metabolizing cytochromes P450. Drug Metab Dispos 2006;34:513-20.
218. Polak T, Weise D, Metzger F, Ehlis AC, Langer JB, Schramm A, *et al*. Vagus nerve somatosensory evoked potentials in Parkinsons disease. J Neurol 2011;258:2276-7.
219. Porter JW, Dustan P, Jaap WC, Patterson KL, Kosmynin V, Meier OW, *et al*. Patterns of spread of coral disease in the Florida Keys. Hydrobiologia 2001;460:1-24.
220. Post SM, Duez H, Gervois PP, Staels B, Kuipers F, Princen HM. Fibrates Suppress Bile Acid Synthesis via Peroxisome Proliferatoractivated receptor-αmediated downregulation of cholesterol 7α-hydroxylase and sterol 27-hydroxylase expression. Arterioscler Thromb Vasc Biol 2001;21:1840-5.
221. Powles SB. Evolved glyphosate-resistant weeds around the world: Lessons to be learnt. Pest Manag Sci 2008;64:360-5.
222. Prouty AM, Schwesinger WH, Gunn JS. Biofilm formation and interaction with the surfaces of gallstones by Salmonella spp. Infect Immun 2002;70:2640-9.
223. Prusiner SB. Molecular biology of prion disease. Science 1991;252:1515-22.
224. Prusiner SB. Biology and genetics of prions causing neurodegeneration. Annu Rev Genet 2013;47:601-23.
225. Pullinger CR, Eng C, Salen G, Shefer S, Batta AK, Erickson SK, *et al*. Human cholesterol 7α-hydroxylase (CYP7A1) deficiency has a hypercholesterolemic phenotype. J Clin Invest 2002;110:109-17.
226. Purcell AE, Jeon OH, Zimmerman AW, Blue ME, Pevsner J. Postmortem brain abnormalities of the glutamate neurotransmitter system in autism. Neurology 2001;57:1618-28.
227. Purdey JM. Ecosystems supporting clusters of sporadic TSEs demonstrate excesses of the radical-generating divalent cation manganese and deficiencies of antioxidant co factors Cu, Se, Fe, Zn. Med Hypotheses 2000;54:278-306.
228. Purdey JM. Elevated levels of ferromagnetic metals in food chains supporting the Guam cluster of neurodegeneration: Do metal nucleated crystal contaminants evoke magnetic fields that initiate the progressive pathogenesis of neurodegeneration? Med Hypotheses 2004;63:793-809.
229. Purdey JM. Metal microcrystal pollutants: The heat resistant, transmissible nucleating agents that initiate the pathogenesis of TSEs? Med Hypotheses 2005;65:448-77.
230. Purgel M, Takács Z, Jonsson CM, Nagy L, Andersson I, Bányai I, *et al*. Glyphosate complexation to aluminium (III). An equilibrium and structural study in solution using potentiometry, multinuclear NMR, ATRFTIR, ESI-MS and DFT calculations. J Inorg Biochem 2009;103:1426-38.
231. Rallis C, Bähler J. Inhibition of TORC1 signaling and increased lifespan: Gained in translation? Aging 2013;5:335-6.
232. Rao AV, Bested AC, Beaulne TM, Katzman MA, Iorio C, Berardi JM, *et al*. A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. Gut Pathog 2009;1:6.
233. Ramachandra R, Namburi RB, Ortega-Martinez O, Shi X, Zaia J, Dupont ST, *et al*. Brittlestars contain highly sulfated chondroitin sulfates/dermatan sulfates that promote fibroblast growth factor 2-induced cell signaling. Glycobiology 2014;24:195-207.
234. Reitsma S, Slaaf DW, Vink H, van Zandvoort MA, oude Egbrink MG. The endothelial glycocalyx: Composition, functions, and visualization. Pflugers Arch 2007;454:345-59.
235. Richard IH. Anxiety disorders in Parkinson's disease. Adv Neurol 2005;96:42-55.
236. Rodionov DA, Vitreschak AG, Mironov AA, Gelfand MS. Comparative genomics of the vitamin B12 metabolism and regulation in prokaryotes. J Biol Chem 2003;278:41148-59.
237. Rolo AP, Oliveira PJ, Moreno AJM, Palmeira CM. Bile acids affect liver mitochondrial bioenergetics: Possible relevance for cholestasis therapy. Toxicol Sci 2000;57:177-85.
238. Román GC, Ghassabian A, Bongers-Schokking JJ, Jaddoe VW, Hofman A, de Rijke YB, *et al*. Association of gestational maternal hypothyroxinemia and increased autism risk. Ann Neurol 2013;74:733-42.
239. Rose S, Melnyk S, Pavliv O, Bai S, Nick TG, Frye RE, *et al*. Evidence of oxidative damage and inflammation associated with low glutathione redox status in the autism brain. Transl Psychiatry 2012;2:e134.
240. Rossi M, Amaretti A, Raimondi S. Folate production by probiotic bacteria. Nutrients 2011;3:118-34.
241. Rossignol DA, Frye RE. Mitochondrial dysfunction in autism spectrum disorders: A systematic review and meta-analysis. Mol Psychiatry 2012;17:290-314.
242. Rossignol DA, Genuis SJ, Frye RE. Environmental toxicants and autism spectrum disorders: A systematic review. Transl Psychiatry 2014;4:e360.
243. Rossignol DA, Frye RE. Evidence linking oxidative stress, mitochondrial

dysfunction, and inflammation in the brain of individuals with autism. Front Physiol 2014;22:150.
244. Roth JA. Homeostatic and toxic mechanisms regulating manganese uptake, retention, and elimination. Biol Res 2006;39:45-57.
245. Rotig A, de Lonlay P, Chretien D, Foury F, Koenig M, Sidi D, *et al*. Aconitase and mitochondrial iron-sulphur protein deficiency in Friedreich ataxia. Nat Genet 1997;17:215-7.
246. Rowe B, Ward LR, Threlfall EJ. Multidrug-resistant Salmonella typhi: A worldwide epidemic. Clin Infect Dis 1997; 24 Suppl 1:S106-9.
247. Saltman PD, Strause LG. The role of trace minerals in osteoporosis. J Am Coll Nutr 1993;12:384-9.
248. Samsel A, Seneff S. Glyphosate's suppression of cytochrome P450 enzymes and amino acid biosynthesis by the gut microbiome: Pathways to modern diseases. Entropy 2013;15:1416-63.
249. Samsel A, Seneff S. Glyphosate, pathways to modern diseases II: Celiac sprue and gluten intolerance. Interdiscip Toxicol 2013;6:159-84.
250. Scales CD Jr, Smith AC, Hanley JM, Saigal CS. Prevalence of kidney stones in the United States. Eur Urol 2012;62:160-5.
251. Schapira AH. Mitochondria in the aetiology and pathogenesis of Parkinsons disease. Lancet Neurol 2008;7:97-109.
252. Schnedler N, Burckhardt G, Burckhardt BC. Glyoxylate is a substrate of the sulfate-oxalate exchanger, SAT-1, and increases its expression in HepG2 cells. J Hepatol 2011;54:513-20.
253. Schinasi L, Leon ME. Non-Hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: A systematic review and meta-analysis. Int J Environ Res Public Health 2014;11:4449-527.
254. Shrader RE, Erway LC, Hurley LS. Mucopolysaccharide synthesis in the developing inner ear of manganese-deficient and pallid mutant mice. Teratology 1973;8:257-66.
255. Seeley M. Unexplained fractures in infants and child abuse: The case for requiring bone-density testing before convicting caretakers. BYU Law Rev 2011;2011:2321. Available from: http://digitalcommons.law.byu.edu/lawreview/vol2011/iss6/13. [Last accessed on 2014 Sep 10].
256. Seiler N. Ammonia and Alzheimers disease. Neurochem Int 2002;41:189-207.
257. Selvam R, Bijikurien T. Methionine feeding prevents kidney stone deposition by restoration of free radical mediated changes in experimental urolithiasis. J Nutr Biochem 1991;2:644-51.
258. Selvam R. Calcium oxalate stone disease: Role of lipid peroxidation and antioxidants. Urol Res 2002;30:35-47.
259. Selvan T, Rajiah K, Nainar MS, Mathew EM. A clinical study on glucosamine sulfate versus combination of glucosamine sulfate and NSAIDs in mild to moderate knee osteoarthritis. ScientificWorldJournal 2012;2012:902676.
260. Seneff S, Lauritzen A, Davidson RM, Lentz-Marino L. Is encephalopathy a mechanism to renew sulfate in autism? Entropy 2013;15:372-406.
261. Séralini GE, Clair E, Mesnage R, Gress S, Defarge N, Malatesta M, *et al*. Republished study: Long-term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. Environ Sci Eur 2014;26:14.
262. Sharma AK, Gaur K, Tiwari RK, Gaur MS. Computational interaction analysis of organophosphorus pesticides with different metabolic proteins in humans. J Biomed Res 2011;25:335-47.
263. Shehata AA, Schrödl W, Aldin AA, Hafez HM, Krüger M. The effect of glyphosate on potential pathogens and beneficial members of poultry microbiota *in vitro*. Curr Microbiol 2013;66:350-8.
264. Shehata AA, Schrödl W, Schledorn P, Krüger M. Distribution of glyphosate in chicken organs and its reduction by humic acid supplementation. J Poult Sci 2014;51:333.
265. Shimmura C, Suda S, Tsuchiya KJ, Hashimoto K, Ohno K, Matsuzaki H, *et al*. Alteration of plasma glutamate and glutamine levels in children with high-functioning autism. PLoS ONE 2011;6:e25340.
266. Shipley MT, Halloran FJ, de la Torre J. Surprisingly rich projection from locus coeruleus to the olfactory bulb in the rat. Brain Res 1985;329:294-9.
267. Sidoryk-Wegrzynowicz M, Aschner M. Manganese toxicity in the central nervous system: The glutamine/glutamate-c-aminobutyric acid cycle. J Intern Med 2013;273:466-77.
268. Soderland P, Lovekar S, Weiner DE, Brooks DR, Kaufman JS. Chronic kidney disease associated with environmental toxins and exposures. Adv Chronic Kidney Dis 2010;17:254-64.
269. Sohler A, Pfeiffer CC. A direct method for the determination of manganese in whole blood. Patients with seizure activity have low blood levels. J Orthomol Psych 1979;8:275-80.
270. Soldin OP, Aschner M. Effects of manganese on thyroid hormone. Neurotoxicology 2007;28:951-6.
271. Ståhlberg D, Reihnér E, Rudling M, Berglund L, Einarsson K, Angelin B. Influence of bezafibrate on hepatic cholesterol metabolism in gallstone patients: Reduced activity of cholesterol 7alpha-hydroxylase. Hepatology 1995;21:1025-30.
272. Stevens DJ, Walter ED, Rodriguez A, Draper D, Davies P, Brown DR, *et al*. Early onset prion disease from octarepeat expansion correlates with copper binding properties. PLoS Pathogens 2009;5:e1000390.
273. Stieger B. Regulation of the expression of the hepatocellular sulfate-oxalate exchanger SAT-1 (SLC26A1) by glyoxylate: A metabolic link between liver and kidney? J Hepatol 2011;54:406-7.
274. Stoyanovsky DA, Huang Z, Jiang J, Belikova NA, Tyurin V, Epperly MW, *et al*. A manganeseporphyrin complex decomposes H2O2, inhibits apoptosis, and acts as a radiation mitigator *in vivo*. ACS Med Chem Lett 2011;2: 814-7.
275. Strange RC, Spiteri MA, Ramachandran S, Fryer AA. Glutathione-S-transferase family of enzymes. Mutat Res 2001;482:21-6.
276. Strause LG, Hegenauer J, Saltman P, Cone R, Resnick D. Effects of long-term dietary manganese and copper deficiency on rat skeleton. J Nutr 1986;116:135-41.
277. Streifel KM, Miller J, Mouneimne R, Tjalkens RB. Manganese inhibits ATP-induced calcium entry through the transient receptor potential channel TRPC3 in astrocytes. Neurotoxicology 2013;34:160-6.
278. Strople J, Lovell G, Heubi J. Prevalence of subclinical vitamin K deficiency in cholestatic liver disease. J Pediatr Gastroenterol Nutr 2009;49:78-84.
279. Subramanian N, Qadri A. Lysophospholipid sensing triggers secretion of flagellin from pathogenic salmonella. Nat Immunol 2006;7:583-9.
280. Svedružić D, Jónsson S, Toyota CG, Reinhardt LA, Ricagno S, Lindqvist Y, *et al*. The enzymes of oxalate metabolism: Unexpected structures and mechanisms. Arch Biochem Biophys 2005;433:176-92.
281. Swerdlow RH, Burns JM, Khan SM. The Alzheimer's disease mitochondrial cascade hypothesis. J Alzheimers Dis 2010;20(Suppl 2):265-79.
282. Tabrizi SJ, Workman J, Hart PE, Mangiarini L, Mahal A, Bates G, *et al*. Mitochondrial dysfunction and free radical damage in the Huntington R6/2 transgenic mouse. Ann Neurol 2000;47:80-6.
283. Takeda A, Kodama Y, Ishiwatari S, Okada S. Manganese transport in the neural circuit of rat CNS. Brain Res Bull 1998;45:149-52.
284. Takeda A, Sawashita J, Okada S. Manganese uptake into rat brain during development and aging. J Neurosci Res 1999;56:93-8.
285. Takeuchi H, Jin S, Wang J, Zhang G, Kawanokuchi J, Kuno R, *et al*. Tumor necrosis factor-alpha induces neurotoxicity via glutamate release from hemichannels of activated microglia in an autocrine manner. J Biol Chem 2006;281:21362-8.
286. Tannock GW. Lactic microbiota of pigs, mice and rats. In: Wood BJ, editor. The lactic acid bacteria in health and disease, Vol. 1. London, United Kingdom: Elsevier Applied Science; 1992. p. 2148.
287. Tash JS, Hidaka H, Means AR. Axokinin phosphorylation by cAMP-dependent protein kinase is sufficient for activation of sperm flagellar motility. J Cell Biol 1986;103:649-55.
288. Teri L, Ferretti LE, Gibbons LE, Logsdon RG, McCurry SM, Kukull WA, *et al*. Anxiety in Alzheimer's disease: Prevalence and comorbidity. J Gerontol A Biol Sci Med Sci 1999;54:M348-52.
289. Thongprakaisang S, Thiantanawat A, Rangkadilok N, Suriyo T, Satayavivad J. Glyphosate induces human breast cancer cells growth via estrogen receptors. Food Chem Toxicol 2013;59:129-36.
290. Thrane VR, Thrane AS, Wang F, Cotrina ML, Smith NA, Chen M, *et al*. Ammonia triggers neuronal disinhibition and seizures by impairing astrocyte potassium buffering. Nat Med 2013;19:1643-8.
291. Tjälve H, Henriksson J, Tallkvist J, Larsson BS, Lindquist NG. Uptake of manganese and cadmium from the nasal mucosa into the central nervous system via olfactory pathways in rats. Pharmacol Toxicol 1996;79:347-56.
292. Tsolis RM, Baumler AJ, Heffron F. Role of Salmonella typhimurium Mn-superoxide dismutase (SodA) in protection against early killing by J774 macrophages. Infect Immun 1995;63:1739-44.
293. Tuchman R, Rapin I. Epilepsy in autism. Lancet Neurol 2002;1:352-8.
294. Tzin V, Galili G. New insights into the shikimate and aromatic amino acids biosynthesis pathways in plants. Mol Plant 2010;3:956-72.
295. Ullah N, Khan FM, Mukhtiar M. Human blood glutathione (GSH) as a tool for arsenic detoxification. Afr J Pharm Pharmacol 2012;6:17-23.

296. Undabeytia T, Morillo E, Maqueda C. FTIR study of glyphosate-copper complexes. J Agric Food Chem 2002;50:1918-21.
297. USDA Pesticide Data Program,Annual Summary Calendar Year 2011.Available from: http://www.ams.usda.gov/pdp. [Last accessed on 2014 May 14].
298. US Patent number 7,771,736 B2. Glyphosate formulations and their use for the inhibition of 5-enolpyruvylshikimate-3-phosphate synthase. Publication date: August 10, 2010. Available from: http://www.google.com/patents/US7771736 [Last accessed on 2014 Jun 30].
299. US Patent application number US8507409. Synergistic combination of a glyphosate compound and DMITS. Publication date: June 06, 2012. Available from: http://www.google.com/patents/US8507409. [Last accessed on 2014 Jun 30].
300. Vielhaber S, Niessen HG, Debska-Vielhaber G, Kudin AP, Wellmer J, Kaufmann J, *et al*. Subfield-specific loss of hippocampal N-acetyl aspartate in temporal lobe epilepsy. Epilepsia 2008;49:40-50.
301. Visner GA, Dougall WC, Wilson JM, Burr IA, Nick HS. Regulation of manganese superoxide dismutase by lipopolysaccharide, interleukin-1, and tumor necrosis factor: Role in the acute inflammatory response. J Biol Chem 1990;265:2856-64.
302. Volkmar FR, Nelson DS. Seizure disorders in autism. J Am Acad Child Adolesc Psychiatry 1990;29:127-9.
303. Wandernoth PM, Raubuch M, Mannowetz N, Becker HM, Deitmer JW, Sly WS, *et al*. Role of carbonic anhydrase IV in the bicarbonate-mediated activation of murine and human sperm. PLoS ONE 2010;5:e15061.
304. Wang G, Fan XN, Tan YY, Cheng Q, Chen SD. Parkinsonism after chronic occupational exposure to glyphosate. Parkinsonism Relat Disord 2011;17:486-7.
305. Watts RJ, Sarasa J, Loge FJ, Teel AL. Oxidative and reductive pathways in manganese-catalyzed Fentons reactions. J Environ Eng 2005;131:158-64.
306. Weinstein DL, Carsiotis M, Lissne CR, O'Brien AD. Flagella help Salmonella ty- phimurium survive within murine macrophages. Infect Immun 1984;46:819-25.
307. Weise E. The starfish are dying, and no one knows why. USA Today. 11:42 a.m. EST Dec. 31, 2013. Available from: http://www.usatoday.com/story/news/nation/2013/12/28/starfish-dying-wasting-disease-mystery/4208859/[Last accessed on 2014 May 10].
308. Wennberg RP. The blood-brain barrier and bilirubin encephalopathy. Cell Mol Neurobiol 2000;20:97-109.
309. Werner P, Pitt D, Raine CS. Multiple sclerosis: Altered glutamate homeostasis in lesions correlates with oligodendrocyte and axonal damage. Ann Neurol 2001;50:169-80.
310. Westmark CJ. Soy infant formula and seizures in children with autism: A retrospective study. PLoS ONE 2014;9:e80488.
311. Wichmann F, Udikovic-Kolic N, Andrew S, Handelsmana J. Diverse antibiotic resistance genes in dairy cow manure. MBio 2014;5:e01017.
312. Willis AW, Evanoff BA, Lian M, Galarza A, Wegrzyn A, Schootman M, *et al*. Metal emissions and urban incident Parkinson disease: A community health study of medicare beneficiaries by using geographic information systems. Am J Epidemiol 2010;172:1357-63.
313. Winter SE, Thiennimitr P, Winter MG, Butler BP, Huseby DL, Crawford RW, *et al*. Gut inflammation provides a respiratory electron acceptor for Salmonella. Nature 2010;467:426-9.
314. Woodburn AT. Glyphosate: Production, pricing and use worldwide. Pest Manag Sci 2000;56:309-12.
315. Xu XC, Brinker RJ, Reynolds TL, Abraham W, Graham JA. Pesticide compositions containing oxalic acid. US Patent number 6,992,045 B2, Monsanto Technology LLC, Jan. 31, 2006.
316. Yao J, Irwin RW, Zhao L, Nilsen J, Hamilton RT, Brinton RD. Mitochondrial bioenergetic deficit precedes Alzheimer's pathology in female mouse model of Alzheimer's disease. Proc Natl Acad Sci USA 2009;106:14670-5.
317. Yang H, Ramani K, Xia M, Ko KS, Li TW, Oh P, *et al*. Dysregulation of glutathione synthesis during cholestasis in mice: Molecular mechanisms and therapeutic implications. Hepatology 2009;49:1982-91.
318. Yokota T, Gots JS. Requirement of adenosine 3',5'-cyclicphosphate for flagella formation in *Escherichia coli* and Salmonella typhimurium. J Bacteriol 1970;103:513-6.
319. Zaidi A, Khan MS, Rizvi PQ. Effect of herbicides on growth, nodulation and nitrogen content of greengram. Agron Sustain Dev 2005;25:497-504.
320. Ziomber A, Thor P, Krygowska-Wajs A, Załęcki T, Moskała M, Romańska I, *et al*. Chronic impairment of the vagus nerve function leads to inhibition of dopamine but not serotonin neurons in rat brain structures. Pharmacol Rep 2012;64:1359-67.

Commentary

Glyphosate-based herbicides (GBH) are the major pesticides used worldwide. Initially patented as a metal ion chelator, glyphosate rapidly jumped to a leading position as an active ingredient of commercial pesticides from the 1970s when Monsanto discovered its herbicidal activities. The herbicidal mode of action of glyphosate is primarily to inhibit the shikimic acid pathway,[2] causing a shortage of aromatic amino acids. Since this biochemical pathway does not exist in vertebrates, it is generally assumed that glyphosate is safe for mammals, including humans.[8] As a consequence, GBH are used in private gardens, city parks and along roads and railway lines, as well as within an agricultural context on food and feed crops. All these diverse applications of GBH have resulted in escalating levels of human exposure and thus body burden.

Glyphosate is an aminophosphonic analog of glycine. The fact that glycine and other amino acids like glutamate function as neurotransmitters and play a crucial role in brain function, makes the potential neurotoxic effects of glyphosate a matter of concern.[5] The potential of glyphosate to act as a neurotoxin is further supported by its structural similarity to the glutamate receptor agonist 2-amino-3-phosphonopropionic acid. Indeed, GBH exposure induces glutamate excitotoxicity through L-VDCC and NMDA receptor activation in immature rat hippocampus, by reducing glutamate uptake and metabolism within glial cells, and by increasing glutamate release in the synaptic cleft.[3] However, the lack of esterase inhibition by glyphosate (the neurotoxic mechanism of most organophosphate compounds), was considered by regulatory authorities as sufficient grounds to avoid having to undertake a complete assessment of glyphosate's neurological effects.[6]

The most recent reevaluation of the acceptable daily intake (ADI) for glyphosate within the European Union (EU) conducted by the German regulatory agency (BfR), states that this has been determined by scrutiny of approximately 450 regulatory toxicological studies and 900 publications from the scientific literature.[4] Based on the review of all these investigations, the BfR concluded that the "no observed

adverse effect level" of glyphosate was in the region of 30–50 mg/kg body weight (bw) per day in rats and the ADI was thus calculated at 0.5 mg/kg bw/day, which constitutes a recommended increase from the current 0.3 mg/kg bw/day. However, these glyphosate ADI values have previously been challenged as review of the same studies and especially extending to feeding studies in animals other than rats, suggested that the current ADI of 0.3 mg/kg bw/day was at least three times higher than what the data suggest should be the case.[1] Nevertheless, with a review of such a relatively large number of studies by the official regulatory authorities, the assessment of the toxicological effects of glyphosate is being considered as complete. The notional strength of glyphosate's safety profile has also resulted in it being neglected in some national wide-scale toxicity testing schemes such as the U.S. Environmental Protection Agency (EPA) ToxCast program.

However, a debate continues as to the soundness of this BfR-led assessment as the studies taken into account were performed with an experimental design adapted to the study of poisoning effects based on the principle of "the dose makes the poison"; that is, the higher the dose the greater the poisoning effect. Of major concern is that none of the studies referred to incorporated testing principles derived from a contemporary understanding of (neuro) endocrinology or developmental epigenetics.

In the classic theory of toxicology, as applied for the study of glyphosate toxicity at a regulatory level, a nontoxic threshold is evidenced by decreasing the level of exposure and assuming that toxic effects observed are a linear response to the dose. Lower doses corresponding to environmental exposures are assumed to be safe and are not tested. However, in contrast to a classical poison, an endocrine disruptive chemical (EDC) will alter the functioning of hormonal systems and induce adverse effects at various dosage levels. Such EDC effects will, in some cases, occur in a nonlinear (non-monotonic) manner at environmentally relevant levels of exposure and will not be observed at higher doses. In addition and not surprisingly, EDC effects can be sex-specific in nature. Such nonmonotonic and sex-specific EDC effects have been extensively described for common pollutants.[7] Although nonmonotonic and sex-specific effects have been reported in many cases with GBH, the regulatory authorities considered these as false positive outcomes rather than a suggestion of potential EDC effects.[4]

Major endpoints of toxicity such as neurodevelopmental, reproductive, and transgenerational effects in humans still needs to be investigated at the glyphosate ADI and other concentrations reflective of human levels of exposure. Given its increasing wide-scale use and consequent rise in exposure, we urgently call for greater research efforts on the toxicology of glyphosate and its commercial herbicide formulations, as well as pesticide neurodevelopmental effects in general. Furthermore, pesticide combinatorial (additive or synergistic) effects remain a poorly investigated subject and area of concern that needs to be addressed. Such studies are particularly relevant to the brain since it is physiologically dependent on neurosteroids, making it potentially very sensitive to endocrine disruptive compounds.

Robin Mesnage, Michael N. Antoniou

Gene Expression and Therapy Group, King's College London, Faculty of Life Sciences and Medicine, Department of Medical and Molecular Genetics, 8th Floor, Tower Wing, Guy's Hospital, Great Maze Pond, London, SE1 9RT, UK.
E-mail: *Michael N. Antoniou - michael.antoniou@kcl.ac.uk

REFERENCES

1. Antoniou M, Habib ME, Howard CV, Jennings RC, Leifert C, Nodari RO, *et al*. Teratogenic Effects of Glyphosate-Based Herbicides: Divergence of Regulatory Decisions from Scientific Evidence. J Environ Anal Toxicol 2012;S4:006.
2. Boocock MR, Coggins JR. Kinetics of 5-enolpyruvylshikimate-3-phosphate synthase inhibition by glyphosate. FEBS Lett 1983;154:127-33.
3. Cattani D, de Liz Oliveira Cavalli VL, Heinz Rieg CE, Domingues JT, Dal-Cim T, Tasca CI, *et al*. Mechanisms underlying the neurotoxicity induced by glyphosate-based herbicide in immature rat hippocampus: Involvement of glutamate excitotoxicity. Toxicology 2014;320:34-45.
4. German Federal Agency BfR. The BfR has finalised its draft report for the re-evaluation of glyphosate. Available from: http://wwwbfrbundde/en/the_bfr_has_finalised_its_draft_report_for_the_re_evaluation_of_glyphosate-188632html 2014. [Last accessed on 2014 Nov 22].
5. Mesnage RS. The Need for a Closer Look at Pesticide Toxicity during GMO Assessment. in Practical Food Safety: Contemporary Issues and Future Directions. In: Bhat R, Gómez-López VM, editors. Chichester, UK: John Wiley and Sons, Ltd.; 2014.
6. U.S.EPA. R.E.D. FACTS - Glyphosate. 1993; EPA-738-F-93-011.
7. Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR Jr, Lee DH, *et al*. Hormones and endocrine-disrupting chemicals: Low-dose effects and nonmonotonic dose responses. Endocr Rev 2012;33:378-455.
8. Williams AL, Watson RE, Desesso JM. Developmental and reproductive outcomes in humans and animals after glyphosate exposure: A critical analysis. J Toxicol Environ Health B Crit Rev 2012;15:39-96.

Poultry, Fisheries & Wildlife Sciences

Hoy et al., Poult Fish Wildl Sci 2015, 3:1
http://dx.doi.org/10.4172/2375-446X.1000132

Research Article Open Access

The High Cost of Pesticides: Human and Animal Diseases

Judy Hoy[1], Nancy Swanson[2] and Stephanie Seneff[3]*

[1]Independent Researcher, Stevensville, MT, USA
[2]Abacus Enterprises, Lummi Island, WA, USA
[3]Computer Science and Artificial Intelligence Laboratory, Massachusetts Institute of Technology, USA

Abstract

A significant degradation in the health of wild animals in Montana has been recorded over the past two decades. We surmise that the health issues are related to pesticide exposure. We present some of the evidence of the deterioration of the health in wildlife, which we used to inspire investigations on human health in the US population. While the animals' exposure is through food, water and air, we believe that human exposure is predominantly through food, as the majority of the population does not reside near agricultural fields and forests.

We have obtained US government data on pesticide usage and on human disease patterns over time from the 1998-2010 hospital discharge data. Since glyphosate is by far the most widely used herbicide, we believe it to be a major source of contamination for humans. Correlations between glyphosate usage and specific health issues, along with the known toxicology profile of glyphosate obtained from the literature, reflect a plausible causal relationship.

Because much of the wildlife data is from deer fawns, most of the human data presented here involve newborn infants, but we also present some data for children 0-15 years old and for the full population (except newborn). We found many diseases and conditions whose hospital discharge rates match remarkably well with the rate of glyphosate usage on corn, soy, and wheat crops. These include head and face anomalies (R=0.95), newborn eye disorders, newborn blood disorders (R=0.92), newborn skin disorders (R=0.96), lymph disorders in children 0-15 (R=0.86) and in the general population except newborn (R=0.89), congenital heart conditions in newborns (R= 0.98), enlarged right ventricle in all age groups except newborn (R=0.96), newborn lung problems (R=0.95), pulmonary bleeding and edema for all age groups except newborn (R=0.97), liver cancer for all age groups except newborn (R=0.93), newborn metabolic disorders (R=0.95) and newborn genitourinary disorders (R=0.96).

Keywords: Glyphosate; Brachygnathia; Hypothyroidism; Congenital heart defects; Thymus; Lymphedema; Hepatic carcinoma; Hypospadias; Genitourinary disorders

Introduction

One of the promises assured with genetically engineered (GE) herbicide-resistant crops was that they would require many fewer pesticides, providing a more sustainable agricultural option. Several GE crops, including cotton, canola, corn, soy, sugar beets and alfalfa, are engineered to withstand direct application of glyphosate, the active ingredient in the pervasive herbicide, Roundup. As a result of the widespread acceptance of GE crops, the increasing practice of using glyphosate for pre-harvest dry-down in grains and legumes, along with the emergence of glyphosate-resistant weeds, the use of glyphosate has skyrocketed since 1996 [1-3].

With the exception of glyphosate, pesticide use on crops was indeed reduced for the first 5 or 6 years after the introduction of these GE crops. Then something happened after about 2002, resulting in a steep increase in glyphosate and 2,4-D applications on corn, soy and potato, along with an increase in dicamba on wheat. This coincides with a steep increase in the number of confirmed cases of glyphosate-resistant weeds [1] as shown in Figure 1.

The active ingredient in the pesticides is usually an acid. To make the pesticides more water soluble and therefore easier to package and distribute, they are chemically altered into a salt or ester formulation. Various salt formulations include isopropylamine, diammonium, monoammonium, or potassium as the cation. Adjuvants are increasingly added to enhance the efficacy of the herbicides, particularly with the advent of the glyphosate-resistant weeds. One adjuvant is oxalic acid or an oxalate salt (potassium oxalate, e.g.) added to the stable salt formulations. For example, a 2006 patent by Xu et al. [4] discloses pesticide compositions, especially storage-stable herbicidal concentrates, containing oxalic acid and glyphosate that allegedly exhibit enhanced efficacy due to the addition of oxalic

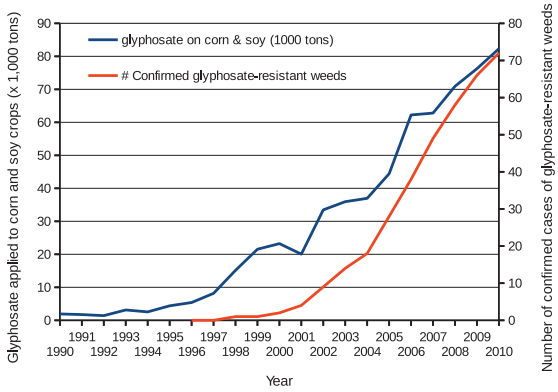


Figure 1: Glyphosate applications to corn, soy and cotton along with the advent of glyphosate-resistant weeds.

***Corresponding author:** Seneff S, Computer Science and Artificial Intelligence Laboratory, Massachusetts Institute of Technology, USA, Tel: +1 617-253-1000; E-mail: Seneff@csail.mit.edu

Received April 16, 2015; **Accepted** May 04, 2015; **Published** May 30, 2015

Citation: Hoy J, Swanson N, Seneff S (2015) The High Cost of Pesticides: Human and Animal Diseases. Poult Fish Wildl Sci 3: 132. doi:10.4172/2375-446X.1000132

Citation: Hoy J, Swanson N, Seneff S (2015) The High Cost of Pesticides: Human and Animal Diseases. Poult Fish Wildl Sci 3: 132. doi:10.4172/2375-446X.1000132

2,4-D (2,4-Dichlorophenoxyacetic acid)		**Year first applied to crop**				
Formulation	**Trade names**	**Corn**	**Soy**	**Potato**	**Winter wheat**	**Spring wheat**
2,4-D sodium salt	2,4-DB, Butoxone®	--	1990	--	--	--
2,4-D isopropylamine salt	Campaign® (2,4-D + glyphosate iso. salt)	2005	--	--	2006	2006
2,4-D tri-isopropanolamine salt	Tordon® (2,4-D + picloram) Banvine® (2,4-D + dicamba)	--	--	--	--	2006
2,4-D dimethylamine salt	Saber®, 2,4-D Amine, Savage®, Weedar® 64	2003	2004	2005	2004	2004
2,4-D 2-ethylhexyl ester	Solve®, Barrage®, Salvo®, Maestro® D, Outlaw®	2005	2005	2005	2006	2006
2,4-D butoxyethyl ester	2,4-D BEE, Agri Star® D-638, Weedone® LV6	2005	2005	--	--	--
Dicamba (3,6-dichloro-2-methoxybenzoate)		**Year first applied to crop**				
Formulation	Trade names	Corn	Soy	Potato	Winter wheat	Spring wheat
Dicamba dymethylamine salt	Diablo®, Banvel® 720, Oracle®, Rifle®, Distinct®, Sterling®, Weedmaster®	1999	--	--	2006	2006
Dicamba diglycolamine salt	Clarity®, Vanquish®	2005	2005	--	2006	2006
Dicamba sodium salt	Status®, Distinct®, Celebrity®, Overdrive®, Yukon®	2001	--	--	2006	2004
Dicamba potassium salt	Marksman® (also contains Atrazine)	1998	--	--	--	--
Glyphosate (N-(phosphonomethyl) glycine)		**Year first applied to crop**				
Formulation	Trade names	Corn	Soy	Potato	Winter wheat	Spring wheat
Glyphosate dimethylamine salt	Roundup®, Rodeo®, Durango DMA®, Duramax®	2003	2002	--	2012	--
Glyphosate isopropylamine salt	Roundup®Ultra, Honcho®, Roundup®Pro, Ranger Pro®, Roundup®Custom, Mad Dog Glyphosate®	2005	2005	2005	2006	2006
Glyphosate potassium salt	Roundup®WeatherMax Roundup®PowerMax Touchdown®, RT Master® II	2010	2012	--	2009	2012

Table 1: Salt and Ester Formulations of Pesticides.

acid. The hypothesis presented for its effect is that it increases cell membrane permeability, suppresses oxidative burst, or increases expression of hydroxyproline-rich glycoproteins. However, it is also likely that it inhibits the breakdown of glyphosate, since oxalate inhibits the breakdown of glyoxylate, which is a disintegration product of glyphosate [5]. This would lead to an accumulation of glyoxylate, a strong glycating agent that would damage membrane fatty acids, explaining the increase in membrane permeability [6]. This patent also discloses that a variety of surfactants, including amines, amine oxides and quaternary ammonium compounds, can be used in combination with oxalic acid for pesticide compositions.

Manufacturers of pesticides do not disclose the adjuvants and surfactants used in any of their products, claiming that they are trade secrets. Only the formulation of the active ingredient can be traced. Table 1 shows the various formulations for 2,4-D, dicamba and glyphosate and when they first appeared in the USDA survey data. When they initially appeared, salts were a small percentage of the total amount of pesticides applied, but within a couple of years (by 2006) nearly all of the applications for a given herbicide were salt formulations. In the pesticide data shown in Figures 2-7, arrows have been superimposed on the graphs indicating when salts were first used and again when nearly all of the formulations used were salts.

One of us (Hoy) has been documenting congenital malformations in Montana wildlife for the past 19 years. In this paper, we present documentation of wildlife deformities and evidence of organ damage. In addition, we obtained corresponding data for human congenital malformations and diseases in newborn infants, along with diseases in children 0-15, and all age groups (except newborn) from the US hospital discharge data. Finally, we obtained pesticide application data on selected crops from the USDA. We show that congenital malformations and wildlife diseases follow the trends for dicamba, 2,4-D, chlorothalonil and glyphosate use. We also show that congenital malformations and other diseases in humans follow the trends in glyphosate use. We hypothesize that the primary exposure route for humans is through food, whereas the primary exposure for animals is not only food but also direct exposure through air and water. Some of these conditions show a steep increase at the same time that the switch to the salt formulations of the herbicides was made.

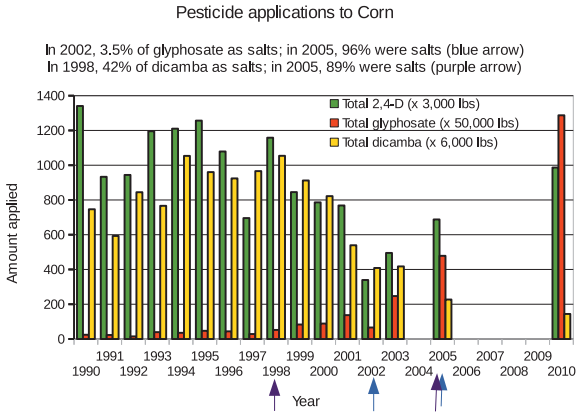


Figure 2: Herbicide applications to corn. Scale varies for each as indicated in the legend. Glyphosate salts were first applied in 2002, almost exclusively applied by 2005. Dicamba salts were first applied in 1998, almost exclusively by 2005. EPTC and alachlor applications have steadily decreased, becoming negligible by 2002 (not shown). Atrazine applications to corn crops have remained approximately constant at about 52.5 million pounds (also not shown).

Data Collection Methods

Pesticides

The United States Department of Agriculture National Agricultural

Citation: Hoy J, Swanson N, Seneff S (2015) The High Cost of Pesticides: Human and Animal Diseases. Poult Fish Wildl Sci 3: 132. doi:10.4172/2375-446X.1000132

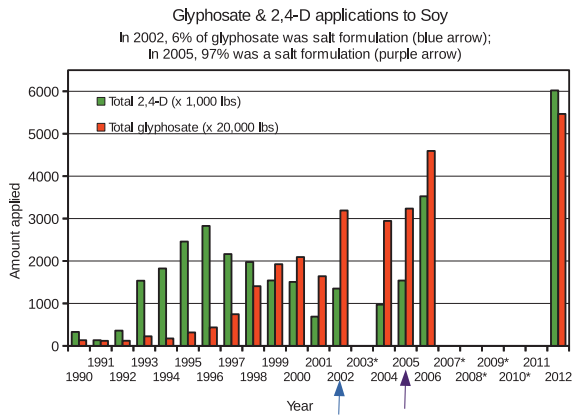


Figure 3: Glyphosate and 2,4-D applications to soy. Scale varies for each as indicated in the legend. Glyphosate salt formulations began to be applied to soy in 2002, almost exclusively by 2005.

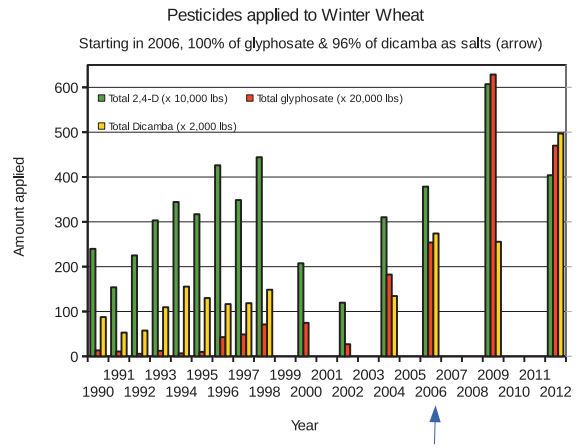


Figure 4: Glyphosate, dicamba and 2,4-D applications to winter wheat. By 2006, nearly all of the glyphosate and dicamba were salt formulations. Scale varies for each as indicated in the legend.

Statistics Service (USDA:NASS) maintains a database of US crops. Every year they randomly select fields of certain crops and send surveys to the persons who manage those fields. Among other things, they ask what herbicides were used, the application rate and how many times was it applied. Surveys are only sent to the states that are the major producers of a given crop, usually accounting for about 90% of the total US acreage planted in that crop. They then perform a statistical analysis and report the total acreage planted, the Percentage of Acres Treated (PAT) with each herbicide for that crop and the application rate per acre per year. One can then calculate the total amount of an herbicide that was applied to that crop in the survey states for that year.

Data files containing the information for pesticide applications are available from 1990-2012 [7]. We extracted the data for glyphosate, 2-4,D, dicamba, chlorothalonil, ETPC (S-Ethyl Dipropylthiocarbamate), atrazine and alachlor applications to corn, soy, potato and wheat crops. Sampling errors for the pesticide application data were less than 5% for most of the pesticides over most of the time period examined. Sampling errors (reported as standard errors) are small (<5%) in both the PAT and the application rate if the PAT is greater than 50%. Sampling errors are 5-10% if the PAT is between 10-50%, while the sampling errors are 10-100% if the PAT is <10%. The PAT for chlorothalonil on potato has exceeded 50% since 1994. The PAT for glyphosate on soy has exceeded 50% since 1998. The PAT for glyphosate on corn has exceeded 50% since 2006. The PAT for 2,4-D on spring wheat exceeds 50% over the entire data range. The PAT for dicamba ranges between 20% and 55% for spring wheat. By 2006, the PAT for glyphosate on spring wheat was 31%, durum wheat was 48% and winter wheat was 15%. Details on the PAT with glyphosate on corn and soy can be found in [3].

Wildlife

Wild ruminates were the primary animals studied. The study area where white-tailed deer (*Odocoileus virginianus*) were examined is Ravalli County, in the far western portion of Montana. The north flowing Bitterroot River forms the Bitterroot Valley (BV) located

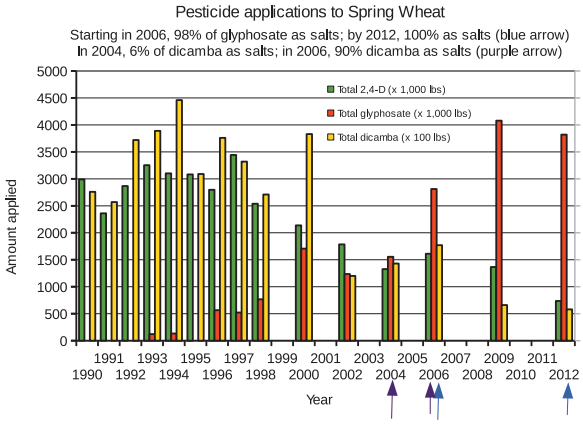


Figure 5: Glyphosate, dicamba and 2,4-D applications to spring wheat (except durum). By 2006, nearly all of the glyphosate and dicamba were salt formulations. Scale varies for each as indicated in the legend.

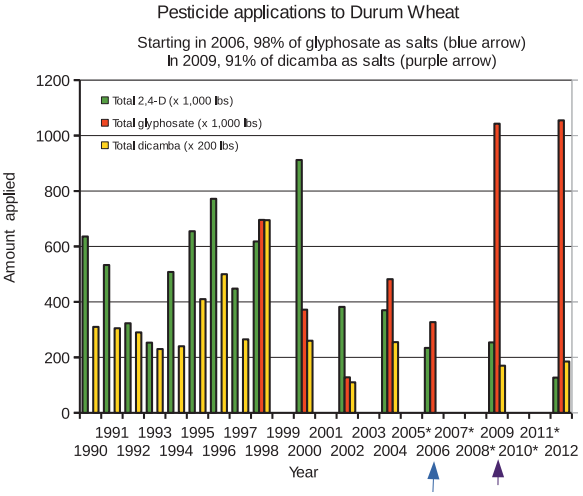


Figure 6: Glyphosate, dicamba and 2,4-D applications to durum wheat. By 2005, nearly all of the glyphosate and dicamba were salt formulations. Scale varies for each as indicated in the legend.

Citation: Hoy J, Swanson N, Seneff S (2015) The High Cost of Pesticides: Human and Animal Diseases. Poult Fish Wildl Sci 3: 132. doi:10.4172/2375-446X.1000132

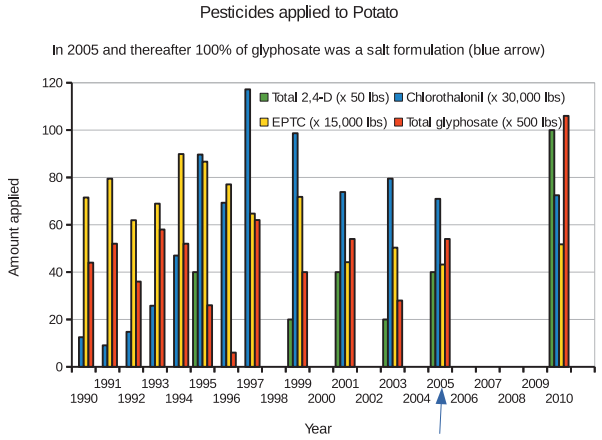


Figure 7: Pesticides applied to potato crops. By 2005, all of the glyphosate was a salt formulation. Scale varies for each as indicated in the legend.

between the Bitterroot Mountains on the west and the Sapphire Range on the east. Riparian vegetation along the river and its tributaries provide cover close to feeding areas in fields for herds of white-tailed deer.

There are two major highways running north and south through the valley on each side of the Bitterroot River, with a network of secondary roads throughout the BV. With the exception of hunting, encounters with vehicles, fences and dogs account for the majority of RC white-tailed deer mortalities.

White-tailed deer that were accident-killed or had been euthanized due to injuries were examined post-mortem from 1995 through 2014. Age, sex, date of examination and several body measurements, including measurement of the mouth and bite and the size of the male sex organs on the external skin were recorded for each animal. In addition, the condition of the heart (normal or enlarged right ventricle) and the lungs (degrees of inflammation symptoms) were recorded. On necropsied newborns, the condition of the thymus was also documented. The year of birth of each white-tailed deer was determined by examining the tooth eruption as outlined by Mosby [8]. Additionally, the following were examined and observations recorded: the conjunctiva of the eyes for blepharitis, the teeth, limbs and hooves for abnormalities.

In addition to white-tailed deer carcasses, heads from hunter-killed white-tailed deer, elk (*Cervis canadensis*), mule deer (*Odocoileus hemionus*), bighorn sheep (*Ovis canadensis*), moose (Alces alces) and pronghorn antelope (*Antilocapra americana*) were examined from animals harvested throughout Montana and surrounding states. Domestic ruminants examined for jaw malocclusions included newborn domestic goats (*Capra aegagrus hircus*) in 2009 and heads from newborn and butchered domestic beef cattle (*Bos taurus*) between 2007 and 2009. Malformations on other vertebrate species were documented with photographs and date of observation. Birth defects were observed on individuals of multiple bird species and on three individuals of the western toad (*Bufo boreas*). Examples of recent eye malformations and liver tumors on various vertebrates were documented with photos.

To quantify brachygnathia superior (BS, or under bite) and mandibular brachygnathia (MB, or overbite), the distance between the extreme anterior of the maxillary pad and the top edge of the central lower incisors was measured in millimeters on all examined white-tailed deer fawns each year from spring of 1995 through spring of 2014.

The heart and lungs were examined on all necropsied deer, both adults and fawns. The severity of the enlargement of the right ventricle of the heart on each deer was designated with a number beginning with 0 for hearts with no enlargement (normal) to 3 for a severely enlarged right ventricle. Similarly, the inflammation of the lungs was recorded with 0 being normal, and 1 through 3 designating the severity of inflammation, and 4 to designate that the animal had died of a hemorrhage in the lungs.

To quantify the genital hypoplasia on male fawns, a measurement from the body wall to the tip of the penis sheath and a measurement of the scrotum from the body wall to the lowest point on the scrotum were taken. To document the misalignment of the hemiscrota, the length from anterior to posterior and the width from side to side of the scrotum were measured. The length of the testes was measured for comparison with the hemiscrota length. Whether the testes were in the hemiscrota or partly or completely ectopic was recorded.

In addition, a study of genital hypoplasia and shortened urogenital distance in male eastern fox squirrel (*Sciurus niger*) populations in Northern Ravalli County, MT is currently in progress. There appear to be significant numbers of similar reproductive defects on examined males of several other Western Montana rodent species, including deer mouse (Peromyscus manipulates), house mouse (*Mus musculus*), red squirrel (*Tamiasciurus hudsonicus*), northern flying squirrel (*Glaucomys sabrinus*), yellow pine chipmunk (*Eutamias dorsalis*) and yellow-bellied marmot (*Marmota flaviventris*).

Humans

Hospital discharge data, containing diagnoses collected from hundreds of hospitals by the United States Centers for Disease Control and Prevention (CDC) can give a snapshot of disease trends over time. These data are available for free download from the Web. Raw data files were available from 1998 through 2010. We downloaded the files and documentation from the CDC website. Each data file contains thousands of discharge records collected from hospitals using a statistically random sampling procedure [9]. The records contain information about the age, sex, race, geographic location and diagnoses for each discharge. The diagnoses are recorded by the International Classification of Diseases, Ninth Revision (ICD-9) codes. Up to seven diagnostic codes can be recorded for each discharge, with the first listed being the primary reason for hospital admission. We included in the set for any particular ICD-9 code any event which mentioned that code as one of the diagnostic codes for the event; i.e., we did not treat the first-mentioned code in any special way.

A computer program was written to query the data file for specific ICD codes for each year. We were interested in disease trends for three distinct age groups: (1) infants (<6 days old), (2) children (6 days–15 years old), and (3) all ages except infant (6 days–100 years old). A rate of increase, as an estimate of prevalence, over time for each particular diagnosis was obtained as follows:

$$\hat{a} = a * T / (t * P)$$

where â is the normalized hospital discharge rate for a disease in a year; a is the total number of the hospital discharge records of the disease in the year computed from the raw files; T represents the total number of hospital discharges in that year in the US; t is the total number of

Citation: Hoy J, Swanson N, Seneff S (2015) The High Cost of Pesticides: Human and Animal Diseases. Poult Fish Wildl Sci 3: 132. doi:10.4172/2375-446X.1000132

hospital discharge records in the sampled hospitals in that same year, which is computed from the raw files; and P is the total population in the US for that year. Population estimates were obtained from the CDC. For the newborn data, we assumed that T/P = 1, i.e. the number of hospital discharges for newborns was equal to the population of newborns.

Using the information in the CDC documentation, we calculated some of the standard errors in these data. The standard errors for the general population were all less than 10%. The largest standard errors were for the acquired hypothyroidism in children, which ranged between 22%-37%. Standard errors for newborns could not be calculated because they were not included in the CDC tables.

Results

US trends in pesticide usage

With the exception of glyphosate, pesticide use on crops decreased for the first 5 or 6 years after the introduction of GE crops in 1996. Survey data from the US Department of Agriculture [7] show that the use of 2,4-D and dicamba on corn steadily decreased starting about 1996 as shown in Figure 2. Applications of EPTC and Alachlor also decreased, but the use of Atrazine has remained constant. The use of 2,4-D on soy also started decreasing in 1996 as shown in Figure 3. In the meantime, glyphosate was being promoted as a pre-harvest treatment to grain, dried pea and bean, and potato crops for more even ripening, dry-down and pre-harvest weed control [10]. The use of 2,4-D and dicamba on wheat decreased, being replaced by glyphosate starting in early to mid 1990s (Figures 4-6). With the exception of fungicides used for potato blight, pesticide applications to potatoes were also decreasing (Figure 7).

After about 2002, there was a steep increase in glyphosate and 2,4-D applications on all of these crops, along with an increase in dicamba on wheat. This coincides with a steep increase in the number of confirmed cases of glyphosate-resistant weeds as shown in Figure 1.

As seen in the Figures, not all crop data were reported for all years. Data for glyphosate applications to corn, soy and wheat were interpolated as outlined in [3] and the results are shown in Figure 8.

Pesticide use in the region of interest

Prior to 1994, there was extensive use of multiple herbicides and other pesticides, especially 2,4-D and dicamba, on wheat, potato and other crops in Idaho, Washington, Oregon and other states upwind of Western Montana as shown in Figures 7 and 8. Glyphosate was also being used prior to 1994, and its use has increased significantly since 1996. The formulation for glyphosate and other commonly used herbicides applied during the growing seasons in 2006 and 2007 and since was changed to salt formulations [4] (we hypothesize that oxalic acid was introduced with these salts as an adjuvant, but this can not be confirmed).

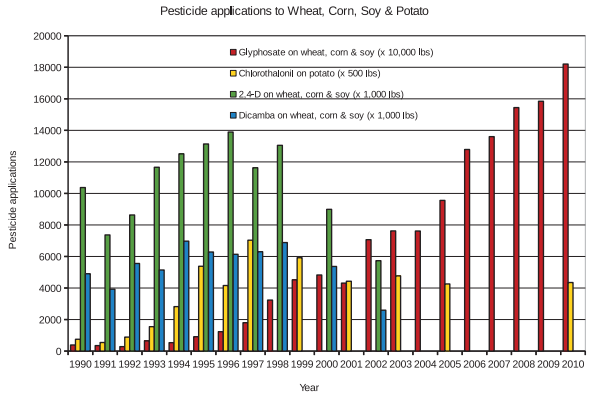


Figure 8: Total glyphosate, 2,4-D and dicamba applied to wheat, corn and soy crops; chlorothalonil applied to potato crops. Glyphosate data has been interpolated. Data for dicamba and 2,4-D were not added in the years when data were not reported.

In addition to glyphosate, 2,4-D and Dicamba as shown in Figure 8, other pesticides were widely used in Western United States prior to 1994, including picloram, atrazine and several organochlorine herbicides. Multiple fungicides were used on over 500,000 acres of potato fields in Idaho, Washington and Oregon. Many types of insecticides were also used in Western Montana and states upwind long before 1994. Even with this extensive exposure to multiple wind drift and locally applied pesticides, almost no birth defects were observed or reported on developing young in Western Montana until 1995. An epidemic of multiple birth defects began being observed on many individuals of domestic and wild animals born that spring [10,11], with a significant increase in many of the birth defects over the study period, despite substantial annual variability.

Development and health issues in wild animals and humans

In the case of the ungulates, we tabulated frequencies of multiple developmental defects as discussed in the Methods section, and noted a general pattern consisting of a high rate of disease early in the study period, a gradual decline until around 2006 and then a generally rising trend subsequently. This is consistent with the trends in pesticide use shown in Figures 2-8. We hypothesize that chlorothalonil on potatoes, along with dicamba and 2,4-D on the other crops, may contribute significantly to the early disease patterns in wildlife, whereas glyphosate is a major factor in the later rise in observed frequency.

We sought human data on disease trends in the hospital discharge data that would correspond as much as possible with the observed defects in the wild animals. This was not always easy, as jaw malocclusion is not reported explicitly in the database, nor is genital malformations. However, there are several malformations of the lower face that are tracked, such as dent facial anomalies (ICD 526), diseases of the jaws (ICD 527), diseases of the salivary glands (ICD 527) and diseases of the oral soft tissues (ICD 528), whose trends can be compared with those observed in the animals with jaw malformations. The plot we obtained for human urogenital disorders encompasses hydrocele (watery fluid around one or both testicles, ICD 778.6); hypospadias (ICD 752.6); and hydronephrosis -- obstruction of urine flow (ICD 591), and other disorders of the kidney and ureter (ICD 593). Thyme involution and dysfunction, notable in postmortem examination of the wild animals, is not normally indicated in ICD-9. Although a code exists for diseases of the thymus (254.8), it is almost never used (only 2 cases among the infant and newborn data in our data set). However, T-lymphocytes mature within the thymus gland, so its impairment can be reasonably linked to immune system disorders. In most other cases, such as the organ tumors, eye deformities, skin disorders, liver cancer and metabolic issues documented on wild and domestic animals, a more direct comparison was possible.

Our results are illustrated in Figures 9-32, and are discussed below in more detail. In addition to plots where we superimpose time trends for human data or wild animal data with pesticide usage, we also provide photos taken of a variety of wild and domestic animals

Citation: Hoy J, Swanson N, Seneff S (2015) The High Cost of Pesticides: Human and Animal Diseases. Poult Fish Wildl Sci 3: 132. doi:10.4172/2375-446X.1000132

Figure 9: Brachygnathia superior and wide lower incisors on ruminant species. A. White-tailed deer fawn skull. B. Mule deer fawn skull. C. Elk calf skull. D. Adult male bighorn sheep skull, showing short narrow premaxillary bone. E. Domestic beef calf skull. F. Skull of an adult male domestic goat.

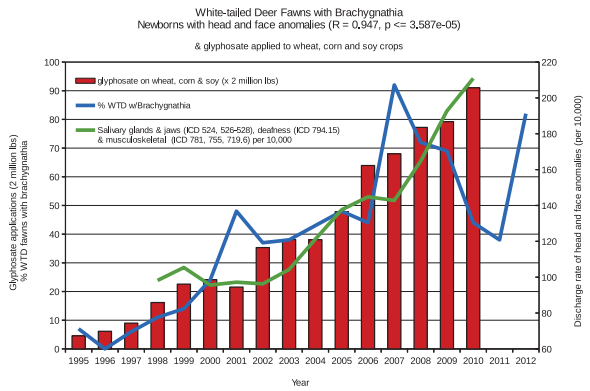


Figure 10: Comparison of hospital discharge rates for congenital facial anomalies with glyphosate applications. The graph shows the percentage of white-tailed deer fawns with brachygnathia superior from 1995-2012; congential facial and musculoskeletal anomalies; and glyphosate applications to wheat, corn and soy crops. The Pearson correlation coefficient between the newborn anomalies and glyphosate applications is R = 0.947.

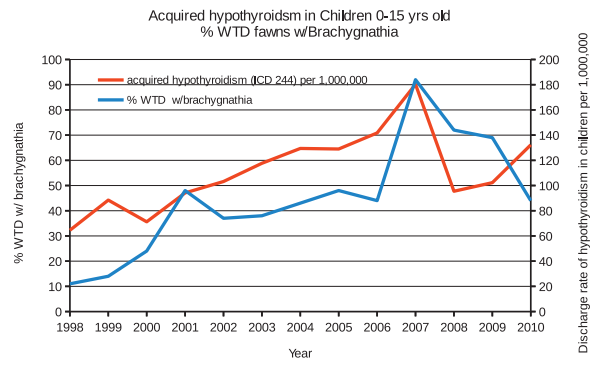


Figure 11: Hospital discharge rates for hypothyroidism in children superimposed with the percentage of WTD with brachygnathia superior.

exhibiting pathologies (Figures 9, 12, 14, 16, 18, 20, 24, 26 and 27).

Congenital head and facial malformations

Brachygnathia superior (BS), the underdevelopment of the upper facial bones of ungulate species, has been photographed in countries around the world and posted on the Internet, usually labeled as under bite. In Montana and throughout the United States, wild and domestic ungulate species appear to have an extremely high prevalence of BS, including our main study species, white-tailed deer [11] as shown in the photos in Figure 9. The percentage of white-tailed deer with BS has increased significantly in this region since 1995, as shown in Figure 10. Figure 10 also shows the prevalence of head, face and musculoskeletal anomalies in newborn infants superimposed with glyphosate applications to wheat, corn and soy crops. The newborn data correlate with glyphosate usage with a Pearson correlation coefficient of R=0.947.

We also noticed that trends in hypothyroidism in children aged 0-15 were rising, and that these patterns aligned very well with the data on brachygnathia in wild animals, both exhibiting a sharp peak in 2007 (Figure 11) approximately coincident with the changeover to salt formulations in the herbicides. Congenital hypothyroidism is common, and it is linked to other congenital disorders, for example hearing loss [12] and renal and urinary tract disorders [13]. According to Kumar, et al. [13], "Congenital hypothyroidism is the most common congenital endocrine disorder, affecting 1 in 3000 to 4000 newborns. Its incidence has increased 138% from 1978 to 2005 in New York State and 73% in the US from 1987 to 2002."

Disorders of the eyes

Figures 12 and 13 depict eye disorders in wild animals and humans respectively. Figure 12 illustrates several cases of various eye deformities in black-billed magpies (*Pica hudsonia*), great horned owls (*Bubo virginianus*), a western toad (*Bufo boreas*), a pygmy goat, and severe blepharitis on a white-tailed deer fawn that were documented by Hoy.

Figure 13 shows the time trends of congenital disorders of the eye

Figure 12: Recent eye malformations in vertebrates. A. Black-billed Magpie fledgling showing a normal-sized eye. B. Blind Black-billed Magpie fledgling right eye, both eyes were underdeveloped. C. Adult 2014 western toad with right eye not formed and left eye normal. D. Pygmy goat born in 2015 with small eye, malformed external ear and BS. E. The normal left eye and eyelids of a Great Horned Owl (GHOW). F. Underdeveloped left eye with malformed eyelids and pupil on 2014 hatch year GHOW. G. Face of a 2013 fledgling GHOW showing the malformed left pupil and malformed eyelids on both eyes. H. The malformed left pupil and eyelids of another 2014 hatch year GHOW. I. The inflamed conjunctiva of a female WTD fawn after exposure to environmental toxins.

Citation: Hoy J, Swanson N, Seneff S (2015) The High Cost of Pesticides: Human and Animal Diseases. Poult Fish Wildl Sci 3: 132. doi:10.4172/2375-446X.1000132

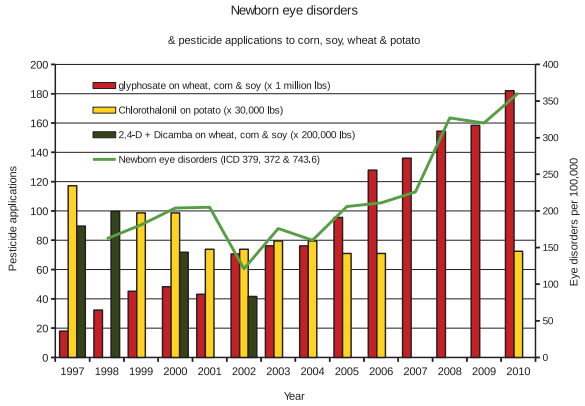


Figure 13: Hospital discharge rate for congenital eye disorders superimposed with pesticide applications to wheat, corn and soy crops. Eye disorders include: congenital anomalies of eyelids lacrimal system and orbit (ICD 743.6); disorders of conjunctiva (ICD 372); other disorders of the eye (ICD 379).

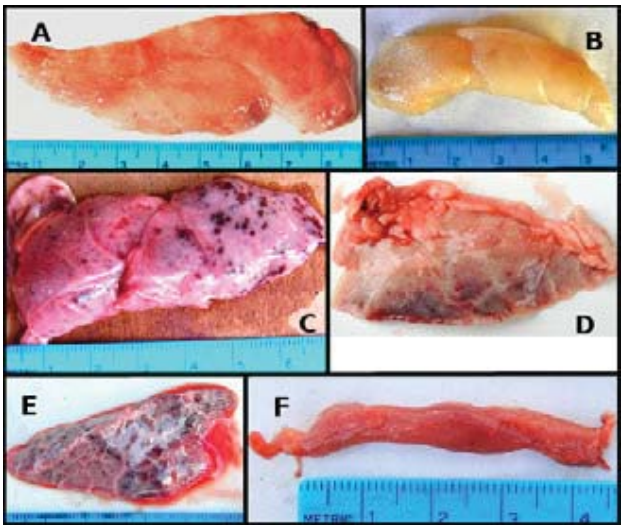


Figure 14: Newborn white-tailed deer thymus conditions. A and B. Normal thymus color and shape. C and D. Thymus with red spots throughout. E. Odd shaped, mostly red thymus. F. Undersized thymus, red throughout.

in newborns, superimposed with pesticide applications to wheat, corn, soy and potato crops. Eye disorders include congenital anomalies of eyelids, lacrimal system and orbit (ICD 743.6), disorders of conjunctiva (ICD 372), and other disorders of the eye (ICD 379). The pattern is somewhat different from that of most other human disease trends we have found, in that it more closely matches some of the time trends for the animal data and the overall pesticide data, not glyphosate alone.

Congenital thymus malformations and impaired immune system

On examined fawns of white-tailed deer, newborn domestic goats and other newborn ruminants, BS and congenital defects of the thymus (Figure 14) increased in spring of 2007 and have remained high since. This is again approximately coincident with the changeover to salt formulations in the herbicides.

There is no data in the hospital discharge records on the thymus. However, since hematopoietic progenitor cells enter the thymus from the blood and then multiply to generate a large population of T-cells, there should be some relationship between thymus impairment and diseases of the blood, especially white blood cells. We combined the following newborn blood disorders: transient neonatal thrombocytopenia (ICD 776.1), cutaneous hemorrhage of fetus or newborn (ICD 772.6), diseases of white blood cells (ICD 288) and nonspecific findings on examination of blood (ICD 790), to form the plot shown in Figure 15. While these conditions are only indirectly related to thymus problems, the trend is well matched to the rise in glyphosate usage on crops (R=0.92; p<8.2E-5). Lymphatic disorders are also rising in the human population, as discussed later in this section.

Newborn skin disorders

In recent years, observation of skin disorders, rash, blistering and

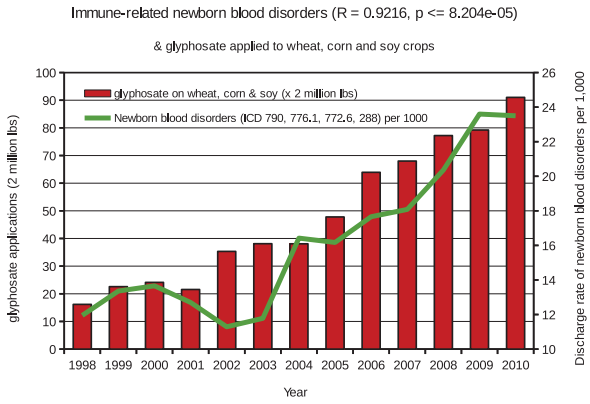


Figure 15: Hospital discharge rates for newborn blood disorders superimposed with glyphosate applications to wheat, corn and soy crops. The blood disorders are: transient neonatal thrombocytopenia (ICD 776.1); cutaneous hemorrhage of fetus or newborn (ICD 772.6); diseases of white blood cells (ICD 288); and nonspecific findings on examination of blood (ICD 790). The Pearson correlation coefficient is R=0.9216.

Figure 16: Skin disorders on wild and domestic mammals. A. Large blisters at the base of the right ear on a male WTD fawn, born 2013. B. Hair loss on shoulders, sides and hind legs of a female WTD fawn after exposure to environmental toxins, born 2003. C. Male WTD fawn inner ear skin with chemical blistering, born 2010. D. Young male eastern fox squirrel's left ear showing severe chemical skin blisters in 2005. E. Adult female dog with chemical blisters, summer 2013. F. Adult female WTD with multiple skin growths in May, 2010.

Citation: Hoy J, Swanson N, Seneff S (2015) The High Cost of Pesticides: Human and Animal Diseases. Poult Fish Wildl Sci 3: 132. doi:10.4172/2375-446X.1000132

skin tumors have been increasing on birds and wild and domestic mammals (Figure 16). We consequently examined data for skin disorders on humans. Figure 17 shows newborn skin disorders and skin disorders for the general population superimposed with glyphosate applications to wheat, corn and soy crops. The newborn skin disorders include: atopic dermatitis (ICD 691); pilonidal cyst (ICD 685); erythema and urticarial (ICD 778.8); vascular hamartomas (benign tumors) (ICD 757.32); pigment anomalies (ICD 757.33); unspecified deformities of hair, skin and nails (ICD 757.9); and meconium staining (ICD 779.84). The Pearson correlation coefficient with glyphosate usage is R=0.963. Skin disorders for the general population include: rash, swelling and changes in skin tone and texture (ICD 782); eczema (ICD 692); and psoriasis (ICD 696). The Pearson correlation coefficient with glyphosate usage is R=0.899.

Lymphatic disorders in the non-newborn populations

The thymus regulates the immune system; therefore, any problems with the thymus will result in a compromised immune system. The human lymphatic disorders, in particular, dramatically increased in 2007 at the same time that almost all of the glyphosate was being used as a salt formulation.

In conjunction with the increase in birth defects after spring of 1995, necropsied wildlife and domestic ruminants of all ages had various degrees of dilation of the lymphatic vessels on the surface of their hearts. The lymphatic vessels on hearts, especially of newborns, were more severely affected beginning in 2007, as illustrated by the last two photos of fawn hearts shown in Figure 18. Data for humans were examined for similar effects on the lymphatic system. The increase in lymphatic disorders among humans is not restricted to the infant population. Figure 19 shows the hospital discharge rate for children aged 0-15 with lymphatic disorders, superimposed with glyphosate applications to wheat, corn and soy crops. The disorders include: lymphedema (ICD 457), lymphocytosis (ICD 288.6), and Castleman's disease (angiofollicular lymph node hyperplasia) (ICD 202). The correlation coefficient is R=0.861. Figure 19 also shows the hospital discharge rate of these same lymphatic disorders over the full age range (except newborn). The correlation coefficient between this and glyphosate applications to wheat, corn, and soy crops is R=0.885.

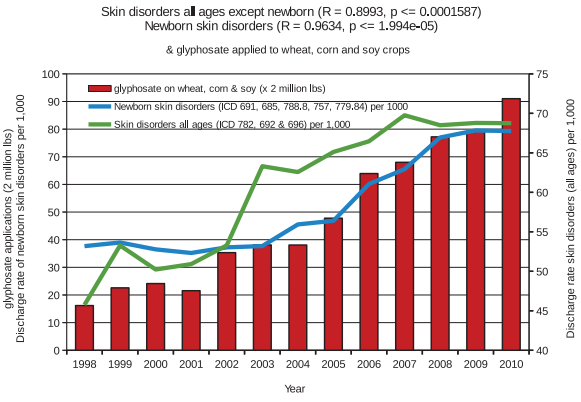


Figure 17: Hospital discharge rates for newborn skin disorders and skin disorders in the general population superimposed with glyphosate applications to wheat, corn and soy crops. The newborn skin disorders are: atopic dermatitis (ICD 691); pilinidal cyst (ICD 685); erythema and urticaria (ICD 778.8); vascular hamartomas (ICD 757.32); pigment anomalies (ICD 757.33); unspecified deformities of hair, skin and nails (ICD 757.9); and meconium staining (ICD 779.84). The Pearson correlation coefficient is R=0.9634. Skin disorders for the general population include: rash, swelling and changes in skin tone and texture (ICD 782); eczema (ICD 692); and psoriasis (ICD 696). The Pearson correlation coefficient is R=0.8993.

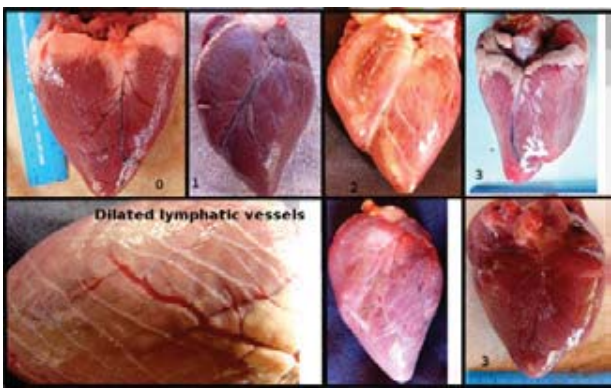


Figure 18: White-tailed deer heart conditions ranked from 0-3. 0. Normal heart. 1. Slightly enlarged right ventricle. 2. Moderately enlarged right ventricle. 3. Severely enlarged right ventricle. Dilated lymphatic vessels on heart surface of newborn fawn and close-up of dilated lymphatic vessels on newborn fawn. Corresponding numbers were used in the field to record the presence or severity of any abnormal heart condition observed.

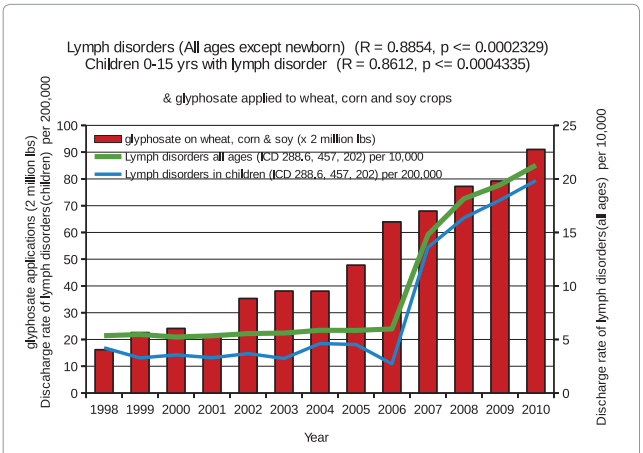


Figure 19: Hospital discharge rates for children with lymphatic disorder and lymphatic disorder in the general population superimposed with glyphosate applications to wheat, corn and soy crops. The lymphatic disorders for children are: lymphedema (ICD 457); Lymphocytosis (ICD 288.6); Castleman's disease (angiofollicular lymph node hyperplasia, ICD 202). The Pearson correlation coefficient is R=0.8612. The lymphatic disorders for the general population are: lymphedema (ICD 457); Lymphocytosis (ICD 288.6); Castleman's disease (angiofollicular lymph node hyperplasia, ICD 202). The Pearson correlation coefficient is R=0.8854.

Diseases and malformations of the heart and lung

On necropsied deer of all ages, the prevalence and severity of enlarged right heart ventricle (Figure 18) and emphysema-like symptoms on lungs (Figure 20) were high in 1998 and 1999, and then decreased until 2005, when these unusual conditions of the heart and lung increased dramatically, as shown graphically in Figure 21. Again, the increase after 2005 is approximately coincident with the switch-over to salt formulations in the herbicides.

Citation: Hoy J, Swanson N, Seneff S (2015) The High Cost of Pesticides: Human and Animal Diseases. Poult Fish Wildl Sci 3: 132. doi:10.4172/2375-446X.1000132

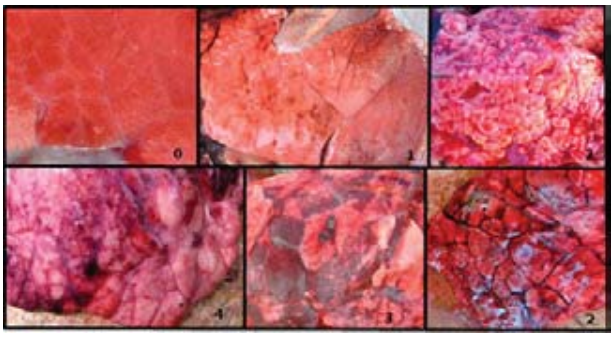


Figure 20: White-tailed deer lung conditions ranked 0-4. 0. Normal lungs. 1. Slightly bumpy on outer lobes . 2. Raised alveoli on much of lung area . 3. Raised alveoli and white areas in lungs. 4. Raised alveoli and bleeding lungs. Corresponding numbers were used in the field to record the presence or severity of any adverse lung conditions observed.

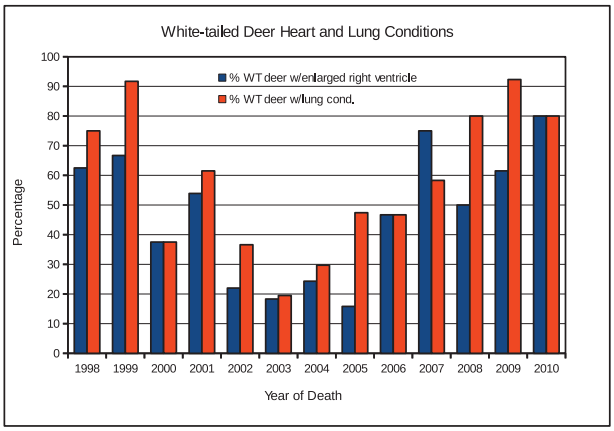


Figure 21: Percentage of white-tailed deer with heart and lung conditions, 1998-2010.

We compared this trend with human data in Figure 22. Both newborn data for congenital heart disorders and data for all ages (except newborn) on enlarged right ventricle show remarkable correspondence with glyphosate usage on core crops. The tabulated newborn heart conditions include: heart murmur (ICD 785.2); ostium secundum type atrial septal defect (ICD 745.5); patent ductus arteriosus (ICD 747.0); pulmonary artery anomalies (ICD 747.3); other congenital anomalies of circulatory system (ICD 747.8); other heart/circulatory conditions originating in the perinatal period (ICD 779.89); and bradycardia (ICD 779.81,427.89). The Pearson correlation coefficient between congenital heart defects and glyphosate applications is R=0.983, and for enlarged right ventricle it is R=0.955.

Figure 23 shows newborn lung conditions superimposed with pulmonary bleeding and edema for all ages (except newborn), and with glyphosate usage on wheat, corn, and soy crops. The newborn lung conditions include: asphyxia and hypoxemia (ICD 799); pulmonary artery anomalies (ICD 747.3); meconium passage during delivery (ICD 763.84); and other respiratory conditions of fetus and newborn (ICD 770). The ICD codes for the full population data are: pulmonary congestion and accumulation of fluid (ICD 514); extrinsic allergic alveolitis (e.g., "farmer's lung", ICD 495); and other diseases of the lung (ICD 518, excluding 518.5, surgery following trauma). The Pearson correlation between the newborn data and glyphosate applications is R=0.949 and for all ages (except newborn) R= 0.971.

Liver disease

An increasing number of mammals and birds have been observed with liver tumors, enlarged liver or liver involution. Figure 24 shows several examples of liver disease in wildlife, including tumor-like growths in a wolf (*Canus lupus*), a domestic goat, a fledgling Rock

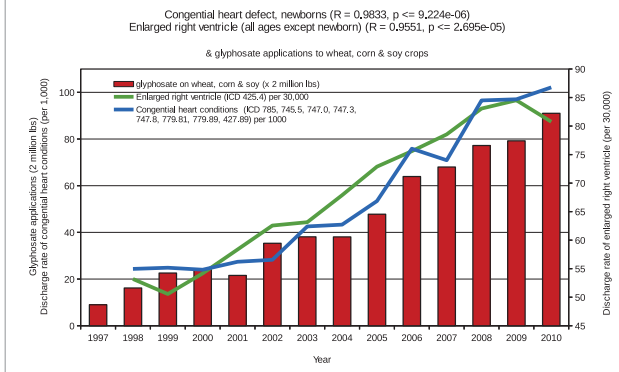


Figure 22: Hospital discharge rates for congenital heart conditions; enlarged right ventricle (ERV) for all ages (except newborn); superimposed with glyphosate applications to wheat, corn and soy crops. The congential heart conditions include: heart murmur (ICD 785.2); ostiumsecundum type atrial septal defect (ICD 745.5); patent ductusarteriosus (ICD 747.0); pulmonary artery anomalies (ICD 747.3); other congenital anomalies of circulatory system (ICD 747.8); other heart/circulatory conditions originating in the perinatal period (ICD 779.89); and bradycardia (ICD 779.81,427.89). The Pearson correlation coefficient between congenital heart defects and glyphosate applications is R=0.9833. The Pearson correlation coefficient between the ERV for all ages and glyphosate applications is R=0.9551.

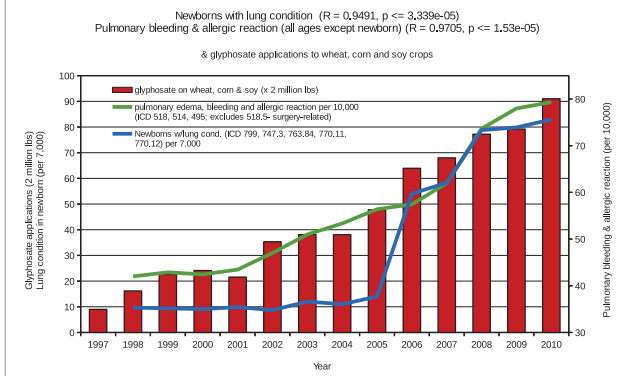


Figure 23: Hospital discharge rates for newborn lung conditions; pulmonary bleeding and edema for all ages (except newborn); superimposed with glyphosate applications to wheat, corn and soy crops. The Pearson correlation coefficient between the pulmonary disorders and glyphosate is R=0.9705. The newborn lung conditions include: asphyxia and hypoxemia (ICD 799); pulmonary artery anomalies ICD 747.3); other respiratory conditions of fetus and newborn (ICD 770); meconium passage during delivery (ICD 763.84); meconium aspiration with and without respiratory symptoms (770.11 and 12). The Pearson correlation coefficient between the newborn lung conditions and glyphosate applications is R=0.9491.

Citation: Hoy J, Swanson N, Seneff S (2015) The High Cost of Pesticides: Human and Animal Diseases. Poult Fish Wildl Sci 3: 132. doi:10.4172/2375-446X.1000132

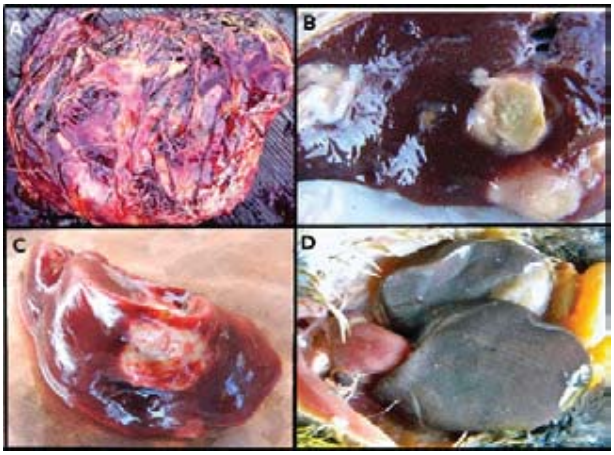


Figure 24: Liver conditions in wildlife. A. Large tumor verified as cancer, 18 cm. (7 in.) in diameter, removed from the outside of the liver on a female grey wolf. B. Tumor-like growths in the liver of an adult female domestic goat. C. Tumor-like growths in the liver of a fledgling Rock Pigeon. D. A Black-billed Magpie fledgling's enlarged, discolored liver.

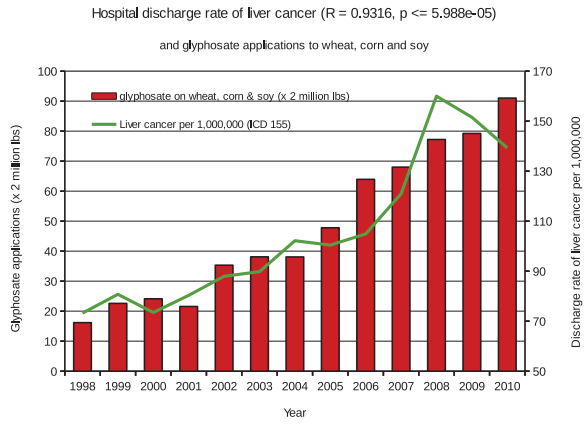


Figure 25: Hospital discharge rates of liver cancer for all ages (except newborn) along with glyphosate applications to wheat, corn and soy crops. The Pearson correlation coefficient between the prevalence of liver cancer and glyphosate applications is R=0.9316.

Pigeon (*Columba livia*), and the enlarged, discolored liver of a Black-billed Magpie fledgling.

Liver cancer in humans has also been increasing in frequency in the United States over the past two decades, with a shift towards relatively younger ages [14]. Similar trends are seen in China [15]. Figure 25 shows the hospital discharge rates of liver cancer in all ages (except newborn), alongside glyphosate usage on core crops. The Pearson correlation coefficient is R=0.932.

Congenital urogenital malformations

Birth defects of the male reproductive organs (Figures 26 and 27) have become common on mammals in Montana and appear to be occurring in some wildlife populations over much of the US [16,17]. The decrease in penis sheath length, scrotum size and the change in testes position in Montana are depicted in Figures 28 and 29. Several of the reproductive malformations have not been well studied, especially misplacement forward of the inguinal lymph node and the left spermatic cord, resulting in misalignment of the testes and corresponding hemiscrota during fetal formation of the scrotal sac (Figure 26C, 26E and 26F). This easily observed reproductive malformation was first reported in a 2002 study of white-tailed deer [10]. It has become very high in prevalence in white-tailed deer (Figure 30), and appears to also be high in several Western Montana rodent species, especially the introduced eastern fox squirrel (Figure 27). In

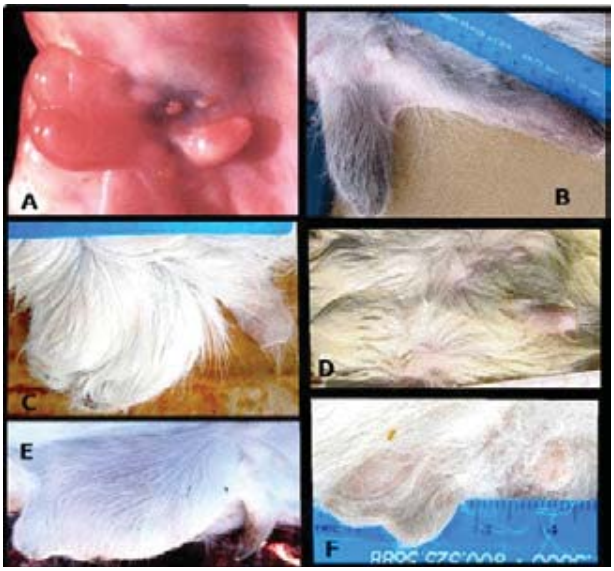


Figure 26: Normal and abnormal white-tailed deer male genitalia. A. White-tailed deer fetus, normal genitalia. B. One-year-old white-tailed deer, normal genitalia. C. One and 1⁄2 year-old white-tailed deer with misaligned hemiscrota and short penis sheath. D. One and 1⁄2 year old white-tailed deer, no scrotum formed on external skin, testes ectopic under the skin (see bumps), short penis sheath. E. Two-year-old white-tailed deer, horizontal misaligned hemiscrota, penis sheath normal. F. Newborn white-tailed deer with misaligned hemiscrota and short penis sheath.

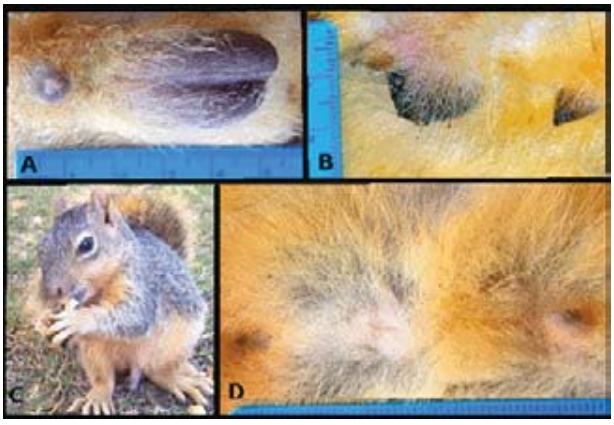


Figure 27: Normal and abnormal eastern fox squirrel male genitalia. A. Normal scrotum, very short penis sheath. B. Scrotum is misaligned with short empty skin flaps, penis sheath very short. C. White spot where scrotum should be formed, penis sheath very short. D. Live juvenile male with normal penis sheath for comparison.

Citation: Hoy J, Swanson N, Seneff S (2015) The High Cost of Pesticides: Human and Animal Diseases. Poult Fish Wildl Sci 3: 132. doi:10.4172/2375-446X.1000132

27 specimens of male eastern fox squirrel examined by Hoy from 2010 through 2014, 78% (21) had no hemiscrota formed and 7% (2) had one hemiscrota formed, all with ectopic testes, leaving only 15% (4) with a normal scrotum containing both testes. On 89%, (24) the penis sheath was less than half normal length.

Figures 28 and 29 show our data collected from WTD from 1995 to 2010 on penis sheath length (Figure 28) and testes position and scrotum length (Figure 29). In almost all years, fewer than half of the animals examined had a normal configuration. Notably, in 2006, 100% of the animals examined had ectopic testes, and more than 90% had a misaligned scrotum.

Figure 31 shows newborn genitourinary disorders compared to glyphosate applications to wheat, corn and soy crops. The human disorders include: hydrocele (watery fluid around the testicles) (ICD 778.6); hypospadias (ICD 752.6); hydronephrosis – obstruction of urine flow (ICD 591); and other disorders of the kidney and ureter (ICD 593). The Pearson correlation coefficient between genitourinary disorders and glyphosate applications is R=0.959.

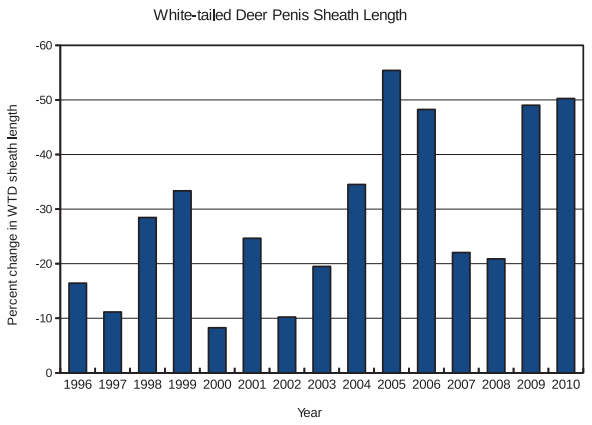


Figure 28: Percent change in white-tailed deer penis sheath length, 1996-2010.

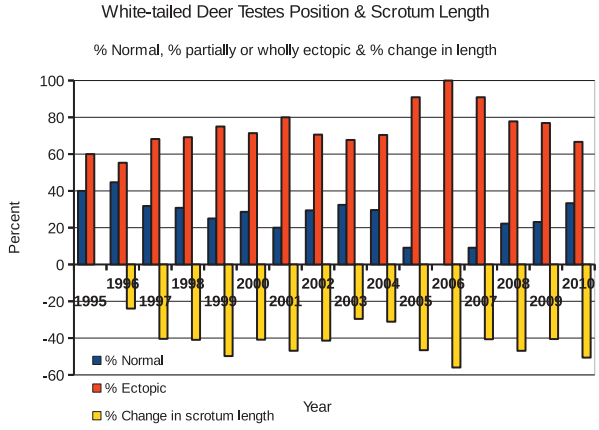


Figure 29: White-tailed deer testes position and scrotum length, 1995-2010.

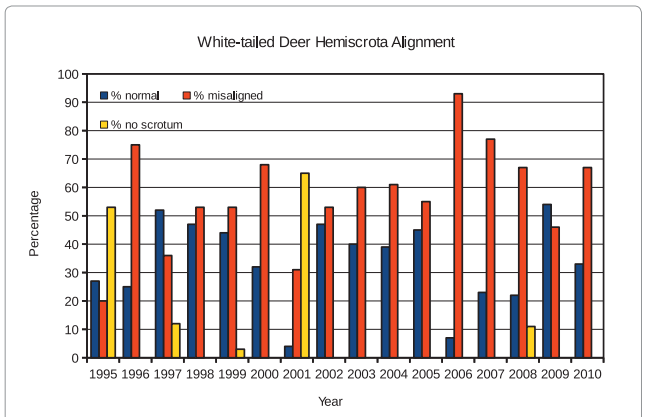


Figure 30: White-tailed deer hemiscrota alignment,1995-2010.

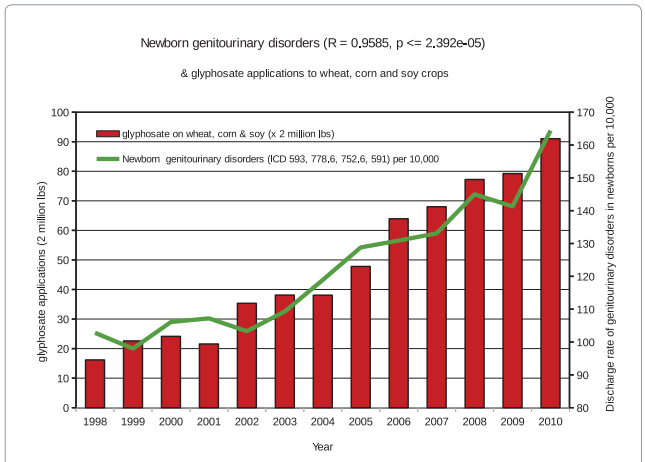


Figure 31: Hospital discharge rates for newborn genitourinary disorders compared to glyphosate applications to wheat, corn and soy crops. The genitourinary disorders include: Other disorders of the kidney and ureter (ICD 593); hydrocele (watery fluid around one or both testicles (ICD 778.6); hypospadias (ICD 752.6); and hydronephrosis- obstruction of urine flow (ICD 591). The Pearson correlation coefficient between the genitourinary disorders and glyphosate use is R=0.9585.

Failure to thrive

Failure to thrive, observed on multiple species of wild newborns, is a recognized problem in livestock, and may well be related to human failure to thrive. For example, porcine periweaning failure-to-thrive syndrome (PFTS) is an increasingly recognized syndrome in the swine industry in North America [17]. It is characterized by anorexia developing within one week of weaning followed by lethargy and, in some cases, death.

We examined the data in human newborns for comparison and found that a number of metabolic disorders have been increasing in frequency in human newborns, as illustrated in Figure 32, in step with glyphosate applications to wheat, corn and soy crops. Included are: disorders of fluid electrolyte and acid/base balance (ICD 276); underweight, feeding problems and fetal malnutrition (ICD 783); disorders of mineral metabolism (ICD 275); slow fetal growth and fetal malnutrition (ICD 764); and other congenital anomalies of the

Citation: Hoy J, Swanson N, Seneff S (2015) The High Cost of Pesticides: Human and Animal Diseases. Poult Fish Wildl Sci 3: 132. doi:10.4172/2375-446X.1000132

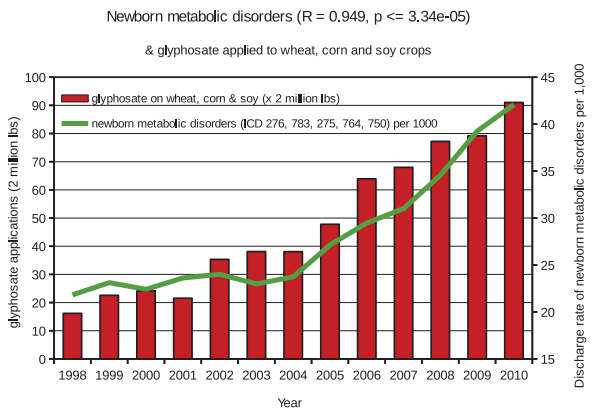


Figure 32: Hospital discharge rates for newborn metabolic disorders compared to glyphosate applications to wheat, corn and soy crops. The metabolic disorders include: disorders of fluid electrolyte and acid-base balance (ICD 276); underweight, feeding problem & fetal malnutrition (ICD 783); disorders of mineral metabolism (ICD 275); slow fetal growth and fetal malnutrition (ICD 764); other congenital anomalies of upper alimentary tract (ICD 750). The Pearson correlation coefficient is R=0.949.

upper alimentary tract (ICD 750). The Pearson correlation coefficient between these two plots is R=0.949.

Discussion

One of us (Hoy) has been documenting health status of wild animals in the mountains of Western Montana for over forty years. She has noticed a significant degradation in health over the past two decades, mainly consistent with mineral deficiencies and thyroid hormone disruption, and she surmises that the health issues are related to exposure to various pesticides being applied to crops in close proximity to the animals' habitat. Besides exposure from nearby applications, many pesticides have been shown to travel on fast-moving weather fronts to come down in rain or snow many hundreds of miles from the application site [18,19]. Low level exposure to 60% of all herbicides applied in the US are known to interfere with thyroid function, in particular 2,4-dichlorophenoxacetic acid (2.4-D) [20]. Glyphosate, another thyroid hormone disrupting herbicide [21], has also been shown to chelate multiple minerals essential to normal fetal development and health of adult animals, and to disrupt retinoic acid [22,23]. A large number of field studies have "found an association between exposure to environmental contaminants and alterations in thyroid gland structure, circulating thyroid hormones and vitamin A (retinoid) status" in multiple populations of wild vertebrates [23]. The proper quantity of minerals, retinoic acid and thyroid hormones are essential to normal development and growth as well as sustaining health during the life of the animal. Thus, exposure to environmental contaminants often results in "reproductive and developmental dysfunction" in all vertebrate classes [24].

In this paper, we present some of the evidence Hoy has gathered, and we use it to inspire investigations on human health status in the general US population. While the animals' exposure is likely mostly through water and air, we believe that human exposure is predominantly through food, as the majority of the population does not reside near agricultural fields. We have obtained government data on pesticide usage from the USDA and on human disease patterns over time from the CDC's hospital discharge data, available from 1998 to 2010. Since glyphosate is by far the most widely used herbicide, we believe it to be a major source of contamination for the humans, and any correlations between glyphosate usage over time and specific health issues is likely to reflect a causal relationship. The research literature can help to clarify whether conditions whose incidence is rising in step with rising glyphosate usage could plausibly be caused by glyphosate, given its known toxicology profile.

Most of our graphs illustrating human disease patterns involve infants, but we also present evidence from children 0-15 and from the full population excepting newborns. We found many diseases and conditions whose hospital discharge rate over the twelve-year period match remarkably well with the rate of glyphosate usage on corn, soy, and wheat crops. These include head and face anomalies (R=0.95) (Figure 10), newborn eye disorders (Figure 13), newborn blood disorders (Figure 15) (R=0.92), newborn skin disorders (R=0.96) and skin disorders in the general population (R=0.90). (Figure 17), lymph disorders in children 0-15 (R=0.86) and in the general population (R=0.89) (Figure 19), congenital heart conditions in newborns (R= 0.98) and enlarged right ventricle in all age groups except newborn (R=0.96) (Figure 22), newborn lung problems (R=0.95) and pulmonary bleeding and edema for all age groups except newborn (R=0.97) (Figure 23), liver cancer (R=0.93) (Figure 25), newborn genitourinary disorders (R=0.96) (Figure 31) and newborn metabolic disorders (R=0.949) (Figure 32).

Glyphosate's established mode of action in killing weeds is through disruption of the shikimate pathway [22,25] whose products, the essential aromatic amino acids, are important precursors to multiple biologically important molecules, including the neurotransmitters dopamine, serotonin, melatonin, and epinephrine, the B vitamin, folate, the molecule nicotoinamide dinucleotide (NAD) involved in many redox reactions, and the tanning pigment, melanin [24,26]. Gut microbes produce the aromatic amino acids using the shikimate pathway, so this ability is impaired in the presence of glyphosate. A general mode of action of glyphosate is that it chelates the soluble ions of many mineral nutrients including calcium, copper, iron, magnesium, nickel and zinc, which are essential cofactors in many specific biochemical reactions [25,27]. Glyphosate has been shown to disrupt the gut microbiome in animals, probably in part through disrupting mineral bioavailability, including manganese, iron, zinc, and cobalt [22,24]. Impaired manganese homeostasis can explain many features of disorders whose incidence is rising in the human population, including autism, Alzheimer's disease, Parkinson's disease, osteoporosis, and rheumatoid arthritis [28]. Multiple pathogenic infections due to gut dysbiosis are a major factor in the decline in orcas (*Orcinus orca*) along the north Pacific coast of the US [29], and glyphosate exposure is a likely contributor.

The newborn is highly susceptible to oxidative stress produced by free radicals [30-32]. An excess of free radicals is implicated in neonatal chronic lung disease [33], which rose sharply in the newborn population in 2006 and was highly correlated with glyphosate usage (Figure 23). Inflammation, hypoxia, ischemia, glutamate, and free iron magnify the effect of free radicals [30]. Glyphosate suppresses the first step in the synthesis of the pyrrole ring, a core structural component of heme [34-36], leading to excess bioavailability of free iron. Glyphosate also, through its chelation of manganese, disrupts the synthesis of glutamine from glutamate, because the enzyme glutamine synthase depends on manganese as a catalyst [28]. Glyphosate can be expected to induce hypoxia by interfering with hemoglobin synthesis. Furthermore, melatonin is a highly effective antioxidant [32], but its

Citation: Hoy J, Swanson N, Seneff S (2015) The High Cost of Pesticides: Human and Animal Diseases. Poult Fish Wildl Sci 3: 132. doi:10.4172/2375-446X.1000132

synthesis depends on the shikimate pathway. Melatonin appears to be both safe and effective as a supplement to treat oxidative stress in newborns [32], and it is possible that melatonin deficiency due to poor bioavailability of its precursor molecule, the shikimate pathway product tryptophan, is contributing to increased oxidative stress in newborns.

Many pesticides, including chlorothalonil and glyphosate, have been shown to work synergistically to more quickly damage vital biological processes in the cells of plants and animals [37,38]. Combinations of pesticides that chelate minerals and disrupt endocrine functions can easily have synergistic effects at extremely low doses that are not predicted by the effects found at higher doses in common toxicity studies. The National Toxicology Program defines the low-dose effects of pesticides we have commonly observed on wildlife as those effects that occur in the range of human exposures or effects observed at doses below those used for traditional toxicological studies [39]. Epidemiological studies present strong evidence that exposures to far lower levels than the concentrations of environmental toxins now found in most air and water samples are associated with diseases and birth defects in all vertebrate classes [15,40]. Glyphosate has been shown to be an endocrine disrupting hormone, able to induce growth of breast cancer tumor cells in concentrations of parts per trillion. This is well below the level usually studied in toxicology investigations [39,41]. Estrogenic compounds like glyphosate can cause sexual reversal during development in alligators, as demonstrated in studies in Florida, particularly if exposure occurs during a critical period of gestation [42].

The patterns over time for the wild animals and the humans are distinctly different, and we believe that the explanation for the high levels of defects in the early years in the wild animals, as contrasted with the humans, are due to exposure to other pesticides besides glyphosate. Between 1997 and 2006 the use of chlorothalonil and other fungicides on potato crops for blight steadily decreased in states directly upwind of our wildlife study area. There was a corresponding observable decrease in the birth defects in mammals and birds in Western Montana. When the more severe birth defects that cause mortality went down, more wild young began to survive, especially those of wild ruminant species in serious decline. By spring of 2006, the facial malformations on grazing animals had decreased to approximately half the 2001 prevalence, and the populations of white-tailed deer and other wild ungulates were steadily going up from 2002 through 2006. However, the wild ungulate populations declined sharply in subsequent years, closely corresponding with the increase in use of glyphosate after 2006 (Figures 2-8).

In addition to the well-documented effect of disrupting normal hormone functions [39], many toxic chemicals, including commonly used herbicides such as 2,4-D, picloram, and glyphosate as well as some fungicides, including chlorothalonil, adversely affect the mitochondria of the cells and disrupt energy metabolism [41,43]. Manganese is a cofactor in the important antioxidant enzyme in mitochondria, manganese superoxide dismutase (Mn-SOD). Mn-SOD plays an important role in defense against inflammation [44], known to be a major factor in cancer. Undoubtedly, such deficits in metabolism would seriously affect the ability of a pregnant female to maintain normal weight and health and would inhibit normal fetal growth, as well as a newborn's ability to maintain heat, energy and normal growth.

Evidence of increased toxicity of glyphosate formulations

The toxicology experiments used by regulatory agents to decide whether to approve a new chemical explicitly require that the active ingredient be evaluated only in isolation [45]. Glyphosate formulations are trade secrets, but they often contain other ingredients that either make glyphosate itself more toxic to cells or are themselves innately toxic [46,47]. Polyethyoxylated tallowamine (POEA) is used in many formulations as a common surfactant to improve glyphosate's effectiveness. By 2006, nearly all of the glyphosate usage was in the form of the salt formulations. Other herbicides were also converted to salt formulations, including 2,4-D and Dicamba. With continuously increasing use of the herbicide salt formulations, the symptoms of fetal hypothyroidism and multiple mineral deficiencies have increased alarmingly in wildlife.

Studies on rat liver mitochondria revealed that Roundup at 15 millimolar concentration collapsed the transmembrane potential, caused mitochondrial swelling and depressed respiration by 40% [48]. Glyphosate alone did not exhibit this effect. In vitro studies showed that only 1 to 3 ppm of POEA is enough to produce toxic effects on cellular respiration and membrane integrity [49]. The lipophilic character of POEA gives it the ability to penetrate cell membranes, and probably also enables glyphosate to gain access to cells. In addition, the salt-based formulations are suspected to be much more deadly to humans who attempt suicide through glyphosate ingestion [49].

Both glyphosate and chlorothalonil suppress cytochrome p450 (CYP) enzyme activity, resulting in a gradual depletion of the vital functions in the liver performed by the CYP enzymes [26,50]. CYP enzymes are responsible for the activation of Vitamin D, and they play a role in the production of bile acids and the synthesis and/or metabolism of cholesterol, testosterone, estrogen, progesterone and other corticosteroids. The suppression of CYP enzymes in the liver can be expected to greatly increase the toxicity of all xenobiotics to the liver, but it also has serious adverse effects on the immune system and other organ functions, including the reproductive organs [28]. While the fatality rate for glyphosate attempted suicide or accidental exposure had been relatively low in earlier reports, a paper published in 2008 claimed a fatality rate of nearly 30% [51]. Symptoms associated with human acute poisoning with glyphosate included respiratory distress, altered consciousness, pulmonary edema, shock, dysrrhythmia, and renal dysfunction. Pulmonary and renal toxicity lead to mortality in humans, following metabolic acidosis and tachycardia [49]. Exposure of glyphosate to piglets in controlled experiments showed that the POEA-based formulation was much more toxic to the piglets [52]. Multiple adverse cardiovascular effects were observed, including pulmonary hypertension, circulatory collapse, and acute metabolic acidosis.

Additionally, in spring of 2006, a relatively new class of neonicotinoid insecticides, which bear a chemical resemblance to nicotine, began being used throughout the US and in other countries. These may well have synergistic effects with glyphosate, due to glyphosate's suppression of CYP enzymes, which are needed for detoxification of neonicotinoids [53]. Our own observations on multiple disease trends in the US population reveal a sharp increase in hospital discharge rates for the health problems addressed herein around the 2006 time frame, which we hypothesize may be connected to the widespread switch to glyphosate salt-based formulations, as well as the introduction of neonicotinoids.

There was a corresponding increase after 2005 of birth defects and serious health problems on white-tailed deer fawns and other animals. This included a significant increase in enlarged right heart ventricle, lung damage, dilated lymphatic vessels on the heart surface and underdeveloped or damaged thymus on newborn white-tailed deer

Citation: Hoy J, Swanson N, Seneff S (2015) The High Cost of Pesticides: Human and Animal Diseases. Poult Fish Wildl Sci 3: 132. doi:10.4172/2375-446X.1000132

necropsied by Hoy. The original formulation of glyphosate had been shown to cause dilated heart on rabbit fetuses, and the percentage of rabbit fetuses with dilated heart was significantly elevated at all dose levels along with skeletal variations, anomalies and malformations [54]. We also observed congenital heart conditions in newborns as well as impaired lung function and enlarged right ventricle in human data (Figures 22 and 23), trending upward in step with glyphosate usage.

An extremely serious health issue with the hooves of wild ruminants began around 2007 in many areas of the United States and Canada. Moose, elk, deer, bighorn sheep and possibly other wild ungulates were observed to have disrupted growth of the keratin of the hooves, causing malformed hooves, severe lameness and resultant mortality. Laminitis has been increasing in horses throughout the United States. The keratin of the hooves of ungulates has a significant amount cholesterol sulfate in its composition, as shown in tests of horse hooves [55]. Impaired cholesterol sulfate synthesis appears to be a primary toxicity path of glyphosate [26].

Below, we will discuss some of our specific findings in more detail and link them to the research literature on animal exposures and on the effects of glyphosate and other pesticides on biological systems.

Congenital head and facial malformations

Glyphosate's mineral chelating effects result in vital minerals being unavailable to the developing cells of vertebrate young. A primary mode of glyphosate action is chelating manganese. The most common birth defect on the white-tailed deer fawns is brachygnathia superior [10], shown to be caused by fetal mineral deficiencies, particularly manganese deficiency [56-58]. This is likely connected to the important role that manganese plays as a catalyst in the production of chondroitin sulfate, which is crucial for bone development [28,59].

Given the documented increase in incidence of underdeveloped facial bones, it appears that young of both bird and mammalian species are being affected by an agent, or more likely a combination of agents, that interfere with bone growth. Our studies on the CDC hospital discharge data revealed that human infants show a rise in disorders of mineral metabolism, specifically for the three minerals, calcium, magnesium, and phosphorus (Figure 32). Human infants are also experiencing an increase in anomalies of the head and face that matches well with glyphosate usage (Figure 10). Many environmental toxins have been shown to interfere with intracellular calcium levels and bone growth in developing animals [60]. Exposure of a mammalian fetus to pesticides more than doubles the risk of mortality due to developmental malformations [61]. Exposure of bird embryos to dioxin resulted in malformed skulls and brains [62]. It is likely that disruption of both calcium and energy metabolism would have an adverse influence on normal ossification, resulting in the underdevelopment of the skull, maxilla, leg bones and more rarely other skeletal bones, as has been observed on wildlife.

Congenital thymus malformations, lymph system and thyroid

The thymus of animals exposed to toxic pesticides is often very obviously damaged upon postmortem examination, as illustrated in Figure 14. Thymus and spleen development take place mainly in the postnatal period, and zinc deprivation during this critical time in mice can result in a markedly reduced size of the thymus [63,64]. Thymus involution due to apoptosis has also been implicated in association with magnesium deficiency [65,66].

Glyphosate's chelating effects on +2 cations could lead to zinc and magnesium deficiency in exposed individuals. Glyphosate has been shown to deplete zinc as well as manganese in glyphosate-resistant soy crops [67]. Studies on rats have shown that melatonin, a product of the shikimate pathway, protects the thymus from oxidative damage [68].

Monsanto's own studies showed that exposure of albino rats to a dust aerosol containing pure glyphosate for four hours led to lesion development on the lungs and thymus in the form of red foci [69]. These align well with the red spots that were observed on thymuses from newborn white-tailed deer in our Figure 14C, 14D and 14E.

Impairment in the thymus logically leads to disorders of the lymph system, which have increased dramatically, especially since 2006, in both children and the general population (except newborn) (Figure 19). Magnesium deficiency is linked to impaired immune function [70].

The thyroid modulates endocrine activity of the thymus, and thymulin levels are correlated with thyroxin 3 (T3) and T4 levels [71]. Human hypothyroidism may therefore be related to the observed defects in thymuses of animals exposed to toxic chemicals. The trend over time of hypothyroidism among children aged 0-15 aligns remarkably well with brachygnathia in deer fawns (Figure 11). Low magnesium was shown to decrease production of the most important form of Vitamin D, essential in bone development [72]. We hypothesize that these two patterns may be linked through manganese dysbiosis. In [28], it was proposed that glyphosate leads to an excess of manganese in the brain stem and a deficiency in the vasculature, due to impaired bile flow in the liver. Excess manganese in the brain stem has been hypothesized to damage thyroid function both through direct damage to the thyroid and through dysregulation of dopaminergic modulation of thyroid hormone synthesis [73].

Newborn rats in a multi-dose study showed developmental effects and delayed sexual maturation at all doses of Chlorothalonil [74]. Chlorothalonil is a nitrile as are its metabolites. It consists of two cyanide molecules attached to a hexachlorobenzene ring. Cyanide has been shown to disrupt thyroid hormone functions, especially during fetal development [75]. Additionally, many herbicides, particularly 2,4-D and Dicamba, disrupt normal thyroid hormone function [20], thus a cumulative or synergistic effect between the organochlorine pesticides and glyphosate should be considered.

Reproductive system

Endocrine disruption is trans-generational because a mother can accumulate toxic chemicals in fat tissues over many years, which are mobilized during pregnancy and lactation, to cause harm to the fetus or infant [76]. Each stage in the development of fetal reproductive organs requires precise amounts of hormones and enzymes, in addition to other factors, such as temperature, in some species of vertebrate. An alarming study of deep-water fish in the Bay of Biscay (northeast Atlantic Ocean) published in March 2015, found a wide variety of inflammatory and degenerative lesions in all species examined, in addition to diseases of the liver, and the first case of an intersex deep-water fish [76]. In a study of pollutants in National Parks, male fish were found to have female sex organs caused by pesticides in high mountain lakes in Glacier National Park, considered to be a pristine area, only150 miles north of our wildlife study area [77].

Conversion of testosterone to estrogen by aromatase depends on CYP enzymes. Aromatase activity is decreased by glyphosate [78]. Glyphosate also decreases serum testosterone concentrations.

Citation: Hoy J, Swanson N, Seneff S (2015) The High Cost of Pesticides: Human and Animal Diseases. Poult Fish Wildl Sci 3: 132. doi:10.4172/2375-446X.1000132

Exposure to the commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology in prepubertal males [79]. Glyphosate also inhibits steroidogenesis and other normal functions of adult male reproductive organs, including the testicular cells [80,81].

Glyphosate caused cytotoxicity to progesterone-producing cells in vitro at levels that were comparable to the allowable levels in drinking water, leading to a decrease in progesterone production, and Roundup was more toxic than glyphosate [82]. Endocrine disrupting effects of Roundup on human female cells, and the activity of the pituitary-derived regulatory gonadotrophin, luteinising hormone (LH), and embryo-derived chorionic gonadotrophin (CG) activity, have not been sufficiently examined and may be contributing to the low reproductive rates in many wildlife species.

Glyphosate working synergistically with other pesticide exposures, disrupting normal hormone and enzyme levels and/or functions at key periods during fetal development, are the most likely cause of the variety of birth defects found in white-tailed deer and other wild ruminant populations since spring 1995 [10,11].

The decreased aromatase activity caused by glyphosate and possibly other pesticides may be responsible for the highly skewed sex ratio in favor of males found in Western Montana white-tailed deer fawns [10]. Studies considering the maternal condition prior to conception provide strong evidence for a relationship between maternal condition and the sex ratio in mammals [83], particularly in wild ungulates such as white-tailed deer [84]. Mineral deficiencies, damaged mitochondria and hormone disruption would certainly have an adverse effect on the condition of a pregnant female, especially in the wild. The sex ratio significantly skewed in favor of males began occurring in the Western Montana white-tailed deer fawns the same spring as the birth defects [85]. Most importantly, in 1995, marine mammals and vertebrates in other areas began being documented with unusual health problems and high rates of mortality in breeding age females and newborns [86,87].

Congenital urogenital malformations

Since 1995, an increasing prevalence of male reproductive malformations [16,24] has been observed on multiple vertebrate species. Analogous birth defects on vertebrates have more recently been shown to be the result of glyphosate exposure [88] as well as other pesticides [42]. Birth defects have been observed on multiple mammalian species [89], including human newborns [90], many individuals of multiple bird species [91,92], on reptiles, particularly alligators [93-95], and on multiple species of amphibian [96-98].

Disrupted development of the male genitalia, resulting in shortening of the penis sheath and/or the scrotum on the external skin, has been shown to be caused by a combination of zinc deficiency, disruption of retinoic acid (Vitamin A), Congenital Fetal Hypothyroidism (CFH) and dihydrotesterone (DHT) disruption, all factors symptomatic of exposure to glyphosate [26,88].

Zinc deficiency in both a pregnant female and her male fetus or fetuses is likely a contributing cause of the shortening of the penis sheath, the underdevelopment of one or both hemiscrota, and possibly of the misalignment of the hemiscrota [99,100]. Cellular zinc levels have a strong influence on the 5-alpha reductase inhibitor, which converts testosterone into DHT. DHT is instrumental in the normal growth and development of external male reproductive organs because DHT bonds to androgen receptors more effectively than other natural androgens [101]. In addition, low levels of retinoic acid (vitamin A) have been connected to zinc deficiency in developing fetuses [102,103]. Also, retinoic acid receptor alpha, a receptor for retinoic acid, has profound effects on vertebrate development by directly regulating gene expression [104].

The disruption of vitamin A caused by ingesting glyphosate [88] would likely have serious effects in the digestive system of ruminants, which may be why they appear to be highly affected by health problems and birth defects. In ruminants, significant amounts of vitamin A are degraded in the rumen, while digestibility of carotene varies in different species [105]. There are also several variables that influence carotene digestibility and vitamin A content in forage including the type of forage, the plant species ingested and the month forage is eaten, being above average during warmer months and below average during the winter. Vitamin A levels depend on adequacy of dietary fat, protein, zinc, phosphorus and antioxidants, which can be seriously lacking in the diets of wild ruminants in winter when the females are carrying developing fetuses. Vitamin A deficiency has been shown to cause lung and liver damage in rats [106].

With increasing use of glyphosate, the amount of glyphosate and other toxins in or on the ingested foliage is likely a primary factor affecting zinc and retinoic acid levels. Depending upon their size, ruminants ingest a large amount of foliage each day, resulting in consumption of biologically significant levels of glyphosate. Cellular zinc levels have a strong influence on the 5-alpha reductase inhibitor, which converts testosterone into DHT. DHT is instrumental in the normal growth and development of external male reproductive organs because DHT bonds to androgen receptors more effectively than other natural androgens [107].

Glyphosate and its synergistic effects with other pesticides, such as Chlorothalonil, are likely closely connected to the increasing prevalence of birth defects and health problems affecting the male reproductive organs since 1995 [10,11,85]. For example, genital hypoplasia, now very common, was almost unknown on white-tailed deer in years prior to 1995 [108] (Figures 28-30). A Danish study showed a steady increase in the incidence of hypospadias in boys from 1977 to 2005 [109]. Glyphosate became available on the market in 1975. Our own data show remarkable correspondence between newborn genitourinary disorders, including hypospadias, and glyphosate usage on crops (Figure 31) (R=0.96).

Thyroid hormone disrupting chemicals act synergistically such that the combined effects are greater than linear. It can be expected that simultaneous fetal exposure to glyphosate and chlorothalonil would synergistically suppress CYP enzymes, as well as thyroid hormone functions, likely resulting in even more severe teratogenic effects than that caused by exposure to either alone. Added to the depletion of cellular zinc and the disruption of gonadotropin expression caused by glyphosate [78,110] the result is a significant assault on the growth and development of the male genitalia.

Conclusion

Something is causing alarming increases in diseases and birth defects in wildlife. Something is causing alarming increases in diseases and birth defects in humans. Our graphs illustrating human disease patterns over the twelve-year period correlate remarkably well with the rate of glyphosate usage on corn, soy, and wheat crops.

Glyphosate is known to chelate vital minerals [US Patent #3160632 A]. Glyphosate is an anti- microbial and biocide [US Patent #20040077608 A1]. Glyphosate has been classified as an endocrine

Citation: Hoy J, Swanson N, Seneff S (2015) The High Cost of Pesticides: Human and Animal Diseases. Poult Fish Wildl Sci 3: 132. doi:10.4172/2375-446X.1000132

disruptor by the Endocrine Society. Glyphosate has been classified as "probably carcinogenic" by the World Health Organization and by the American Cancer Society. Glyphosate interferes with the shikimate pathway, essential to healthy gut microbes. Glyphosate inhibits the CYP enzyme activity, which is vital to a healthy functioning liver.

The strong correlations between glyphosate usage and disease patterns, the highly significant p-values and the known toxicological profile of glyphosate indicate that glyphosate is likely a major factor in the increases in the serious issues with human health documented here.

Our over-reliance on chemicals in agriculture is causing irreparable harm to all beings on this planet, including the planet herself. Most of these chemicals are known to cause illness, and they have likely been causing illnesses for many years. But until recently, the herbicides have never been sprayed directly on food crops, and never in this massive quantity. We must find another way.

References

1. Benbrook CB (2012) Impacts of genetically engineered crops on pesticide use in the U.S. – the first sixteen years. Environmental Sciences Europe 24: 2190-4715.
2. Monsanto B (2012) Preharvest Staging Guide.
3. Swanson N, Leu A, Abrahamson J, Wallet B (2014) Genetically engineered crops, glyphosate and the deterioration of health in the United States of America. J Org Syst 9: 6-37.
4. Xu XC, Brinker N, Leu A, Abrahamson J, Wallet B W, Graham JA (2006) Pesticide compositions containing oxalic acid. U.S. Patent No. 6,992,045.
5. Duncan RJS, Tipton KF (1969) The Oxidation and Reduction of Glyoxylate by Lactic Dehydrogenas.e. European J. Biochem 11: 58-61.
6. Cochrane SM, Robinson GB (1995) In vitro glycation of glomerular basement membrane alters its permeability: a possible mechanism in diabetic complications. FEBS Lett 375: 41-44.
7. USDA:NASS (2013) Agricultural Chemical Usage - Field Crops and Potatoes. USDA Economics, Statistics and Market Information System. Albert R. Mann Library. Cornell University.
8. Mosby HS (1963) Wildlife Investigational Techniques. Wildlife Society Inc., Bethesda, MD p: 171-184.
9. Dennison BA, Jenkins PL, Rockwell HL (2000) Development and validation of an instrument to assess child dietary fat intake. Prev Med 31: 214-224.
10. Hoy JA, Hoy RD, Seba D, Kerstetter TH (2002) Genital abnormalities in white-tailed deer (Odocoileus virginianus) in west-central Montana: Pesticide exposure as a possible cause. J Environ Biol 23: 189-197.
11. Hoy JA, Haas GT, Hoy RD, Hallock P (2011) Observations of brachygnathia superior in wild ruminants in Western Montana, USA. Wildl Biol Pract 7: 15-29.
12. Schroeder K (2005) The effect of hypothyroidism on hearing loss susceptibility. Hearing Journal 58: 10-12.
13. Kumar J, Gordillo R, Kaskel FJ, Druschel CM, Woroniecki RP (2009) Increased prevalence of renal and urinary tract anomalies in children with congenital hypothyroidism. J Pediatr 154: 263-266.
14. El-Seraga HB (2004) Hepatocellular carcinoma: Recent trends in the United States. Gastroenterology 127: 527-534.
15. Chen JG, Zhang SW (2011) Liver cancer epidemic in China: past, present and future. Semin Cancer Biol 21: 59-69.
16. Lyons G (2008) Effects of pollutants on the reproductive health of male vertebrate wildlife - males under threat. CHEMTrust.
17. Huang Y, Gauvreau H, Harding J (2012) Diagnostic investigation of porcine periweaning failure-to-thrive syndrome: lack of compelling evidence linking to common porcine pathogens. J Vet Diagn Invest 24: 96-106.
18. Chernyak SM, Rice CP, McConnell LL (1996) Evidence of currently used pesticides in air, ice, fog, seawater and surface microlayer in the Bering and Chukchi seas. Mar Pollut Bull 32: 410-419.
19. Seba DB, Prospero JM (1971) Pesticides in the lower atmosphere of the northern equatorial Atlantic Ocean. Atmos Environ 5: 1043-1050.
20. Román GC (2007) Autism: transient in utero hypothyroxinemia related to maternal flavonoid ingestion during pregnancy and to other environmental antithyroid agents. J Neurol Sci 15;262: 15-26.
21. Howe CM, Berrill M, Pauli BD, Helbing CC, Werry K, et al. (2004) Toxicity of glyphosate-based pesticides to four North American frog species. Environ Toxicol Chem 23: 1928-1938.
22. Huber DM (2010) What's new in agricultural chemical and crop interactions. Fluid Journal 18:1-3.
23. Rolland RR (2000) A review of chemically-induced alterations in thyroid and vitamin A status from field studies of wildlife and fish. J Wildlife Dis 36: 15-35.
24. Hamlin HJ, Guillette LJ Jr (2010) Birth defects in wildlife: the role of environmental contaminants as inducers of reproductive and developmental dysfunction. Syst Biol Reprod Med 56: 113-121.
25. Steinrücken HC, Amrhein N (1980) The herbicide glyphosate is a potent inhibitor of 5-enolpyruvylshikimic acid-3-phosphate synthase. Biochemical and Biophysical Research Communications 94: 1207-1212.
26. Samsel A, Seneff S (2013) Glyphosates suppression of cytochrome P450 enzymes and amino acid biosynthesis by the gut microbiome: pathways to modern diseases. Entropy 15: 1416-1463.
27. Johal GS, Huber DM (2009) Glyphosate effects on diseases of plants. European Journal of Agronomy 31: 144-152.
28. Samsel A, Seneff S (2015) Glyphosate, pathways to modern diseases III: Manganese neurological diseases, and associated pathologies. Surg Neurol Int 6: 45.
29. Schroeder JP, Raverty S, Zabek E, Cameron CE, Eshghi A, et al. (2009) Investigation into the Microbial Culture and Molecular Screening of exhaled breaths of Endangered Southern Resident Killer Whales (SRKW) and Pathogen Screening of the Sea Surface Microlayer (SML) in Puget Sound. Proceedings of the Puget Sound Georgia Basin Ecosystem 1-8.
30. Perrone S, Negro S, Tataranno ML, Buonocore G (2010) Oxidative stress and antioxidant strategies in newborns. J Matern Fetal Neonatal Med 23: 63-65.
31. Friel JK, Friesen RW, Harding SV, Roberts LJ (2004) Evidence of oxidative stress in full-term healthy infants. Pediatric Research 56: 878-882.
32. Gitto E, Pellegrino S, Gitto P, Barberi I, Reiter RJ (2009) Oxidative stress of the newborn in the pre- and postnatal period and the clinical utility of melatonin. Journal of Pineal Research 46: 128-39.
33. Ogihara T, Okamoto R, Kim HS, Nagai A, Morinobu T, et al. (1996) New evidence for the involvement of oxygen radicals in triggering neonatal chronic lung disease. Pediatr Res 39: 117-119.
34. Cole DJ (1985) Mode of action of glyphosate - A literature analysis. In: Grossbard E, Atkinson D, Editors. The herbicide glyphosate. Butterworths, London. Pp: 48-74.
35. Kearney PC, Kaufman DD, Editors. Herbicides Chemistry: Degradation and Mode of Action. USA: CRC Press; 1988.
36. Zaidi A, Khan MS, Rizvi PQ (2005) Effect of herbicides on growth, nodulation and nitrogen content of greengram. Agron Sustain Dev 25: 497-504.
37. DeLorenzo ME, Serrano L (2003) Individual and mixture toxicity of three pesticides; atrazine, chlorpyrifos, and chlorothalonil to the marine phytoplankton species Dunaliella tertiolecta. J Environ Sci Health B 38: 529-38.
38. Pettis JS, Lichtenberg EM, Andree M, Stitzinger J, Rose R, et al. (2013) Crop pollination exposes honey bees to pesticides which alters their susceptibility to the gut pathogen Nosema ceranae. PLOS ONE 8: e70182.
39. Colborn T, vom Saal, FS, Soto AM (1993) Developmental effects of endrocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect. 101: 378-384.
40. Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs Jr. DR, et al. (2012) Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocrine Reviews. 33: 378-455.
41. Thongprakaisang S, Thiantanawat A, Rangkadilok N, Suriyo T, Satayavivad J (2013) Glyphosate induces human breast cancer cells growth via estrogen receptors. Food and Chemical Toxicology 59: 129-136.

Citation: Hoy J, Swanson N, Seneff S (2015) The High Cost of Pesticides: Human and Animal Diseases. Poult Fish Wildl Sci 3: 132. doi:10.4172/2375-446X.1000132

42. Guillette LJ Jr, Crain DA, Rooney AA, Pickford DB (1995) Organization versus activation: the role of endocrine-disrupting contaminants (EDCs) during embryonic development in wildlife. Environ Health Perspect 103: 157-164.

43. Oakes DJ, Pollak JK (1999) Effects of a herbicide formulation, Tordon 75D(R), and its individual components on the oxidative functions of mitochondria. Toxicology 136: 41-52.

44. Li C, Zhou HM (2011) The Role of Manganese Superoxide Dismutase in Inflammation Defense. Enzyme Research.

45. Swanson NS, Ho MW (2014) Scandal of Glyphosate Reassessment in Europe. Inst for Sci in Soc 63:8-9.

46. Mesnage RB, Bernay B, Séralini GE (2013) Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. Toxicology 313: 122-128.

47. Mesnage R, Defarge N, Spiroux de Vendômois J, Séralini G-E (2014) Major Pesticides are more toxic to human cells than their declared active principles. BioMed Research International.

48. Peixoto F (2005) Comparative effects of the Roundup and glyphosate on mitochondrial oxidative phosphorylation. Chemosphere 61: 1115-22.

49. Lee HL, Guo HR (2011) The Hemodynamic Effects of the Formulation of Glyphosate- Surfactant Herbicides. Chapter 25 in Sonia Soloneski and Marcelo L. Larramendy, Ed. Herbicides, Theory and Applications. InTech.

50. Suzuki T, Nojiri H, Isono H, Ochi T (2004) Oxidative damages in isolated rat hepatocytes treated with the organochlorine fungicides captan, dichlofluanid and chlorothalonil. Toxicology 204: 97107.

51. Lee CH, Shih CP, Hsu KH, Hung DZ, Lin CC (2008) The early prognostic factors of glyphosate-surfactant intoxication. The American Journal of Emergency Medicine 26(3): 75-281.

52. Lee HL, Kan CD, Tsai CL, Liou MJ, Guo HR (2009) Comparative effects of the formulation of glyphosate-surfactant herbicides on hemodynamics in swine. Clin Toxicol (Phila) 47: 651-658.

53. Markussen MDK, Kristensen M (2010) Cytochrome P450 monooxygenase-mediated neonicotinoid resistance in the house fly Musca domestica L. Pesticide Biochemistry and Physiology 98: 50-58.

54. Rapporteur member state, Germany (1998) Monograph on Glyphosate. Annex B5: Toxicology and Metabolism. In: Glyphosate DAR, released by German government agency BVL on CD 3: 45.

55. Wertz PW, Downing DT (1984) Cholesteryl sulfate: the major polar lipid of horse hoof. J Lipid Res 45: 1320-1323.

56. Hansen SL, Spears JW, Lloyd KE, Whisnant CS (2006) Feeding a low manganese diet to heifers during gestation impairs fetal growth and development. Journal of Dairy Science 89: 4305.

57. Hansen SL, Trakooljul N, Liu HC, Moeser AJ, Spears JW (2009) Iron transporters are differentially regulated by dietary iron, and modifications are associated with changes in manganese metabolism in young pigs. J Nutr 139:1474-9.

58. Staley GP, van der Lugt JJ, Axsel G, Loock AH (1994) Congenital skeletal malformations in Holstein calves associated with putative manganese deficiency. J South Afr Vet Assoc 65: 73-78.

59. Wilson DG, Phamluong K, Lin WY, Barck K, Carano RAD, et al. (2012) Chondroitin sulfate synthase 1 (Chsy1) is required for bone development and digit patterning. Developmental Biology 363: 413-425.

60. Cox C (1998) Herbicide Factsheet: Clopyralid. J Pest Reform 18: 15-19.

61. Pastore LM, Hertz-Picciotto I, Beaumont JJ (1997) Risk of stillbirth from occupational and residential exposures. Occup Environ Med 54: 511-518.

62. Raloff J (1997) Dioxin's fowl deed: Misshapen brains. Science News 15: 133.

63. Beach RS, Gershwin ME, Hurley LS (1979) Altered thymic structure and mitogen responsiveness in postnatally zinc-deprived mice. Dev Comp Immunol 3: 725-738.

64. Beach RS, Gershwin ME, Hurley LS (1982) Growth and development in postnatally zinc-deprived mice. J Nutr 110: 201-211.

65. Malpuech-Brugère C, Nowacki W, Gueux E, Kuryszko J, Rock E, et al. (1999) Accelerated thymus involution in magnesium-deficient rats is related to enhanced apoptosis and sensitivity to oxidative stress British Journal of Nutrition 81: 405-411.

66. Mazur A, Malpuech-Brugère C, Nowacki W, Gueux E, Kuryszko J, et al. (1997) Increased apoptosis and free radical production in thymus of magnesium-deficient rats: implications to enhanced thymus involution and immunity. In Theophanides, T and Anastassopoulou, J (Eds.) Magnesium: Current Status and New Developments. Kluwer Academic Publishers, Dordrecht, the Netherlands 313-315.

67. Bott S, Tesfamariam T, Candan H, Cakmak I, Rmheld V, et al. (2008) Glyphosate-induced impairment of plant growth and micronutrient status in glyphosate-resistant soybean (Glycine max L.). Plant Soil 312: 185-194.

68. Sokolovic D, Djordjevic B, Kocic G, Veljkovic A, Marinkovic M, et al. (2013) Melatonin protects rat thymus against oxidative stress caused by exposure to microwaves and modulates proliferation/apoptosis of thymocytes. Gen Physiol Biophys 32: 79-90.

69. Monsanto Company Report No. IR-82-192 (IRDC #401-188). Glyphosate Intermediate: Acute Inhalation Toxicity Study in Rats.

70. Tam M, Gómez, M González-Gross S, Marcos A (2003) Possible roles of magnesium on the immune system. European Journal of Clinical Nutrition 57: 1193-1197.

71. Fabris N, Mocchegiani E, Mariotti S, Pacini F, Pinchera A (1986) Thyroid function modulates thymic endocrine activity. J Clin Endocrinol Metab 62: 474-478.

72. Saggese G, Bertelloni S, Baroncelli GI, Federico G, Calisti L, et al. (1989) Bone demineralization and impaired mineral metabolism in insulin-dependent diabetes mellitus. A possible role of magnesium deficiency. Helv Paediatr Acta. 43: 405-414.

73. Soldin OP, Aschner M (2007) Effects of manganese on thyroid hormone homeostasis: potential links. Neurotoxicology 28: 951-956.

74. De Castro LS, Heloísa Chiorato S (2007) Effects of separate and combined exposure to the pesticides methamidophos and chlorothalonil on the development of suckling rats. Int J Hyg Environ Health 210: 169-176.

75. Soto-Blanco B, Gorniak SL (2004) Prenatal toxicity of cyanide in goats; model for teratological studies in ruminants. Theriogenology 62: 1012-1026.

76. Feist SW, Stentiford GD, Kent ML, Santos AR, Lorance P (2015) Histopathological assessment of liver and gonad pathology in continental slope fish from the northeast Atlantic Ocean Marine Environmental Research 106.

77. Landers DH, Simonich S, Jaffe D, Geiser L, Campbell DH, et al. (2010) The Western Airborne Contaminant Assessment Project (WACAP): An Interdisciplinary Evaluation of the Impacts of Airborne Contaminants in Western US National Parks. Environ Sci Technol 44: 855-859.

78. Romano MA, Romano RM, Santos LD, Wisniewski P, Campos DA, et al. (2012) Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression. Arch Toxicol 86: 663-673.

79. Romano RM, Romano MA, Bernardi MM, Furtado PV, Oliveira CA (2010) Prepubertal exposure to commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology. Arch Toxicol 84: 309-317.

80. Clair E, Mesnage R, Travert C, Séralini GE (2012) A glyphosate-based herbicide induces in mature rat testicular cells in vitro, and testosterone decrease at lower levels. Toxicol Vitro 26: 269-279.

81. Walsh LP, McCormick C, Martin C, Stocco DM (2000) Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression. Environ Health Perspect 108: 769.

82. Young F, Ho D, Glynn D, Edwards V (2015) Endocrine disruption and cytotoxicity of glyphosate and roundup in human JAr cells in vitro. Integr Pharm Toxicol Gentocicol 1: 12-19.

83. Sheldon BC, West SA (2004) Maternal dominance, maternal condition, and offspring sex ratio in ungulate mammals. Am Nat 163: 40-54.

84. Burke RL, Birch JM (1995) White-tailed deer vary offspring sex-ratio according to maternal condition and age. Ecological Research 10: 351-357.

85. Frost K, Ruhter M, Zhang D (2008) Baseline risk assessment Bitterroot Valley, Montana. School of Public and Environmental Affairs, Indiana University, Bloomington, Indiana.

86. Estes JA (2002) What's wrong with the California Sea Otter. U.S. Department of Interior, USGS Monthly Newsletter Sound Waves.

Citation: Hoy J, Swanson N, Seneff S (2015) The High Cost of Pesticides: Human and Animal Diseases. Poult Fish Wildl Sci 3: 132. doi:10.4172/2375-446X.1000132

87. Bain DE, Balcomb III KC (1999) Populations of southern resident killer whales (Orcinus orca) from 1960-1999. Center for Whale Research.

88. Paganelli A, Gnazzo V, Acosta H, López SL, Carrasco AE (2010) Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling. Chem Res Toxicol 23: 1586-1595.

89. Carter L (1999) Mickey/Minnie. Earth Island Journal 14: 4.

90. Antoniou M, Habib MEM, Howard CV, Jennings RC, Leifert C, et al. (2012) Teratogenic effects of glyphosate-based herbicides: Divergence of regulatory decisions from scientific evidence. J Environ Anal Toxicol S: 4.

91. Handel CM, Pajot LM, Matsuoka SM, Van Hemert C, Terenzi J, et al. (2010) Epizootic of beak deformities among wild birds in Alaska: an emerging disease in North America? Auk 127: 882-898.

92. Van Hemert C, Handel CM (2010) Beak deformities in northwestern crows: Evidence of a multispecies epizootic. Auk 127: 746-751.

93. Matter JM, Crain DA, Pickford DB, Rainwater TR, Reynolds KD, et al. (1998) Effects of endocrine-disrupting contaminants in reptiles: alligators. In: Kendall R, Dickerson R, Giesy J, Suk WP, editors. Principles and processes for evaluation endocrine disruption in wildlife. SETAC Press, Pensacola, FL. P: 267-289.

94. Guillette LJ Jr, Gross TS, Masson GR, Matter JM, Percival HF, Woodward AR (1994) Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. Environ Health Perspect 102: 680-688.

95. Guillette LJ Jr, Pickford DB, Crain DA, Rooney AA, Percival HF (1996) Reduction in penis size and plasma testosterone concentrations in juvenile alligators living in a contaminated environment. Gen Comp Endocrinol 101: 32-42.

96. Hayes T, Haston K, Tsui M, Hoang A, Haeffele C, Vonk A (2003) Atrazine-Induced Hermaphroditism at 0.1 ppb in American Leopard Frogs (Rana pipiens): Laboratory and Field Evidence Environ Health Perspect 111: 568-575.

97. Ouellet M, Bonin J, Rodrique J, DesGranges JL, Lair S (1997) Hindlimb deformities (Ectromelia, Ectrodactyly) in free-living anurans from agricultural habitats. J Wildlife Dis 33: 95-104.

98. Biek R, Funk WC, Maxell BA, Mills LS (2002) What is missing in amphibian decline research: Insights from ecological sensitivity analysis. Cons Biol 16: 728-734.

99. Martin GB, White CL, Markey CM, Blackberry MA (1994) Effects of dietary zinc deficiency on the reproductive system of young male sheep: testicular growth and the secretion of inhibin and testosterone. J Reprod Fertil 101: 87-96.

100. Om A, Chung K (1996) Dietary zinc deficiency alters 5a-reduction and aromatization of testosterone and androgen and estrogen receptors in rat liver. J Nutr 126: 842-848.

101. Dean A, Smith LB, Macpherson S, Sharpe RM (2012) The effect of dihydrotestosterone exposure during or prior to the masculinization programming window on reproductive development in male and female rats. Int J Androl 35: 330-339.

102. Smith JC Jr. (1980) The vitamin A-zinc connection: a review. Ann. NY Acad Sci 355: 62-75.

103. Uriu-Adams JY, Keen CL (2010) Zinc and reproduction: effects of zinc deficiency on prenatal and early postnatal development. Birth Defects. Res. B Dev. Reprod. Toxicol 89: 313-25.

104. Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, et al. (2013) Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol 26: 878-895.

105. Rode LM, McAllister TA, Cheng KJ (1990) Microbial degradation of vitamin a in rumen fluid from steers fed co ncentrate, hay or straw diets. Canadian Journal of Animal Science 70: 227-233.

106. Baybutt RC, Hu L, Molteni A (2000) Vitamin A deficiency injures lung and liver parenchyma and impairs function of rat type II pneumocytes. J Nutr 130: 1159-1165.

107. Marburger RG, Robinson RM, Thomas JW (1967) Genital hypoplasia of white-tailed deer. J Mammal 48: 674-676.

108. Lund L, Engebjerg MC, Pedersen L, Ehrenstein V, Nørgaard M, et al. (2009) Prevalence of hypospadias in Danish boys: a longitudinal study, 1977-2005. European Urology 55: 1022-1026.

109. Crofton KM, Craft ES, Hedge JM, Gennings C, Simmons JE, et al. (2005) Thyroid- Hormone Disrupting Chemicals: Evidence for Dose-Dependent Additivity or Synergism. Environ Health Perspect 113: 1549-1554.

110. Dallegrave E, Mantese FD, Oliveira RT, Andrade AJ, Dalsenter PR, et al. (2007) Pre- and postnatal toxicity of the commercial glyphosate formulation in Wistar rats. Arch Toxicol 81: 665-673.

Citation: Hoy J, Swanson N, Seneff S (2015) The High Cost of Pesticides: Human and Animal Diseases. Poult Fish Wildl Sci 3: 132. doi:10.4172/2375-446X.1000132

Glyphosate, Roundup, Glyphosate-Tolerance GM Soybeans, Chemical Extracted Soybean Food Oil/Soybean Powder Cause Serious Harm to Health of American/Chinese People

Reference Information

June 18, 2014

Compiled and translated by
I-wan, Chen (cheniwan@cei.gov.cn)
(A British citizen living, studying and working in China for 64 years)
Advisor, Committee of Disaster History for
China Disaster Prevention Association

(Advisor Chen's blog in China has posted 1192 papers on relevant issues: http://blog.sina.com.cn/cheniwan)

Index

Twelve Health Deteriorate Trends in Both USA and China

Index

1）USA Infertility Reaching 15%, China Closely Following Infertility Patients Already Exceeding 50 Million

2）Precocious puberty Rapidly Increase in both USA and China

3）USA and China's Children Asthma Incident Rate Increase Year by Year, Even if Air Quality is Improved

4）USA and China's Children with Chronic Diseases Rapidly Increase: The percentage of children with a current chronic disease rose to 25% in 2000-2006, the percentage of kids who had ever had a chronic illness grew to 52% in the same time in USA; In China, among children, youth and youngsters, incident rate of high blood pressure 9%, lipid disorders 9.8%; based on survey in a number of provinces and cities, the incident rate of pre-school age children with high blood pressure has already reached 2% - 4%, and for school children already 4% - 9%!

5）USA and China's Patients Reason Unclear Increase During Past Ten Years: 44% of Americans in 2005 had at least one chronic medical condition; Chinese residents with chronic disease is already 20%, the total number of determined diagnosed chronic patients reach 260 million. During past ten years, there are 10 million chronic disease patents newly diagnosed each year.

6) 8.3% Americans Have Diabetes, 9.7% Chinese Have Diabetes Exceeding USA, the Risk is Very High for the "East Asian Weakling" Nightmare to Arrive Again for China!

7）About one in every 33 babies (about 3%) is born with a birth defect, including approx 1% diagnosed with congenital heart defects in USA; the incidence of newborns with defects in China at present is about 5.6%, the incidence of newborns with defects shows a trend of increase, increased from 877,000 in 1996 to 1.499 million in 2010, increase rate 70.9%, with congenital heart disease becomes the number one defect!

8) In USA, 78% increase of autism during 2007 - 2010, In China, autism rate in various areas has reached 1.5%, increased 10 times over 20 years, environmental, chemical, biological factors pointed as the culprit!

9) Studies indicate possible links between Alzheimer's disease and Parkinson's disease, indicate 10% increase of annual incidence of Alzheimer's disease from 2000 to 2010 in USA; During the past 20 years (1998 - 2008), the average ages of Alzheimer's disease patients has advanced from 65 years to 55 years; In the USA, 50,000-60,000 new cases of Parkinson disease are diagnosed each year; Based on most conservative estimations, the incidence of Parkinson disease during recent 20 years in China has at least increased over 20 times!

10) 1988-2004 USA incidence of primary liver cancer rapidly rose 90%, China's liver cancer patients accounts for 54.26% of World's total

11) Children cancer increasing, especially leukemias, dramatically increased in USA and China.

12) Inflammatory Bowel Diseases rapidly increased 65 percent increase from 2000 to 2009, and increased 12 times in Shanghai from 2003 to 2013!

1）USA Infertility Reaching 15%, China Closely Following Infertility Patients Already Exceeding 50 Million

USA infertility continue increases: 1995 10%; 2002 12%; 2009 15%

- **1995:** According to National Survey of Family Growth, CDC 1995, **approximately 6.1 million women and their partners in the U.S. are affected by infertility, i.e., 10% of the total reproductive-age population**. [2]
- **2002: Infertility affects about 7.3 million women and their partners in the U.S. -- about 12% of the reproductive-age population** (Source: *National Survey of Family Growth, CDC 2002*). [3]
- **2009**: **Based on reported cases alone, almost 15 percent of adult Americans experience infertility.**Despite medical advances in the treatment of infertility over the last two decades, that rate has not declined. In fact, most experts believe it has risen. Meanwhile, as the population of the United States has grown since 1970, the number of infertile couples has more than doubled. **At present, an estimated 9 million individuals are affected by infertility.** [4]

China：In China, the rate of infertile couples has reached 1/8, the number of infertile patients has exceeded 50 million!

- Wu Jing-chun, Executive Committee Member of China Women Federation, former Deputy Director of China Birth Planning Commission, on the "Sino-U.S. Infertile Academic Forum" held in Dec., 2011 stated, due to various factors and work pressure, the ratio of infertile couples have reached 1/8, infertile patients have exceeded 50 million in China, and are gradually increasing. She said, a birth crises is approaching. [5]
- Prof. Chen Qiu-bo, honorary director, Hangzhou Guangren Infertility Research Institute, in March 2012 pointed out: Statistics show, the infertility incidence among appropriate age couples is sharply increasing, the average incidence in the country is 12.5%-15%, meaning, one infertility among each 8 couples. [6]

[1] Brady E. Hamilton, Paul D. Sutton, Recent Trends in Births and Fertility Rates Through June 2011
http://www.cdc.gov/nchs/data/hestat/births_june_2011/births_june_2011.htm

[2] women-health-info, Infertility statistics
http://www.women-health-info.com/331-Infertility-general.html

[3] American Society for Reproductive Medicine: Quick Facts About Infertility
http://www.asrm.org/detail.aspx?id=2322

[4] A. Toth, Chapter 1 New Hope for Reproductive Health，Infertility Solution Book, 2009
http://www.fertilitysolution.com/Fertility-Solution-Book/Chapter-1-New-Hope-for-Reproductive-Health.html

[5] China News Website, 2011-12-26, Executive Committee Member of China Women Federation: Infertile patients have exceeded 50 million in China:
http://www.chinanews.com/jk/2011/12-26/3560743.shtml

[6] Zhejiang Online Health website, 2012 China Xihu Infertility Diagnosis & Treatment Senior Forum is held in Hangzhou, 2012-03-20
http://health.zjol.com.cn/05zjhealth/system/2012/03/20/018338078.shtml

2）Precocious puberty Rapidly Increase in both USA and China

USA:

About 15 percent of American girls now begin puberty by age 7, according to a study of 1,239 girls published last year in the journal Pediatrics. One in 10 white girls begin developing breasts by that age - twice the rate seen in a 1997 study. Among black girls, 23 percent hit puberty by age 7. [1]

China:

- The age of children in Guangzhou entering puberty has from average 13 years old advanced to 11 years; Based on study of a few thousand children in Guangdong province, the rate of "early puberty" has increased from 0.5% in 1994 increased to 1.3% in 2004, the average rate of "early puberty" in China is 1%. [2]
- Early puberty rate survey of 23 thousand children in Zhejiang coast area reveals: 84 children were identified early puberty, 9 boys and 75 girls, indicating rate of early puberty 0.38%, and girls with early puberty is 7.3 times of boys.
- The survey also revealed that the rate of early puberty is 0.45% in cities and towns, higher than 0.32% in rural areas; 0.50% in economic developed areas, and 0.32% in undeveloped areas; 0.52% in areas close to highways and industrial polluted areas, and 0.28% in less polluted areas.

[1] Jennifer Ashton, Puberty starting earlier for many girls: study,
CBS News, April 12, 2011

http://www.cbsnews.com/2100-500172_162-20053084.html

[2] Puberty of children in Guangzhou have advanced two years, rate of "early puberty" is also rather high, Information Times, 2004-09-12
http://news.163.com/40912/2/1021BNN80001122B.html

[3] Jin Li-peng, Early puberty rate survey announced for children in Zhejiang coast area, girl with early puberty 7.3 times of boys, Hangzhou network - Municipality Express, 2005-05-30
http://www.zjol.com.cn/05zjnews/system/2005/05/30/006122163.shtml

3）USA and China's Children Asthma Incident Rate Increase Year by Year, Even if Air Quality is Improved

USA:

- CHICAGO (Reuters) - About 25 million Americans, or 1 in 12 people, have asthma, a figure that is rising despite efforts to control key asthma triggers such as indoor smoking, U.S. government researchers said on Tuesday.
- According to the U.S. Centers for Disease Control and Prevention, an additional 4.3 million people were diagnosed with asthma from 2001 to 2009. The life-long disease causes wheezing, tightness in the chest, coughing and shortness of breath.
- "Despite the fact that outdoor air quality has improved, we've reduced two common asthma triggers - secondhand smoke and smoking in general -- asthma is increasing," Paul Garbe, chief of the CDC's Air Pollution and Respiratory Health Branch, said in a statement.
- Asthma diagnoses increased among all demographic groups between 2001 and 2009, but children were hit hardest, with asthma affecting 9.6 percent of children, compared with 7.7 percent of U.S. adults.

China:

It is learn that there is close to 300 million asthma patients, China has close to 30 million asthma patients, incident rate is between 1% to 4%, children below 16 years old have an even higher incident rate. [7] The national average level is 1.84%. [9]
According to the national epidemiology investigation on children, the asthma incident rate has increased 60% during the past 10 years. [6]

Beijing: In Beijing, the children asthma incident rate among 0-14 year age group has already exceeded 2%, meaning each middle school, primary school class on average has one asthma child. [6]

Nanjing: The Nanjing Children Hospital every ten years carries out sample survey on incidents of children asthma. Zhao De-yu told the journalist: "According to our investigation, in 2000 the incident of children asthma in the 0 – 14 age group was about 2.3%, by 2010 has exceeded 3%." Zhao is Director, Internal Medicine, Nanjing Children Hospital, as well as vice director of pediatric specialist, Pediatric Sub-division, Nanjing medical association. [7]
Zhao De-yu reported, the incident of asthma increases year by year. In 1990, the asthma incident in Nanjing was 1.4%, but by 2000 has almost doubled, reaching 2.3%. "Our Asthma outpatient service earlier received about 40 – 60 patients each morning. Since the past 1 – 2 years, each weekend morning could reach 200 patients."

Jinan: The journalist learnt at a number of hospitals during activities seeing patients on volunteer basis, due to environment and other factors, the incidents of children asthma has significantly increased compared with 10 years ago. [8]
On the "World Asthma Day" (2011-05-04) during activities seeing patients on volunteer basis, the Shandong Provincial Hospital and the Shangdong University Qilu Children's Hospital examined 164 children, and 46 of them were diagnosed with asthma.

Xiamen: According to the national epidemiology investigation on children in 2000, children's asthma incident rate in Xiamen is 3.96%, much higher than the national average level of 1.84%. [9]

[1] Health News, U.S. Asthma Rates Rising but Reasons Unclear: CDC, 2011-05-16
http://www.healthnews.com/en/news/US-Asthma-Rates-Rising-but-Reasons-UnclearCDC/1QYyd9qej3SegShH3yNK4F/

[6] Qianlong website, 2011-5-02, Asthma Rate in Beijing exceeds 2%
http://news.cntv.cn/20110502/103535.shtml

[7] People's website, 2012-03-14, Asthma Rate in Nanjing 2%, specialists suggestion identify the allergen to prevent http://js.people.com.cn/html/2012/03/14/89516.html

[8] Jinnan Times, 2011-05-04, Prevalence of asthma clearly increases in Jinnan
http://news.163.com/11/0504/04/736CG7TT00014AED.html

[9] Southeast Website, 2011-05-01, Xiamen Children Asthma Incident Rate Much Highter Than National Level, Caused by Room Dust and Acarids
http://news.66163.com/2011-05-01/513214.shtml

4）USA and China's Children with Chronic Diseases Rapidly Increase: The percentage of children with a current chronic disease rose to 25% in 2000-2006, the percentage of kids who had ever had a chronic illness grew to 52% in the same time in USA; In China, among children, youth and youngsters, incident rate of high blood pressure 9%, lipid disorders 9.8%; based on survey in a number of provinces and cities, the incident rate of pre-school age children with high blood pressure has already reached 2% - 4%, and for school children already 4% - 9%!

USA

- A follow-up study of 5,001 children from 1988 to 2006 and followed each child for six years, published by *Journal of the American Medical Association* in Feb. 2010 shows: More than half of children ages 8 to 14 have had a long-term health problem at some point, such as obesity, asthma, a learning disability or other ailment. [1] Though the percentage of children with a current chronic disease rose to 25% in 2000-2006, the percentage of kids who had ever had a chronic illness grew to 52% in the same time. [1-2]
- Much of the increase in chronic diseases was a result of obesity, says author Jeanne Van Cleave of the MassGeneral Hospital for Children in Boston.[1]
- In another study published in Academic Pediatrics, an estimated 43% of US children (32 million) currently have at least 1 of 20 chronic health conditions assessed, increasing to 54.1% when overweight, obesity, or being at risk for developmental delays are included. This tells us matters are much worse now–perhaps 50% are now chronically ill not counting obesity. [2]
- Furthermore, as this study is based on latest data 2007 data, 4 years old data, thus the situation in 2012 should be even worst![2]
- The study published by the June 2011 issue of Academic Pediatrics also said, developmental disability is on the rise in the U.S. Between 1997 and 2008, the number of school-age children diagnosed with autism, ADHD, or another developmental disability rose by about 17 percent. It also said roughly 15 percent of kids – nearly 10 million – have such a disability.[2-3]
- These rates will continue to increase, in view of one of the authors[2], since nothing is really changing in terms of the amount of unhealthy, industrially processed foods families and children eat, the poor USDA dietary guidelines being promoted as healthy, the very high rate of vaccinations and their damages inflicted, a continuing flow of pharmaceuticals, and of course, other environmental and lifestyle factors. So in five years we may be looking at 60-70% of American children being chronically ill and even more obesity and infertility.[1]
- A further study published by the Archives of Psychiatry in March and it found that the U.S. has the highest in the world lifetime rate of bipolar disorder at 4.4%, and India the lowest, with 0.1%, as reported by CNN. In other words, the U.S. has 44 times more bipolars (manic-depressives) than India. Bipolar disorder is characterized by cycles of depression and mania, a euphoric, high-energy state that can result in heightened levels of creativity or output as well as erratic or risky behavior. People with bipolar disorder are at high risk of substance abuse and suicide, and treatment includes psychiatric care and medication. [2]
- Boys had a higher prevalence overall and for a number of select disabilities compared with girls. Hispanic children had the lowest prevalence for a number of disabilities compared with non-Hispanic white and black children. Low income and public health insurance were associated with a higher prevalence of many disabilities. Prevalence of any developmental disability increased from 12.84% to 15.04% over 12 years., i.e. a 17% increase. Autism, attention deficit hyperactivity disorder, and other developmental delays increased, whereas hearing loss showed a significant decline. These trends were found in all of the sociodemographic subgroups, except for autism in non-Hispanic black children. [4-5]
- "We don't know for sure why the increase happened," study author Sheree Boulet of the Centers for Disease Control and Prevention, told Reuters. [4]
- But Philip Landrigan of the Mount Sinai School of Medicine in New York City, told USA Today that improvements in diagnosis can't fully explain the increase. Research suggests that environmental chemicals - including pesticides and the phthalates found in soft plastics - can affect kids' mental development, he said. [4]

China:

- The result of the recently announced "2010 China's Urban Health Survey" indicates, chronic disease incidents in China appear with a trend towards younger ages. Sanitation specialists suggest, should enhance guidance and interference to individual lifestyle. Mortality caused by chronic diseases accounts to 80% in China. As social pressure increases, medium-age and young people with chronic disease begins to increase, with a trend of which incident rates gradually increase year by year. Specialists consider, compared with infectious disease, the latent period of chronic disease is rather long, thus should give emphasis on prevention. [6]

- China carries out national resident nutrition and health condition survey every 10 years. According to the recent survey, obesity and overweight already become an outstanding health problem among urban children, youth and youngsters. The result of the 2005 national student corporeity and health survey also indicated, the obesity rate and overweight rate of urban boys 7 – 22 years age has already reached 13.25% and 11.39%, 1.4% and 2.7% respectively higher than 2000. [7]
- Yang Xiao-guang, researcher, China CDC, considers, it is estimated that the incidents of overweight and associated chronic diseases shall increase substantially during the next 10 – 20 years. If powerful interference actions are not adopted, the ratio of residents with normal body weight shall reduce from 70% at present to 1/3. [7]
- In China, within every 10 diabetes patient, one of them is a youth or youngster, with type 2 diabetes increasing most rapidly. Estimated accordingly, China already has close to 10 million youth and youngsters with diabetes. With Beijing as example, within the 6 – 18 year age group of urban and rural children and youngsters, the incident rate of diabetes is 0.57%; the incident rate of impaired fasting glucose (IFG) i.e. pre-diabetes, has already reached 1.35%. Since the 80s of the past century, the incident rate of diabetes in China almost doubles every 10 years. [8]
- The Beijing Health Bureau organized a number of hospitals to carry out an overall survey of overweight, high blood pressure, type 2 diabetes and lipid disorders among age group below 18 years. The result shows: Among children, youth and youngsters, incident rate of high blood pressure 9%, lipid disorders 9.8%. This means, within every 100 children there is about 9 children with high blood pressure, and about 10 children with dyslipidemia. And among children with diabetes, those with type 2 diabetes already accounted to 43%, compared with less than 5% 10 years ago. Specialist analysis, the overweight is the key for causing the increasing trend of these diseases. In Beijing, the rate of overweight among children, youth and youngsters, is already close to 10%, meaning a 47% increase since 2000. [9]
- Prof. Wang Wen, National Cardiovascular Center, Fuwai Hospital of China Academy of Medical Science, points out, based on survey in a number of provinces and cities, the incident rate of pre-school age children with high blood pressure has already reached 2% - 4%, and for school children already 4% - 9%, in which overweight children are majority. In USA and Japan, the incident rate for children respectively is 14.1% and 13.3%. High blood pressure is quietly stealing Children's health. [10]

References

[1] Liz Szabo, More children have chronic diseases; study cites obesity, USA Today,2010-02-17
http://www.usatoday.com/news/health/2010-02-17-chronic17_st_N.htm

[2] Augie, Alarming New Studies: 50% of U.S. Children Have Chronic Disease/Disorders, 21% Developmentally Disabled, Journal Living Food, 2011-5-26
http://journal.livingfood.us/2011/05/26/alarming-new-studies-50-of-u-s-children-have-chronic-illnesses-21-developmentally-disabled/

[3] Christina D. Bethell at el., A National and State Profile of Leading Health Problems and Health Care Quality for US Children: Key Insurance Disparities and Across-State Variations, Academic Pediatrics, 2011 May-Jun;11(3 Suppl):S22-33.
http://www.ncbi.nlm.nih.gov/pubmed/21570014

[4] David W Freeman, Developmental disability on rise in U.S. kids: Why?, CBS, 2011-05-23
http://www.cbsnews.com/8301-504763_162-20065315-10391704.html

[5] Coleen A. Boyle at al., Prevalence of Autism Spectrum Disorders in Hispanic and Non-Hispanic White Children, Pediatrics 2012; 129:3 e629-e635
http://pediatrics.aappublications.org/content/early/2011/05/19/peds.2010-2989.abstract

[6] China radio website, 2011-10-20, Prevalence of chronic disease appear with trend of lower age http://www.chinanews.com/jk/2011/10-20/3403689.shtml

[7] Jiaxing online health frequency, 2011-01-06, Obesity disturbs Chinese, chronic disease caused increases
http://www.cnjxol.com/health/news/content/2011-01/06/content_1573035.htm

[8] People's website, 2010-06-01, China ranks the No.1 nation in diabetes, children's diabetes doubled in ten years
http://news.xinhuanet.com/health/2010-06/01/c_12164803.htm

[9] Beijing TV website, 2008-04-06, children chronic disease, prevalence increasing
http://www.btv.org/btvweb/07btv1/2008-04/06/content_295166.htm

[10] Life Times, 2011-06-13, Obesity children take care of high blood pressure, maintaining one hour exercise each date is helpful for prevention
http://health.sina.com.cn/d/2011-06-13/074322630082.shtml

5）USA and China's Patients Reason Unclear Increase During Past Ten Years: 44% of Americans in 2005 had at least one chronic medical condition; Chinese residents with chronic disease is already 20%, the total number of determined diagnosed chronic patients reach 260 million. During past ten years, there are 10 million chronic disease patents newly diagnosed each year.

USA:

- Based on government survey data, 44 percent of Americans in 2005 had at least one chronic medical condition, which could include diabetes, high blood pressure, high cholesterol levels, cancer, arthritis, heart failure and others. That compares to 41 percent in 1996. The percentage of Americans with three or more chronic illnesses rose even more sharply, from 7 percent in 1996 to 13 percent in 2005. [1]

China:

- At the same time our nation's economy enjoys rapid development, we are also encountered by the heavy burden of chronic diseases, of which incidences is quickly increasing, and appearing among younger people. "During past ten years, the number of diagnosed chronic disease patients has increased 14.3%, which include diabetes increasing over three times, high blood pressure increasing over 1.5 times, cerebrovascular disease increasing one time, coronary heat disease increasing 63%, cancer increasing 60%." Result of the 4th National Sanitation Service General Survey show, the percentage of Chinese residents with chronic disease is already 20%, the total number of determined diagnosed chronic patients reach 260 million. During past ten years, there are 10 million chronic disease patents newly diagnosed each year. Chronic diseases account for the cause of 85% mortality, and 69% of the medical care financial burden. Chronic diseases bring substantial burden to family life, sanitation service system and public fiancé, especially have serious effect on the low income population, According to statistic, the percentage of chronic disease medical treatment cost within the total sanitation expenditures has already from 47.4% in 1990 increased to 70% in 2008, amounting to about RMB850 billion (approx. USD133 billion)，have already become a serious public sanitation problem and social problem. The WHO estimates, China's direct medical costs for chronic diseases by 2015 will exceed USD500 billion.
- Report "China Chronic Desease Report and New Developments in International Chronic Disease Prevention and Control" by Wang Shi-yong, Senior health specialist, World Bank China Office, revealed:
- Shi Xiao-ming, Chief, Chronic Disease Community Section, CDC, revealed: At present China has 305 million obese, 120 million overweight, 236 million high blood pressure, 32.92 million high cholesterin and 96.81 million diabetes patients.
- China's middle and old age people have on average 3.1 types of deseases.
- In April, 2011, the Ministry of Health issued The China Chronic Disease Report. The Report shows that the health of Chinese residents is facing serious challenge by chronic diseases. The first 4th causes of deaths are cerebrovascular disease, cancer, respiratory system disease and heart disease, all chronic disease. [2]
- Chronic disease is one of the greatest challenges to health of mankind. At the same time our nation's economy enjoys rapid development, we are also encountered by the heavy burden of chronic diseases, of which incidences is quickly increasing, and appearing among younger people. The number of already diagnosed chronic patients exceeds 260 million. Chronic diseases account for the cause of 85% mortality, and 69% of the medical care financial burden. Chronic diseases bring substantial burden to family life, sanitation service system and public fiancé, especially have serious effect on the low income population, have already become a serious public sanitation problem and social problem. [3]
- Li Lian-da, Academician, China Academy of Engineering, revealed: "During past ten years, the number of diagnosed chronic disease patients has increased 14.3%, which include diabetes increasing over three times, high blood pressure increasing over 1.5 times, cerebrovascular disease increasing one time, coronary heat disease increasing 63%, cancer increasing 60%." [4]
- Result of the 4th National Sanitation Service General Survey show, the percentage of Chinese residents with chronic disease is already 20%, the total number of determined diagnosed chronic patients reach 260 million. During past ten years, there are 10 million chronic disease patents newly diagnosed each year. Chronic disease already account as the cause of over 80% mortality.
- "In our nation, chronic disease already forms a major threat to public health." Kong Ling-zhi, Deputy Director, China CDC, says. According to statistic, the percentage of chronic disease medical treatment cost within the total sanitation expenditures has already from 47.4% in 1990 increased to 70% in 2008, amounting to about RMB850 billion (approx. USD133 billion).
- The WHO estimates, China's direct medical costs for chronic diseases by 2015 will exceed USD500 billion.

References

[1] **Source: Reuters 2009-01-06 More Americans getting multiple chronic illnesses**
http://www.reuters.com/article/2009/01/06/us-usa-chronic-idUSTRE5050S920090106

[2] Chongqing Evening News, 2011-12-07, Ministry of Health Minister Stated That Mortality Rate of Chronic Disease is Above 85%
http://news.qq.com/a/20111207/001112.htm

[3] China News Website, 2012-01-05, China's Chronic Disease Patients Exceed 260 million, incidences increasing, with trend towards younger people
http://news.qq.com/a/20120105/001110.htm

[4] Hebei Medicines, 2009-01-05, China's Chronic Disease Incidents Increased 14% During Past Ten Years.
http://news.9939.com/jbyw/2009/0105/279107.shtml

[5] Xinhua Website, 2011-03-24, WHO: Five Years Later China's Medical Costs for Chronic Diseases will Exceed USD500 Billion.
http://news.qq.com/a/20110324/001210.htm

6) 8.3% Americans Have Diabetes, 9.7% Chinese Have Diabetes Exceeding USA, the Risk is Very High for the "East Asian Weakling" Nightmare to Arrive Again for China!

USA：[1]

- Total prevalence of diabetes
- **Total:** 25.8 million children and adults in the United States—8.3% of the population—have diabetes.
- **Diagnosed:** 18.8 million people; **Undiagnosed:** 7.0 million people; **Prediabetes:** 79 million people*
- **New Cases:** 1.9 million new cases of diabetes are diagnosed in people aged 20 years and older in 2010.
- **Under 20 years of age**
- 215,000, or 0.26% of all people in this age group have diabetes
- About 1 in every 400 children and adolescents has diabetes
- **Age 20 years or older:** 25.6 million, or 11.3% of all people in this age group have diabetes
- **Age 65 years or older:** 10.9 million, or 26.9% of all people in this age group have diabetes
- **Men:** 13.0 million, or 11.8% of all men aged 20 years or older have diabetes
- **Women:** 12.6 million, or 10.8% of all women aged 20 years or older have diabetes
- **Race and ethnic differences in prevalence of diagnosed diabetes**
- After adjusting for population age differences, 2007-2009 national survey data for people diagnosed with diabetes, aged 20 years or older include the following prevalence by race/ethnicity: 7.1% of non-Hispanic whites; 8.4% of Asian Americans; 12.6% of non-Hispanic blacks; 11.8% of Hispanics
- Among Hispanics rates were: 7.6% for Cubans; 13.3% for Mexican Americans; 13.8% for Puerto Ricans

China:

In China, the rate of diabetes has increased ten fold during past 10 years, reached 9.7% at present, with about 150 million categorized under high risk of the illness! [2]

On 2011-11-24, Ji Li-nong, Director Committee Member of the Diabetes Sub-society of the China Medicine Society, at the No.15th national academic conference of the Diabetes Sub-society of the China Medicine Society (CDS2011) present the specialty speech. Ji Li-nong reported that **China's diabetes patient rate has doubled within the last decade, reached now 9.7%, making China the largest diabetes patient nation of the world, higher than the global average diabetes rate of 6.4%. At the same time, the diabetes high risk population is also expanding, about 150 million people at present.**

Shanghai's survey indicates: Diabetes patients in Shanghai during 2002 – 2009 rapidly increased 60%.

Xu Wanghong at el., Public Health College of Fudan University, jointly with Shanghai CDC, developed surveys of two groups of population which indicated, during 2002 – 2009, there was rapid increase in diabetes patients in Shanghai, diagnosed diabetes patients rose 45%, undiagnosed diabetes patients rose 13%. [3]

China becomes world's largest diabetes nation [4]

- China now has more people with diabetes than any other country, a new report shows, making it clear that the nation's soaring economic growth is taking a toll on public health.
- According to the report, more than 92 million adults in China have diabetes, and nearly 150 million more are well on their way to developing it. The disease is more common in people with large waistlines and in those who live in cities, the report indicates.
- The Federation projected last year that some 435 million people would have diabetes by 2030. "With this new study, we're going to have to rerun our estimate," Whiting told Reuters Health.
- The report, published Thursday in the New England Journal of Medicine, is based on

a nationally representative sample of more than 46,000 people who were tested for diabetes. [5]

- Based on their findings, the researchers calculate that about 50 million men and 42 million women have diabetes, or almost 1 in 10 adults. And in most cases, the disease is undiagnosed.
- The prevalence is twice as high as estimates suggested by previous studies, which did not use World Health Organization-recommended tests to diagnose diabetes, and is similar to US numbers. It would place China far ahead of India, whose estimated 50 million diabetics lands it a dubious second place in terms of the total number of people with diabetes.
- "These data really show diabetes has become a major epidemic in China," Dr. Jiang He, of Tulane University in New Orleans, told Reuters Health.
- **He, who worked on the new study with colleagues in China, noted that diabetes is a risk factor for heart disease, which is now the biggest killer in the country. "We basically need to make diabetes one of the top priorities for public health in China," he said.**

References

[1] 1. CDC：Data from the 2011 National Diabetes Fact Sheet (released Jan. 26, 2011) http://www.diabetes.org/diabetes-basics/diabetes-statistics/

[2] Xinhua News Agency, 2011-11-26, Title: China's diabetes patient rate has reached 9.7% http://www.gov.cn/jrzg/2011-11/26/content_2003793.htm

[3] Chinese Diabetes Society, China Medical Society, 2012-03-19, Shanghai CDC population survey indicates diabetes patients within 8 years increase 60% in Shanghai http://cdschina.org/news_show.jsp?id=1213.html

[4] Reuters，March 25，China becomes world's new diabetes capital http://www.reuters.com/article/2010/03/24/us-china-diabetes-idUSTRE62N66220100324

[5] Wenying Yang at el. for the China National Diabetes and Metabolic Disorders Study Group，Prevalence of Diabetes among Men and Women in China, N Engl J Med 2010; 362:1090-1101March 25, 2010 http://www.nejm.org/doi/full/10.1056/NEJMoa0908292

7）About one in every 33 babies (about 3%) is born with a birth defect, including approx 1% diagnosed with congenital heart defects in USA; the incidence of newborns with defects in China at present is about 5.6%, the incidence of newborns with defects shows a trend of increase, increased from 877,000 in 1996 to 1.499 million in 2010, increase rate 70.9%, with congenital heart disease becomes the number one defect!

USA：

- In the United States, about one in every 33 babies (about 3%) is born with a birth defect. Birth defects are one of the leading causes of infant deaths, accounting for more than 20% of all infant deaths. [1]
- Congenital heart defects are diagnosed in approximately 1% of births in the United States and account for the largest proportion of infant mortality attributable to birth defects. [2]
- Congenital heart defects affect approximately 1% of live births, of which 25% are estimated to be critical and require surgery or catheterization within the first year of life.[3]
- In Michigan, the prevalence of CHDs reported by 1 year of age for whites increased slightly from about 99 cases per 10,000 live births in 1992 to about 135 cases per 10,000 live births in 2006, i.e. an increase of 36.36%. [4]
- Congenital heart defects (CHDs) account for 24% of infant deaths due to birth defects. In the United States, about 4,800 (or 11.6 per 10,000) babies born every year have one of seven *critical congenital heart defects* (CCHDs. [5]
- In 2003, congenital cardiovascular defects contributed only 0.5 percent of CVD deaths; however, they remain a leading cause of death in infants and children. Among children ages 5–11, data from the Bogalusa Heart Study have shown that 27.1 percent now have one or more risk factors for heart disease and that an additional 6.9 percent have two or more risk factors. Taken together, these epidemiological findings are great cause for alarm. [6]
- In the United States, hospital costs for people with a heart defect were about $1.4 billion in one year. Families and the government share the burden of these costs, which means that all taxpayers are affected. Other costs due to lost wages or work limitations can affect families and businesses as well.[7]
- CDC: The cause of most congenital heart defects is unknown. [7]

China:

- It is estimated that the incidence of newborns with defects in China at present is about 5.6%, the number of newborns each year is about 900,000, which includes about 25,000 newborns with clinical apparent defects at born. **[8]**
- During recent years, the incidence of newborns with defects shows a trend of increase, increased from 877,000 in 1996 to 1.499 million in 2010, increase rate 70.9%. In 2010, the first 5 types of defects of newborns identified by hospital

examination are congenital heart disease, many fingers (toe), total cleft lip, congenital hydrocephalus and a neural tube defects. The defects account for 49.1% of all defects of newborns, in which congenital heart disease becomes the number one defect. [9]

- The sequence of birth defects in the national infant deaths, ranked No.4 in 2000, which advanced to No.2 in 2011, reaching 19.1%. **[8]**
- In 2011, congenital heart defects account to 26.7% of all the newborns found with defects during monitoring. **[8]**
- During 2000 – 2011, the incidence of perinatal congenital heart disease is on the rise. In 2011, the average incidence of newborn congenital heart defects is 3.56 times of 2000, 4.41 times in urban areas, and 2.97 times in rural areas. **[7]**
- The national prevalence of perinatal birth defects decreased to 145.64 and 145.06 per 10,000 in 2012 and 2013 respectively, compared with 153.23 in 2011, meaning at least 16,000 fewer children with birth defects in the past two years, said Zhang Shikun, director of the commission's maternal and child health service bureau, at a press conference. [10]

References:

[1] CDC, Birth Defects Data & Statistics
http://www.cdc.gov/ncbddd/birthdefects/data.html

[2] Racial Differences by Gestational Age in Neonatal Deaths Attributable to Congenital Heart Defects—United States, 2003-2006, CDC Morbidity and Mortality Weekly Report JAMA. 2010;304(18):2006-2008.
http://jama.ama-assn.org/content/304/18/2006.full

[3] CDC: Key Findings: Estimating the impact of newborn screening for critical congenital heart defects in the United States
http://www.cdc.gov/ncbddd/heartdefects/features/keyfindings-screening-impact-cchd.htm
l

[4] Michigan Department of Community Health Bureau of Epidemiology， Birth Defects Prevalence and Mortality in Michigan, 1992-2006， 2011 Annual Report
http://www.michigan.gov/documents/mdch/Birth_Defect_Annual_Report_352411_7.pdf

[5] CDC：Screening for Critical Congenital Heart Defects
http://www.cdc.gov/ncbddd/pediatricgenetics/cchdscreening.Html

[6] George A. Mensah, David W. Brown, An Overview Of Cardiovascular Disease Burden In The United States, Health Aff January 2007 vol. 26 no. 1 38-48
http://content.healthaffairs.org/content/26/1/38.full

[7] CDC: Five Facts about Congenital Heart Defects
http://www.cdc.gov/Features/HeartDefects/

[8] "China Newborn Defect Prevention And Treatment Report (2012)"signed on Sept. 13 2012
http://www.moh.gov.cn/publicfiles///business/cmsresources/mohjcg/cmsrsdocument/doc16048.pdf

[9] Jinghua Times, 2011-09-22, Title: Our nation's newborn with defects has increased 70.9%, is related to elimination of compulsive health check before marriage.
http://health.people.com.cn/GB/15721842.html

[10] Xinhua, China sees drop in birth defect rate, 2014-5-29
http://www.globaltimes.cn/content/862910.shtml

8) In USA, 78% increase of autism during 2007 - 2010, In China, autism rate in various areas has reached 1.5%, increased 10 times over 20 years, environmental, chemical, biological factors pointed as the culprit!

USA:

- CDC estimates (2010) 1 in 88 children (11.3 per 1,000) has been identified with an autism spectrum disorder (ASD). [1]
- This marks a 23% increase since our last report in 2009. And, a 78% increase since our first report in 2007. Some of the increase is due to the way children are identified, diagnosed and served in their local communities, although exactly how much is due to these factors in unknown. [1]
- ASDs are almost 5 times more common among boys (1 in 54) than among girls (1 in 252). [1]
- The largest increases over time were among Hispanic children (110%) and black children (91%). [1]
- California autism prevalence more than tripled from 1987 to 1998 [2]
- The California scientists "were at a loss to explain the reasons for what they called an epidemic of autism" in 2002 [2]
- "The causes of autism to be more environmentally influenced than previously

thought" – Findings by Canadian Scientists in 2012 [4]

- Published in the January 2009 issue of the journal Epidemiology, results from the study also suggest that research should shift from genetics to the host of chemicals and infectious microbes in the environment that are likely at the root of changes in the neurodevelopment of California's children. [5]
- "It's time to start looking for the environmental culprits responsible for the remarkable increase in the rate of autism in California," said UC Davis M.I.N.D. Institute researcher Irva Hertz-Picciotto, a professor of environmental and occupational health and epidemiology and an internationally respected autism researcher. [5]
- The incidence of autism by age six in California has increased from fewer than nine in 10,000 for children born in 1990 to more than 44 in 10,000 for children born in 2000. [5]

China:

- In China, there are now over 1 million children with autism, which is rapidly increasing, the autism rate in various areas has reached 1.5%, increased 10 times over 20 years! [6-7]
- Changchun: Autism prevalence rate 1.5% for children age 2 – 6 years [9]
- Harbin: Autism prevalence rate apparent increase trend, 2.27% for children age 2 – 6 years. [10]
- Shenzhen：Autism prevalence rate 1.32% for children, five folds of the national average rate 0.025%.[11]
- Nanjing: Autism rate of children have increased to 10 times within 20 years. "Specialists indicate, the fundamental reason causing autism is still not so clear at present, and there is not special effective drugs, early education is the main means of interference."[12]
- Shenyang: Children with autism have increased at the rate of 300 – 400 each year, indicating a trend of rapidly increasing! [13]
- Shanghai: Shanghai has over 8,000 autism patients, Children autism rate has reached 0.6% [14]

References:

[1] CDC：New Data on Autism Spectrum Disorders, 2010
http://www.cdc.gov/Features/CountingAutism/

[2] SANDRA BLAKESLEE, **INCREASE IN AUTISM BAFFLES SCIENTIST,** New York Times, 2002-10-18
http://www.nytimes.com/2002/10/18/us/increase-in-autism-baffles-scientists.html?pagewanted=all&src=pm

[3] Thomas Insel, Autism Prevalence: More Affected or More Detected?, The National Institute of Mental Health (NIMH), March 29, 2012
http://www.nimh.nih.gov/about/director/2012/autism-prevalence-more-affected-or-more-detected.shtml

[4] Kim, Causes of Autism: Environmental Versus Genetic Factors, International Center for Autism Research & Education, 2012-01-19
http://www.icare4autism.org/news/2012/01/causes-of-autism-environmental-versus-genetic-factors/

[5] Science Daily, California's Autism Increase Not Due To Better Counting, Diagnosis, 2009-01-08
http://www.sciencedaily.com/releases/2009/01/090108095429.htm

[6] Science Daily, Don't let autism take away their joyful childhood, 2009-08-11
http://www.stdaily.com/kjrb/content/2009-08/11/content_92182.htm

[7] Li Jing, Investigation report about autism children (sections), Beijing Autism Recovery Association, 2009-6-17
http://www.guduzheng.net/2009/6/20908.html

[9] China Jilin Website, Changchun autism children prevalence show increase trend, 2011-02-18
http://www.chinajilin.com.cn/content/2011-02/18/content_2184003.htm

[10] Harbin News Website, Harbin children autism rate increasing, ratio of autism between boys and girls is 7:1, 2011-04-02 http://www.jk396.com/xexl/2/942.html

[11] Nanfang City Daily, Shenzhen children autism prevalence rate is five fold of national level, 2007-12-21 http://news.sina.com.cn/c/2007-12-21/012913116221s.shtml

[12] China Chinese Medicine Newpaper, Nanjing children's autism rate increasing, increased 10 times within 20 years, 2008-04-18
http://www.chinanews.com/jk/ysbb/news/2008/04-18/1224952.shtml

[13] Shenyang Daily, Trend of rapidly increasing focuses on today's "Children's autism", 2005-10-20 http://news.sina.com.cn/c/2005-10-20/10107218716s.shtml

[14] (Shanghai) Xinmin Weekly, Autism becomes the No.1 children illness, survey said autism rate has reached 0.6%, 2010-08-25
http://news.sina.com.cn/h/sd/2010-08-25/112020972095.shtml

9) Studies indicate possible links between Alzheimer's disease and Parkinson's disease, indicate 10% increase of annual incidence of Alzheimer's disease from 2000 to 2010 in USA; During the past 20 years (1998 - 2008), the average ages of Alzheimer's disease patients has advanced from 65 years to 55 years; In the USA, 50,000-60,000 new cases of Parkinson disease are diagnosed each year; Based on most conservative estimations, the incidence of Parkinson disease during recent 20 years in China has at least increased over 20 times!

USA:

- Studies Investigate Links Between Parkinson's Disease and Alzheimer's Disease
- Parkinson's disease (PD) and Alzheimer's disease (AD) are common neurodegenerative diseases associated with aging. People can develop both PD and AD, leading researchers to consider whether there are links between the two diseases. Two new studies have found that the two diseases do not appear to share genetic characteristics but may be linked by proteins. [1]
- In the genetics study, published in the August 5 online edition of *JAMA Neurology*, researchers analyzed genome-wide association (GWA) studies of PD and AD to look for genetic variants shared by both diseases. The study was led by author Valentina Moskvina, Ph.D., of the Cardiff University School of Medicine in Wales, in collaboration with an international team that included researchers from the National Institutes of Health. The team reviewed GWA studies of thousands of people with AD and PD, to see if any genetic variants increased the risk of both diseases. [2]
- The second study, published in the July 3 online edition of *Cell* by a team from the University of Pennsylvania led by Virginia Lee, Ph.D., investigated proteins found in AD and PD. Most neurodegenerative diseases are characterized by the accumulation of abnormal forms of certain proteins – for example, tau and amyloid in AD and alpha-synuclein in PD. Studies have shown some overlap in the pathology of AD and PD: more than 50 percent of people with AD show alpha-synuclein deposits and people with PD commonly have tau deposits. To better understand any relationship, researchers conducted experiments with alpha-synuclein involving mice and cell cultures.[3]

USA: Alzheimer's disease

- Alzheimer's disease is the most common type of dementia. "Dementia" is an umbrella term describing a variety of diseases and conditions that develop when nerve cells in the brain (called neurons) die or no longer function normally. The death or malfunction of neurons causes changes in one's memory, behavior and ability to think clearly. In Alzheimer's disease, these brain changes eventually impair an individual's ability to carry out such basic bodily functions as walking and swallowing. Alzheimer's disease is ultimately fatal. [4]
- An estimated 5.2 million Americans of all ages have Alzheimer's disease in 2013. This includes an estimated 5 million people age 65 and older [5], A1and approximately 200,000 individuals under age 65 who have younger-onset Alzheimer's. [6] [4]
- In 2000, there were an estimated 411,000 new cases of Alzheimer's disease. For 2010, that number was estimated to be 454,000 (a 10 percent increase); [4]
- By 2025, the number of people age 65 and older with Alzheimer's disease is estimated to reach 7.1 million — a 40 percent increase from the 5 million age 65 and older currently affected. [7]

USA: Parkinson's disease

- Parkinson's disease (PD) is a chronic and progressive movement disorder, meaning that symptoms continue and worsen over time. Parkinson's involves the malfunction and death of vital nerve cells in the brain, called neurons. Parkinson's primarily affects neurons in the an area of the brain called the substantia nigra. Some of these dying neurons produce dopamine, a chemical that sends messages to the part of the brain that controls movement and coordination. As PD progresses, the amount of dopamine produced in the brain decreases, leaving a person unable to control movement normally. [8]
- Parkinson's disease is the 14th leading cause of death in the United States, according to the Centers for Disease Control and Prevention's annual analysis of mortality data. The CDC also released data this week showing there was a 4.6% increase in deaths attributable to Parkinson's disease in 2010 (the most recent year for which they have data). [9] [10]
- Between 500,000 and 1.5 million Americans live with Parkinson's, a disease for which there is no cure or treatment to stop the progression. [9]
- Incidence of Parkinson's increases with age, but an estimated four percent of people with PD are diagnosed before the age of 50. [11]

- In the United States, 50,000-60,000 new cases of PD are diagnosed each year, adding to the one million people who currently have PD. [12]

China: **Alzheimer disease**

- According to a meta-analysis of 89 studies published June 7 in a China-themed issue of the Lancet, the number of people with dementia in the world's most populated country soared from 3.7 million in 1990 to 9.2 million in 2010. The figures eclipse those from the World Alzheimer Report 2012, which estimated 5.4 million dementia cases in China in 2010. Moreover, a report on global disease burden in the special Lancet issue found that the number of deaths in China due to Alzheimer's disease and other dementias doubled between 1990 and 2010, while mortality rates, especially among women, fell steeply during the same period. With continuing growth of China's aging population, these findings suggest the nation is heading for a bigger dementia burden than anticipated. [13] [14]
- The dementia study was led by senior investigators Wei Wang of Capital Medical University in Beijing, and Harry Campbell and Igor Rudan at the University of Edinburgh Medical School in the U.K. Christopher Murray of the University of Washington in Seattle led the analysis of China health trends with collaborators from China and Australia. [13] [14]
- A epidemiology survey report on Alzheimer's disease in Shanghai issued by public health departments show, prevalence of old aged older than 65 years old is 6%, above 80 years old exceeds 30%, prevalence of old aged above 65 years old increases at a rate of 1.4% per annum. [15]
- Survey data in Tianjin, Shanghai, Guangzhou and other large cities show: During the past 20 years (1998 - 2008), the average ages of Alzheimer's disease patients has advanced from 65 years to 55 years, i.e. "younger" 10 years. [16]

China: Parkinson disease

- During education activities in Shanghai on the "World Parkinson Day", Prof. Jiang Yu-ping, secretary, neurology committee of China Medical Society stated, the incidence of Parkinson disease during recent 20 years has at least increased over 20 times, such increase might be associated with environment and food factors. [16]
- Prof. Jiang said, based on the statistics of 1986, the incidence of Parkinson disease in China is about 0.047%, and according to Shanghai statistics of 2000, the incidence of Parkinson disease has reached 1.14%. However, national-wide statistics indicate incidence of Parkinson disease has reached about 2%. Based on most conservative estimations, the incidence of Parkinson disease during recent 20 years has at least increased over 20 times. [16]
- Date provided by the Shanghai Municipality Health Education Institute claims, Epidemiological statistics show that China at present (2007) has about 1.7 million Parkinson disease patients. [16]
- "Parkinson's disease is a kind of neurological system diseases, with higher incidence in older age. However, there is a trend of Parkinson disease developing in younger age patients. Before the diagnosed age was more over 70 years old, quite a number of Parkinson disease patients begin to appear in the 30 - 50 age group, it has become the 3rd killer of middle to older age people. [17]

[1] Parkinson's Disease Foundation, Studies Investigate Links Between Parkinson's Disease and Alzheimer's Disease Oct 29 2013 http://www.pdf.org/en/science_news/release/pr_1379607636

[2] Moskvina V, Harold, D, Russo G, et al. (2013) Analysis of Genome-Wide Association Studies of Alzheimer Disease and of Parkinson Disease to Determine If These 2 Diseases Share a Common Genetic Risk. *JAMA Neurol*DOI: 10.1001/jamaneurol.2013.448http://dx.doi.org/10.1001/jamaneurol.2013.448

[3] Lee, V MY, Guo JL, Daniels JP, et al. (2013) Distinct Alpha-Synuclein Strains Differentially Promote Tau Inclusions in Neurons. Cell 154, 103-117. DOI:10.1016/j.cell.2013.05.057 http://dx.doi.org/10.1016/j.cell.2013.05.057

[4] Alzheimer Association, 2013 Alzheimer's Disease Acts And Figures http://www.alz.org/downloads/facts_figures_2013.pdf

[5] Hebert LE, Weuve J, Scherr PA, Evans DA. Alzheimer's disease in the United States (2010-2050) estimated using the 2010 Census. Neurology. Published online before print, Feb. 6, 2013. www.neurology.org/content/early/2013/02/06/WNL.0b013e31828726f5.abstract.

[6] Alzheimer's Association. Early-onset dementia: A national challenge, a future crisis

[7] Hebert LE, Beckett LA, Scherr PA, Evans DA. Annual incidence of Alzheimer disease in the United States projected to the years 2000 through 2050. Alzheimer Dis Assoc Disord 2001;15(4):169–73. 109. Corrada MM, Brookm

[8] Parkinson's Disease Foundation, What is Parkinson's Disease? http://www.mayoclinic.org/diseases-conditions/parkinsons-disease/basics/definition/con-20028488

[9] Parkinson's Action Network, CDC Lists Parkinson's as the 14th Leading Cause of

Death in America
http://www.parkinsonsaction.org/news/cdc-lists-parkinsons-14th-leading-cause-death-america

[10] **CDC:** QuickStats: Age-Adjusted Death Rates* for Parkinson Disease --- United States, 1973--2003†, February 17, 2006
HTTP://WWW.CDC.GOV/MMWR/PREVIEW/MMWRHTML/MM5506A7.HTM

[11] Parkinson's Disease Foundation, Statistics on Parkinson's,
http://www.pdf.org/en/parkinson_statistics

[12] National Parkinson Foundation, Parkinson's Disease Overview
http://www.parkinson.org/parkinson-s-disease.aspx

[13] **Biomedical Research Forum--**ALZFORUM, Prevalence of Dementia, AD, in China Eclipses Predictions, 14 Jun 2013
http://www.alzforum.org/news/research-news/prevalence-dementia-ad-china-eclipses-predictions

[14] Chan KY, et al., Epidemiology of Alzheimer's disease and other forms of dementia in China, 1990-2010: a systematic review and analysis. Lancet. 2013 Jun 8;381(9882):2016-23
http://www.ncbi.nlm.nih.gov/pubmed/23746902

[15] Sohu Health, Incidence of Alzheimer's disease in old age in Shanghai increases, prevalence among old age above 80 years old reaches 30%.
http://health.sohu.com/20081013/n259991123.shtml

[16] Souhu Health, Specialists: incidence of Parkinson disease during recent 20 years has at least increased over 20 times, 2007-04-12
http://health.sohu.com/20070412/n249373631.shtml

[17] Parkinson treatment center, the "old aged disease" becoming younger, 2012-07-31
http://www.999brain.com/NewsMessage/Show-4-0-3-11519-166-2.html

10) 1988-2004 USA incidence of primary liver cancer rapidly rose 90%, China's liver cancer patients accounts for 54.26% of World's total

USA:

- The incidence of primary liver cancer rose modestly between 1979 and 1988 (14.5 percent) and more rapidly subsequently (90 percent over the period 1988–2004) (Figure 1). Liver cancer was one of the most lethal digestive system cancers, although 5-year survival did increase nearly fourfold during this period, albeit to only 8 percent. [1]
- The number of new cases of hepatocellular carcinoma (HCC), a type of primary liver cancer, has increased in the U.S. over the past several years, reaching an incidence rate of 3.2 cases per 100,000 persons in 2006, according to the latest figures reported by the Centers for Disease Control and Prevention (CDC) in the May 7, 2010 issue of Morbidity and Mortality Weekly Report. [2]

China:

- At present, cancer prevalence in China is 285.91/100,000, on average every minute everyday 6 persons are diagnosed with malignant cancer. [3]
- Supervision data shows, colon cancer rises faster in the urban areas, as well as thyroid cancer. At present, in urban areas, the prevalence of thyroid cancer already ranks No.4. [3]
- National cancer mortality rates has reached 180.54/100,000, reaching 2.7 million deaths caused cancer ear year. The national rate of mortality caused by cancer in China is 13%, meaning cancer causes one of every 7 - 8 deaths. [3]
- The mortality rate of men caused by cancer is higher than women, with ratio of 1.68/1. [3]
- Regarding kinds of cancer, lung cancer ranks No.1, followed by stomach cancer, then colon cancer, liver cancer and then esophageal cancer. The top ten kinds of cancer amount to 76.39% of all kinds of cancer. [3]
- The cancer with highest mortality rate still is lung cancer, followed by liver cancer, stomach cancer, esophageal cancer and colon cancer. [3]
- Lung cancer has the highest mortality rate in both men and women. The kinds of death causing cancer in men then include liver cancer, stomach cancer, esophageal cancer and colon cancer. In women then include stomach cancer, liver cancer, colon cancer and breast cancer. [3]
- Primary liver cancer in our country is one of the common malignant tumors. Has high mortality rate, ranking No.3 following malignant tumor in the stomach and in the esophagus; but, in the rural areas ranking 2nd place following cancer in the stomach. In China, about 110,000 people die from liver cancer each year, accounting 45% of the total number of deaths caused by liver cancer worldwide. [4]
- China's liver cancer situation is very grim, in 2000 306,000 patients developed liver cancer, and 306,000 mortalities from liver cancer, which accounted for 54.26% of

world liver cancer patients, and 54.64% of all mortalities caused by liver cancer over the world. [5]

- In characteristic of regional distribution of liver cancer in China is higher incidents in coastal regions than inland regions; higher incidents in Southeast and Northeast regions than Northwest, North and Southwest regions of China, and higher incidents in coastal islands and river estuaries regions. [5]
- According to cancer register data 1998-2002 from nationwide 30 cities and counties, China's top three liver cancer areas for men are Qidong of Jiangsu Province, Haimen of Jiangsu Province, and Fushui of Guangxi Autonomous Region, and top three liver cancer areas for female are Qidong of Jiangsu Province, Haimen of Jiangsu Province, and Yanting of Sichuan Province. [5]
- From 1972 to 1999, although the total number of liver cancer patients in Shanghai increased significantly, but age standardized incidence rate reduced, for male and female reduced 26% and 28% respectively. [5]
- In 2006, the Ministry of Health and Ministry of Science & Technology led a national survey on nationwide mortality causes. The Department of Health of the Guangxi Autonomous Region entrusted the Cancer Hospital attached to the Guangxi Medical University to carry out review mortality causes from 9 locations. Survey data indicate, the first 3 causes of death of population in Guangxi respectively are malignancy cancers, respiration system diseases and cerebrovascular disease. During the 70s of the last century, the mortality rate caused by malignancy cancers was 38.29/100,000, during the 90s was 80.48/100,000, and at present is 112.0/100,000, indicating apparent increase. [6]
- The mortality rate of 112.0/100,000, to the over 50 million population of Guangxi means about 50,000 people die from malignancy cancers, the prevalence increase ranks No.1 in China, which makes Guangxi one of the regions with the highest liver cancer and nasopharynx prevalence rate in China. [6]
- 370,000 new liver cancer patients and 536,000 new lung cancer patients appear each year, which is increasing at the pace of 5% each year in China. [6]
- Epidemiology investigation indicates: The liver cancer incidence for males are 26.23/100,000, and for females 11.09/100,000 in Tianjin. [7]

[1] Everhart JE, editor. The burden of digestive diseases in the United States. US Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases. Washington, DC: US Government Printing Office, 2008; NIH Publication No. 09-6443.
http://www3.niddk.nih.gov/Burden_of_Digestive_Diseases/index.shtml#CHAPTER9

[2] Liz Highleyman, CDC Reports Increasing Incidence of Liver Cancer, Mostly Due to Chronic Hepatitis B and C, 2010
http://www.hivandhepatitis.com/hep_c/news/2010/0615_2010_b.html

[3] Sanxiang Metropolis Daily, 2012 Cancer Registration Report, 2013-01-15
http://news.sina.com.cn/w/2013-01-15/075926036542.shtml

[4] Health China, Primary Liver Cancer Incidence, 2010-11-02
http://health.china.com.cn/html/ganai/yfxga/201011/02-77769.html

[5] Jiujiu Anti-Cancer Website Specialist Group, What characteristics does China's primary liver cancer have for its distribution?, Jiujiu Anti-Cancer Website, 2011-07-18
http://www.99kangai.com/html/azzt/ganai/gacs/2011/0718/64005.html

[6] Guangxi News Website-Current Times Life Newspaper, malignancy cancers becomes the No.1 killer of Guangxi residents, prevalence increase ranks No.1 in China, 2010-02-28
http://www.gxnews.com.cn/staticpages/20100228/newgx4b89c584-2722100.shtml

[7] City Express, Epidemiology investigation: Tianjin has more male liver cancer patients than women, 2011-08-28
http://www.chinadaily.com.cn/dfpd/tianjin/2011-08-28/content_3624967.html

11) Children cancer increasing, especially leukemias, dramatically increased in USA and China.

USA:

About 12,500 children and adolescents under the age of 20 years are diagnosed with cancer each year. Childhood cancers remain a leading cause of childhood deaths in the United States, despite the fact that advances in health care and treatment have dramatically increased survival from these cancers. [1]

The most common childhood cancers are leukemias, cancers of the blood cells. There are different kinds of childhood leukemia. The most common kinds are acute lymphocytic leukemia (ALL) and acute myelogenous leukemia (AML). Brain and other nervous system cancers are the second most common types of childhood cancers. Of the 12 major types of childhood cancer, leukemias and brain and other nervous system cancers account for 40% of all cases among children less than 20 years of age. Other childhood cancers, in decreasing order of occurrence, include lymphomas, sympathetic nervous system

cancers, soft tissue sarcomas, renal tumors (Wilms tumor and renal carcinoma), bone tumors (osteosarcoma and Ewing sarcoma), malignant germ cell tumors, retinoblastomas, hepatic tumors, and other malignancies. Researchers have studied childhood cancers for many years but still do not know a lot about what causes them. Finding the causes of childhood cancers is challenging because cancer in children is rare and each type of cancer may have different factors that lead to its development. It is especially difficult to identify environmental factors related to childhood cancer because environmental exposures to the parent, the child in the womb, or the child after birth may play a role. Childhood cancers, like adult cancers, may be the result of a mix of genetic, environmental, and behavioral causes, not just one factor by itself. [1]

China:

Tumor prevalence in Children shows tread of increase, on average there is one child tumor patient among ever 10,000 children. The causes of death among children deaths under 14 years old, malignant tumor already ranks No.2. Among all kinds of children tumors, leukemia, brain tumor, malignant lymphoma and neuroblastoma rank the top four, leukemia accounts to 1/3 of all children tumor patients. [2]

Based on survey in a number of cities, the incidence of children tumor each year is 10 per each 100,000. China has about 400 million children, thus new children tumor incidence could reach over 30,000 children. [2]

The increasing trend of children malignant tumors is apparent, with a higher rate in boys than girls. Two age peaks are apparent, 42% develop tumors before 3 years old, thereafter with a lower rate, the 2nd peak appears during period of puberty. [2]

[1] CDC: Childhood Cancers and the Environment
http://ephtracking.cdc.gov/showChildhoodCancer.action

[2] Chengdu Daily, Children cancer indcidence increases, how to protect children, 2013-02-20
http://www.cdrb.com.cn/html/2013-02/20/content_1794291.htm

12) Inflammatory Bowel Diseases rapidly increased 65 percent increase from 2000 to 2009, and increased 12 times in Shanghai from 2003 to 2013!

USA

- Inflammatory Bowel Diseases (IBD) is a broad term that describes conditions with chronic or recurring immune response and inflammation of the gastrointestinal tract. The two most common inflammatory bowel diseases are ulcerative colitis and Crohn's disease. [1]
- Both illnesses have one strong feature in common. They are marked by an abnormal response by the body's immune system. Normally, the immune cells protect the body from infection. In people with IBD, however, the immune system mistakes food, bacteria, and other materials in the intestine for foreign substances and it attacks the cells of the intestines. In the process, the body sends white blood cells into the lining of the intestines where they produce chronic inflammation. When this happens, the patient experiences the symptoms of IBD. [1]
- The peak age of onset for IBD is 15 to 30 years old, although it may occur at any age. About 10% of cases occur in individuals younger than 18 years. [1]
- CDC claims: Precise incidence and prevalence of Crohn's disease and ulcerative colitis have been limited by (1) a lack of gold standard criteria for diagnosis; (2) inconsistent case ascertainment; and (3) disease misclassification. The data that does exist suggest that the worldwide incidence rate of ulcerative colitis varies greatly between 0.5–24.5/100,000 persons, while that of Crohn's disease varies between 0.1–16/100,000 persons worldwide, with the prevalence rate of IBD reaching up to 396/100,000 persons. It is estimated that as many as 1.4 million persons in the United States suffer from these diseases. [1]
- A new study found, the largest investigation to date has found a dramatic increase in the number of hospitalizations for children with inflammatory bowel disease (IBD) during the past decade in the United States. [2]
- The new study, published online and scheduled for the August 2013 print issue of the Journal of Investigative Medicine, found a 65 percent increase in IBD hospital discharges from 2000 to 2009. The number increased from 11,928 discharges in 2000 to 19,568 discharges in 2009. [2]

China:

- During past 10 years (2003 - 2013), the incidence of inflammatory Bowel Disease in children below 14 years old increased 12 times. [3]
- A study published in Aug. 2013 by Inflammatory Bowel Disease Previously confirms: A disease of the West and rarely seen in China, inflammatory bowel disease (IBD) is now increasing in incidence in China. However, its true incidence is unknown. Conclusion: There is a substantial incidence of IBD in China.[4]

[1] CDC, Inflammatory Bowel Disease (IBD)
http://www.cdc.gov/ibd/

[2] University Hospitals Case Medical Center, Dramatic increase in hospitalization of US children with inflammatory bowel disease, Science Daily, USA, June 25, 2013
http://www.sciencedaily.com/releases/2013/06/130625141208.htm

[3] Health Daily, incidence of inflammatory Bowel Disease in children increased 12 times, 2013-03-01http://health.qq.com/a/20130301/000024.htm

[4] Zhao J et al., First prospective, population-based inflammatory bowel disease incidence study in mainland of China: the emergence of "western" disease. Inflamm Bowel Dis. 2013 Aug;19(9):1839-45.
http://www.ncbi.nlm.nih.gov/pubmed/23669403

13) Sudden cardiac arrest (SCA) increasing, including in younger people in both USA and China.

USA
CDC confirms:

- About 600,000 people die of heart disease in the United States every year–that's 1 in every 4 deaths. [1]
- Heart disease is the leading cause of death for both men and women. More than half of the deaths due to heart disease in 2009 were in men. [1]
- Coronary heart disease is the most common type of heart disease, killing nearly 380,000 people annually. [1]
- Every year about 720,000 Americans have a heart attack. Of these, 515,000 are a first heart attack and 205,000 happen in people who have already had a heart attack.[2]

The Pediatric Sudden Cardiac Arrest report issued in 2012 by American Academy of Pediatrics reports: [3]

- CDC has estimated that every year in the United States, approximately 2000 patients younger than 25 years will die of SCA. [4]
- Other older reports estimate the frequency of SCA in children and adolescents to be between 0.8 and 6.2 per 100 000 per year. [5] [6] [7] [8] [9]
- Two studies suggest that the frequency of SCA in adolescents and young adults actually may be increasing. [10] One study published in 2003 estimated Sudden cardiac deaths rise 10% in young Americans. [11]

China:

- The number of sudden cardiac arrest deaths reach 544,000 each year, i.e. over 1000 people die of SCA each day, with higher incidence in male than female. [12]
- According to media reports, in 1998 the "120" Emergency Center in Beijing received over 80 sudden cardiac arrest death young to middle age patients, this number rapidly increased to over 270 in 2011. [13]
- Other media reported, the percentage of sudden cardiac arrest deaths at age lower than 50 years old reached 12.65%, increased 1.7% compared with 1998. [13]
- During the 1st quarter of 2013, the "120" Emergency Center in Qingdao registered 848 sudden cardiac arrest deaths, the age of most of them were between 35 - 59 years old, accounting to 25% - 35% of the total visits by the center. [14]

References:

[1] Murphy SL, Xu JQ, Kochanek KD. Deaths: Final data for 2010. *Natl Vital Stat Rep*. 2013;61(4).http://www.cdc.gov/nchs/data/nvsr/nvsr61/nvsr61_04.pdf[PDF-3M]

[2] Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Blaha MJ, et al. Heart disease and stroke statistics—2014 update: a report from the American Heart Association. *Circulation*. 2014 ;128.

[3] American Academy of Pediatrics, Pediatric Sudden Cardiac Arrest (Report), e March 26, 2012
http://pediatrics.aappublications.org/content/129/4/e1094.full.pdf

[4]. Kung HC, Hoyert DL, Xu J, Murphy SL.Deaths: final data for 2005. Natl Vital Stat Rep. 2008;56(10):1–120

[5] Corrado D, Basso C, Schiavon M, Thiene G.Screening for hypertrophic cardiomyopathy in young athletes. N Engl J Med. 1998;
339(6):364–369

[6] Driscoll DJ, Edwards WD. Sudden unexpected death in children and adolescents. J Am Coll Cardiol. 1985;5(suppl 6):118B–121B

[7] Wren C. Sudden death in children and adolescents. Heart. 2002;88(4):426–431

[8] Neuspiel DR, Kuller LH. Sudden and unexpected natural death in childhood and adolescence. JAMA. 1985;254(10):1321–1325

[9] Shen WK, Edwards WD, Hammill SC, Bailey KR, Ballard DJ, Gersh BJ. Sudden unexpected nontraumatic death in 54 young adults: a 30-year population-based study. Am J Cardiol. 1995;76(3):148–152

[10] Spurgeon D. Sudden cardiac deaths rise 10% in young Americans. BMJ. 2001;322

(7286):573

[11] SoRelle R. Jump in sudden deaths reported in younger people during past decade. Circulation. 2001;103(10):e9019–e9021

[12] News Evening News, At least over 1000 people suddenly die each day in China, incidence higher in male than female., 2012-10-27
http://news.sohu.com/20121027/n355856691.shtml

[13] Sina Health, Why does sudden death catch on young people, 2012-07-19
http://health.sina.com.cn/d/2012-07-19/120840838.shtml

[14] Qilu Daily News, 70% of Qingdao's citizens have cardiac problems, mental stimulation induced by sudden death, 2013-07-26
http://qd.ifeng.com/fgqd/detail_2013_07/26/1039063_0.shtml

Common in both USA and China: Exposure to glyphosate and n-hexane residues through environment pollution, food and chemical extracted food oil

China:

- **Largest producer of glyphosate of the world.**
- **Largest exporter of glyphosate of the world, including export of glyphosate to Monsanto to produce Roundup formula herbicide.**
- **Massive environment pollution caused by illegal discharge of toxic industrial waste from production of glyphosate.**
- **China is major importer of Monsanto's Roundup, which accounts to 80% of China's herbicide market.**
- **China widely applies Roundup and other glyphosate-based herbicide, causing widespread pollution.**
- **China is largest importer of RR soybeans, RR canola processed into RR soybean food oil and RR canola food oil, flooding the Chinese market**
- **Chinese researchers identified AMPA, metabolites of glyphosate, toxic, in RR soybean food oil.**
- **RR soybean protein powder, containing higher levels of glyphostate, is added to sausages, ham, frozen food, cakes, cookies, bread, even infant formula milk powder and wheat-flour in China.**
- **About 90% of food oil in China, are produced by chemical extraction, using n-hexane as oil extracting solvent, results most food oil contains n-hexane residue, including food oil process from soybeans, canola, corn, rice husks.**
- **Most soybean protein powder in China is processed from soybean cake, the side product of chemical extracted soybean oil.**
- **According to Chinese national standards, up to <50 mg/kg n-hexane residue is allowed in food oil, and up to <500 mg/kg n-hexane residue is allowed in food grade soybean protein powder added to infant formula milk powder.**
- **"EFSA allows chemical extracted RR soybean food oil and soybean protein powder to be added to infant formula milk powder", is the main reason China's Food & Drug Administration Bureau allows such criminal practice!**

Eleven studies by Chinese scholars reveal harm caused by glyphosate

Eleven scientific studies by Chinese scholars reveal that glyphosate cause damage to proteins and lipids, cause apoptosis and necrosis of liver cells with obvious damage, cause mutagenic and reproductive toxicity, capable of causing birth defects to human offspring.

1) (1996)草甘膦与试验鼠"肝微粒体蛋白含量明显减少…蛋白含量减少可能与肝细胞受损致使合成蛋白能力下降有关"；

1)(1996) Glyphosate and test rat "Liver microsomal protein content decreased significantly … protein content reduction might be associated with protein synthesis ability reduction caused by impaired liver cell damage."

邬惠琼，草甘膦对大鼠细胞色素 P450 2B1 和 P450 2C11 基因表达的影响，《卫生毒理学杂志》1996 年第 10 卷第 4 期，231-234 页
（作者单位：武汉同济医科大学公共卫生学院环境毒理学研究室。）

Wu Hui-qiong, Glyphosate impact on rat cytochrome P450 2 B1 and P450 2 c11 gene expression, Health Toxicology Journal, 1996 10(4): 231-234 [Chinese]
(Organization: Environment Toxicology Research Section, Public Health College, Wuhan Tongji Medical University.)
http://www.cnki.com.cn/Article/CJFDTotal-WSDL604.004.htm

2) (2000) "对黄鳝具有明显的遗传学损伤作用"；

2) (2000) "causes obvious genetic damage to yellow eel"

耿德贵等，除草剂农达对黄鳝致突变性研究，《徐州师范大学学报(自然科学版)》 2000 年 02 期

Geng De-gui et al., Study of Herbicide Roundup impact on yellow eel mutagenic, Journal of Xuzhou Normal University (Natural Science Edition), 2000(2)
http://www.cnki.com.cn/Article/CJFDTotal-XZSX200002018.htm

3) (2001) 不同浓度草甘膦药物"对蟾蜍的红细胞微核率和核异常率均有一定程度的影响"；

3) (2001) Different concentration of glyphosate "causes certain degree of effect on RBC micronucleus rate and the rate of nuclear anomalies of toads"

南旭阳，除草剂“草甘膦”对鲫鱼外周血红细胞微核及核异常的影响[J]. 安徽师范大学学报：自然科学版，2001，24(4)：329-331.

Nan Xu-yang, Impact of glyphosate herbicide on carp peripheral blood erythrocyte micronucleus and nuclear anomalies, Journal of Anhui Normal University (Natural Science Edition), 2001,24(4): 329-331 [Chinese]
http://www.cqvip.com/qk/97138X/200006/4887295.html

4) (2002) **“对鲫鱼的血红蛋白、红细胞和白细胞影响较大”**

4) (2002) “causes rather large effect on Crucian carp hemoglobin, red blood cells and white blood cells”

南旭阳，除草剂草甘膦对鲫鱼血细胞及血红蛋白影响的研究，甘肃科学学报，2002（4）
(就职机构：浙江温州师范学院生物与环境科学系)
(Organization: Biology & Environment Science Dept., Zhejiang Wenzhou Normal College)

Nan Xu-yang, Study of impact of glyphosate herbicide on carp blood cells and hemoglobin, Gansu Science, 2002(4)
http://www.cnki.com.cn/Article/CJFDTotal-GSKX200204015.htm

5) (2003) **“对泥鳅具有一定的生理毒性”；**

5) (2003) **“causes certain degree of biological toxicity to loaches”.**

南旭阳，张艳丹，黄小莲．除草剂“ 草甘膦” 对泥鳅白细胞的影响[J]，温州师范学院 学报：自然科学版，2003,24(2):72-74 就职机构：温州师范学院生物与环境科学学院

Nan Xu-yang et al., Impact of glyphosate herbicide on loach white blood cells, Journal of Wenzhou Normal University: (Natural Science Edition), 2003,24(2): 72-74 [Chinese]
http://www.cnki.com.cn/Article/CJFDTotal-WZSF200302019.htm

6) (2008) **“对小鼠具有生殖毒性并具有一定的致突变作用”；**

6) (2008) **“causes reproduction toxicity to mice with a certain mutagenic effect”.**

中国学者康菊芳 et al.，草甘膦对小鼠的致突变作用研究（《癌变.畸变.突变》，2008 年 03 期）

Kang Ju-fang et al., Study of glyphosate effect causing mutagenic on rats, Carcinogenesis, Teratogenesis & Mutagenesis, 2008(3)

http://www.cnki.com.cn/Article/CJFDTotal-ABJB200803018.htm

7) (2008) **“能引起人肝细胞存活率下降,细胞膜通透性增加,抑制细胞离子转运,诱发 DNA 损伤,线粒体膜电位降低,Cyt C、AIF 等凋亡因子泄漏,使细胞产生凋亡和坏死,对肝细胞具有明显的损伤作用”；**

7) (2008) **“Can lead to liver cell survival rate decrease, cell membrane permeability increase, inhibit cell ion transport, induce DNA damage, mitochondrial membrane potential decreased, leakage of Cyt C, AIF apoptosis factors, causes cell apoptosis and necrosis, obvious damage to liver cells”.**

王非，农达 41％草甘膦对人 L02 肝细胞损伤的研究（中南大学硕士论文）2008

Wang Fei, Study of Roundup 41% causing damage to human L02 liver cells, Master's thesis, Zhongnan University, 2008
http://cdmd.cnki.com.cn/article/cdmd-10533-2008165795.htm

8) (2010) **“可引起小鼠精子数目减少、精子畸形率增加,以及附睾和睾丸重量及其系数下降，提示农达对雄性小鼠具有明显的生殖毒性作用”；**

8) (2010) Could cause mice sperm number reduce, sperm deformity rate increase, epididymis and testis weight and coefficient decline, suggesting Roundup causes obvious reproductive toxicity in male mice”.

黄婷，农达对雄性生殖细胞的毒性作用及其机制的初步研究，中南大学（硕士论文），2010

Huang Ting, Preliminary study of Roundup’s toxicity effect and mechanism on male reproductive cells, Master's thesis, Zhongnan University
http://cdmd.cnki.com.cn/Article/CDMD-10533-2010187394.htm
全文链接：http://www.doc88.com/p-974197814056.html

9) (2010) **“草甘膦对海胆胚胎各发育期具有一定的急性毒性”；**

9) (2010) “Glyphosate causes certain acute toxicity to sea urchin embryos during

different phases of development".

李娇 et al.，8 种常见农药对海胆胚胎各发育期的急性毒性， 生态毒理学报，2010(2)
(机构：大连海洋大学、农业部海洋水产增养殖学与生物技术重点开放实验室)
(Organization: Marine Fisheries Aquaculture & Key Laboratory of Biotechnology, Dalian Marine University and Ministry of Agriculture)

Li Qiao, et al., Acute toxicity of eight types of pesticides to sea urchin embryos during different phases of development".
http://d.wanfangdata.com.cn/Periodical_cyyhj201002014.aspx

10) (2011) **"草甘膦能降低小鼠的总抗氧化能力,损伤蛋白质和脂质,造成机体的氧化损伤,导致各种疾病的发生"；**

10) (2011) **"Glyphosate can reduce total antioxidant capacity, damage protein and lipid, cause oxidative damage of the body, cause development of various diseases".**

赵伟，曹曦予，吴艳萍，等．草甘膦致小鼠机体氧化损伤作用的研究[J]。毒理学杂志，2011,25(5):364-366

Zhao Wei et al., Study of **oxidative damage of the body** caused by glyphosate, Toxiology Journal, 2011,25(5):364-366 [Chinese]
http://www.cnki.com.cn/Article/CJFDTotal-WSDL201105013.htm

11) (2012) **"草甘膦对小鼠具有生殖毒性并具有一定的致突变作用"。**

11) (2012) **"Glyphosate causes reproductive toxicity to mice and has certain mutagenic effect".**

俞慧;江城梅;赵文红;草甘膦毒性作用研究进展[J];蚌埠医学院学报;2012 年 06 期
Yu Hui et al., Progress in study of glyphosate toxicity, 2012(6)
http://www.cnki.com.cn/Article/CJFDTOTAL-BANG201206050.htm
http://www.doc88.com/p-666125982792.html

Twenty-four international and Chinese and scientific studies and epidemiology studies prove that hexane residuals and benzene residuals cause reproduction toxicity to both female and males

Summary:

- Hexane residuals were found in urine of fathers, mothers and children exposed to application or mixture of glyphosate-based herbicide Roundup, meaning that glyphosate can enter blood circulation of males, females and children.
- Hexane residuals are found can through blood circulation can pass through the placenta and enter into fetus of pregnant rats.
- Hexane is a hormone interrupter, which demonstrates effects harmful to human health at very low dose levels, even by exposure of breathing air with low levels of hexane content.
- Hexane, as well as benzene, i.e. residual contained in hexane solvent produced for vegetable food oil extraction, show that they specifically attack and cause damage to the female reproduction system, as well as attack and cause damage to the male reproduction system and sperms.

[Note: Benzene is a residue of the hexane solvent used for vegetable oil extraction, thus is studied together with the effects of hexane.]

1) (1987) :"Testicular damage was induced in rats by respiratory treatment with *n*-hexane at a concentration of 5000 ppm. …after interruption of the treatment, the testicular lesions became increasingly severe, up to complete atrophy of the seminiferous tubules, suggesting an irreversible sterility of the treated animals."

C. De Martino et al., Effects of respiratory treatment with *N*-hexane on rat testis morphology: I. A light microscopic study, Experimental and Molecular Pathology,Volume 46, Issue 2, April 1987, Pages 199 – 216
F. Angelini Research Institute, Viale Amelia 70, 00181, Rome, Italy
http://www.sciencedirect.com/science/article/pii/0014480087900669

2) (1989): "after cessation of a 61-day inhalation exposure to 1000 ppm n-hexane…. Severe testicular atrophy involving the seminiferous tubules with loss of the nerve growth factor (NGF) immunoreactive germ cell line was found. Total loss of the germ cell line was found in a fraction of animals up to 14 months post-exposure, indicating permanent testicular damage."

Arch Toxicol. 1989;63(4):296-307.

Testicular atrophy and loss of nerve growth factor-immunoreactive germ cell line in rats exposed to n-hexane and a protective effect of simultaneous exposure to toluene or xylene.

Nylén P, Ebendal T, Eriksdotter-Nilsson M, Hansson T, Henschen A, Johnson AC, Kronevi T, Kvist U, Sjöstrand NO, Höglund G, et al.

Department of Neuromedicine, National Institute of Occupational Health, Solna, Sweden.

http://www.ncbi.nlm.nih.gov/pubmed/2764718

3) (1990) Prenatal exposure to hexane was found to cause "persistence of reduced body growth … effects of malnutrition were added to the solvent-induced retardation. This resulted in an extreme delay in tissue maturation accompanied by a retarded cell maturation. …"

Gisela Stoltenburg-Didinger at el., Neurotoxicity of organic solvent mixtures: Embryotoxicity and fetotoxicity, Neurotoxicology and Teratology**,** Volume 12, Issue 6, November–December 1990, Pages 585–589
http://www.sciencedirect.com/science/article/pii/089203629090066L

4) (1991): "Benzine might have effects on the reproduction function of female workers and intelligence development of their offsprings."

Yu Dong-xue, Liu Rui-hua, Effects of benzene compounds in female reproductive function and their offspring, Industrial Health & Professional Disease, 1991(6) [Chinese]
http://www.cnki.com.cn/Article/CJFDTotal-GYWZ199106010.htm

5. (1995): "The results of the study support the hypothesis that daily or high exposure to organic solvents is associated with reduced fertility."

Am J Ind Med. 1995 May;27(5):699-713.

Reduced fertility among women exposed to organic solvents.

Sallmén M, Lindbohm ML, Kyyrönen P, Nykyri E, Anttila A, Taskinen H, Hemminki K.

Department of Epidemiology and Biostatistics, Institute of Occupational Health, Helsinki, Finland.

http://www.ncbi.nlm.nih.gov/pubmed/7611306

6. (1997): "Our results support the hypothesis that spontaneous abortion may be an adverse effect of exposure to high levels of organic aliphatic solvents in women employed in shoe manufacture."

Int Arch Occup Environ Health 1997;69 (5):311-6.
Risk of spontaneous abortion and maternal exposure to organic solvents in the shoe industry.
Agnesi R , **Valentini F** , **Mastrangelo G** .
ULSS 13 Dipartimento di Prevenzione, Dolo, Italy.
http://pubmed.cn/9192214

6. (1997): "Exposure to more than the highest allowable concentration and lower than the highest allowable concentration of benzene and its homologue has certain effect on female menstruation and pregnancy"

Jiang Qi-de et al., The effect of exposure to different concentration of benzene on the female menstruation and pregnancy outcome, Industrial Health & Professional Disease, 1997(2) [Chinese]
http://www.cnki.com.cn/Article/CJFDTOTAL-GYWZ199702024.htm

7. (1997): "benzene mixture causes harm to genetic material of workers. The degree of harm indicates a trend increases with duration of exposure."

Zhao Guo-hua et al., Observation of exposure to low concentration benzene compounds in workers cell micronucleus rate and chromosome fracture rate, Modern Preventive Medicine, 1997(4) [Chinese]
http://www.cnki.com.cn/Article/CJFDTOTAL-XDYF704.004.htm

8. (1997): "Pregnant woman's exposure to benzene mixture is the main factor effecting weight and length of newborns."

Chen Da-fang, et al., The effect of pregnant women exposed to benzene mixture compounds on fetal growth and development, Disease Control Journal, 1997(4) [Chinese]
http://www.cnki.com.cn/Article/CJFDTotal-JBKZ199704008.htm

9. (1998): "The findings of the study provide limited support for the hypothesis that paternal exposure to organic solvents might be associated with decreased fertility."

Sallmén M et al., Time to pregnancy among the wives of men exposed to organic solvents. Occup Environ Med 1998 Jan;55 (1): 24-30
Finnish Institute of Occupational Health, Department of Epidemiology and Biostatistics, Helsinki, Finland.
http://pubmed.cn/9536159

10. (2000): "Exposure to low concentration of mixed benzene maybe correlated with the declined quality of semen and VEFL in occupational workers, and urinary t, t-MA and beta-hCG can be used as biomarkers of exposure and effect, respectively."

[Wang S, Chen H, Wang X. Studies on relationship between exposure to low concentration of mixed benzene and lower quality of semen and very early fetal loss]. Zhonghua Yu Fang Yi Xue Za Zhi. 2000 Sep;34(5):271-3.
School of Public Health, Nanjing Medical University, Nanjing 210029, China.
英译文：http://www.ncbi.nlm.nih.gov/pubmed/11372393

11. (2000): "N-hexane induces chromosome damage on poisoned patients"

Liang Wei-hui et al., Peripheral blood micronucleus tests of occupational n-hexane poisoned patients, China Academy of Science Shanghai Metallurgical Research Institute; Material physics and chemistry PhD thesis, 2000 [Chinese]
http://www.chemyq.com/health/ep109/1083892_A7928.htm

12. (2001): "Exposure to low level of mixed benzene in workspaces could interrupt the function of hypothalamic-pituitary-ovarian axis and affect their normal levels of FSH, PdG and E1C."

Chen Hai-yan et al., Effects of low concentration mixed benzene on female reproductive hormones, China Preventative Medicine Journal, 2001(2) [Chinese]
http://www.cnki.com.cn/Article/CJFDTotal-ZHYF200102003.htm
English: http://www.ncbi.nlm.nih.gov/pubmed/11413688

13. (2004): "Female, age between 17 to 22, average 18. Past history all indicate healthy … monitoring shows, the main composition of the evaporated fume from the resins is hexane … 11 girls among this group all appeared with no periods or out of order."

Xu Xue-chun, Clinical observation analysis of chronic n-hexane poisoning, Industrial Health & Occupational Disease, 2004(3) [Chinese]

http://www.cnki.com.cn/Article/CJFDTotal-GYWZ200403019.htm

14. (2004): "Professional exposure to benzene could cause problems with periods or reproduction functions."

Huo Zhong-li, Pu Xiao-yan, Influence of benzene and its homologue on reproductive function of occupational female, China's Maternity & Child Care, 2004(4),39-40
http://www.chemdrug.com/databases/7_41_akjrtegreeveuyow.html

15. (2006): "Results suggest that maternal occupational exposure to organic solvents during pregnancy may play a role in the aetiology of oral clefts."

Occup Environ Med 2006 Sep;63 (9):617-23.
Chevrier C et al., Occupational exposure to organic solvent mixtures during pregnancy and the risk of non-syndromic oral clefts. Occup Environ Med 2006 Sep;63 (9):617-23.
Inserm U625, Rennes, France. cecile.chevrier@rennes.inserm.fr
http://pubmed.cn/16644895

16. (2007) : "The findings provide further evidence that exposure to organic solvents is hazardous for female reproduction. The observed association may be related to any of the following solvents commonly used in shoe manufacturing: n-hexane and hexane isomers, toluene, acetone, ethyl acetate and dichloromethane."

Occup Environ Med. 2008 Aug;65(8):518-24. Epub 2007 Nov 7.
Sallmén M, Reduced fertility among shoe manufacturing workers. Occup Environ Med. 2008 Aug;65(8):518-24. Epub 2007 Nov 7.
Sallmén M, Neto M, Mayan ON.
Centre of Expertise for Health and Work Ability, Finnish Institute of Occupational Health, Topeliuksenkatu 41 aA, FI-00250 Helsinki, Finland. Markku.Sallmen@ttl.fi
http://www.ncbi.nlm.nih.gov/pubmed/17989205

17. (2007): "Shows that n-hexane caused different levels of lipid peroxidation damage. The microscopic observation 7 d group micro-structure of germ cells shows apparent anomalies in the development of gonad. Conclusion: N-hexane suction cause certain damage to gonad and developing germ cells of rat, lipid peroxidation damage may be one of the mechanism of such toxic effects."

Cao Jing-ting et al., N-hexane static type causes gonad damage to SD rats, China Labor Health Occupational Disease Journal, 2007(5) [Chinese]
http://d.wanfangdata.com.cn/Periodical_zhldwszyb200705005.aspx

18. (2007): "Benzine, ….. causes abnormal changes to periods, incidence shows positive relevance with the number of years of professional exposure; incidence of natural miscarriage, newborn low weight are both higher than control group."

Xing Liang-hong, Deng Xiao-mei, Survey of female menstruation and fertility working with benzene exposure in leather shoe manufacturer at Shandong Rizhao city, Preventative Medicine Forum, 2007(10)
http://www.cnki.com.cn/Article/CJFDTotal-YXWX200710034.htm

19. (2009): "1. N-hexane has obvious female gonads toxicity, N-hexane can decrease the organ weights and organ coefficients of ovaries, the period of estrous stages in rats decreased significantly, the constituent rate of primordial follice, atrestic folicle, mature follicle and corpus uterum/albiacans number were in disorder. N-hexane can decrease the level of serum P4, N-hexane can inhibit P4 and E2 release in the ovaries, having the direct adverse effects, which might be the important mechanism in endocrine disruption …4. N-hexane can decrease the reaction of super gonadotrophic hormones, but it not sure of the reaction of normal gonadotrophic hormones. 4. N-hexane can decrease the reaction of super gonadotrophic hormones, but it not sure of the reaction of normal gonadotrophic hormones.

Pang Fen, N-hexane (female) female sex gonad reproductive toxicity and reproductive endocrine disruption studies, (Master degree thesis, 2009-03-01), Health Toxicology, Fujian Medical University
http://d.wanfangdata.com.cn/Thesis_Y1487285.aspx

20. (2009): "Hexane could cause obvious female gonads toxicity, ovaries is one of the main toxicity organ targets."

Ou Yang-jiang et al., N-hexane gonad toxic effect on female rats, Haixia Preventive Medical Journal, 2009(4)
http://www.cnki.com.cn/Article/CJFDTotal-HXYF200904003.htm

21. (2010) "Hexane demonstrates obvious female reproduction gonads interruption, can cause abnormal changes in period of estrous stages, cause Ovarian sex hormone synthesis dysfunction of granulosa cells; N-hexane through effect StAR protein, P450scc and P450arom expression in the process of female, progestational hormone synthesis of ovarian granulosa cells, thus further interfere synthesis of both male and female, progestational hormone. This may be one of the important mechanisms how n-hexane causes disruption of reproductive endocrine."

Ou Yang-jiang, Zhang Wen-chang, Studies of N-hexane's disruption of ovarian female male, progestational hormone synthesis and mechanism, Biochemical/Industrial & Health Toxicology Academic Conference Proceedings, 2010.
http://cpfd.cnki.com.cn/Article/CPFDTOTAL-ZGDV201007001088.htm

22. (2010): "N-hexane affected mice oocyte GVBD rate, and inhibit its nuclear mature oocytes."

Liu Jin, Zhang Wen-chang, N-hexane toxic effect study of oocyte maturation, Biochemical/Industrial & Health Toxicology Academic Conference Proceedings, 2010.
http://cpfd.cnki.com.cn/Article/CPFDTOTAL-ZGDV201007001095.htm

23. (2010): N-hexane reproductive endocrine disruption (like estrus cycle disorder, sex hormone level change, etc), is caused not by the influence of sex hormone receptor expression in mice. ... Its reproductive secrete interference effect may be through the influence of the combination of sex hormone ligand and receptor or other signaling pathways."

Liu Jin, Zhang Wen-chang, The chronic exposure of n-hexane to uterus, ovarian hormone receptor expression in mice, Biochemical/Industrial & Health Toxicology Academic Conference Proceedings, 2010.
http://cpfd.cnki.com.cn/Article/CPFDTOTAL-ZGDV201007001095.htm

24. (2011): "1. N-hexane can inhibit mouse oocyte mature, including nuclear maturity and maturity; 2,5-adipic ketone is an effect how n-hexane affects the main active metabolite of oocyte nucleus maturity. 2. Mitochondrial damage and early apoptosis of oocytes, might be one of the important factors of n-hexane inhibition of mouse oocytes mature; 3. N-hexane can induce ovarian granulosa cell apoptosis."

Liu Jin, Studies of N-hexane effect on oocyte maturation and granulosa cell apoptosis and its mechanism, PhD thesis, Fujian Medical University, 2011
http://cdmd.cnki.com.cn/Article/CDMD-10392-1011154760.htm

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 179691, 8 pages
http://dx.doi.org/10.1155/2014/179691

Research Article

Major Pesticides Are More Toxic to Human Cells Than Their Declared Active Principles

Robin Mesnage,[1] Nicolas Defarge,[1] Joël Spiroux de Vendômois,[2] and Gilles-Eric Séralini[1]

[1] *University of Caen, Institute of Biology, CRIIGEN and Network on Risks, Quality and Sustainable Environment MRSH-CNRS, Esplanade de la Paix, 14032 Caen Cedex, France*
[2] *CRIIGEN, 40 rue Monceau, 75008 Paris, France*

Correspondence should be addressed to Gilles-Eric Séralini; criigen@unicaen.fr

Received 28 October 2013; Accepted 11 December 2013; Published 26 February 2014

Academic Editor: Bruno C. Cavalcanti

Pesticides are used throughout the world as mixtures called formulations. They contain adjuvants, which are often kept confidential and are called inerts by the manufacturing companies, plus a declared active principle, which is usually tested alone. We tested the toxicity of 9 pesticides, comparing active principles and their formulations, on three human cell lines (HepG2, HEK293, and JEG3). Glyphosate, isoproturon, fluroxypyr, pirimicarb, imidacloprid, acetamiprid, tebuconazole, epoxiconazole, and prochloraz constitute, respectively, the active principles of 3 major herbicides, 3 insecticides, and 3 fungicides. We measured mitochondrial activities, membrane degradations, and caspases 3/7 activities. Fungicides were the most toxic from concentrations 300–600 times lower than agricultural dilutions, followed by herbicides and then insecticides, with very similar profiles in all cell types. Despite its relatively benign reputation, Roundup was among the most toxic herbicides and insecticides tested. Most importantly, 8 formulations out of 9 were up to one thousand times more toxic than their active principles. Our results challenge the relevance of the acceptable daily intake for pesticides because this norm is calculated from the toxicity of the active principle alone. Chronic tests on pesticides may not reflect relevant environmental exposures if only one ingredient of these mixtures is tested alone.

1. Introduction

Pesticides are used throughout the world as mixtures called formulations. They contain adjuvants, which are often kept confidential and are called inerts by the manufacturing companies, plus a declared active principle (AP), which is the only one tested in the longest toxicological regulatory tests performed on mammals. This allows the calculation of the acceptable daily intake (ADI)—the level of exposure that is claimed to be safe for humans over the long term—and justifies the presence of residues of these pesticides at "admissible" levels in the environment and organisms. Only the AP and one metabolite are used as markers, but this does not exclude the presence of adjuvants, which are cell penetrants. Our previous investigation showed unexpected APs for human cell toxicity in the adjuvants of glyphosate-based herbicides [1]. Ethoxylated adjuvants found in glyphosate-based herbicides were up to 10.000 times more toxic than the so-called active AP glyphosate [1] and are better candidates for secondary side effects. This may explain in vivo long-term toxicity from 0.1 ppb of the formulation and other toxicities that were not explained by a consideration of glyphosate alone [2–5]. These adjuvants also have serious consequences to the health of humans and rats in acute exposures [6, 7]. These findings prompted us to investigate the presence of similar toxic molecules in other classes of pesticides.

The regulatory system assumes that the AP designed to specifically target plants, insects or fungi is the most toxic compound of a formulation to nontarget species. Thus long-term regulatory tests are performed on this substance alone. In this paper, we tested to what extent the AP or adjuvants in present formulations account for the toxicity of 9 major pesticides: 3 herbicides, 3 insecticides, and 3 fungicides.

We have thus selected 9 APs of herbicides, insecticides, or fungicides of different classes (Table 1) used for agricultural or domestic purposes, from the major pesticides

Table 1: Summary of the pesticides tested. We have tested 9 APs of major herbicides, insecticides, or fungicides of different classes, used worldwide for agricultural or domestic purposes. Concentrations of the APs are indicated in parenthesis. Adjuvants are reported where they are mentioned on the material safety data sheet (MSDS).

	Pesticide classes	Active principles	(g/L)	Formulations	Declared adjuvants
Herbicides	Phosphonoglycine	Glyphosate	450	Roundup GT+	Ethoxylated etheralkylamine
	Phenylurea	Isoproturon	500	Matin EL	Unknown
	Synthetic auxin	Fluroxypyr (ester 1-methylheptyl)	200	Starane 200	Solvent naphtha; alkyl-aryl sulfonates
Insecticides	Carbamate	Pirimicarb	500	Pirimor G	Docusate sodium; benzenesulfonic acid
	Neonicotinoid	Imidacloprid	200	Confidor	1-Methyl-2-pyrrolidinone
	Neonicotinoid	Acetamiprid	5	Polysect Ultra	1,2-Benzisothiazoline-3-one; ethanol
Fungicides	Triazole	Tebuconazole	250	Maronee	N,N-Dimethyldecanamide
	Triazole	Epoxiconazole	125	Opus	Solvent naphtha; fatty alcohol ethoxylated
	Imidazole	Prochloraz	450	Eyetak	Solvent naphtha; xylene; isobutanol

used worldwide [8, 9]. First we tested Roundup and its AP, glyphosate. Upon the introduction of herbicide tolerant genetically modified organisms (GMOs), designed to tolerate Roundup and to accumulate unusual levels of its residues, Roundup quickly became the major pesticide in the world and a major food or feed contaminant [10]. Two other herbicides of a different class were tested: isoproturon (phenylurea) is the second most widely used AP of herbicides in Europe in the control of annual grasses and broad-leaved weeds in cereals and a major water contaminant [11]; and fluroxypyr (a synthetic auxin) is used as an AP on noncrop areas and also for agricultural use on wheat, barley, corn, and oats. Forest services are expanding its use as an alternative to other pesticides known to be toxic [12]. However, it is poorly studied and its effects on human cells were never published before. Among the insecticides chosen, pirimicarb (a carbamate), used specifically to target aphids, is the most representative AP in this family for cereal production and garden insect control worldwide [13]. Neonicotinoids are the largest selling insecticides worldwide and are marketed in more than 120 countries for use on more than 140 crops [14]. Their spectrum of biological efficacy covers a broad range of target pests such as whiteflies, lepidopteran, and coleopteran species. We tested the major neonicotinoid, the AP imidacloprid, which is widely used for seed dressing. Its toxicity against bees is widely admitted [15], but little is known about the effects of its adjuvants. We also tested the AP acetamiprid, another neonicotinoid advocated to replace imidacloprid [16]. Azole-type fungicides are applied every year on field crops, fruit trees, vegetables, and grassgrowing areas [17]. We tested the two most popular triazole APs, epoxiconazole and tebuconazole. Finally, prochloraz (imidazole) was tested because it is the main fungicide sprayed on cereals in Europe [8].

We used the embryonic (HEK293), placental (JEG3), and hepatic (HepG2) human cell lines because they are well characterized and validated as useful models to test toxicities of pesticides [18–20], corresponding to what is observed on fresh tissue or primary cells [21–23]. These cell lines are even in some instances less sensitive than primary cells [24, 25] and therefore do not overestimate cellular toxicity. We assayed their mitochondrial succinate dehydrogenase (SD) activity (MTT assay) after 24 h pesticide exposure, which is one of the most accurate cytotoxicity assays for measuring the toxicity of pesticide adjuvants such as surfactants [26]. Cytotoxicity was confirmed by the measurement of apoptosis and necrosis, respectively, by caspases 3/7 activation [27] and adenylate kinase leakage after membrane alterations [28]. Each AP was tested from levels below its ADI to its solubility limit in our system. The formulations containing adjuvants were tested at the same levels.

2. Materials and Methods

2.1. Chemicals. The 9 Aps, glyphosate (N-phosphonomethyl glycine, G, CAS: 1071-83-6), isoproturon (3-(4-isopropylphenyl)-1,1-dimethylurea, CAS: 34123-59-6), fluroxypyr 1-methylheptyl ester (((4-Amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy)acetic acid, 1-methylheptyl ester, CAS: 81406-37-3), acetamiprid (N-[(6-chloro-3-pyridyl) methyl]-N′-cyano-N-methyl-acetamidine, CAS: 135410-20-7), imidacloprid (1-((6-chloro-3-pyridinyl)methyl)-4,5-dihydro-N-nitro-1H-imidazol-2-amine, CAS: 105827-78-9), pirimicarb (2-dimethylamino-5,6-dimethyl-4-pyrimidinyl dimethylcarbamate, CAS: 23103-98-2), prochloraz (N-propyl-N-(2,4,6-trichlorophenoxy) ethyl-imidazole-1-carboxamide, CAS: 67747-09-5), epoxiconazole (1-{[3-(2-Chlorophenyl)-2-(4-fluorophenyl)-2-oxiranyl]methyl}-1H-1,2,4-triazole, CAS: 135319-73-2), tebuconazole (1-(4-Chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazole-1-ylmethyl)pentane-3-ol, CAS: 107534-96-3), and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), as well as all other compounds, unless otherwise noted, were obtained from Sigma-Aldrich. Formulations were available on the market: Roundup GT+ (approval 2020448), Matin EL (2020328), Starane 200 (8400600), Pirimor G (7500569), Confidor (9200543), Polysect Ultra SL (2080018), Maronee (2000420), Opus (9200018), and Eyetak (9400555). MTT was prepared as a 5 mg/mL stock solution in phosphate-buffered saline, filtered through a 0.22 μm filter before use, and diluted to 1 mg/mL in a serum-free medium.

2.2. Cell Lines and Treatments. The human embryonic kidney 293 cell line (HEK 293, ECACC 85120602) was provided by Sigma-Aldrich (Saint-Quentin Fallavier, France). The hepatoma cell line HepG2 was provided by ECACC

http://dx.doi.org/10.1155/2014/179691

(85011430). JEG3 cell line (ECACC 92120308) was provided by CERDIC (Sophia-Antipolis, France). Cells were grown in phenol red-free EMEM (Abcys, Paris, France) containing 2 mM glutamine, 1% nonessential amino acid, 100 U/mL of antibiotics (a mixture of penicillin, streptomycin, and fungizone) (Lonza, Saint Beauzire, France), 10 mg/mL of liquid kanamycin (Dominique Dutscher, Brumath, France), and 10% Fetal Bovine Serum (PAA, les Mureaux, France). JEG3 cells were supplemented with 1 mM sodium pyruvate. Cells were grown with this medium at 37°C (5% CO_2, 95% air) during 48 h to 80% confluence, then washed, and exposed 24 h with serum-free EMEM to the APs or the formulations. Before treatment, all the pesticides were solubilized in a 100% DMSO solution, then diluted in serum-free medium to reach 0.5% DMSO (which had been previously proven not to be cytotoxic for the cells), and adjusted to a similar pH. This model was validated [29] and cytotoxic effects were similar in presence of serum but delayed by 48 h.

2.3. Cytotoxicity Measurement. After treatments, succinate dehydrogenase (SD) activity assay (MTT) [30] was applied as described previously [25]. Integrity of mitochondrial dehydrogenase enzymes indirectly reflects the cellular mitochondrial respiration. The optical density was measured at 570 nm using a Mithras LB 940 luminometer (Berthold, Thoiry, France). The bioluminescent ToxiLight bioassay (Lonza, Saint Beauzire, France) was applied for the membrane degradation assessment, by the intracellular adenylate kinase (AK) release in the medium; this is described as a necrosis marker [28]. Finally, the apoptotic cell death was evaluated with the Caspase-Glo 3/7 assay (Promega, Paris, France). Luminescence was measured using a Mithras LB 940 luminometer (Berthold, Thoiry, France). These methods were previously described [25].

2.4. Statistical Analysis. The experiments were repeated at least 3 times in different weeks on 3 independent cultures ($n = 9$). All data were presented as the means ± standard errors (SEMs). LC50 values were the best-fitted value of a nonlinear regression using sigmoid (5-parameter) equation with the GraphPad Prism 5 software. The differential effects between APs and formulations are measured by the surfaces between the curves by the calculation of integrals with ImageJ software [31]. Statistical differences of necrosis and apoptosis assays were calculated by a nonparametric Mann-Whitney test with the GraphPad Prism 5 software.

3. Results

All formulations were cytotoxic and far more toxic than their APs, except for isoproturon and its formulated pesticide Matin which were both not soluble over 100 ppm. As a matter of fact, Matin does not have any declared adjuvant (Table 1). On human cells, among the tested products, fungicides were the most toxic (Figure 1), being cytotoxic from doses 300–600 times lower than agricultural dilutions, followed by herbicides (Figure 2) (except Matin) and then insecticides (Figure 3). JEG3 was the most sensitive cell line, the LC50 being on average, respectively, 7% and 23% lower than for HEK293 and HepG2, the least sensitive. The LC50 is calculated over 24 h. In all cell types, fungicides were the most toxic (mean LC50 12 ppm). They were followed by the herbicide Roundup (LC50 63 ppm), twice as toxic as Starane, and more than 10 times as toxic as the 3 insecticides, which represent the less toxic group (mean LC50 720 ppm). The APs of fungicides were the only APs that were toxic alone in our system, from 50 ppm in JEG3 for prochloraz, but they were still less toxic than their formulations.

In fact, 8 formulations out of 9 were clearly on average several hundred times more toxic than their APs, ranging from 2-3 times more toxic for pirimicarb or prochloraz to 1056 times more toxic for tebuconazole. Results were similar for all cell types.

This was even better understood by the differential measurement of the cytotoxicity through membrane disruption (Figure 4) or caspases activation (Figure 5). For the three cell lines, membrane disruptions are comparable. Most of the pesticides were necrotic and more necrotic than their APs except for Eyetak whose active principle prochloraz is the main toxicant of the formulation. We have not obtained relevant results with Pirimor because a green dye in the formulated product prevents the lecture of luminescence. Differential effects on apoptosis (Figure 5) were less obvious. With the formulated herbicides and insecticides, apoptosis levels are mostly decreased because of the prevailing effects of necrosis. This is not the case with fungicides which are apoptotic depending on the cell line. JEG3 cell lines are the most sensitive to apoptosis, in particular with fluroxypyr, pirimicarb, tebuconazole, and prochloraz. Overall, adjuvants in pesticides are thus far from inerts but cell membrane disruptors and induce in addition mitochondrial alterations.

4. Discussion

This is the first time that all these formulated pesticides have been tested on human cells well below agricultural dilutions. The three different cell types reacted very similarly and the toxicities were observed on several biomarkers; this confirmed our results. Moreover, these are very consistent with several studies on cell lines [1, 25], where placental JEG3 cells were found to be the most sensitive. In this study [1], adjuvants were also more cytotoxic through the disruption of membrane and mitochondrial respiration than from an activation of apoptotic pathways. Primary cells are in some case up to 100 times more sensitive, for instance, neonate umbilical cord vein cells [25]. We also study here short exposures (24 h), but we have previously demonstrated a time-amplifying effect: the differential toxicity between the AP glyphosate and Roundup is increased by 5 times in 72 h [29]. It appears that, with cell lines and short exposures, we underestimate by far the direct toxicity of the products in the long term. In this case in vivo, the metabolism may reduce the toxic effect, but this can be compensated or amplified by bioaccumulation and/or the combined effect of the AP with adjuvants. For instance, in this experiment, after 24 h, 63 ppm of Roundup was found to be toxic to cells, but in our previous

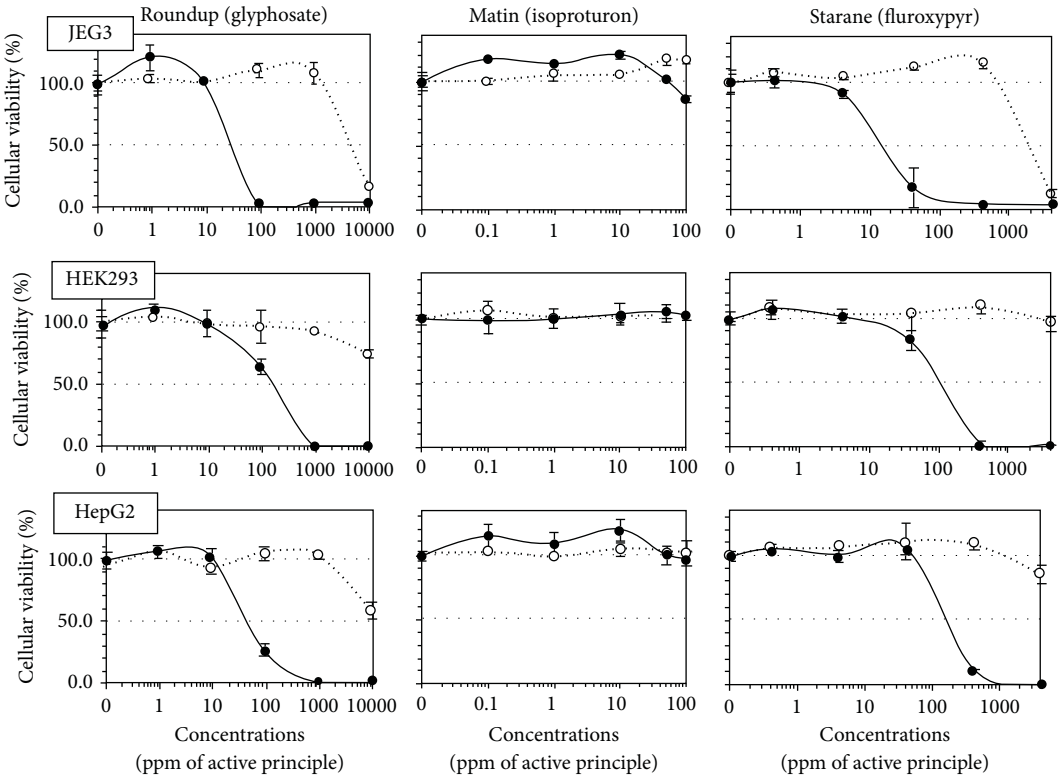


Figure 1: Differential cytotoxic effects between formulations of herbicides and their active principles (APs) on HepG2, HEK293, and JEG3 human cell lines. Effects on the mitochondrial succinate dehydrogenase (SD) activity, reflecting cell respiration inhibition, were measured in percentage of control in serum-free medium after 24 h of exposure. The concentrations in ppm are dilutions of each AP (dotted line) and their equivalent in formulation with adjuvants (solid line). All formulations are more toxic than their APs, except for isoproturon. SEMs are shown in all instances ($n = 9$).

experiment, after two years in rats, only 0.1 ppb of Roundup was found to be sufficient to provoke pathologies [2].

Adjuvants in pesticides are generally declared as inerts, and for this reason they are not tested in long-term regulatory experiments. It is thus very surprising that they amplify up to 1000 times the toxicity of their APs in 100% of the cases where they are indicated to be present by the manufacturer (Table 1). In fact, the differential toxicity between formulations of pesticides and their APs now appears to be a general feature of pesticides toxicology. As we have seen, the role of adjuvants is to increase AP solubility and to protect it from degradation, increasing its half-life, helping cell penetration, and thus enhancing its pesticidal activity [32] and consequently side effects. They can even add their own toxicity [1]. The definition of adjuvants as "inerts" is thus nonsense; even if the US Environmental Protection Agency has recently changed the appellation for "other ingredients," pesticide adjuvants should be considered as toxic "active" compounds.

In the scientific literature, in contrast with regulatory beliefs, some harmful effects of the adjuvants present in this study are reported. In the formulations (Table 1) Starane 200, Opus, and Eyetak, the adjuvants include solvent naphtha (a petroleum distillate), which is known to have developmental effects in rodents [33]. Xylene (in Eyetak) has long been associated with cardiac and central nervous system diseases in humans [34]. 1-Methyl-2-pyrrolidinone (in Confidor) is a developmental toxicant and caused malformations, incomplete ossification of skull, and decreased fetal body weights in rats [35]. N,N-Dimethyldecanamide (Maronee adjuvant) has been characterized as a developmental toxicant in rodents [36] but is insufficiently studied for reproductive toxicity. The distinction between AP and "declared inert" compounds appears to be a regulatory assumption with no toxicological basis, from this experiment and others. Even industry and regulators contradict themselves in the classification of APs and inert compounds. For example, 1,2-benzisothiazoline-3-one is classed as an inert ingredient in the pesticide Polysect in particular and as an active ingredient in cleaning products [37].

All this does not exclude the toxicity of APs alone. Glyphosate inserted in the aromatase active site of mammalian cells disrupts steroidogenesis [23]. Imidacloprid alters the developing immunity in rats [38]. Fluroxypyr (ester 1-methylheptyl) has never been tested in human cells before this study but appears to be toxic from 22 ppm in formulation; its ADI is only 0.8 ppm/day (DG SANCO, 2013). It also appears here that prochloraz is the main toxicant of the tested formulation.

It is commonly believed that Roundup is among the safest pesticides. This idea is spread by manufacturers, mostly in the reviews they promote [39, 40], which are often cited

http://dx.doi.org/10.1155/2014/179691

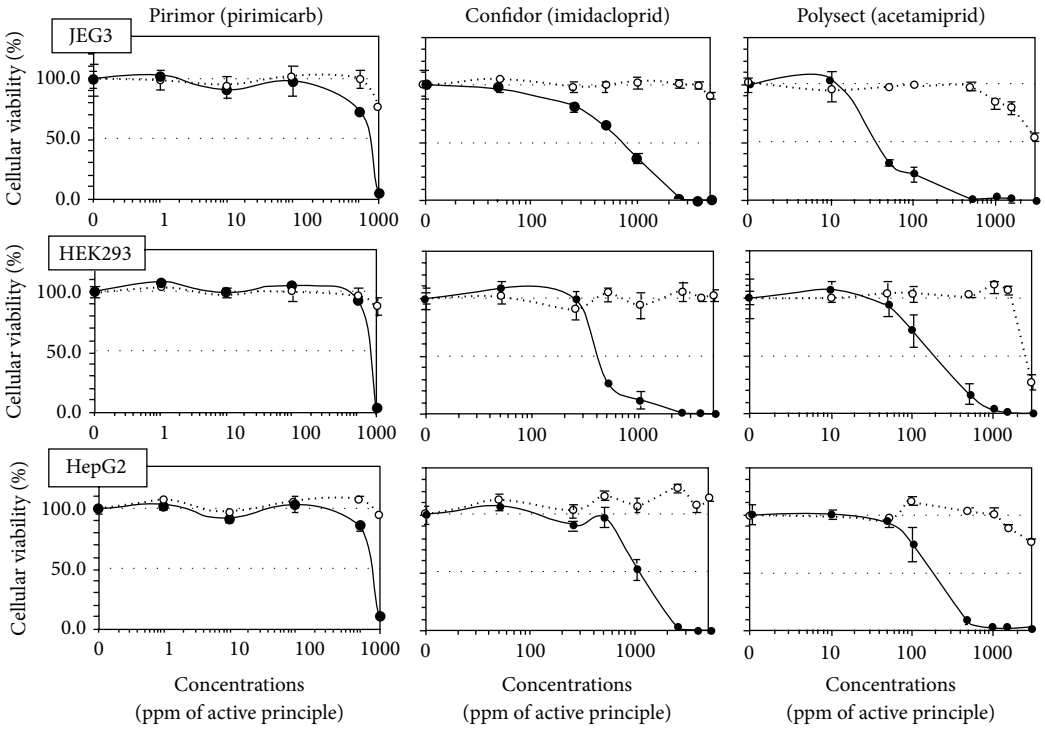


Figure 2: Differential cytotoxic effects between formulations of insecticides and their APs on HepG2, HEK293, and JEG3 human cell lines. The three described human cell lines were used in the conditions of Figure 1 and the results were almost identical. All formulations (solid line) are more toxic than their APs (dotted line); APs are slightly cytotoxic. SEMs are shown in all instances ($n = 9$).

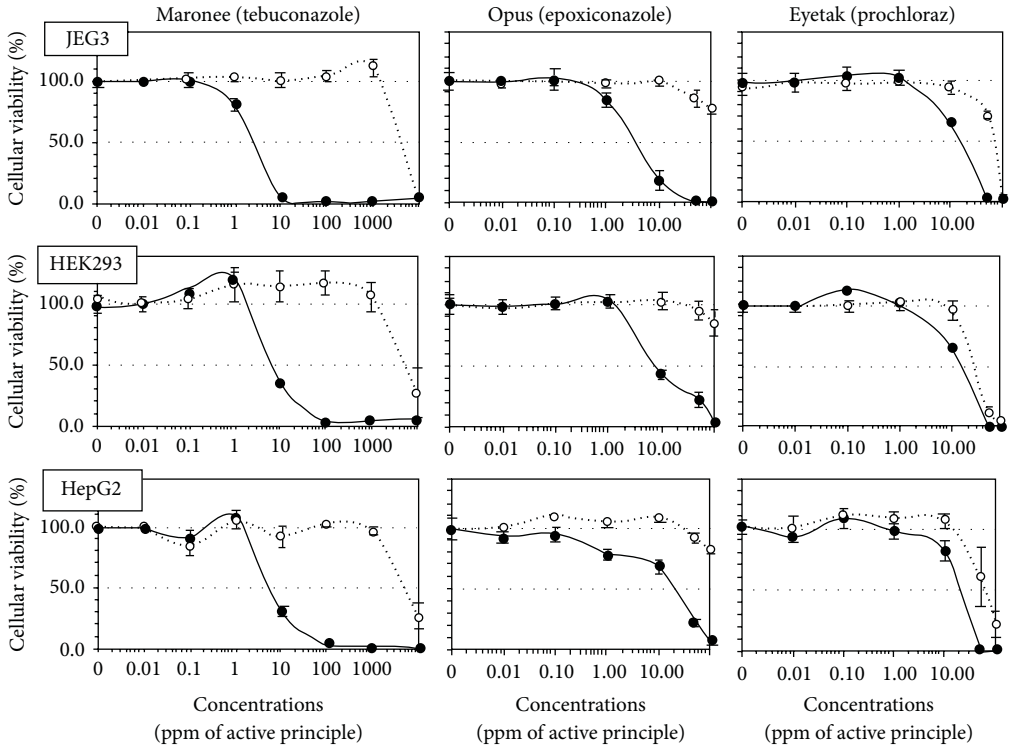


Figure 3: Differential cytotoxic effects between formulations of fungicides and their APs on HepG2, HEK293, and JEG3 human cell lines. The three described human cell lines were used in the culture conditions of Figure 1, and the results were almost identical. All formulations (solid line) are more cytotoxic than their APs (dotted line). Maronee is the most toxic compound tested from 1 ppm in JEG3. SEMs are shown in all instances ($n = 9$).

http://dx.doi.org/10.1155/2014/179691

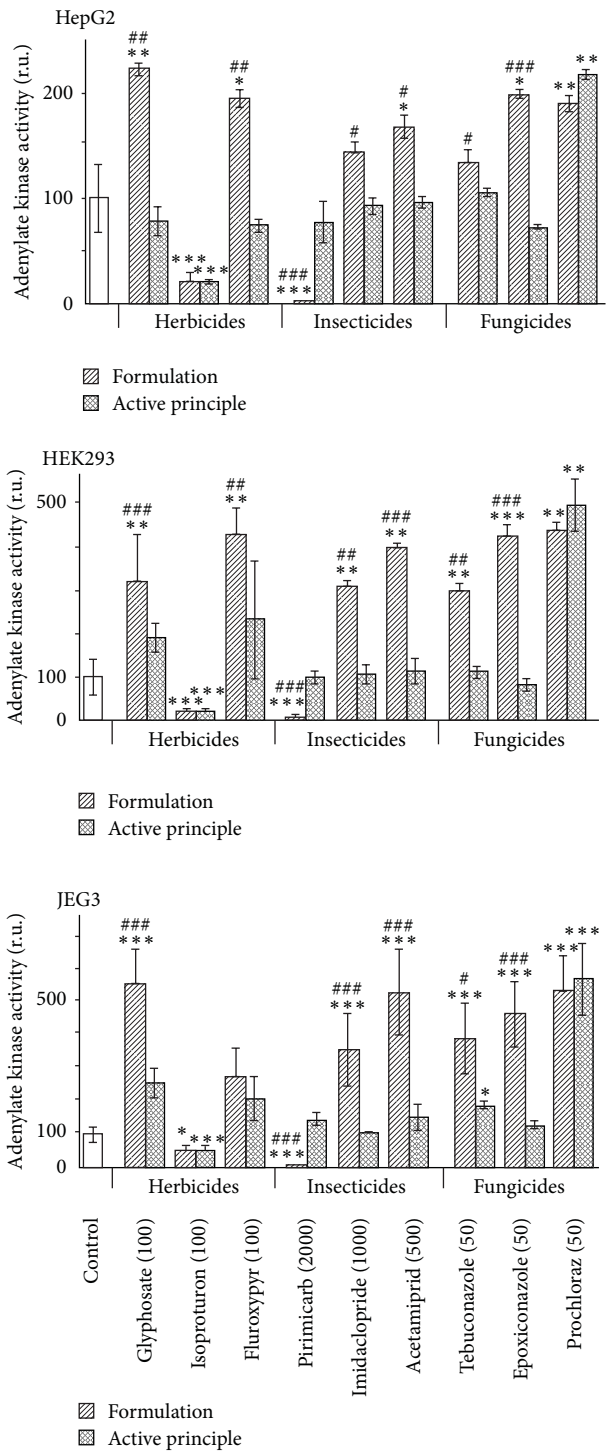


Figure 4: Differential necrotic effects between formulations and their APs. The three described human cell lines were used in the culture conditions of Figure 1. We have chosen the doses at the first differential effects measured by MTT assay. Formulations (stripped columns, expressed in ppm of the AP) are generally more cytotoxic than their APs (dashed columns) due to a necrotic effect of adjuvants. SEMs are shown in all instances ($n = 9$). For the comparison of each AP or formulation to the control (white column), $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ in a nonparametric Mann-Whitney test. # symbol is used similarly for comparisons between APs and their formulations.

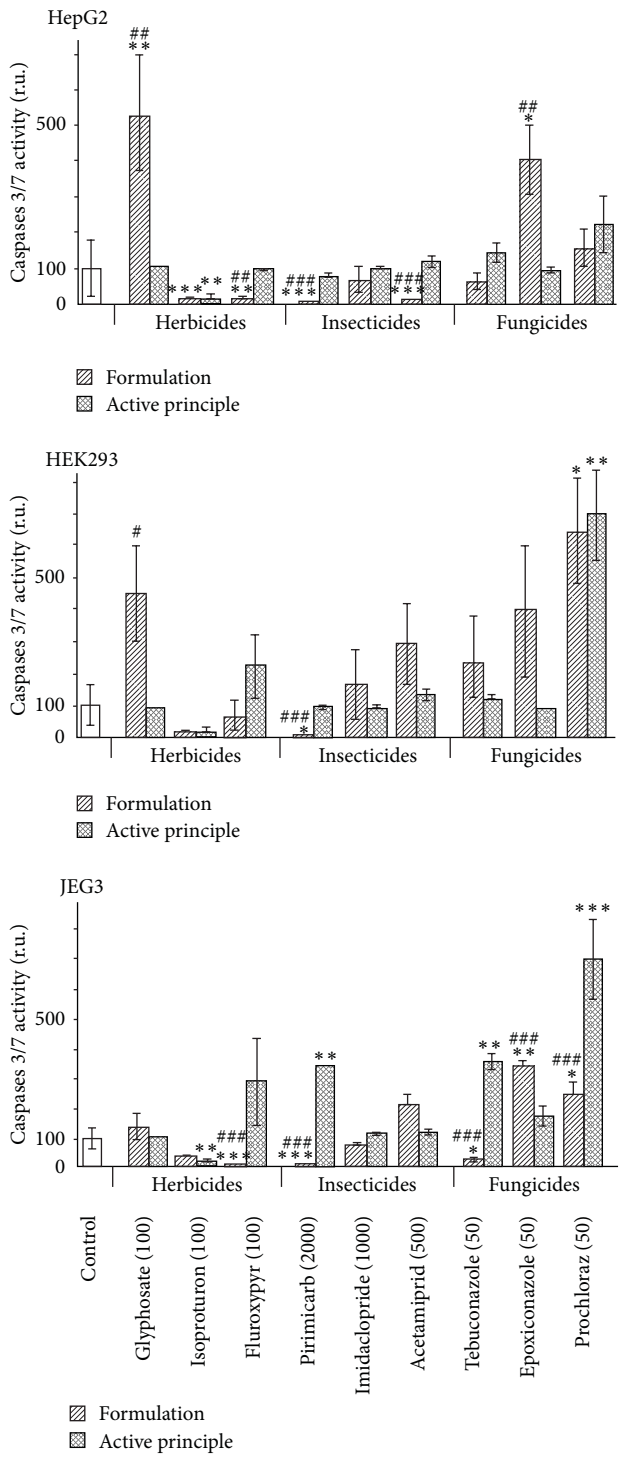


Figure 5: Differential apoptotic effects between formulations and their APs. The three described human cell lines were used in the culture conditions of Figure 1. We have chosen the doses at the first differential effects measured by MTT assay. SEMs are shown in all instances ($n = 9$). For the comparison of each AP or formulation to the control (white column), $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ in a nonparametric Mann-Whitney test. # symbol is used similarly for comparisons between APs and their formulations.

in toxicological evaluations of glyphosate-based herbicides. However, Roundup was found in this experiment to be 125 times more toxic than glyphosate. Moreover, despite its reputation, Roundup was by far the most toxic among the herbicides and insecticides tested. This inconsistency between scientific fact and industrial claim may be attributed to huge economic interests, which have been found to falsify health risk assessments and delay health policy decisions [41].

In conclusion, our results challenge the relevance of the ADI, because it is calculated today from the toxicity of the AP alone in vivo. An "adjuvant factor" of at least a reduction by 100 can be applied to the present calculation of the ADI if this is confirmed by other studies in vivo. As an example, the present ADI for glyphosate is 0.3 ppm; for glyphosate-based herbicides it would be 3 ppb or less. However, this will never replace the direct study of the commercial formulation with its adjuvants in regulatory tests. Anyway, an exposure to a single formulated pesticide must be considered as coexposure to an active principle and the adjuvants. In addition, the study of combinatorial effects of several APs together may be very secondary if the toxicity of the combinations of each AP with its adjuvants is neglected or unknown. Even if all these factors were known and taken into account in the regulatory process, this would not exclude an endocrine-disrupting effect below the toxicity threshold. The chronic tests of pesticides may not reflect relevant environmental exposures if only one ingredient is tested alone.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors acknowledge the Regional Council of Low Normandy for Robin Mesnage fellowship and the Charles Leopold Mayer (FPH) and Denis Guichard Foundations, together with CRIIGEN, for structural support. They are equally thankful to Malongo, Lea Nature, and the JMG Foundation for their help.

References

[1] R. Mesnage, B. Bernay, and G. E. Seralini, "Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity," *Toxicology*, vol. 313, no. 2-3, pp. 122–128, 2013.

[2] G. E. Seralini, R. Mesnage, N. Defarge et al., "Answers to critics: why there is a long term toxicity due to NK603 Roundup-tolerant genetically modified maize and to a Roundup herbicide," *Food and Chemical Toxicology*, vol. 53, pp. 461–468, 2013.

[3] C. Gasnier, C. Dumont, N. Benachour, E. Clair, M. Chagnon, and G. Séralini, "Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines," *Toxicology*, vol. 262, no. 3, pp. 184–191, 2009.

[4] M. Peluso, A. Munnia, C. Bolognesi, and S. Parodi, "32P-post-labeling detection of DNA adducts in mice treated with the herbicide Roundup," *Environmental and Molecular Mutagenesis*, vol. 31, no. 1, pp. 55–59, 1998.

[5] L. P. Walsh, C. McCormick, C. Martin, and D. M. Stocco, "Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression," *Environmental Health Perspectives*, vol. 108, no. 8, pp. 769–776, 2000.

[6] S. M. Bradberry, A. T. Proudfoot, and J. A. Vale, "Glyphosate poisoning," *Toxicological Reviews*, vol. 23, no. 3, pp. 159–167, 2004.

[7] A. Adam, A. Marzuki, H. A. Rahman, and M. A. Aziz, "The oral and intratracheal toxicities of ROUNDUP and its components to rats," *Veterinary and Human Toxicology*, vol. 39, no. 3, pp. 147–151, 1997.

[8] European Commission, "The use of plant protection products in the European Union," 2007, http://epp.eurostat.ec.europa.eu/.

[9] US EPA, "Pesticide Industry Sales and Usage," 2012, http://www.epa.gov/opp00001/pestsales/.

[10] A. Székács and B. Darvas, "Forty years with glyphosate," in *Herbicides-Properties, Synthesis and Control of Weeds*, M. N. A. E.-G. Hasaneen, Ed., InTech, Rijeka, Croatia, 2012.

[11] Commissariat Général au Développement Durable, *Les Pesticides Dans Les Milieux Aquatiques*, Études et Documents, Paris, France, 2010.

[12] P. R. Durkin, "Fluroxypyr human health and ecological risk assessment report," SERA TR-052-13-03a, USDA Forest service, Washington, DC, USA, 2009.

[13] J. Vera Candioti, G. S. Natale, S. Soloneski, A. E. Ronco, and M. L. Larramendy, "Sublethal and lethal effects on Rhinella Arenarum (Anura, Bufonidae) tadpoles exerted by the pirimicarb-containing technical formulation insecticide Aficida®," *Chemosphere*, vol. 78, no. 3, pp. 249–255, 2010.

[14] M. L. Ambrose, *Characterization of the Insecticidal Properties of Acetamiprid Under Field and Laboratory Conditions*, Faculty of North Carolina State University, Raleigh, NC, US, 2003.

[15] L. Dicks, "Bees, lies and evidence-based policy," *Nature*, vol. 494, no. 7437, p. 283, 2013.

[16] Y. Aliouane, A. K. El Hassani, V. Gary, C. Armengaud, M. Lambin, and M. Gauthier, "Subchronic exposure of honeybees to sublethal doses of pesticides: effects on behavior," *Environmental Toxicology and Chemistry*, vol. 28, no. 1, pp. 113–122, 2009.

[17] Agropages, "Triazole Fungicides Insight-Market overview," 2013, http://www.agropages.com/BuyersGuide/category/Triazole-Fungicides-Insight.html#smmn1.

[18] R. J. Letcher, I. Van Holsteijn, H. Drenth et al., "Cytotoxicity and aromatase (CYP19) activity modulation by organochlorines in human placental JEG-3 and JAR choriocarcinoma cells," *Toxicology and Applied Pharmacology*, vol. 160, no. 1, pp. 10–20, 1999.

[19] C. Urani, M. Doldi, S. Crippa, and M. Camatini, "Human-derived cell lines to study xenobiotic metabolism," *Chemosphere*, vol. 37, no. 14-15, pp. 2785–2795, 1998.

[20] G. G. Kuiper, J. G. Lemmen, B. Carlsson et al., "Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β," *Endocrinology*, vol. 139, no. 10, pp. 4252–4263, 1998.

[21] J. Krijt, I. Van Holsteijn, I. Hassing, M. Vokurka, and B. J. Blaauboer, "Effect of diphenyl ether herbicides and oxadiazon on porphyrin biosynthesis in mouse liver, rat primary hepatocyte culture and HepG2 cells," *Archives of Toxicology*, vol. 67, no. 4, pp. 255–261, 1993.

[22] I. Nakagawa, M. Suzuki, N. Imura, and A. Naganuma, "Enhancement of paraquat toxicity by glutathione depletion in mice in vivo and in vitro," *Journal of Toxicological Sciences*, vol. 20, no. 5, pp. 557–564, 1995.

[23] S. Richard, S. Moslemi, H. Sipahutar, N. Benachour, and G. Seralini, "Differential effects of glyphosate and roundup on human placental cells and aromatase," *Environmental Health Perspectives*, vol. 113, no. 6, pp. 716–720, 2005.

[24] B. L'Azou, P. Fernandez, R. Bareille et al., "In vitro endothelial cell susceptibility to xenobiotics: comparison of three cell types," *Cell Biology and Toxicology*, vol. 21, no. 2, pp. 127–137, 2005.

[25] N. Benachour and G. Séralini, "Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells," *Chemical Research in Toxicology*, vol. 22, no. 1, pp. 97–105, 2009.

[26] H. C. Korting, S. Schindler, A. Hartinger, M. Kerscher, T. Angerpointner, and H. I. Maibach, "MTT-assay and neutral red release (NRR)-assay: relative role in the prediction of the irritancy potential of surfactants," *Life Sciences*, vol. 55, no. 7, pp. 533–540, 1994.

[27] J. J. Liu, W. Wang, D. T. Dicker, and W. S. El-Deiry, "Bioluminescent imaging of TRAIL-induced apoptosis through detection of caspase activation following cleavage of DEVD-aminoluciferin," *Cancer Biology & Therapy*, vol. 4, no. 8, pp. 885–892, 2005.

[28] S. P. Crouch, R. Kozlowski, K. J. Slater, and J. Fletcher, "The use of ATP bioluminescence as a measure of cell proliferation and cytotoxicity," *Journal of Immunological Methods*, vol. 160, no. 1, pp. 81–88, 1993.

[29] N. Benachour, H. Sipahutar, S. Moslemi, C. Gasnier, C. Travert, and G. E. Séralini, "Time- and dose-dependent effects of roundup on human embryonic and placental cells," *Archives of Environmental Contamination and Toxicology*, vol. 53, no. 1, pp. 126–133, 2007.

[30] T. Mosmann, "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays," *Journal of Immunological Methods*, vol. 65, no. 1-2, pp. 55–63, 1983.

[31] C. A. Schneider, W. S. Rasband, and K. W. Eliceiri, "NIH Image to ImageJ: 25 years of image analysis," *Nature Methods*, vol. 9, no. 7, pp. 671–675, 2012.

[32] M. Marutani and V. Edirveerasingam, "Influence of irrigation methods and an adjuvant on the persistence of carbaryl on pakchoi," *Journal of Environmental Quality*, vol. 35, no. 6, pp. 1994–1998, 2006.

[33] R. H. McKee, Z. A. Wong, S. Schmitt et al., "The reproductive and developmental toxicity of high flash aromatic naphtha," *Toxicology and Industrial Health*, vol. 6, no. 3-4, pp. 441–460, 1990.

[34] J. M. Langman, "Xylene: its toxicity, measurement of exposure levels, absorption, metabolism and clearance," *Pathology*, vol. 26, no. 3, pp. 301–309, 1994.

[35] A. M. Saillenfait, F. Gallissot, I. Langonné, and J. P. Sabaté, "Developmental toxicity of N-methyl-2-pyrrolidone administered orally to rats," *Food and Chemical Toxicology*, vol. 40, no. 11, pp. 1705–1712, 2002.

[36] US Environmental Protection Agency (EPA), "Hazard Characterization Document-N, N-Dimethylalkanamides Category," 2011, http://www.epa.gov/chemrtk/hpvis/hazchar/Category_N,N-Dimethylalkanamides_September_2011 .pdf.

[37] US Environmental Protection Agency (EPA), *Reregistration Eligibility Decision (RED) For Benzisothiazoline-3-One*, US Environmental Protection Agency (EPA), Boston, Mass, USA, 2005.

[38] L. Gawade, S. S. Dadarkar, R. Husain, and M. Gatne, "A detailed study of developmental immunotoxicity of imidacloprid in Wistar rats," *Food and Chemical Toxicology*, vol. 51, pp. 61–70, 2013.

[39] G. M. Williams, R. Kroes, and I. C. Munro, "Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans," *Regulatory Toxicology and Pharmacology*, vol. 31, no. 2, part 1, pp. 117–165, 2000.

[40] A. L. Williams, R. E. Watson, and J. M. Desesso, "Developmental and reproductive outcomes in humans and animals after glyphosate exposure: a critical analysis," *Journal of Toxicology and Environmental Health B*, vol. 15, no. 1, pp. 39–96, 2012.

[41] European Environment Agency, "Late lessons from early warnings: science, precaution, innovation," EEA Report, European Environment Agency, Copenhagen, Denmark, 2013.

Toxicology xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity

R. Mesnage [a,b], B. Bernay [c], G.-E. Séralini [a,b,*]

[a] University of Caen, EA2608, Institute of Biology, Risk Pole CNRS, Esplanade de la Paix, 14032 Caen, Cedex, France
[b] CRIIGEN, 40 rue de Monceau, 75008 Paris, France
[c] Proteogen, SFR 146 ICORE, University of Caen, France

ARTICLE INFO

Article history:
Received 27 April 2012
Received in revised form 30 August 2012
Accepted 10 September 2012
Available online xxx

Keywords:
Pesticide
Glyphosate
POEA
Adjuvant
Roundup
Human cells

ABSTRACT

Pesticides are always used in formulations as mixtures of an active principle with adjuvants. Glyphosate, the active ingredient of the major pesticide in the world, is an herbicide supposed to be specific on plant metabolism. Its adjuvants are generally considered as inert diluents. Since side effects for all these compounds have been claimed, we studied potential active principles for toxicity on human cells for 9 glyphosate-based formulations. For this we detailed their compositions and toxicities, and as controls we used a major adjuvant (the polyethoxylated tallowamine POE-15), glyphosate alone, and a total formulation without glyphosate. This was performed after 24 h exposures on hepatic (HepG2), embryonic (HEK293) and placental (JEG3) cell lines. We measured mitochondrial activities, membrane degradations, and caspases 3/7 activities. The compositions in adjuvants were analyzed by mass spectrometry. Here we demonstrate that all formulations are more toxic than glyphosate, and we separated experimentally three groups of formulations differentially toxic according to their concentrations in ethoxylated adjuvants. Among them, POE-15 clearly appears to be the most toxic principle against human cells, even if others are not excluded. It begins to be active with negative dose-dependent effects on cellular respiration and membrane integrity between 1 and 3 ppm, at environmental/occupational doses. We demonstrate in addition that POE-15 induces necrosis when its first micellization process occurs, by contrast to glyphosate which is known to promote endocrine disrupting effects after entering cells. Altogether, these results challenge the establishment of guidance values such as the acceptable daily intake of glyphosate, when these are mostly based on a long term in vivo test of glyphosate alone. Since pesticides are always used with adjuvants that could change their toxicity, the necessity to assess their whole formulations as mixtures becomes obvious. This challenges the concept of active principle of pesticides for non-target species.

1. Introduction

Pesticide formulations are mixtures of adjuvants and so-called "active principles" on plants for herbicides, and insects for insecticides, etc. The supposed specificity of active principles on their targets does not mean a priori that they are the most toxic compounds of the formulations on human cells. Numerous mammalian (Colborn et al., 1993) and other animal studies (Hawthorne and Dively, 2011) evidenced side effects for pesticides. The toxicology of mixtures cannot be fully understood without knowing the differential toxicity of the various compounds of the formulations and their combined effects. Surprisingly, to measure their side effects, the active principles of pesticides are generally tested alone at a regulatory level in long-term mammalian trials, although their adjuvants are developed at least to enhance their stability and penetration into cells. However, most of the adjuvants are classified as inert.

Here we tested the differential and combined cytotoxicity of the major pesticides in the world which are glyphosate-based herbicides (GBH), and analyzed their composition and mechanisms of action. The residues of the GBH such as Roundup (R) are also among the first contaminants of ground and surface waters (IFEN, 2006), and of some food and feed because they are present since more than 15 years in around two third of genetically modified (GM) cultivated edible plants, because they are designed at least to tolerate R (James, 2011). Glyphosate (G) is toxic in plant cells by inhibition of 5-enolpyruvylshikimate-3-phosphate synthase used as a first step in aromatic amino acid synthesis (Boocock and Coggins, 1983). Adjuvants considered as inert include, according to the formulations, surfactants like POEAs (polyethoxylated alkylamines,

* Corresponding author. Tel.: +33 2 31 56 56 84; fax: +33 2 31 56 53 20.
E-mail address: criigen@unicaen.fr (G.-E. Séralini).

0300-483X/$ – see front matter © 2012 Published by Elsevier Ireland Ltd.
http://dx.doi.org/10.1016/j.tox.2012.09.006

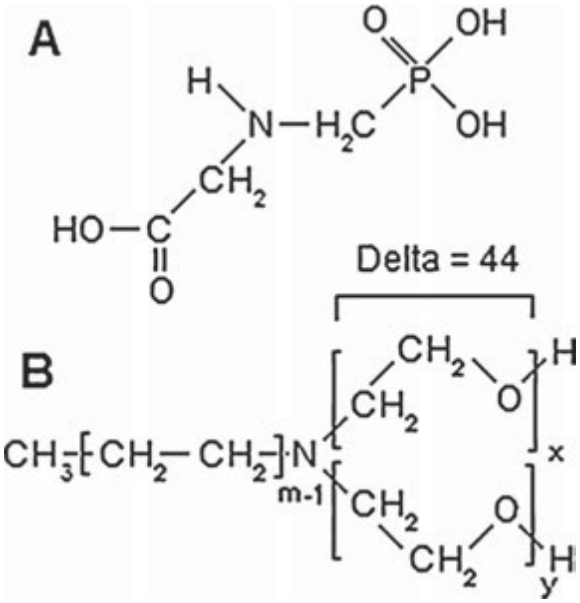


Fig. 1. Structures of glyphosate (A) and POEAs (B). Glyphosate is the N-(phosphonomethyl)glycine, C3H8NO5P). Di-ethoxylates of tallowamines adjuvants (C_mNEO_n, $n = x + y$) such as POEA are characterized by their oxide/tallowamine ratio. The delta of 44 (—CH_2—CH_2—O—) corresponded to the increment of the different peaks observed in mass spectrometry. Length of the more abundant tallowamine part in the adjuvant mixture corresponded to the maximal *m/z* of the spectrum.

Fig. 1), isobutane, light petroleum distillate, etc. that may induce among other DNA damages (Cox, 2004). However G is still generally hypothesized to be the active ingredient for non-target side effects. Unexpected side effects of G-based formulations were evidenced on non-target species, among other endocrine disruptions during spermatogenesis or pregnancy (Beuret et al., 2005; Clair et al., 2012; Dallegrave et al., 2007; Daruich et al., 2001; Oliveira et al., 2007; Romano et al., 2011; Savitz et al., 1997; Yousef et al., 1995). This may be related to adjuvants in formulation. They are indeed more and more considered as responsible for GBH toxicity (Mesnage et al., 2010; Williams et al., 2012), but the mechanistic and the nature of the cytotoxic agent(s) on human cells are still unknown. This is a general question that can arise for all pesticides.

The detailed known composition indicate that major adjuvants are ethoxylated, such as POEAs which are themselves mixtures of di-ethoxylates of tallowamines characterized by their oxide/tallowamine ratio. POEA commonly used in GBH is the POE (15) tallowamine (POE-15). We thus compared the toxicity and the composition of 9 formulations varying in adjuvants contents: Roundup Ultra, Roundup GT, Roundup GT+, Roundup Bioforce, Roundup 3plus, Glyphogan, Topglypho 360, Clinic E.V., and Bayer GC. For controls, we tested a formulation containing POE-15 without G (Genamin T200), and POE-15 alone. The compositional analysis of these products was performed by a non-quantitative mass spectrometry (MALDI-TOF MS/MS), considered as the best way to analyze pesticides formulations (Corbera et al., 2010; Cserháti and Forgács, 1997). Physico-chemical properties of POE-15 were approached by the measurements of its critical micelle concentration (CMC), determined by absorption changes in its presence of Coomassie blue CBB R-250.

We used HEK293, JEG3 and HepG2 cell lines, three models where unexpected effects of GBH have already been demonstrated (Benachour and Seralini, 2009; Gasnier et al., 2009). JEG3 cells are a useful model for examining placental toxicity (Letcher et al., 1999), and HepG2 for hepatic toxicity (Urani et al., 1998). HEK293 were chosen because of the sensitivity of embryonic cells, Roundup causing pregnancy outcomes (Savitz et al., 1997). Moreover, we have demonstrated that these cell lines are even less sensitive than primary cells (Benachour and Seralini, 2009; L'Azou et al., 2005), and therefore are possibly representative of a real cellular toxicity. For cytotoxicity measurements, we assayed mitochondrial succinate dehydrogenase (SD) activity (MTT assay), G and its formulations are indeed known to target mitochondria (Astiz et al., 2009; Peixoto, 2005). Cytotoxicity was also characterized by the measurement of apoptosis and necrosis, respectively by caspases 3/7 activation (Liu et al., 2005) and adenylate kinase leakage after membrane alterations (Crouch et al., 1993).

Overall, we questioned if an active toxic principle in a target species may be always generalized as such in a non target one, and thus if the regulatory toxicological tests on active principles alone are relevant.

2. Materials and methods

2.1. Chemicals

Glyphosate (N-phosphonomethyl glycine, G, CAS: 1071-83-6) was purchased from Sigma–Aldrich (Saint Quentin Fallavier, France). GBH formulations available on the market were by alphabetical order: Bayer GC (12.5% of G, 1–5% of POE-15, homologation 05873567), Clinic EV (42% of G, 11% of POE-15, homologation 9900039), Genamin T200 (60–80% of POE-15, homologation 8500170), Glyphogan (39–43% of G, 13–18% of POE-15, homologation 9100537), Roundup Grand Travaux (400 g/L of G, R GT, homologation 8800425), Roundup Grand Travaux plus (450 g/L of G, 90 g/L of ethoxylated etheralkylamine (EtO-EA), R GT+, homologation 2020448), Roundup Ultra (41.5% of G, 16% surfactant, homologation 9700259), Roundup Bioforce (360 g/L of G, homologation 9800036), Roundup 3plus (170 g/L of G, 8% surfactant homologation 9300241), Topglypho 360 (360 g/L of G, homologation 2000254). POE-15 (CAS: 61791-26-2) was purchased from ChemService (West Chester, PA, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and all other compounds, otherwise noticed, were obtained from Sigma–Aldrich. MTT was prepared as a 5 mg/mL stock solution in phosphate-buffered saline, filtered through a 0.22 μm filter before use, and diluted to 1 mg/mL in a serum-free medium.

2.2. Cell lines and treatments

The human embryonic kidney 293 cell line (HEK 293, ECACC 85120602), was provided by Sigma–Aldrich (Saint-Quentin Fallavier, France). The hepatoma cell line HepG2 was provided by ECACC (85011430). JEG3 cell line (ECACC 92120308) was provided by CERDIC (Sophia-Antipolis, France). Cells were grown in phenol red-free EMEM (Abcys, Paris, France) containing 2 mM glutamine, 1% non-essential amino acid, 100 U/mL of antibiotics (a mixture of penicillin, streptomycin and fungizone) (Lonza, Saint Beauzire, France), 10 mg/mL of liquid kanamycin (Dominique Dutscher, Brumath, France) and 10% Fetal Bovine Serum (PAA, les Mureaux, France). JEG3 cells were supplemented with 1 mM sodium pyruvate. Cells were grown with this medium at 37 °C (5% CO_2, 95% air) during 48 h to 80% confluence, and then washed and exposed 24 h with serum-free EMEM to various chemicals. This model was validated (Benachour et al., 2007) since cytotoxic effects were similar in presence of serum but delayed by 48 h. The dilutions of formulated herbicides, adjuvants and G alone were prepared in serum free medium as stock solutions at a similar pH.

2.3. Cytotoxicity biomarkers

After treatments, the following tests were applied: succinate dehydrogenase (SD) activity assay (MTT) (Mosmann, 1983). Integrity of mitochondrial dehydrogenase enzymes indirectly reflects the cellular mitochondrial respiration. The optical density was measured at 570 nm using a Mithras LB 940 luminometer (Berthold, Thoiry, France). The bioluminescent ToxiLight bioassay (Lonza, Saint Beauzire, France) was applied for the membrane degradation assessment, by the intracellular adenylate kinase (AK) release in the medium; this is described as a necrosis marker (Crouch et al., 1993). Finally, the apoptotic cell death was evaluated with the Caspase-Glo 3/7 assay (Promega, Paris, France). Luminescence was measured using a Mithras LB 940 luminometer (Berthold, Thoiry, France). These methods were previously described (Benachour and Seralini, 2009).

2.4. Mass spectrometry (MS)

MS experiments were carried out on an AB Sciex 5800 proteomics analyzer equipped with TOF TOF ion optics and an OptiBeam™ on-axis laser irradiation with 1000 Hz repetition rate. The system was calibrated immediately before analysis with a mixture of des-Arg-Bradykinin, Angiotenin I, Glu1-Fibrinopeptide B, ACTH (18–39), ACTH (7–38) and mass precision was better than 50 ppm. A 0.8 μL volume of the GBH solution diluted 100 times in water was mixed with 1.6 μL volumes

of solutions of α-cyano-4-hydroxycinnamic acid matrix prepared in 50% ACN with 0.1% TFA. The mixture was spotted on a stainless steel Opti-TOF™ 384 targets; the droplet was allowed to evaporate before introducing the target into the mass spectrometer. Acquisitions were taken in manual and automatic modes. A laser intensity of 3000 was typically employed for ionizing. MS spectra were acquired in the positive reflector mode by summarizing 1000 single spectra (5 × 200) in the mass range from 100 to 2000 Da. MS/MS spectra were acquired in the positive MS/MS reflector mode by summarizing a maximum of 2500 single spectra (10 × 250) with a laser intensity of 3900. For the tandem MS experiments, the acceleration voltage applied was 1 kV and air was used as the collision gas. Gas pressure medium was selected as settings.

2.5. Critical micelle concentrations (CMC) determinations

CMC determinations were performed and adapted according to (Samsonoff et al., 1986). CMC was measured by the incorporation of Coomassie brilliant blue R-250 (CBB-R250) in micelles formed by serial dilutions of detergents. The CCB-R250 reagent was prepared as previously described (Bradford, 1976). Varying concentrations of adjuvants were added in a volume of 1 mL, 100 μL of CBB-R250 was added to make a final concentration of 80 μg/mL. Solutions were shaken and distributed in 96 well-plates in triplicate. Absorption was then measured against a water blank at 600 nm using a Mithras LB 940 luminometer (Berthold, Thoiry, France). The validation of the technique was performed with triton X-100, with a CMC of 0.15–0.20 mM (Courtney et al., 1986).

2.6. Statistical analysis

The experiments were repeated at least 3 times in different weeks on 3 independent cultures ($n = 9$). LC_{50} values were calculated by a nonlinear regression using sigmoid (5-parameters) equation with the GraphPad software. All data were presented as the means ± standard errors (SEMs). Statistical differences were determined by Student's t-test using significant levels with $p < 0.01$ (**) and $p < 0.05$ (*).

3. Results

Here we studied for the first time the precise involvement of the adjuvants and G in GBH induced toxicity, on three human cell lines from different embryonic origins (kidney, liver, and placenta) in order to test their specificities. We first compared mitochondrial respiration (SD activity) in presence of 9 formulated mixtures of G and adjuvants, G alone, formulating agents without G (Genamin), and a major adjuvant of some formulations, POE-15 (Fig. 2). All chemicals are cytotoxic, inducing similar dose-dependent patterns on HEK293, HepG2, and JEG3 in 24 h. JEG3 were up to 2-fold more sensitive to treatments than HEK293 and HepG2 in comparison to control. We observed for all cell lines different ranges of toxicities

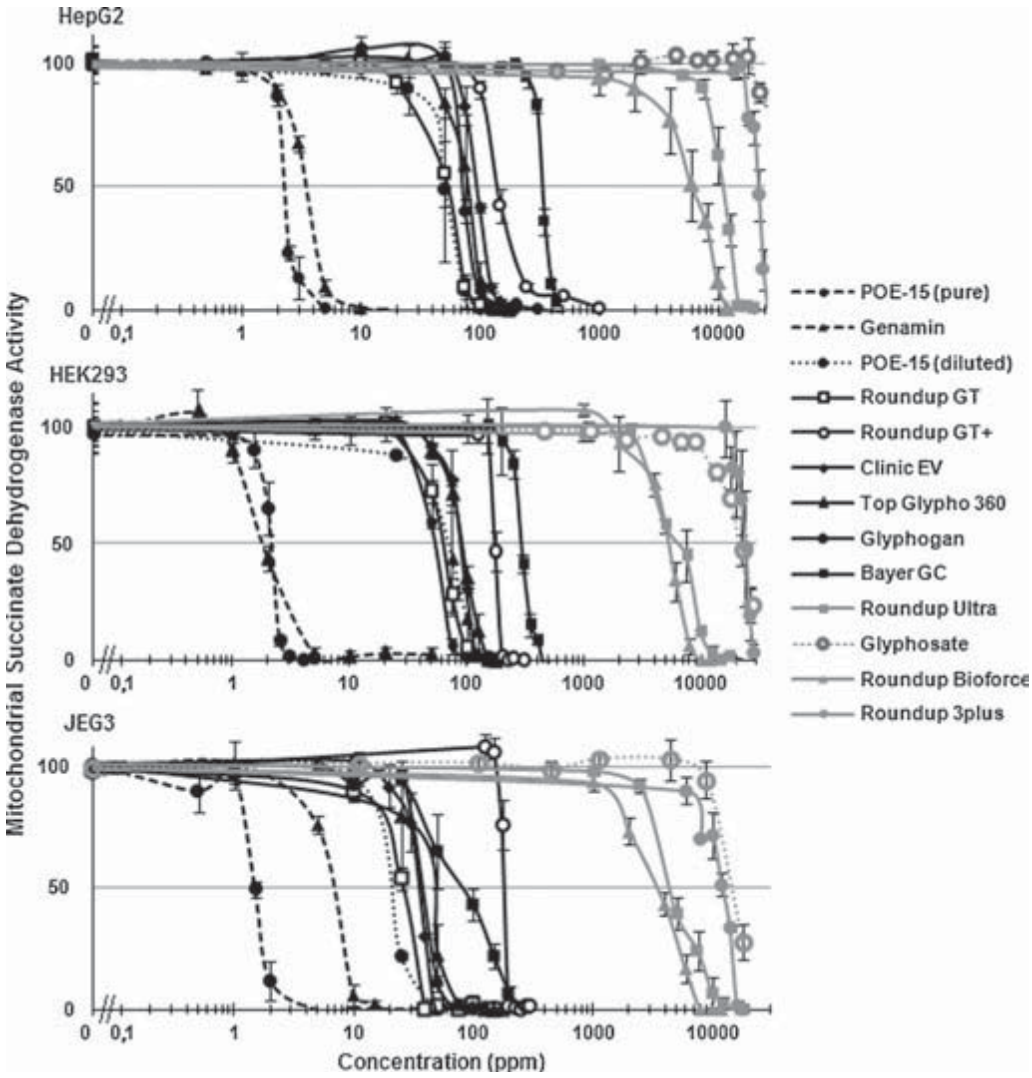


Fig. 2. Dose-dependent cytotoxic effects of glyphosate-based herbicides (GBH) or glyphosate (G) and adjuvants alone (POE-15 and Genamin) on HepG2, HEK293 and JEG3 human cell lines. Effects on the mitochondrial succinate dehydrogenase (SD) activity, reflecting cell respiration inhibition, were measured in % of control in serum-free medium after 24 h of exposure. The concentrations in ppm are dilutions of each mixture in the commercial formulation (considered as 100%). The adjuvants POE-15 and Genamin alone (a mixture containing 785 g/L of POE-15, no G) were the most toxic. The middle group approximately 100-fold less toxic was composed by GBH: Roundup GT, Roundup GT+, and Clinic EV, Top Glypho 360, Glyphogan, Bayer GC. The less toxic group was formed by Roundup Ultra, Bioforce and 3plus. SEMs are shown in all instances ($n = 9$).

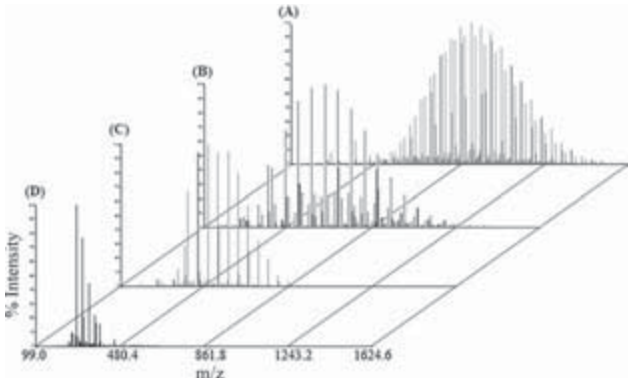


Fig. 3. MALDI-TOF analysis of glyphosate-based herbicides (GBH) main adjuvants. (A) POE-15 spectrum was centered on 900*m/z* (increment delta 44, Fig. 1), all other herbicides (group A, see Table 1) declaring a POEA adjuvant had the same spectrum. In addition, identification was confirmed by MS/MS fragmentation. (B) The 3 Roundup Ultra, Bioforce and 3plus contained another common adjuvant (600*m/z*, delta 58). (C) Adjuvants of Roundup GT+ (500*m/z*, delta 44, Fig. 1) were declared as ethoxylated etheralkylamines (EtO-EA). (D) Adjuvants of Roundup GT (300*m/z*, delta 44, Fig. 1) were identified as POE-2.

allowing the classification of the products tested as follows. The most toxic were the adjuvants alone POE-15 ($LC_{50} \sim 1$–2 ppm; agricultural dilutions: 1–2% of the herbicide formulation containing adjuvants) and Genamin, themselves around 100-fold more toxic than a middle group with the majority of formulations (6, with among them R GT and GT+). This middle group is again 100-fold more toxic than the third one which includes R Ultra, R Bioforce, R 3plus and finally G alone. Moreover, POE-15 diluted to the concentration at which it is present in Clinic E.V. (a formulation from the middle group) presented a similar toxicity than this GBH and to the middle group in general. It thus appears to be the toxic principle in human cells. In addition, we also demonstrate that two formulations claiming a similar concentration of G (360 g/L) and different adjuvants (16% of POEA or other adjuvants), Glyphogan and R Ultra respectively, exhibited very different toxicities, 150-fold stronger on average for Glyphogan on the 3 cell lines (Fig. 2). Thus some other adjuvants appear also to have some toxicity.

To check the composition in adjuvants we studied all the formulations by MALDI-TOF MS/MS (Fig. 3). Knowing that the specificities of MALDI-TOF ionization did not detect G but adjuvants, we separated 4 groups of adjuvants: (A) with a spectrum centered on 900*m/z*, POE-15 and Genamin, and those present in 4 formulations of the middle group thus containing also POE-15, (B) those contained in the third less toxic group with a spectrum centered on 600*m/z* corresponding to another common adjuvant, and (C) and (D), two other adjuvants in the formulations of the middle group, respectively in (C) R GT+ (500*m/z*) and (D) R GT (300*m/z*). The belonging of each product to each group was further confirmed by analysis of fragmentation spectra, giving for instance for ions of group A: 840.6, 858.7, 884.7, 902.8*m/z*. All these spectra corresponded to the family of alkylamines. The POE-15 had a peak increment of 44 (delta) like all group A (Table 1). The same delta in C and D were characteristic of an ethoxylated chain. C was an ethoxylated etheralkylamine, D was confirmed by fragmentation to be identical to POE-2; and a delta of 58 corresponded to another non ethoxylated adjuvant in group B. We summarized these findings with LC_{50} values (Table 1).

We then tested the linearity of the toxicity in function of G or ethoxylated adjuvants concentrations (Fig. 4). The cytotoxicity induced by GBH is not linear to G concentrations ($R^2 \sim 0.3$, Fig. 4A), but only to the 3 ethoxylated adjuvants ($R^2 > 0.93$, Fig. 4B), and not to the non-ethoxylated one, and this is obtained with all cell lines. Ethoxylated adjuvants can thus be considered as the active principle of the toxicity of GBH in human cells.

In order to understand the mechanism of action of adjuvants, three other experiments were performed. First, the critical micelle concentration (CMC) of POE-15 was determined by absorption changes of CBB R-250 (Fig. 5). The method was validated by the measurement of the CMC of the triton X-100 (0.15–0.20 mM (Courtney et al., 1986)). We evidenced a micellization of POE-15 beginning at 3 ppm, similarly to toxicity thresholds (Fig. 2). POE-15 thus appears to be able to disrupt the cellular membranes by micellization with the lipid bilayer around the CMC. This was even better understood by the differential measurement of the cytotoxicity through membrane disruption or caspases activation (Fig. 6). For the three cell lines, results are almost comparable: POE-15 and R GT+ (containing also an ethoxylated adjuvant) induced more necrosis (Fig. 6A) by membrane alterations rather than apoptosis (Fig. 6B), even if present. By contrast, G induced only apoptosis at higher levels. Ethoxylated adjuvants are thus not inert at all but cell membrane disruptors, and then induce severe mitochondrial alterations.

4. Discussion

This study unravels the differential nature and cytotoxicity of the main compounds from the major herbicide formulations in the world. These formulations are conceived to enhance the pesticide activity through mixtures of adjuvants and G. The latter is the active principle toxic in plants; in this study we checked how this

Table 1
Main spectral and toxicological characteristics of the herbicides (GBH) and adjuvants tested. Groups corresponded to spectra of adjuvants contained in products according to Fig. 3. Contents in glyphosate and adjuvants were indicated by manufacturers (except for POE-2) and identified by MS/MS as revealed by *m/z* and delta measurements. LC50 (ppm) are calculated from Fig. 2. nd: non detected; nk: not known.

Group	Products tested	Glyphosate (g/L)	Adjuvants	*m/z* (MS)	Delta (MS)	LC50 HepG2 (ppm)	LC50 HEK293 (ppm)	LC50 JEG3 (ppm)
A	Topglypho 360	360	15% POE-15	900	44	79	89	37
	Glyphogan	360	13–18% POE-15	900	44	59	54	30
	Clinic E.V	360	11% POE-15	900	44	94	89	34
	Bayer GC	96	1–5% POE-15	900	44	333	290	84
	Genamin	0	60–80% POE-15	900	44	4	2	7
	POE-15	0	POE-15	900	44	2	2	1
B	R Ultra	360	16% nk	600	58	11,000	6395	4477
	R Bioforce	360	nk	600	58	6106	5043	3560
	R 3plus	170	nk	600	58	22,000	24,000	1200
C	Roundup GT+	450	7.5% Eto-EA	500	44	145	170	115
D	Roundup GT	400	POE-2	300	44	53	62	32
	Glyphosate	>95%	nd	nd	nd	nd	19,323	1192

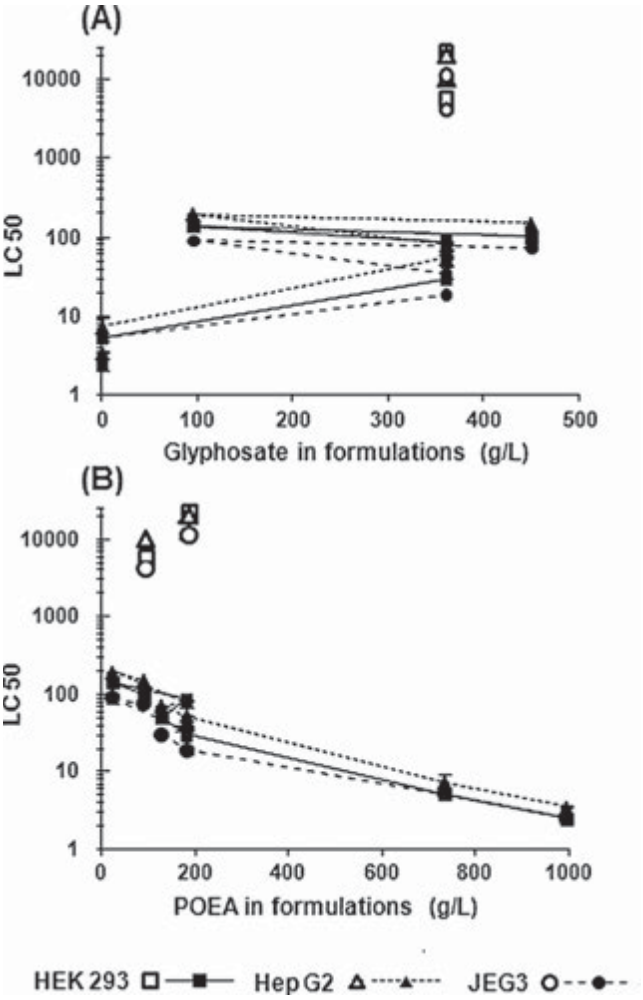


Fig. 4. Toxicity of glyphosate in formulations (A) measured by LC50, and of adjuvants in glyphosate-based herbicides (B) on the three human cell lines described. The effects on the mitochondrial succinate dehydrogenase (SD) activity were measured to calculate the LC50s (ppm) and compiled to be compared in relation to glyphosate or adjuvants concentrations. The form of the symbols is related to the cell lines (squares for HEK293, triangles for HepG2 and circles for JEG3). For colors, black dots are ethoxylated adjuvants, white dots are others. The three described human cell lines were used in the conditions of Fig. 2 and the results were almost identical. The linear correlation was not obtained (A) between glyphosate concentration and toxicity (coefficient of determination is 0.36 for HEK293, 0.35 for HepG2 and 0.29 for JEG3), but was demonstrated between the concentrations in the formulations of ethoxylated adjuvants (B) and toxicity (coefficient of determination is 0.94 for HEK293, 0.97 for HepG2 and 0.93 for JEG3). SEMs are represented in all instances ($n = 9$).

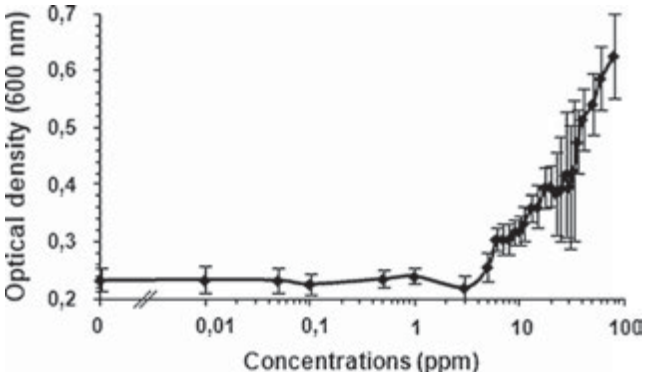


Fig. 5. Critical Micelle Concentration (CMC) of the POE-15 determined by absorption changes of Coomassie Brilliant Blue R-250. CBB R-250 was added to serial dilutions of POE-15 in serum-free medium. D.O. at 600 nm was measured with a spectrophotometer. A major breakpoint was evidenced in the curve around 3 ppm, at the CMC. SEMs are shown in all instances ($n = 9$).

active principle is differentially toxic on non-target organisms in comparison to the so-called inert adjuvants in numerous formulations.

Here we demonstrate that all formulations are more toxic than G alone on three human cell lines as previously underlined (Benachour and Seralini, 2009; Richard et al., 2005). Then for the first time we separated experimentally three groups of formulations differentially toxic according to the amount of ethoxylated adjuvants. The 3 less toxic formulations (like G alone) were demonstrated to contain no ethoxylated adjuvants by mass spectrometry, and are around 10,000 times less toxic on mitochondrial activity than POE-15 alone, the major adjuvant. All the other formulations were toxic proportionally to the dilutions of POE-15 or other ethoxylated adjuvants in the formulations, in a linear manner to some extent; in fact G does not buffer or amplify direct POE-15 toxicity.

Thus POE-15 appears to be clearly the toxic principle in human cells. It begins to be active with negative effects on cellular respiration and membrane integrity between 1 and 3 ppm, when its first micellization process occurs in this work. This membrane disruption then lead to the necrotic adjuvant-linked effects observed, amplifying the necrosis/apoptosis ratio by contrast to G at higher levels as shown. Accordingly, it was found (Chamel and Gambonnet, 1997) that a CMC of the $C_{18}NEO_{20}$ congener of a POEA is around 2 ppm. Its partition coefficient measured at around 1.7 confirmed its lipophilic character and its ability to penetrate the cells. It is known that ethoxylated adjuvants can insert in cells membranes, disrupting their structure and functions as previously shown in bacteria (Nobels et al., 2011). This is a general property of surfactants (Boeije et al., 2006). We notice that among different class of surfactants, ethoxylated adjuvants are of the more toxic, even potentially genotoxic (Nobels et al., 2011). Importantly, this is not only observed in vitro because when rats are treated with G, R and POEA, the latter was also found to be the most toxic compound (Adam et al., 1997), even in other animal models (Marc et al., 2005). This was demonstrated for other pesticides (Eddleston et al., 2012). Generally, the question of the toxicity of adjuvants in pesticides is more and more recognized (Brausch and Smith, 2007; Krogh et al., 2003; Tsui and Chu, 2003).

This does not exclude cellular endocrine disruptions below these levels that may not be due to POE-15 alone (or other ethoxylated adjuvants), but that occur through glyphosate entering in aromatase active site for instance (Richard et al., 2005) or in androgen receptor which is inhibited from 0.2 ppm of G in adjuvants (Gasnier et al., 2009). It should not be forgotten that G has its own toxicity and may also exert long term or chronic toxicity. The active principle G alone has been evidenced to cause oxidative stress (Astiz et al., 2009; Cavusoglu et al., 2011), endocrine disruption (Clair et al., 2012), or developmental effects (Marc et al., 2005). G was even recently described as a teratogen (Paganelli et al., 2010). In this case we have a model of multiple combined negative effects (through different cellular metabolic endpoints) caused by the main pesticide mixtures, which are the formulations themselves. This is true even if the activities of ethoxylated adjuvants on endocrine disruption must be still detailed in the future.

These results were obtained in vitro; cellular cultures replace whenever it is possible animal experimentation (Hartung, 2009). Our study was performed during 24 h and does not anticipate the elimination or the possible bioaccumulation and long term combined effects with other xenobiotics. R human cellular effects indeed increased according to time (Benachour et al., 2007) and radiolabeled G accumulated in cells within 48 h, suggesting a

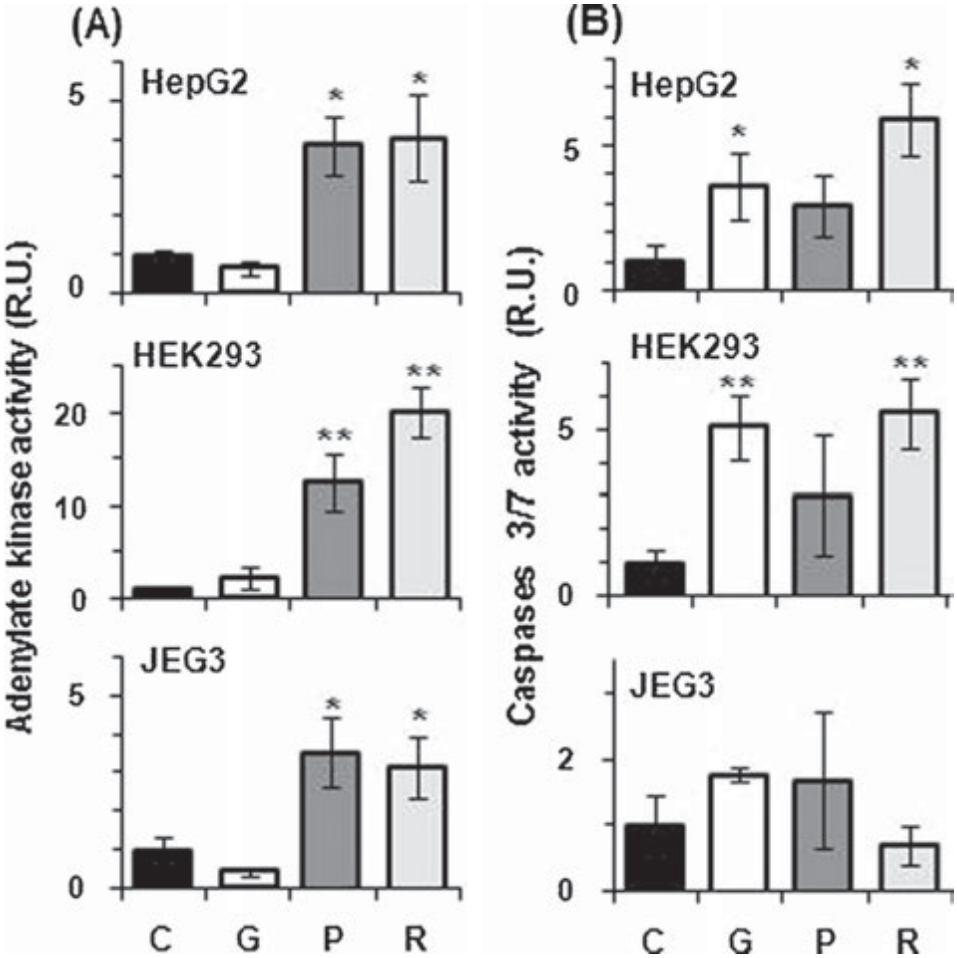


Fig. 6. Cytotoxic effects of control (C), glyphosate (G), POE-15 (P) and Roundup GT+ (R). Cell membrane integrity reflecting necrosis (A) was measured by adenylate kinase leakage (active in the medium), and apoptosis (B) by caspases 3/7 activities, both expressed in relative units (RU) after 24 h of treatments like in Fig. 2. To understand the mechanism of cytotoxicity, the concentrations in products were those inducing 80% of the general cytotoxicity in MTT assay. SEMs are shown in all instances ($n = 12$, $^{*}p < 0.05$; $^{**}p < 0.01$).

bioaccumulation of low concentrations of G (Gasnier et al., 2011). R adjuvants may also form adducts and link to DNA avoiding a direct elimination (Peluso et al., 1998).

However, our lowest thresholds of toxicities and endocrine disruptions may be comparable to the range of environmental/occupational exposures. A farmer or a gardener spraying a GBH may be punctually exposed to 5000 ppm, and even regularly by occupational exposure. As a matter of fact G varied from 3 to 233 ppb in farmers urine (Acquavella et al., 2004), this may be in addition to a chronic dietary/drink exposure of G found up to 70 ppb in serum of non-occupationally exposed women (Aris and Leblanc, 2011).

In conclusion, pesticide formulations should be studied as mixtures for toxic effects. The multiple combined effects could induce pathologies on a long term. Here we can question the use of ethoxylated adjuvants in herbicide formulations, since they appear as active principles for human cell toxicity. This leads also to challenge guidance values such as the acceptable daily intake (ADI) of G, which is calculated with pure G in long term toxicological tests in vivo (German Federal Agency CPFS, 1998), while G is always used with adjuvants that are not immediately biodegradable (Banduhn and Frazier, 1978) and could change its toxicity. This will be also important for other active principles of pesticides, and thus their ADI can be overestimated. The necessity of studying formulations as mixtures is common to all pesticides. The pathological consequences of exposure to chronic toxicities of whole formulations could be tested with mammals over a 2-year period. This implies a complete shift in the concepts underlying chemical toxicology, which could come from mixtures studies.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

We gratefully thank Angelo San Filippo for technical support for adjuvants isolation. We acknowledge the Regional Council of Low Normandy for R.M. fellowship, but also the Charles Leopold Mayer (FPH) and Denis Guichard Foundations, together with CRIIGEN, for structural supports.

References

Acquavella, J.F., Alexander, B.H., Mandel, J.S., Gustin, C., Baker, B., Chapman, P., Bleeke, M., 2004. Glyphosate biomonitoring for farmers and their families: results from the Farm Family Exposure Study. Environ. Health Perspect. 112, 321–326.
Adam, A., Marzuki, A., Abdul Rahman, H., Abdul Aziz, M., 1997. The oral and intratracheal toxicities of ROUNDUP and its components to rats. Vet. Hum. Toxicol. 39, 147–151.
Aris, A., Leblanc, S., 2011. Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. Reprod. Toxicol. 31, 528–533.

Astiz, M., de Alaniz, M.J., Marra, C.A., 2009. Effect of pesticides on cell survival in liver and brain rat tissues. Ecotoxicol. Environ. Saf. 72, 2025–2032.
Banduhn, M., Frazier, H., 1978. G 3780A surfactant: biodegradation in nature waters. Report No. MSL-0488. Monsanto Agricultural Research Department, St. Louis.
Benachour, N., Sipahutar, H., Moslemi, S., Gasnier, C., Travert, C., Seralini, G.E., 2007. Time- and dose-dependent effects of roundup on human embryonic and placental cells. Arch. Environ. Contam. Toxicol. 53, 126–133.
Benachour, N., Seralini, G.E., 2009. Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. Chem. Res. Toxicol. 22, 97–105.
Beuret, C.J., Zirulnik, F., Gimenez, M.S., 2005. Effect of the herbicide glyphosate on liver lipoperoxidation in pregnant rats and their fetuses. Reprod. Toxicol. 19, 501–504.
Boeije, G.M., Cano, M.L., Marshall, S.J., Belanger, S.E., Van Compernolle, R., Dorn, P.B., Gumbel, H., Toy, R., Wind, T., 2006. Ecotoxicity quantitative structure–activity relationships for alcohol ethoxylate mixtures based on substance-specific toxicity predictions. Ecotoxicol. Environ. Saf. 64, 75–84.
Boocock, M.R., Coggins, J.R., 1983. Kinetics of 5-enolpyruvylshikimate-3-phosphate synthase inhibition by glyphosate. FEBS Lett. 154, 127–133.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 72, 248–254.
Brausch, J.M., Smith, P.N., 2007. Toxicity of three polyethoxylated tallowamine surfactant formulations to laboratory and field collected fairy shrimp, *Thamnocephalus platyurus*. Arch. Environ. Contam. Toxicol. 52, 217–221.
Cavusoglu, K., Yapar, K., Oruc, E., Yalcin, E., 2011. Protective effect of *Ginkgo biloba* L. leaf extract against glyphosate toxicity in Swiss albino mice. J. Med. Food 14, 1263–1272.
Chamel, A., Gambonnet, B., 1997. Sorption and diffusion of an ethoxylated stearic alcohol and an ethoxylated stearic amine into and through isolated plant cuticles. Chemosphere 34, 1777–1786.
Clair, E., Mesnage, R., Travert, C., Seralini, G.E., 2012. A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells in vitro, and testosterone decrease at lower levels. Toxicol. In Vitro 26, 269–279.
Colborn, T., vom Saal, F.S., Soto, A.M., 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ. Health Perspect. 101, 378–384.
Corbera, M., Simonet, B.M., Salvado, V., Hidalgo, M., 2010. Characterisation of alkylamine ethoxylates (ANEOs) in commercial herbicide formulations using liquid chromatography/electrospray ionisation mass spectrometry. Rapid Commun. Mass Spectrom. 24, 2931–2937.
Courtney, H.S., Simpson, W.A., Beachey, E.H., 1986. Relationship of critical micelle concentrations of bacterial lipoteichoic acids to biological activities. Infect. Immun. 51, 414–418.
Cox, C., 2004. Herbicide factsheet—glyphosate. J. Pesticide Reform 24, 10–15.
Crouch, S.P., Kozlowski, R., Slater, K.J., Fletcher, J., 1993. The use of ATP bioluminescence as a measure of cell proliferation and cytotoxicity. J. Immunol. Methods 160, 81–88.
Cserháti, T., Forgács, E., 1997. Separation and quantitative determination of non-ionic surfactants used as pesticide additives. J. Chromatogr. A 774, 265–279.
Dallegrave, E., Mantese, F.D., Oliveira, R.T., Andrade, A.J., Dalsenter, P.R., Langeloh, A., 2007. Pre- and postnatal toxicity of the commercial glyphosate formulation in Wistar rats. Arch. Toxicol. 81, 665–673.
Daruich, J., Zirulnik, F., Gimenez, M.S., 2001. Effect of the herbicide glyphosate on enzymatic activity in pregnant rats and their fetuses. Environ. Res. 85, 226–231.
Eddleston, M., Street, J.M., Self, I., Thompson, A., King, T., Williams, N., Naredo, G., Dissanayake, K., Yu, L.M., Worek, F., John, H., Smith, S., Thiermann, H., Harris, J.B., Eddie Clutton, R., 2012. A role for solvents in the toxicity of agricultural organophosphorus pesticides. Toxicology 294, 94–103.
Gasnier, C., Dumont, C., Benachour, N., Clair, E., Chagnon, M.C., Seralini, G.E., 2009. Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. Toxicology 262, 184–191.
Gasnier, C., Laurant, C., Decroix-Laporte, C., Mesnage, R., Clair, E., Travert, C., Seralini, G.E., 2011. Defined plant extracts can protect human cells against combined xenobiotic effects. J. Occup. Med. Toxicol. 6, 3.
German Federal Agency CPFS, 1998. Monograph on Glyphosate. Released by the German Federal Agency for Consumer Protection and Food Safety. Annex B-5: Toxicology and Metabolism, p. 136.
Hartung, T., 2009. Toxicology for the twenty-first century. Nature 460, 208–212.
Hawthorne, D.J., Dively, G.P., 2011. Killing them with kindness? In-hive medications may inhibit xenobiotic efflux transporters and endanger honey bees. PLoS ONE 6, e26796.
IFEN, 2006. Report on Pesticides in Waters. Data 2003–2004.
James, C., 2011. Global Status of Commercialized Biotech/GM Crops: 2009. ISAAA Brief 43.
Krogh, K.A., Halling-Sorensen, B., Mogensen, B.B., Vejrup, K.V., 2003. Environmental properties and effects of nonionic surfactant adjuvants in pesticides: a review. Chemosphere 50, 871–901.
L'Azou, B., Fernandez, P., Bareille, R., Beneteau, M., Bourget, C., Cambar, J., Bordenave, L., 2005. In vitro endothelial cell susceptibility to xenobiotics: comparison of three cell types. Cell Biol. Toxicol. 21, 127–137.
Letcher, R.J., van Holsteijn, I., Drenth, H.J., Norstrom, R.J., Bergman, A., Safe, S., Pieters, R., van den Berg, M., 1999. Cytotoxicity and aromatase (CYP19) activity modulation by organochlorines in human placental JEG-3 and JAR choriocarcinoma cells. Toxicol. Appl. Pharmacol. 160, 10–20.
Liu, J.J., Wang, W., Dicker, D.T., El-Deiry, W.S., 2005. Bioluminescent imaging of TRAIL-induced apoptosis through detection of caspase activation following cleavage of DEVD-aminoluciferin. Cancer Biol. Ther. 4, 885–892.
Marc, J., Le Breton, M., Cormier, P., Morales, J., Belle, R., Mulner-Lorillon, O., 2005. A glyphosate-based pesticide impinges on transcription. Toxicol. Appl. Pharmacol. 203, 1–8.
Mesnage, R., Clair, E., Séralini, G.E., 2010. Roundup in genetically modified plants: regulation and toxicity in mammals. Theor. Ökol. 16, 31–33.
Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.
Nobels, I., Spanoghe, P., Haesaert, G., Robbens, J., Blust, R., 2011. Toxicity ranking and toxic mode of action evaluation of commonly used agricultural adjuvants on the basis of bacterial gene expression profiles. PLoS ONE 6, e24139.
Oliveira, A.G., Telles, L.F., Hess, R.A., Mahecha, G.A., Oliveira, C.A., 2007. Effects of the herbicide Roundup on the epididymal region of drakes *Anas platyrhynchos*. Reprod. Toxicol. 23, 182–191.
Paganelli, A., Gnazzo, V., Acosta, H., Lopez, S.L., Carrasco, A.E., 2010. Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling. Chem. Res. Toxicol. 23, 1586–1595.
Peixoto, F., 2005. Comparative effects of the Roundup and glyphosate on mitochondrial oxidative phosphorylation. Chemosphere 61, 1115–1122.
Peluso, M., Munnia, A., Bolognesi, C., Parodi, S., 1998. 32P-postlabeling detection of DNA adducts in mice treated with the herbicide Roundup. Environ. Mol. Mutagen. 31, 55–59.
Richard, S., Moslemi, S., Sipahutar, H., Benachour, N., Seralini, G.E., 2005. Differential effects of glyphosate and roundup on human placental cells and aromatase. Environ. Health Perspect. 113, 716–720.
Romano, M.A., Romano, R.M., Santos, L.D., Wisniewski, P., Campos, D.A., de Souza, P.B., Viau, P., Bernardi, M.M., Nunes, M.T., de Oliveira, C.A., 2011. Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression. Arch. Toxicol. 86, 663–673.
Samsonoff, C., Daily, J., Almog, R., Berns, D.S., 1986. The use of coomassie brilliant blue for critical micelle concentration determination of detergents. J. Colloid Interface Sci. 109, 325–329.
Savitz, D.A., Arbuckle, T., Kaczor, D., Curtis, K.M., 1997. Male pesticide exposure and pregnancy outcome. Am. J. Epidemiol. 146, 1025–1036.
Tsui, M.T., Chu, L.M., 2003. Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. Chemosphere 52, 1189–1197.
Urani, C., Doldi, M., Crippa, S., Camatini, M., 1998. Human-derived cell lines to study xenobiotic metabolism. Chemosphere 37, 2785–2795.
Williams, A.L., Watson, R.E., Desesso, J.M., 2012. Developmental and reproductive outcomes in humans and animals after glyphosate exposure: a critical analysis. J. Toxicol. Environ. Health B: Crit. Rev. 15, 39–96.
Yousef, M.I., Salem, M.H., Ibrahim, H.Z., Helmi, S., Seehy, M.A., Bertheussen, K., 1995. Toxic effects of carbofuran and glyphosate on semen characteristics in rabbits. J. Environ. Sci. Health B 30, 513–534.

GLYPHOSATE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 1071-83-6 (acid); also relevant:
38641-94-0 (glyphosate-isopropylamine salt)
40465-66-5 (monoammonium salt)
69254-40-6 (diammonium salt)
34494-03-6 (glyphosate-sodium)
81591-81-3 (glyphosate-trimesium)

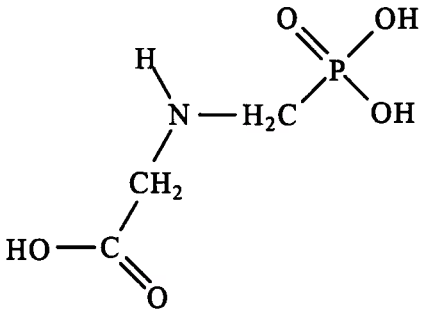
Chem. Abstr. Serv. Name: *N*-(phosphonomethyl)glycine

Preferred IUPAC Name: *N*-(phosphonomethyl)glycine

Synonyms: Gliphosate; glyphosate; glyphosate hydrochloride; glyphosate [calcium, copper (2+), dilithium, disodium, magnesium, monoammonium, monopotassium, monosodium, sodium, or zinc] salt

Trade names: Glyphosate products have been sold worldwide under numerous trade names, including: Abundit Extra; Credit; Xtreme; Glifonox; Glyphogan; Ground-Up; Rodeo; Roundup; Touchdown; Tragli; Wipe Out; Yerbimat (Farm Chemicals International, 2015).

1.1.2 Structural and molecular formulae and relative molecular mass



Molecular formula: $C_3H_8NO_5P$
Relative molecular mass: 169.07

Additional information on chemical structure is also available in the PubChem Compound database (NCBI, 2015).

1.1.3 Chemical and physical properties of the pure substance

Description: Glyphosate acid is a colourless, odourless, crystalline solid. It is formulated as a salt consisting of the deprotonated acid of glyphosate and a cation (isopropylamine, ammonium, or sodium), with more than one salt in some formulations.

Solubility: The acid is of medium solubility at 11.6 g/L in water (at 25 °C) and insoluble in common organic solvents such as acetone, ethanol, and xylene; the alkali-metal and

amine salts are readily soluble in water (Tomlin, 2000).

Volatility: Vapour pressure, 1.31×10^{-2} mPa at 25 °C (negligible) (Tomlin, 2000).

Stability: Glyphosate is stable to hydrolysis in the range of pH 3 to pH 9, and relatively stable to photodegradation (Tomlin, 2000). Glyphosate is not readily hydrolysed or oxidized in the field (Rueppel *et al.* 1977). It decomposes on heating, producing toxic fumes that include nitrogen oxides and phosphorus oxides (IPCS, 2005).

Reactivity: Attacks iron and galvanized steel (IPCS, 2005).

Octanol/water partition coefficient (P): log P, < –3.2 (pH 2–5, 20 °C) (OECD method 107) (Tomlin, 2000).

Henry's law: $< 2.1 \times 10^{-7}$ Pa m^3 mol^{-1} (Tomlin, 2000).

Conversion factor: Assuming normal temperature (25 °C) and pressure (101 kPa), mg/m^3 = 6.92 × ppm.

1.1.4 Technical products and impurities

Glyphosate is formulated as an isopropylamine, ammonium, or sodium salt in water-soluble concentrates and water-soluble granules. The relevant impurities in glyphosate technical concentrates are formaldehyde (maximum, 1.3 g/kg), *N*-nitrosoglyphosate (maximum, 1 mg/kg), and *N*-nitroso-*N*-phosphonomethylglycine (FAO, 2000). Surfactants and sulfuric and phosphoric acids may be added to formulations of glyphosate, with type and concentration differing by formulation (IPCS, 1994).

1.2 Production and use

1.2.1 Production

(a) Manufacturing processes

Glyphosate was first synthesized in 1950 as a potential pharmaceutical compound, but its herbicidal activity was not discovered until it was re-synthesized and tested in 1970 (Székács & Darvas, 2012). The isopropylamine, sodium, and ammonium salts were introduced in 1974, and the trimesium (trimethylsulfonium) salt was introduced in Spain in 1989. The original patent protection expired outside the USA in 1991, and within the USA in 2000. Thereafter, production expanded to other major agrochemical manufacturers in the USA, Europe, Australia, and elsewhere (including large-scale production in China), but the leading preparation producer remained in the USA (Székács & Darvas, 2012).

There are two dominant families of commercial production of glyphosate, the "alkyl ester" pathways, predominant in China, and the "iminodiacetic acid" pathways, with iminodiacetic acid produced from iminodiacetonitrile (produced from hydrogen cyanide), diethanol amine, or chloroacetic acid (Dill *et al.*, 2010; Tian *et al.*, 2012).

To increase the solubility of technical-grade glyphosate acid in water, it is formulated as its isopropylamine, monoammonium, potassium, sodium, or trimesium salts. Most common is the isopropylamine salt, which is formulated as a liquid concentrate (active ingredient, 5.0–62%), ready-to-use liquid (active ingredient, 0.5–20%), pressurized liquid (active ingredient, 0.75–0.96%), solid (active ingredient, 76–94%), or pellet/tablet (active ingredient, 60–83%) (EPA, 1993a).

There are reportedly more than 750 products containing glyphosate for sale in the USA alone (NPIC, 2010). Formulated products contain various non-ionic surfactants, most notably polyethyloxylated tallowamine (POEA), to

facilitate uptake by plants (Székács & Darvas, 2012). Formulations might contain other active ingredients, such as simasine, 2,4-dichlorophenoxyacetic acid (2,4-D), or 4-chloro-2-methylphenoxyacetic acid (IPCS, 1996), with herbicide resistance driving demand for new herbicide formulations containing multiple active ingredients (Freedonia, 2012).

(b) Production volume

Glyphosate is reported to be manufactured by at least 91 producers in 20 countries, including 53 in China, 9 in India, 5 in the USA, and others in Australia, Canada, Cyprus, Egypt, Germany, Guatemala, Hungary, Israel, Malaysia, Mexico, Singapore, Spain, Taiwan (China), Thailand, Turkey, the United Kingdom, and Venezuela (Farm Chemicals International, 2015). Glyphosate was registered in over 130 countries as of 2010 and is probably the most heavily used herbicide in the world, with an annual global production volume estimated at approximately 600 000 tonnes in 2008, rising to about 650 000 tonnes in 2011, and to 720 000 tonnes in 2012 (Dill *et al.*, 2010; CCM International, 2011; Hilton, 2012; Transparency Market Research, 2014).

Production and use of glyphosate have risen dramatically due to the expiry of patent protection (see above), with increased promotion of non-till agriculture, and with the introduction in 1996 of genetically modified glyphosate-tolerant crop varieties (Székács & Darvas, 2012). In the USA alone, more than 80 000 tonnes of glyphosate were used in 2007 (rising from less than 4000 tonnes in 1987) (EPA, 1997, 2011). This rapid growth rate was also observed in Asia, which accounted for 30% of world demand for glyphosate in 2012 (Transparency Market Research, 2014). In India, production increased from 308 tonnes in 2003–2004, to 2100 tonnes in 2007–2008 (Ministry of Chemicals & Fertilizers, 2008). China currently produces more than 40% of the global supply of glyphosate, exports almost 35% of the global supply (Hilton, 2012), and reportedly has sufficient production capacity to satisfy total global demand (Yin, 2011).

1.2.2 Uses

Glyphosate is a broad-spectrum, post-emergent, non-selective, systemic herbicide, which effectively kills or suppresses all plant types, including grasses, perennials, vines, shrubs, and trees. When applied at lower rates, glyphosate is a plant-growth regulator and desiccant. It has agricultural and non-agricultural uses throughout the world.

(a) Agriculture

Glyphosate is effective against more than 100 annual broadleaf weed and grass species, and more than 60 perennial weed species (Dill *et al.*, 2010). Application rates are about 1.5–2 kg/ha for pre-harvest, post-planting, and pre-emergence use; about 4.3 kg/ha as a directed spray in vines, orchards, pastures, forestry, and industrial weed control; and about 2 kg/ha as an aquatic herbicide (Tomlin, 2000). Common application methods include broadcast, aerial, spot, and directed spray applications (EPA, 1993a).

Due to its broad-spectrum activity, the use of glyphosate in agriculture was formerly limited to post-harvest treatments and weed control between established rows of tree, nut, and vine crops. Widespread adoption of no-till and conservation-till practices (which require chemical weed control while reducing soil erosion and labour and fuel costs) and the introduction of transgenic crop varieties engineered to be resistant to glyphosate have transformed glyphosate to a post-emergent, selective herbicide for use on annual crops (Duke & Powles, 2009; Dill *et al.* 2010). Glyphosate-resistant transgenic varieties have been widely adopted for the production of corn, cotton, canola, and soybean (Duke & Powles, 2009). Production of such crops accounted for 45% of worldwide demand for glyphosate in 2012 (Transparency Market Research, 2014). However, in Europe,

where the planting of genetically modified crops has been largely restricted, post-harvest treatment is still the most common application of glyphosate (Glyphosate Task Force, 2014). Intense and continuous use of glyphosate has led to the emergence of resistant weeds that may reduce its effectiveness (Duke & Powles, 2009).

(b) Residential use

Glyphosate is widely used for household weed control throughout the world. In the USA, glyphosate was consistently ranked as the second most commonly used pesticide (after 2,4-D) in the home and garden market sector between 2001 and 2007, with an annual use of 2000–4000 tonnes (EPA, 2011).

(c) Other uses

Glyphosate was initially used to control perennial weeds on ditch banks and roadsides and under power lines (Dill *et al.*, 2010). It is also used to control invasive species in aquatic or wetland systems (Tu *et al.*, 2001). Approximately 1–2% of total glyphosate use in the USA is in forest management (Mance, 2012).

Glyphosate has been used in a large-scale aerial herbicide-spraying programme begun in 2000 to reduce the production of cocaine in Colombia (Lubick, 2009), and of marijuana in Mexico and South America (Székács & Darvas, 2012).

(d) Regulation

Glyphosate has been registered for use in at least 130 countries (Dill *et al.*, 2010). In the USA, all uses are eligible for registration on the basis of a finding that glyphosate "does not pose unreasonable risks or adverse effects to humans or the environment" (EPA, 1993a). A review conducted in 2001 in connection with the registration process in the European Union reached similar conclusions regarding animal and human safety, although the protection of groundwater during non-crop use was identified as requiring particular attention in the short term (European Commission, 2002).

Nevertheless, as worldwide rates of adoption of herbicide-resistant crops and of glyphosate use have risen in recent years (Duke & Powles, 2009), restriction of glyphosate use has been enacted or proposed in several countries, although documented actions are few. In 2013, the Legislative Assembly of El Salvador voted a ban on the use of pesticides containing glyphosate (República de El Salvador, 2013). Sri Lanka is reported to have instituted a partial ban based on an increasing number of cases of chronic kidney disease among agricultural workers, but the ban was lifted after 2 months (ColomboPage, 2014). The reasons for such actions have included the development of resistance among weed species, as well as health concerns.

No limits for occupational exposure were identified by the Working Group.

1.3 Measurement and analysis

Several methods exist for the measurement of glyphosate and its major metabolite aminomethyl phosphonic acid (AMPA) in various media, including air, water, urine, and serum (Table 1.1). The methods largely involve derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl) to reach sufficient retention in chromatographic columns (Kuang *et al.*, 2011; Botero-Coy *et al.*, 2013). Chromatographic techniques that do not require derivatization and enzyme-linked immunosorbent assays (ELISA) are under development (Sanchís *et al.*, 2012).

Table 1.1 Methods for the analysis of glyphosate

Sample matrix	Assay procedure	Limit of detection	Reference
Water	HPLC/MS (with online solid-phase extraction)	0.08 µg/L	Lee *et al.* (2001)
	ELISA	0.05 µg/L	Abraxis (2005)
	LC-LC-FD	0.02 µg/L	Hidalgo *et al.* (2004)
	Post HPLC column derivatization and FD	6.0 µg/L	EPA (1992)
	UV visible spectrophotometer (at 435 ng)	1.1 µg/L	Jan *et al.* (2009)
Soil	LC–MS/MS with triple quadrupole	0.02 mg/kg	Botero-Coy *et al.* (2013)
Dust	GC-MS-MID	0.0007 mg/kg	Curwin *et al.* (2005)
Air	HPLC/MS with online solid-phase extraction	0.01 ng/m³	Chang *et al.* (2011)
Fruits and vegetables	HILIC/WAX with ESI-MS/MS	1.2 µg/kg	Chen *et al.* (2013)
Field crops (rice, maize and soybean)	LC–ESI-MS/MS	0.007–0.12 mg/kg	Botero-Coy *et al.* (2013b)
Plant vegetation	HPLC with single polymeric amino column	0.3 mg/kg	Nedelkoska & Low (2004)
Serum	LC–MS/MS	0.03 µg/mL 0.02 µg/mL (aminomethylphosphonic acid) 0.01 µg/mL (3-methylphosphinicopropionic acid)	Yoshioka *et al.* (2011)
Urine	HPLC with post-column reaction and FD	1 µg/L	Acquavella *et al.* (2004)
	ELISA	0.9 µg/L	Curwin *et al.* (2007)

ELISA, enzyme-linked immunosorbent assay; ESI-MS/MS, electrospray tandem mass spectrometry; FD, fluorescence detection; GC-MS-MID, gas chromatography-mass spectrometry in multiple ion detection mode; HILIC/WAX, hydrophilic interaction/weak anion-exchange liquid chromatography; HPLC/MS, high-performance liquid chromatography with mass spectrometry; HPLC, high-performance liquid chromatography; LC-ESI–MS/MS, liquid chromatography-electrospray–tandem mass spectrometry; LC–LC, coupled-column liquid chromatography; LC–MS/MS, liquid chromatography–tandem mass spectrometry

1.4 Occurrence and exposure

1.4.1 Exposure

(a) Occupational exposure

Studies related to occupational exposure to glyphosate have included farmers and tree nursery workers in the USA, forestry workers in Canada and Finland, and municipal weed-control workers in the United Kingdom (Centre de Toxicologie du Québec, 1988; Jauhiainen *et al.*, 1991; Lavy *et al.*, 1992; Acquavella *et al.*, 2004; Johnson *et al.*, 2005). Para-occupational exposures to glyphosate have also been measured in farming families (Acquavella *et al.*, 2004; Curwin *et al.*, 2007). These studies are summarized in Table 1.2.

(b) Community exposure

Glyphosate can be found in soil, air, surface water, and groundwater (EPA, 1993a). Once in the environment, glyphosate is adsorbed to soil and is broken down by soil microbes to AMPA (Borggaard & Gimsing, 2008). In surface water, glyphosate is not readily broken down by water or sunlight (EPA, 1993a). Despite extensive worldwide use, there are relatively few studies

Table 1.2 Occupational and para-occupational exposure to glyphosate

Industry, country, year	Job/process	Results	Comments/additional data	Reference
Forestry				
Canada, 1986		Arithmetic mean of air glyphosate concentrations:	Air concentrations of glyphosate were measured at the work sites of one crew (five workers) during ground spraying 268 urine samples were collected from 40 workers; glyphosate concentration was above the LOD (15 µg/L) in 14%	Centre de Toxicologie du Québec (1988)
	Signaller	Morning, 0.63 µg/m³ Afternoon, 2.25 µg/m³		
	Operator	Morning, 1.43 µg/m³ Afternoon, 6.49 µg/m³		
	Overseer	Morning, 0.84 µg/m³ Afternoon, 2.41 µg/m³		
	Mixer	Morning, 5.15 µg/m³ Afternoon, 5.48 µg/m³		
Finland, year NR	Workers performing silvicultural clearing (n = 5)	Range of air glyphosate concentrations, < 1.25–15.7 µg/m³ (mean, NR)	Clearing work was done with brush saws equipped with pressurized herbicide sprayers Air samples were taken from the workers' breathing zone (number of samples, NR) Urine samples were collected during the afternoons of the working week (number, NR) Glyphosate concentrations in urine were below the LOD (10 µg/L)	Jauhiainen *et al.* (1991)
USA, year NR	Workers in two tree nurseries (n = 14)	In dermal sampling, 1 of 78 dislodgeable residue samples were positive for glyphosate The body portions receiving the highest exposure were ankles and thighs	Dermal exposure was assessed with gauze patches attached to the clothing and hand rinsing Analysis of daily urine samples repeated over 12 weeks was negative for glyphosate	Lavy *et al.* (1992)
Weed control				
United Kingdom, year NR	Municipal weed control workers (n = 18)	Median, 16 mg/m³ in 85% of 21 personal air samples for workers spraying with mechanized all-terrain vehicle Median, 0.12 mg/m³ in 33% of 12 personal air samples collected from workers with backpack with lance applications	[The Working Group noted that the reported air concentrations were substantially higher than in other studies, but was unable to confirm whether the data were for glyphosate or total spray fluid] Dermal exposure was also measured, but reported as total spray fluid, rather than glyphosate	Johnson *et al.* (2005)

Table 1.2 (continued)

Industry, country, year	Job/process	Results	Comments/additional data	Reference
Farming				
USA, 2001	Occupational and para-occupational exposure of 24 farm families (24 fathers, 24 mothers and 65 children). Comparison group: 25 non-farm families (23 fathers, 24 mothers and 51 children)	Geometric mean (range) of glyphosate concentrations in urine: Non-farm fathers, 1.4 µg/L (0.13–5.4) Farm fathers, 1.9 µg/L (0.02–18) Non-farm mothers, 1.2 µg/L (0.06–5.0) Farm mothers, 1.5 µg/L (0.10–11) Non-farm children, 2.7 µg/L (0.10–9.4) Farm children, 2.0 µg/L (0.02–18)	Frequency of glyphosate detection ranged from 66% to 88% of samples (observed concentrations below the LOD were not censored). Detection frequency and geometric mean concentration were not significantly different between farm and non-farm families (observed concentrations below the LOD were not censored)	Curwin *et al.* (2007)
USA, year NR	Occupational and para-occupational exposures of 48 farmers, their spouses, and 79 children	Geometric mean (range) of glyphosate concentration in urine on day of application: Farmers, 3.2 µg/L (< 1 to 233 µg/L) Spouses, NR (< 1 to 3 µg/L) Children, NR (< 1 to 29 µg/L)	24-hour composite urine samples for each family member the day before, the day of, and for 3 days after a glyphosate application. Glyphosate was detected in 60% of farmers' samples, 4% of spouses' samples and 12% of children's samples the day of spraying and in 27% of farmers' samples, 2% of spouses' samples and 5% of children's samples 3 days after	Acquavella *et al.* (2004)

LOD, limit of detection; ND, not detected; NR, not reported

on the environmental occurrence of glyphosate (Kolpin *et al.*, 2006).

(i) Air

Very few studies of glyphosate in air were available to the Working Group. Air and rainwater samples were collected during two growing seasons in agricultural areas in Indiana, Mississippi, and Iowa, USA (Chang *et al.*, 2011). The frequency of glyphosate detection ranged from 60% to 100% in air and rain samples, and concentrations ranged from < 0.01 to 9.1 ng/m^3 in air samples and from < 0.1 to 2.5 µg/L in rainwater samples. Atmospheric deposition was measured at three sites in Alberta, Canada. Rainfall and particulate matter were collected as total deposition at 7-day intervals throughout the growing season. Glyphosate deposition rates ranged from < 0.01 to 1.51 µg/m^2 per day (Humphries *et al.*, 2005).

No data were available to the Working Group regarding glyphosate concentrations in indoor air.

(ii) Water

Glyphosate in the soil can leach into groundwater, although the rate of leaching is believed to be low (Borggaard & Gimsing, 2008; Simonsen *et al.*, 2008). It can also reach surface waters by direct emission, atmospheric deposition, and by adsorption to soil particles suspended in runoff water (EPA, 1993a; Humphries *et al.*, 2005). Table 1.3 summarizes data on concentrations of glyphosate or AMPA in surface water and groundwater.

(iii) Residues in food and dietary intake

Glyphosate residues have been measured in cereals, fruits, and vegetables (Table 1.4). Residues were detected in 0.04% of 74 305 samples of fruits, vegetables, and cereals tested from 27 member states of the European Union, and from Norway, and Iceland in 2007 (EFSA, 2009). In cereals, residues were detected in 50% of samples tested in Denmark in 1998–1999, and in 9.5% of samples tested from member states of the European Union, and from Norway and Iceland in 2007 (Granby & Vahl, 2001; EFSA, 2009). In the United Kingdom, food sampling for glyphosate residues has concentrated mainly on cereals, including bread and flour. Glyphosate has been detected regularly and usually below the reporting limit (Pesticide Residues Committee, 2007, 2008, 2009, 2010). Six out of eight samples of tofu made from Brazilian soy contained glyphosate, with the highest level registered being 1.1 mg/kg (Pesticide Residues Committee, 2007).

(iv) Household exposure

In a survey of 246 California households, 14% were found to possess at least one product containing glyphosate (Guha *et al.*, 2013).

(v) Biological markers

Glyphosate concentrations in urine were analysed in urban populations in Europe, and in a rural population living near areas sprayed for drug eradication in Colombia (MLHB, 2013; Varona *et al.*, 2009). Glyphosate concentrations in Colombia were considerably higher than in Europe, with means of 7.6 ng/L and 0.02 µg/L, respectively (Table 1.5). In a study in Canada, glyphosate concentrations in serum ranged from undetectable to 93.6 ng/mL in non-pregnant women (n = 39), and were undetectable in serum of pregnant women (n = 30) and fetal cord serum (Aris & Leblanc, 2011).

1.4.2 Exposure assessment

Exposure assessment methods in epidemiological studies on glyphosate and cancer are discussed in Section 2.0 of the *Monograph* on Malathion, in the present volume.

Table 1.3 Concentration of glyphosate and AMPA in water

Country, year of sampling	Number of samples/setting	Results	Comments/additional data	Reference
USA, 2002	51 streams/agricultural areas (154 samples)	Maximum glyphosate concentration, 5.1 µg/L Maximum AMPA concentration, 3.67 µg/L	The samples were taken following pre- and post-emergence application and during harvest season Glyphosate detected in 36% of samples; AMPA detected in 69% of samples	Battaglin *et al.*, (2005)
USA, 2002	10 wastewater treatment plants and two reference streams (40 samples)	Glyphosate, range ≤ 0.1–2 µg/L AMPA, range ≤ 0.1–4 µg/L	AMPA was detected more frequently (67.5%) than glyphosate (17.5%)	Kolpin *et al.* (2006)
Canada, 2002	3 wetlands and 10 agricultural streams (74 samples)	Range, < 0.02–6.08 µg/L	Glyphosate was detected in most of the wetlands and streams (22% of samples)	Humphries *et al.* (2005)
Colombia, year NR	5 areas near crops and coca eradication (24 samples)	Maximum concentration, 30.1 µg/L (minimum and mean, NR)	Glyphosate detected in 8% of samples (MDL, 25 µg/L)	Solomon *et al.*, (2007)
Denmark, 2010–2012	4 agricultural sites (450 samples)	Range, < 0.1–31.0 µg/L	Glyphosate detected in 23% of samples; AMPA detected in 25% of samples	Brüch *et al.* (2013)

AMPA, aminomethylphosphonic acid; MDL, method detection limit; NR, data not reported

Table 1.4 Concentrations of glyphosate in food

Country, year	Type of food	Results	Comments/additional data	Reference
Denmark, 1998, 1999	Cereals	> 50% of samples had detectable residues Means: 0.08 mg/kg in 1999 and 0.11 mg/kg in 1998	49 samples of the 1998 harvest 46 samples of the 1999 harvest	Granby & Vahl (2001)
27 European Union member states, Norway and Iceland, 2007	350 different food commodities	0.04% of 2302 fruit, vegetable and cereal samples 9.5% of 409 cereal samples	74 305 total samples	EFSA (2009)
Australia, 2006	Composite sample of foods consumed in 24 hours	75% of samples had detectable residues Mean, 0.08 mg/kg Range, < 0.005 to 0.5 mg/kg	20 total samples from 43 pregnant women	McQueen *et al.* (2012)

Table 1.5 Concentrations of glyphosate and AMPA in urine and serum in the general population

Country, period	Subjects	Results	Comments/additional data	Reference
Urine				
18 European countries, 2013	162 individuals	Arithmetic mean of glyphosate concentration: 0.21 µg/L (maximum, 1.56 µg/L) Arithmetic mean of AMPA concentration: 0.19 µg/L (maximum, 2.63 µg/L)	44% of samples had quantifiable levels of glyphosate and 36% had quantifiable levels of AMPA	MLHB (2013)
Colombia, 2005–2006	112 residents of areas sprayed for drug eradication	Arithmetic mean (range) of glyphosate concentration: 7.6 µg/L (ND–130 µg/L) Arithmetic mean (range) of AMPA concentration: 1.6 µg/L (ND–56 µg/L)	40% of samples had detectable levels of glyphosate and 4% had detectable levels of AMPA (LODs, 0.5 and 1.0 µg/L, respectively) Urinary glyphosate was associated with use in agriculture	Varona *et al.* (2009)
Serum				
Canada, NR	30 pregnant women and 39 non-pregnant women	ND in serum of pregnant women or cord serum; Arithmetic mean, 73.6 µg/L, (range, ND–93.6 µg/L) in non-pregnant women	No subject had worked or lived with a spouse working in contact with pesticides LOD, 15 µg/L	Aris & Leblanc (2011)

AMPA, aminomethylphosphonic acid; LOD, limit of detection; ND, not detected; NR, not reported

2. Cancer in Humans

2.0 General discussion of epidemiological studies

A general discussion of the epidemiological studies on agents considered in Volume 112 of the *IARC Monographs* is presented in Section 2.0 of the *Monograph* on Malathion.

2.1 Cohort studies

See Table 2.1

The Agricultural Health Study (AHS), a large prospective cohort study conducted in Iowa and North Carolina in the USA, is the only cohort study to date to have published findings on exposure to glyphosate and the risk of cancer at many different sites (Alavanja *et al.*, 1996; NIH, 2015) (see Section 2.0 of the *Monograph* on Malathion, in the present volume, for a detailed description of this study).

The enrolment questionnaire from the AHS sought information on the use of 50 pesticides (ever or never exposure), crops grown and livestock raised, personal protective equipment used, pesticide application methods used, other agricultural activities and exposures, nonfarm occup ational exposures, and several lifestyle, medical, and dietary variables. The duration (years) and frequency (days per year) of use was investigated for 22 of the 50 pesticides in the enrolment questionnaire. [Blair *et al.* (2011) assessed the possible impact of misclassification of occupational pesticide exposure on relative risks, demonstrating that nondifferential exposure misclassification biases relative risk estimates towards the null in the AHS and tends to decrease the study power.]

The first report of cancer incidence associated with pesticide use in the AHS cohort considered cancer of the prostate (Alavanja *et al.*, 2003). Risk estimates for exposure to glyphosate were not presented, but no significant exposure–response association with cancer of the prostate was found. In an updated analysis of the AHS (1993 to 2001), De Roos *et al.* (2005a) (see below) also found no association between exposure to glyphosate and cancer of the prostate (relative risk, RR, 1.1; 95% CI, 0.9–1.3) and no exposure–response trend (*P* value for trend = 0.69).

De Roos *et al.* (2005a) also evaluated associations between exposure to glyphosate and the incidence of cancer at several other sites. The prevalence of ever-use of glyphosate was 75.5% (> 97% of users were men). In this analysis, exposure to glyphosate was defined as: (a) ever personally mixed or applied products containing glyphosate; (b) cumulative lifetime days of use, or "cumulative exposure days" (years of use × days/year); and (c) intensity-weighted cumulative exposure days (years of use × days/year × estimated intensity level). Poisson regression was used to estimate exposure–response relations between exposure to glyphosate and incidence of all cancers combined, and incidence of 12 cancer types: lung, melanoma, multiple myeloma, and non-Hodgkin lymphoma (see Table 2.1) as well as oral cavity, colon, rectum, pancreas, kidney, bladder, prostate, and leukaemia (results not tabulated). Exposure to glyphosate was not associated with all cancers combined (RR, 1.0; 95% CI, 0.9–1.2; 2088 cases). For multiple myeloma, the relative risk was 1.1 (95% CI, 0.5–2.4; 32 cases) when adjusted for age, but was 2.6 (95% CI, 0.7–9.4) when adjusted for multiple confounders (age, smoking, other pesticides, alcohol consumption, family history of cancer, and education); in analyses by cumulative exposure-days and intensity-weighted exposure-days, the relative risks were around 2.0 in the highest tertiles. Furthermore, the association between multiple myeloma and exposure to glyphosate only appeared within the subgroup for which complete data were available on all the covariates; even without any adjustment, the risk of multiple myeloma associated with glyphosate use was increased by twofold among the smaller subgroup with available covariate data

Table 2.1 Cohort studies of cancer and exposure to glyphosate

Reference, study location, enrolment period/follow-up, study-design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
De Roos *et al.* (2005a) Iowa and North Carolina, USA 1993–2001	54 315 (after exclusions, from a total cohort of 57 311) licensed pesticide applicators Exposure assessment method: questionnaire; semi-quantitative assessment from self-administered questionnaire	Lung	Ever use	147	0.9 (0.6–1.3)	Age, smoking, other pesticides, alcohol consumption, family history of cancer, education	AHS Cancer sites investigated: lung, melanoma, multiple myeloma and NHL (results tabulated) as well as oral cavity, colon, rectum, pancreas, kidney, bladder, prostate and leukaemia (results not tabulated) [Strengths: large cohort; specific assessment of glyphosate; semiquantitative exposure assessment. Limitations: risk estimates based on self-reported exposure; limited to licensed applicators; potential exposure to multiple pesticides]
			Cumulative exposure days:				
			1–20	40	1 (ref.)		
			21–56	26	0.9 (0.5–1.5)		
			57–2678	26	0.7 (0.4–1.2)		
			Trend-test *P* value: 0.21				
		Melanoma	Ever use	75	1.6 (0.8–3)		
			1–20	23	1 (ref.)		
			21–56	20	1.2 (0.7–2.3)		
			57–2678	14	0.9 (0.5–1.8)		
			Trend-test *P* value: 0.77				
		Multiple myeloma	Ever use	32	1.1 (0.5–2.4)	Age only (results in this row only)	
			Ever use	32	2.6 (0.7–9.4)		
			1–20	8	1 (ref.)		
			21–56	5	1.1 (0.4–3.5)		
			Trend-test *P* value: 0.27				
		NHL	Ever use	92	1.1 (0.7–1.9)		
			1–20	29	1 (ref.)		
			21–56	15	0.7 (0.4–1.4)		
			57–2678	17	0.9 (0.5–1.6)		
			Trend-test *P* value: 0.73				

Table 2.1 (continued)

Reference, study location, enrolment period/follow-up, study-design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Flower *et al.* (2004) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up, 1975–1998	21 375; children (aged < 19 years) of licensed pesticide applicators in Iowa (n = 17 357) and North Carolina (n = 4018) Exposure assessment method: questionnaire	Childhood cancer	Maternal use of glyphosate (ever) Paternal use of glyphosate (prenatal)	13 6	0.61 (0.32–1.16) 0.84 (0.35–2.34)	Child's age at enrolment	AHS Glyphosate results relate to the Iowa participants only [Strengths: Large cohort; specific assessment of glyphosate. Limitations: based on self-reported exposure; potential exposure to multiple pesticides; limited power for glyphosate exposure]
Engel *et al.* (2005) Iowa and North Carolina, USA Enrolment, 1993–1997 follow-up to 2000	30 454 wives of licensed pesticide applicators with no history of breast cancer at enrolment Exposure assessment method: questionnaire	Breast	Direct exposure to glyphosate Husband's use of glyphosate	82 109	0.9 (0.7–1.1) 1.3 (0.8–1.9)	Age, race, state	AHS [Strengths: large cohort; specific assessment of glyphosate. Limitations: based on self-reported exposure; limited to licensed applicators; potential exposure to multiple pesticides]
Lee *et al.* (2007) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up to 2002	56 813 licensed pesticide applicators Exposure assessment method: questionnaire	Colorectum Colon Rectum	Exposed to glyphosate Exposed to glyphosate Exposed to glyphosate	225 151 74	1.2 (0.9–1.6)	Age, smoking, state, total days of any pesticide application	AHS [Strengths: large cohort. Limitations: based on self-reported exposure, limited to licensed applicators, potential

Table 2.1 (continued)

Reference, study location, enrolment period/follow-up, study-design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Andreotti *et al.* (2009) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up to 2004 Nested case–control study	Cases: 93 (response rate, NR); identified from population-based state-cancer registries. Incident cases diagnosed between enrolment and 31 December 2004 (> 9 years follow-up) included in the analysis. Participants with any type of prevalent cancer at enrolment were excluded. Vital status was obtained from the state death registries and the National Death Index. Participants who left North Carolina or Iowa were not subsequently followed for cancer occurrence. Controls: 82 503 (response rate, NR); cancer-free participants enrolled in the cohort Exposure assessment method: questionnaire providing detailed pesticide use, demographic and lifestyle information. Ever-use of 24 pesticides and intensity-weighted lifetime days [(lifetime exposure days) × (exposure intensity score)] of 13 pesticides was assessed	Pancreas (C25.0–C25.9)	Ever exposure to glyphosate Low (< 185 days) High (≥ 185 days) Trend-test *P* value: 0.85	55 29 19	1.1 (0.6–1.7)	Age, smoking, diabetes	AHS [Strengths: large cohort. Limitations: based on self-reported exposure; limited to licensed applicators; potential exposure to multiple pesticides]

AHS, Agricultural Health Study; NHL, non-Hodgkin lymphoma; NR, not reported

(De Roos *et al.*, 2005b). [The study had limited power for the analysis of multiple myeloma; there were missing data on covariates when multiple adjustments were done, limiting the interpretation of the findings.] A re-analysis of these data conducted by Sorahan (2015) confirmed that the excess risk of multiple myeloma was present only in the subset with no missing information (of 22 cases in the restricted data set). In a subsequent cross-sectional analysis of 678 male participants from the same cohort, Landgren *et al.* (2009) did not find an association between exposure to glyphosate and risk of monoclonal gammopathy of undetermined significance (MGUS), a premalignant plasma disorder that often precedes multiple myeloma (odds ratio, OR, 0.5; 95% CI, 0.2–1.0; 27 exposed cases).

Flower *et al.* (2004) reported the results of the analyses of risk of childhood cancer associated with pesticide application by parents in the AHS. The analyses for glyphosate were conducted among 17 357 children of Iowa pesticide applicators from the AHS. Parents provided data via questionnaires (1993–1997) and the cancer follow-up (retrospectively and prospectively) was done through the state cancer registries. Fifty incident childhood cancers were identified (1975–1998; age, 0–19 years). For all the children of the pesticide applicators, risk was increased for all childhood cancers combined, for all lymphomas combined, and for Hodgkin lymphoma, compared with the general population. The odds ratio for use of glyphosate and risk of childhood cancer was 0.61 (95% CI, 0.32–1.16; 13 exposed cases) for maternal use and 0.84 (95% CI, 0.35–2.34; 6 exposed cases) for paternal use. [The Working Group noted that this analysis had limited power to study a rare disease such as childhood cancer.]

Engel *et al.* (2005) reported on incidence of cancer of the breast among farmers' wives in the AHS cohort, which included 30 454 women with no history of cancer of the breast before enrolment in 1993–1997. Information on pesticide use and other factors was obtained at enrolment by self-administered questionnaire from the women and their husbands. A total of 309 incident cases of cancer of the breast were identified until 2000. There was no difference in incidence of cancer of the breast for women who reported ever applying pesticides compared with the general population. The relative risk for cancer of the breast among women who had personally used glyphosate was 0.9 (95% CI, 0.7–1.1; 82 cases) and 1.3 (95% CI, 0.8–1.9; 109 cases) among women who never used pesticides but whose husband had used glyphosate. [No information on duration of glyphosate use by the husband was presented.] Results for glyphosate were not further stratified by menopausal status.

Lee *et al.* (2007) investigated the relationship between exposure to agricultural pesticides and incidence of cancer of the colorectum in the AHS. A total of 56 813 pesticide applicators with no prior history of cancer of the colorectum were included in this analysis, and 305 incident cancers of the colorectum (colon, 212; rectum, 93) were diagnosed during the study period, 1993–2002. Most of the 50 pesticides studied were not associated with risk of cancer of the colorectum, and the relative risks with exposure to glyphosate were 1.2 (95% CI, 0.9–1.6), 1.0 (95% CI, 0.7–1.5), and 1.6 (95% CI, 0.9–2.9) for cancers of the colorectum, colon, and rectum, respectively.

Andreotti *et al.* (2009) examined associations between the use of pesticides and cancer of the pancreas using a case–control analysis nested in the AHS. This analysis included 93 incident cases of cancer of the pancreas (64 applicators, 29 spouses) and 82 503 cancer-free controls who completed the enrolment questionnaire. Ever-use of 24 pesticides and intensity-weighted lifetime days [(lifetime exposure days) × (exposure intensity score)] of 13 pesticides were assessed. Risk estimates were calculated controlling for age, smoking, and diabetes. The odds ratio for ever- versus never-exposure to glyphosate was

1.1 (95% CI, 0.6–1.7; 55 exposed cases), while the odds ratio for the highest category of level of intensity-weighted lifetime days was 1.2 (95% CI, 0.6–2.6; 19 exposed cases).

Dennis *et al.* (2010) reported that exposure to glyphosate was not associated with cutaneous melanoma within the AHS. [The authors did not report a risk estimate.]

2.2 Case–control studies on non-Hodgkin lymphoma, multiple myeloma, and leukaemia

2.2.1 Non-Hodgkin lymphoma

See Table 2.2

(a) Case–control studies in the midwest USA

Cantor *et al.* (1992) conducted a case–control study of incident non-Hodgkin lymphoma (NHL) among males in Iowa and Minnesota, USA (see the *Monograph* on Malathion, Section 2.0, for a detailed description of this study). A total of 622 white men and 1245 population-based controls were interviewed in person. The association with farming occupation and specific agricultural exposures were evaluated. When compared with non-farmers, the odds ratios for NHL were 1.2 (95% CI, 1.0–1.5) for men who had ever farmed, and 1.1 (95% CI, 0.7–1.9; 26 exposed cases; adjusted for vital status, age, state, cigarette smoking status, family history of lymphohaematopoietic cancer, high-risk occupations, and high-risk exposures) for ever handling glyphosate. [There was low power to assess the risk of NHL associated with exposure to glyphosate. There was no adjustment for other pesticides. These data were included in the pooled analysis by De Roos *et al.* (2003).]

Brown *et al.* (1993) reported the results of a study to evaluate the association between multiple myeloma and agricultural risk factors in the midwest USA (see the *Monograph* on Malathion, Section 2.0, for a detailed description of this study). A population-based case–control study of 173 white men with multiple myeloma and 650 controls was conducted in Iowa, USA, an area with a large farming population. A non-significantly elevated risk of multiple myeloma was seen among farmers compared with never-farmers. The odds ratio related to exposure to glyphosate was 1.7 (95% CI, 0.8–3.6; 11 exposed cases). [This study had limited power to assess the association between multiple myeloma and exposure to glyphosate. Multiple myeloma is now considered to be a subtype of NHL.]

De Roos *et al.* (2003) used pooled data from three case–control studies of NHL conducted in the 1980s in Nebraska (Zahm *et al.*, 1990), Kansas (Hoar *et al.*, 1986), and in Iowa and Minnesota (Cantor *et al.*, 1992) (see the *Monograph* on Malathion, Section 2.0, for a detailed description of these studies) to examine pesticide exposures in farming as risk factors for NHL in men. The study population included 870 cases and 2569 controls; 650 cases and 1933 controls were included for the analysis of 47 pesticides controlling for potential confounding by other pesticides. Both logistic regression and hierarchical regression (adjusted estimates were based on prior distributions for the pesticide effects, which provides more conservative estimates than logistic regression) were used in data analysis, and all models were essentially adjusted for age, study site, and other pesticides. Reported use of glyphosate as well as several individual pesticides was associated with increased incidence of NHL. Based on 36 cases exposed, the odds ratios for the association between exposure to glyphosate and NHL were 2.1 (95% CI, 1.1–4.0) in the logistic regression analyses and 1.6 (95% CI, 0.9–2.8) in the hierarchical regression analysis. [The numbers of cases and controls were lower than those in the pooled analysis by Waddell *et al.* (2001) because only subjects with no missing data on pesticides were included. The strengths of this study when compared with other studies are that it was large,

Table 2.2 Case–control studies of leukaemia and lymphoma and exposure to glyphosate

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
USA							
Brown *et al.* (1990) Iowa and Minnesota, USA 1981–1983	Cases: 578 (340 living, 238 deceased) (response rate, 86%); cancer registry or hospital records Controls: 1245 (820 living, 425 deceased) (response rate, 77–79%); random-digit dialling for those aged < 65 years and Medicare for those aged ≥ 65 years Exposure assessment method: questionnaire	Leukaemia	Any glyphosate	15	0.9 (0.5–1.6)	Age, vital status, state, tobacco use, family history lymphopoietic cancer, high-risk occupations, high risk exposures	[Strengths: large population based study in a farming area. Limitations: not controlled for exposure to other pesticides. Limited power for glyphosate exposure]
Cantor *et al.* (1992) Iowa and Minnesota, USA 1980–1982	Cases: 622 (response rate, 89.0%); Iowa health registry records and Minnesota hospital and pathology records Controls: 1245 (response rate, 76–79%); population-based; no cancer of the lympho-haematopoietic system; frequency-matched to cases by age (5-year group), vital status, state. Random-digit dialling (aged < 65 years); Medicare records (aged ≥ 65 years); state death certificate files (deceased subjects) Exposure assessment method: questionnaire; in-person interview	NHL	Ever handled glyphosate	26	1.1 (0.7–1.9)	Age, vital status, state, smoking status, family history lymphopoietic cancer, high-risk occupations, high-risk exposures	Data subsequentially pooled in De Roos *et al.* (2003); white men only [Strengths: large population-based study in farming areas. Limitations: not controlled for exposure to other pesticides. Limited power for glyphosate exposure]

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Brown *et al.* (1993) Iowa, USA 1981–1984	Cases: 173 (response rate, 84%); Iowa health registry Controls: 650 (response rate, 78%); Random-digit dialling (aged < 65 years) and Medicare (aged > 65 years) Exposure assessment method: questionnaire	Multiple myeloma	Any glyphosate	11	1.7 (0.8–3.6)	Age, vital status	[Strengths: population-based study. Areas with high prevalence of farming. Limitations: limited power for glyphosate exposure]
De Roos *et al.* (2003) Nebraska, Iowa, Minnesota, Kansas, USA 1979–1986	Cases: 650 (response rate, 74.7%); cancer registries and hospital records Controls: 1933 (response rate, 75.2%); random-digit dialling, Medicare, state mortality files Exposure assessment method: questionnaire; interview (direct or next-of-kin)	NHL	Any glyphosate exposure	36	2.1 (1.1–4)	Age, study area, other pesticides	Both logistic regression and hierarchical regression were used in data analysis, the latter providing more conservative estimates [Strengths: increased power when compared with other studies, population-based, and conducted in farming areas. Advanced analytical methods to account for multiple exposures] Included participants from Cantor *et al.* (1992), Zahm *et al.* (1990), Hoar *et al.* (1986), and Brown *et al.* (1990)

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Lee *et al.* (2004a) Iowa, Minnesota and Nebraska, USA 1980–1986	Cases: 872 (response rate, NR); diagnosed with NHL from 1980 to 1986 Controls: 2381 (response rate, NR); frequency-matched controls Exposure assessment method: questionnaire; information on use of pesticides and history of asthma was based on interviews	NHL	Exposed to glyphosate – non-asthmatics Exposed to glyphosate – asthmatics	53 6	1.4 (0.98–2.1) 1.2 (0.4–3.3)	Age, vital status, state	177 participants (45 NHL cases, 132 controls) reported having been told by their doctor that they had asthma
Canada							
McDuffie *et al.* (2001) Canada 1991–1994	Cases: 517 (response rate, 67.1%), from cancer registries and hospitals Controls: 1506 (response rate, 48%); random sample from health insurance and voting records Exposure assessment method: questionnaire, some administered by telephone, some by post	NHL	Exposed to glyphosate Unexposed > 0 and ≤ 2 days > 2 days	51 464 28 23	1.2 (0.83–1.74) 1 1.0 (0.63–1.57) 2.12 (1.2–3.73)	Age, province of residence	Cross-Canada study [Strengths: large population based study. Limitations: no quantitative exposure data. Exposure assessment by questionnaire. Relatively low participation]

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Karunanayake *et al.* (2012) Six provinces in Canada (Quebec, Ontario, Manitoba, Saskatchewan, Alberta, and British Columbia) 1991–1994	Incident cases: 316 (response rate, 68.4%); men aged ≥ 19 years; ascertained from provincial cancer registries, except in Quebec (hospital ascertainment) Controls: 1506 (response rate, 48%); matched by age ± 2 years to be comparable with the age distribution of the entire case group (HL, NHL, MM, and STS) within each province of residence. Potential controls (men aged ≥ 19 years) selected at random within age constraints from the provincial health insurance records (Alberta, Saskatchewan, Manitoba, Quebec), computerized telephone listings (Ontario), or voters' lists (British Columbia) Exposure assessment method: questionnaire; stage 1 used a self-administered postal questionnaire; and in stage 2 detailed pesticide exposure information was collected by telephone interview	HL (ICDO2 included nodular sclerosis (M9656/3; M9663/3; M9664/3; M9665/3; M9666/3; M9667/3), lymphocytic predominance (M9651/3; M9657/3; M9658/3; M9659/3), mixed cellularity (M9652/3), lymphocytic depletion (M9653/3; M9654/3), miscellaneous (other M9650-M9669 codes for HL)	Glyphosate-based formulation Glyphosate-based formulation	38 38	1.14 (0.74–1.76) 0.99 (0.62–1.56)	Age group, province of residence Age group, province of residence, medical history	Cross Canada study Based on the statistical analysis of pilot study data, it was decided that the most efficient definition of pesticide exposure was a cumulative exposure ≥ 10 hours/year to any combination of pesticides. This discriminated (a) between incidental, bystander, and environmental exposure vs more intensive exposure, and (b) between cases and controls [Strengths: large study. Limitations: low response rates]

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kachuri *et al.* (2013) Six Canadian provinces (British Columbia, Alberta, Saskatchewan, Manitoba, Ontario and Quebec) 1991–1994	Cases: 342 (response rate, 58%); men aged ≥ 19 years diagnosed between 1991 and 1994 were ascertained from provincial cancer registries except in Quebec, where ascertained from hospitals Controls: 1357 (response rate, 48%); men aged ≥ 19 years selected randomly using provincial health insurance records, random digit dialling, or voters' lists, frequency-matched to cases by age (±2 years) and province of residence Exposure assessment method: questionnaire	Multiple myeloma	Glyphosate use	32	1.19 (0.76–1.87)	Age, province of residence, use of a proxy respondent, smoking status, medical variables, family history of cancer	Cross-Canada study [Strengths: population-based case–control study. Limitations: relatively low response rates]
			Use of glyphosate (> 0 and ≤ 2 days per year)	15	0.72 (0.39–1.32)		
			Use of glyphosate (> 2 days per year)	12	2.04 (0.98–4.23)		
Sweden							
Nordström *et al.* (1998) Sweden 1987–1992	Cases: 111 (response rate, 91%); 121 HCL cases in men identified from Swedish cancer registry Controls: 400 (response rate, 83%); 484 (four controls/case) matched for age and county; national population registry Exposure assessment method: questionnaire; considered exposed if minimum exposure of 1 working day (8 h) and an induction period of at least 1 year	HCL	Exposed to glyphosate	4	3.1 (0.8–12)	Age	Overlaps with Hardell *et al.* (2002). HCL is a subtype of NHL [Strengths: population-based case–control study. Limitations: Limited power. There was no adjustment for other exposures]

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Hardell & Eriksson (1999) Northern and middle Sweden 1987–1990	Cases: 404 (192 deceased) (response rate, 91%); regional cancer registries Controls: 741 (response rate, 84%); live controls matched for age and county were recruited from the national population registry, and deceased cases matched for age and year of death were identified from the national registry for causes of death Exposure assessment method: questionnaire	NHL (ICD-9 200 and 202)	Ever glyphosate – univariate Ever glyphosate – multivariate	4 NR	2.3 (0.4–13) 5.8 (0.6–54)	Not specified in the multivariable analysis	Overlaps with Hardell *et al.* (2002) [Strengths: population-based study. Limitations: few subjects were exposed to glyphosate and the study had limited power. Analyses were "multivariate" but covariates were not specified]
Hardell *et al.* (2002) Sweden; four Northern counties and three counties in mid Sweden 1987–1992	Cases: 515 (response rate, 91% in both studies); Swedish cancer registry Controls: 1141 (response rates, 84% and 83%%); national population registry Exposure assessment method: questionnaire	NHL and HCL	Ever glyphosate exposure (univariate) Ever glyphosate exposure (multivariate)	8 8	3.04 (1.08–8.5) 1.85 (0.55–6.2)	Age, county, study site, vital status, other pesticides in the multivariate analysis	Overlaps with Nordström *et al.* (1998) and Hardell & Eriksson (1999). [Strengths: large population-based study. Limitations: limited power for glyphosate exposure]

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Eriksson *et al.* (2008) Sweden. Four health service areas (Lund, Linkoping, Orebro and Umea) 1999–2002	Cases: 910 (response rate, 91%); incident NHL cases were enrolled from university hospitals Controls: 1016 (response rate, 92%); national population registry Exposure assessment method: questionnaire	NHL	Any glyphosate	29	2.02 (1.1–3.71)	Age, sex, year of enrolment	[Strengths: population-based case-control. Limitations: limited power for glyphosate]
			Any glyphosate*	29	1.51 (0.77–2.94)		* Exposure to other pesticides (e.g. MPCA) controlled in the analysis
			≤ 10 days per year use	12	1.69 (0.7–4.07)		
			> 10 days per year use	17	2.36 (1.04–5.37)		
		NHL	1–10 yrs	NR	1.11 (0.24–5.08)		
			> 10 yrs	NR	2.26 (1.16–4.4)		
		B-cell lymphoma	Exposure to glyphosate	NR	1.87 (0.998–3.51)		
		Lymphocytic lymphoma/B-CLL	Exposure to glyphosate	NR	3.35 (1.42–7.89)		
		Diffuse large B-cell lymphoma	Exposure to glyphosate	NR	1.22 (0.44–3.35)		
		Follicular, grade I–III	Exposure to glyphosate	NR	1.89 (0.62–5.79)		
		Other specified B-cell lymphoma	Exposure to glyphosate	NR	1.63 (0.53–4.96)		
		Unspecified B-cell lymphoma	Exposure to glyphosate	NR	1.47 (0.33–6.61)		
		T-cell lymphoma	Exposure to glyphosate	NR	2.29 (0.51–10.4)		
		Unspecified NHL	Exposure to glyphosate	NR	5.63 (1.44–22)		

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Other studies in Europe							
Orsi *et al.* (2009) France 2000–2004	Cases: 491 (response rate, 95.7%); cases (244 NHL; 87 HL; 104 LPSs; 56 MM) were recruited from main hospitals of the French cities of Brest, Caen, Nantes, Lille, Toulouse and Bordeaux, aged 20–75 years; ALL cases excluded Controls: 456 (response rate, 91.2%); matched on age and sex, recruited in the same hospitals as the cases, mainly in orthopaedic and rheumatological departments and residing in the hospital's catchment area Exposure assessment method: questionnaire	NHL	Any glyphosate exposure	12	1.0 (0.5–2.2)	Age, centre, socioeconomic category (blue/ white collar)	[Limitations: limited power for glyphosate]
		HL	Any exposure to glyphosate	6	1.7 (0.6–5)		
		LPS	Any exposure to glyphosate	4	0.6 (0.2–2.1)		
		MM	Any exposure to glyphosate	5	2.4 (0.8–7.3)		
		All lymphoid neoplasms	Any exposure to glyphosate	27	1.2 (0.6–2.1)		
		NHL, diffuse large cell lymphoma	Occupational use of glyphosate	5	1.0 (0.3–2.7)		
		NHL, follicular lymphoma	Occupational exposure to glyphosate	3	1.4 (0.4–5.2)		
		LPS/CLL	Occupational exposure to glyphosate	2	0.4 (0.1–1.8)		
		LPS/HCL	Occupational exposure to glyphosate	2	1.8 (0.3–9.3)		

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Cocco *et al.* (2013) Czech Republic, France, Germany, Italy, Ireland and Spain 1998–2004	Cases: 2348 (response rate, 88%); cases were all consecutive adult patients first diagnosed with lymphoma during the study period, resident in the referral area of the participating centres Controls: 2462 (response rate, 81% hospital; 52% population); controls from Germany and Italy were randomly selected by sampling from the general population and matched to cases on sex, 5-year age-group, and residence area. The rest of the centres used matched hospital controls, excluding diagnoses of cancer, infectious diseases and immunodeficiency diseases Exposure assessment method: questionnaire; support of a crop-exposure matrix to supplement the available information, industrial hygienists and occupational experts in each participating centre reviewed the general questionnaires and job modules to assess exposure to pesticides	B-cell lymphoma	Occupational exposure to glyphosate	4	3.1 (0.6–17.1)	Age, sex, education, centre	EPILYMPH case-control study in six European countries

ALL, acute lymphocytic leukaemia; B-CLL, chronic lymphocytic leukaemia; CLL, chronic lymphocytic leukaemia; HCL, hairy cell leukaemia; HL, Hodgkin lymphoma; LPS, lymphoproliferative syndrome; MCPA, 2-methyl-4-chlorophenoxyacetic acid; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; NR, not reported; ref., reference; STS, soft tissue sarcoma

population-based, and conducted in farming areas. Potential confounding from multiple exposures was accounted for in the analysis.]

Using the data set of the pooled population-based case–control studies in Iowa, Minnesota, and Nebraska, USA, Lee *et al.* (2004a) investigated whether asthma acts as an effect modifier of the association between pesticide exposure and NHL. The study included 872 cases diagnosed with NHL from 1980 to 1986 and 2381 frequency-matched controls. Information on use of pesticides and history of asthma was based on interviews. A total of 177 subjects (45 cases, 132 controls) reported having been told by their doctor that they had asthma. Subjects with a history of asthma had a non-significantly lower risk of NHL than non-asthmatics, and there was no main effect of pesticide exposure. In general, asthmatics tended to have larger odds ratios associated with exposure to pesticides than non-asthmatics. There was no indication of effect modification: the odds ratio associated with glyphosate use was 1.4 (95% CI, 0.98–2.1; 53 exposed cases) among non-asthmatics and 1.2 (95% CI, 0.4–3.3; 6 exposed cases) for asthmatics, when compared with non-asthmatic non-exposed farmers). [This analysis overlapped with that of De Roos *et al.* (2003).]

(b) *The cross-Canada case–control study*

McDuffie *et al.* (2001) studied the associations between exposure to specific pesticides and NHL in a multicentre population-based study with 517 cases and 1506 controls among men of six Canadian provinces (see the *Monograph* on Malathion, Section 2.0, for a detailed description of this study). Odds ratios of 1.26 (95% CI, 0.87–1.80; 51 exposed cases; adjusted for age and province) and 1.20 (95% CI, 0.83–1.74, adjusted for age, province, high-risk exposures) were observed for exposure to glyphosate. In an analysis by frequency of exposure to glyphosate, participants with > 2 days of exposure per year had an odds ratio of 2.12 (95% CI, 1.20–3.73, 23 exposed cases) compared with those with some, but ≤ 2 days of exposure. [The study was large, but had relatively low participation rates.]

Kachuri *et al.* (2013) investigated the association between lifetime use of pesticides and multiple myeloma in a population-based case–control study among men in six Canadian provinces between 1991 and 1994 (see the *Monograph* on Malathion, Section 2.0, for a detailed description of this study). Data from 342 cases of multiple myeloma and 1357 controls were obtained for ever-use of pesticides, number of pesticides used, and days per year of pesticide use. The odds ratios were adjusted for age, province of residence, type of respondent, smoking and medical history. The odds ratio for ever-use of glyphosate was 1.19 (95% CI, 0.76–1.87; 32 cases). When the analysis was conducted by level of exposure, no association was found for light users (≤ 2 days per year) of glyphosate (OR, 0.72; 95% CI, 0.39–1.32; 15 exposed cases) while the odds ratio in heavier users (> 2 days per year) was 2.04 (95% CI, 0.98–4.23; 12 exposed cases). [The study had relatively low response rates. Multiple myeloma is now considered a subtype of NHL.]

(c) *Case–control studies in Sweden*

Nordström *et al.* (1998) conducted a population case–control study in Sweden on hairy cell leukaemia (considered to be a subgroup of NHL). The study included 121 cases in men and 484 controls matched for age and sex. An age-adjusted odds ratio of 3.1 (95% CI, 0.8–12; 4 exposed cases) was observed for exposure to glyphosate. [This study had limited power to detect an effect, and there was no adjustment for other exposures.]

Hardell & Eriksson (1999) reported the results of a population-based case–control study on the incidence of NHL in men associated with pesticide exposure in four northern counties in Sweden. Exposure data was collected by questionnaire (also supplemented by telephone interviews) from 404 cases (192 deceased) and 741

controls (matched by age, sex, county, and vital status). Increased risks of NHL were found for subjects exposed to herbicides and fungicides. The odds ratio for ever-use of glyphosate was 2.3 (95% CI, 0.4–13; 4 exposed cases) in a univariate analysis, and 5.8 (95% CI, 0.6–54) in a multivariable analysis. [The exposure frequency was low for glyphosate, and the study had limited power to detect an effect. The variables included in the multivariate analysis were not specified. This study may have overlapped partially with those of Hardell *et al.* (2002).]

Hardell *et al.* (2002) conducted a pooled analysis of two case–control studies, one on NHL (already reported in Hardell & Eriksson, 1999) and another on hairy cell leukaemia, a subtype of NHL (already reported by Nordström *et al.*, 1998). The pooled analysis of NHL and hairy cell leukaemia was based on 515 cases and 1141 controls. Increased risk was found for exposure to glyphosate (OR, 3.04; 95% CI, 1.08–8.52; 8 exposed cases) in the univariate analysis, but the odds ratio decreased to 1.85 (95% CI, 0.55–6.20) when study, study area, and vital status were considered in a multivariate analysis. [The exposure frequency was low for glyphosate and the study had limited power. This study partially overlapped with those of Hardell & Eriksson (1999) and Nordström *et al.* (1998).]

Eriksson *et al.* (2008) reported the results of a population based case–control study of exposure to pesticides as a risk factor for NHL. Men and women aged 18–74 years living in Sweden were included from 1 December 1999 to 30 April 2002. Incident cases of NHL were enrolled from university hospitals in Lund, Linköping, Örebro, and Umeå. Controls (matched by age and sex) were selected from the national population registry. Exposure to different agents was assessed by questionnaire. In total, 910 (91%) cases and 1016 (92%) controls participated. Multivariable models included agents with statistically significant increased odds ratios (MCPA, 2-methyl-4-chlorophenoxyacetic acid), or with an odds ratio of > 1.50 and at least 10 exposed subjects (2,4,5-T and/or 2,4-D; mercurial seed dressing, arsenic, creosote, tar), age, sex, year of diagnosis or enrolment. The odds ratio for exposure to glyphosate was 2.02 (95% CI, 1.10–3.71) in a univariate analysis, and 1.51 (95% CI, 0.77–2.94) in a multivariable analysis. When exposure for more than 10 days per year was considered, the odds ratio was 2.36 (95% CI, 1.04–5.37). With a latency period of > 10 years, the odds ratio was 2.26 (95% CI, 1.16–4.40). The associations with exposure to glyphosate were reported also for lymphoma subtypes, and elevated odds ratios were reported for most of the cancer forms, including B-cell lymphoma (OR, 1.87; 95% CI, 0.998–3.51) and the subcategory of small lymphocytic lymphoma/chronic lymphocytic leukaemia (OR, 3.35; 95% CI, 1.42–7.89; [not adjusted for other pesticides]). [This was a large study; there was possible confounding from use of other pesticides including MCPA, but this was considered in the analysis.]

(d) Other case–control studies in Europe

Orsi *et al.* (2009) reported the results of a hospital-based case–control study conducted in six centres in France between 2000 and 2004. Incident cases with a diagnosis of lymphoid neoplasm aged 20–75 years and controls of the same age and sex as the cases were recruited in the same hospital, mainly in the orthopaedic and rheumatological departments during the same period. [The Working Group noted that the age of case eligibility was given in the publication as 20–75 years in the materials and methods section, but as 18–75 years in the abstract.] Exposures to pesticides were evaluated through specific interviews and case-by-case expert reviews. The analyses included 491 cases (244 cases of NHL, 87 cases of Hodgkin lymphoma), 104 of lymphoproliferative syndrome, and 56 cases of multiple myeloma), and 456 age- and sex-matched controls. Positive associations between some subtypes and occupational exposure to several pesticides

were noted. The odds ratios associated with any exposure to glyphosate were 1.2 (95% CI, 0.6–2.1; 27 exposed cases) for all lymphoid neoplasms combined, 1.0 (95% CI, 0.5–2.2; 12 exposed cases) for NHL, 0.6 (95% CI, 0.2–2.1; 4 exposed cases) for lymphoproliferative syndrome, 2.4 (95% CI, 0.8–7.3) for multiple myeloma, and 1.7 (95% CI, 0.6–5.0; 6 exposed cases) for Hodgkin lymphoma, after adjusting for age, centre, and socioeconomic category ("blue/white collar").

Cocco *et al.* (2013) reported the results of a pooled analysis of case–control studies conducted in six European countries in 1998–2004 (EPILYMPH, Czech Republic, France, Germany, Ireland, Italy, and Spain) to investigate the role of occupational exposure to specific groups of chemicals in the etiology of lymphoma overall, B-cell lymphoma, and its most prevalent subtypes. A total of 2348 incident cases of lymphoma and 2462 controls were recruited. Controls from Germany and Italy were randomly selected by sampling from the general population, while the rest of the centres used matched hospital controls. Overall, the participation rate was 88% for cases, 81% for hospital controls, and 52% for population controls. An occupational history was collected with farm work-specific questions on type of crop, farm size, pests being treated, type and schedule of pesticide use. In each study centre, industrial hygienists and occupational experts assessed exposure to specific groups of pesticides and individual compounds with the aid of agronomists. [Therefore any exposure misclassification would be non-differential.] Analyses were conducted for lymphoma and the most prevalent lymphoma subtypes adjusting for age, sex, education, and centre. Lymphoma overall, and B-cell lymphoma were not associated with any class of the investigated pesticides, while the risk of chronic lymphocytic leukaemia was elevated among those ever exposed to inorganic and organic pesticides. Only for a few individual agrochemicals was there a sizeable number of study subjects to conduct a meaningful analysis, and the odds ratio for exposure to glyphosate and B-cell lymphoma was 3.1 (95% CI, 0.6–17.1; 4 exposed cases and 2 exposed controls). [The study had a very limited power to assess the effects of glyphosate on risk of NHL.]

2.2.2 Other haematopoietic cancers

Orsi *et al.* (2009) also reported results for Hodgkin lymphoma (see Section 2.2.1).

Karunanayake *et al.* (2012) conducted a case–control study of Hodgkin lymphoma among white men, aged 19 years or older, in six regions of Canada (see the Malathion *Monograph*, Section 2.0, for a detailed description of this study). The analysis included 316 cases and 1506 age-matched (± 2 years) controls. Based on 38 cases exposed to glyphosate, the odds ratios were 1.14 (95% CI, 0.74–1.76) adjusted for age and province, and 0.99 (95% CI, 0.62–1.56) when additionally adjusted for medical history variables.

Brown *et al.* (1990) evaluated exposure to carcinogens in an agricultural setting and the relationship with leukaemia in a population-based case–control interview study in Iowa and Minnesota, USA, including 578 white men with leukaemia and 1245 controls. The exposure assessment was done with a personal interview of the living subjects or the next-of-kin. Farmers had a higher risk of all leukaemias compared with non-farmers, and associations were found for exposure to specific animal insecticides, including the organophosphates crotoxyphos, dichlorvos, famphur, pyrethrins, and methoxychlor. The odds ratio for glyphosate was 0.9 (95% CI, 0.5–1.6; 15 exposed cases; adjusted for vital status, age, state, tobacco use, family history of lymphopoietic cancer, high-risk occupations, and high-risk exposures). [This was a large study in an agricultural setting, but had limited power for studying the effects of glyphosate use.]

2.3 Case–control studies on other cancer sites

2.3.1 Cancer of the oesophagus and stomach

Lee *et al.* (2004b) evaluated the risk of adenocarcinomas of the oesophagus and stomach associated with farming and agricultural pesticide use. The population-based case–control study was conducted in eastern Nebraska, USA. Subjects of both sexes diagnosed with adenocarcinoma of the stomach (n = 170) or oesophagus (n = 137) between 1988 and 1993 were enrolled. Controls (n = 502) were randomly selected from the population registry of the same geographical area. The response rates were 79% for cancer of the stomach, 88% for cancer of the oesophagus, and 83% for controls. Adjusted odds ratios were estimated for use of individual and chemical classes of insecticides and herbicides, with non-farmers as the reference category. No association was found with farming or ever-use of insecticides or herbicides, or with individual pesticides. For ever-use of glyphosate, the odds ratio was 0.8 (95% CI, 0.4–1.4; 12 exposed cases) for cancer of the stomach, and 0.7 (95% CI, 0.3–1.4; 12 exposed cases) for oesophageal cancer. [The study was conducted in a farming area, but the power to detect an effect of glyphosate use was limited.]

2.3.2 Cancer of the brain

Ruder *et al.* (2004) conducted a case–control study on glioma among nonmetropolitan residents of Iowa, Michigan, Minnesota, and Wisconsin in the Upper Midwest Health Study, USA. The study included 457 cases of glioma and 648 population-based controls, all adult men. Exposure assessment was done with interviews of the subject or the relatives. The response rates were 93% and 70% for cases and controls, respectively. No association were found with any of the pesticides assessed, including glyphosate. [Glyphosate use was assessed, but specific results were not presented.]

Carreón *et al.* (2005) evaluated the effects of rural exposures to pesticides on risk of glioma among women aged 18–80 years who were nonmetropolitan residents of Iowa, Michigan, Minnesota, and Wisconsin in the Upper Midwest Health Study, USA. A total of 341 cases of glioma and 528 controls were enrolled. A personal interview was carried out for exposure assessment. The response rates were 90% and 72%, respectively. After adjusting for age, age group, education, and farm residence, no association with glioma was observed for exposure to several pesticide classes or individual pesticides. There was a reduced risk for glyphosate (OR, 0.7; 95% CI, 0.4–1.3; 18 exposed cases). These results were not affected by the exclusion of proxy respondents (43% of cases, 2% of controls).

Lee *et al.* (2005) evaluated the association between farming and agricultural pesticide use and risk of adult glioma in a population-based case–control study in eastern Nebraska, USA. Cases of glioma were in men and women (n = 251) and were compared with population controls from a previous study (n = 498). A telephone interview was conducted for 89% of the cases and 83% of the controls. Adjusted odds ratios for farming and for use of individual and chemical classes of insecticides and herbicides were calculated using non-farmers as the reference category. Among men, ever living or working on a farm and duration of farming were associated with significantly increased risks of glioma, but the positive findings were limited to proxy respondents. Among women, there were no positive associations with farming activities among self or proxy respondents. Some specific pesticide families and individual pesticides were associated with significantly increased risks among male farmers, but most of the positive associations were limited to proxy respondents. There was a non-significant excess risk with glyphosate use for the overall group (OR, 1.5; 95% CI, 0.7–3.1; 17 exposed cases), but there was inconsistency between observations for self-respondents (OR,

0.4; 95% CI, 0.1–1.6) and observations for proxy respondents (OR, 3.1; 95% CI, 1.2–8.2). [The study had limited power to detect an effect of glyphosate use, and the inconsistencies for self and proxy respondents made the results difficult to interpret.]

2.3.3 Soft tissue sarcoma

Pahwa *et al.* (2011) reported the results of the soft tissue sarcoma component of the cross-Canada study in relation to specific pesticides, including 357 cases of soft tissue sarcoma and 1506 population controls from 1991–1994. The fully adjusted odds ratio for glyphosate use was 0.90 (95% CI, 0.58–1.40).

2.3.4 Cancer of the prostate

Band *et al.* (2011) report results of a case–control study including 1516 patients with cancer of the prostate (ascertained by the cancer registry of British Columbia, Canada, for 1983–90) and 4994 age-matched controls with cancers at all other cancer sites excluding lung and unknown primary site. Agricultural exposures were assessed by job-exposure matrix. A total of 60 cases were exposed to glyphosate (adjusted OR, 1.36; 95% CI, 0.83–2.25).

2.3.5 Childhood cancer

Parental exposure to pesticides, including glyphosate, was assessed in a population-based case–control study of childhood leukaemia in Costa Rica (Monge *et al.*, 2007). However, associations of childhood cancer with glyphosate were reported only for an "other pesticides" category that also included paraquat, chlorothalonil, and other chemicals. [Because glyphosate was not specifically assessed, this study was not evaluated by the Working Group.]

2.4. Meta-analyses

Schinasi & Leon (2014) conducted a systematic review and meta-analysis of NHL and occupational exposure to agricultural pesticides, including glyphosate. The meta-analysis for glyphosate included six studies (McDuffie *et al.*, 2001; Hardell *et al.*, 2002; De Roos *et al.*, 2003; 2005a; Eriksson *et al.*, 2008; Orsi *et al.*, 2009) and yielded a meta risk-ratio of 1.5 (95% CI, 1.1–2.0). [The Working Group noted that the most fully adjusted risk estimates from the articles by Hardell *et al.* (2002) and Eriksson *et al.* (2008) were not used in this analysis. After considering the adjusted estimates of the two Swedish studies in the meta-analysis, the Working Group estimated a meta risk-ratio of 1.3 (95% CI, 1.03–1.65), $I^2 = 0\%$, P for heterogeneity 0.589.]

3. Cancer in Experimental Animals

3.1 Mouse

See Table 3.1

3.1.1 Dietary administration

Groups of 50 male and 50 female CD-1 mice [age not reported] were given diets containing glyphosate (purity, 99.7%) at a concentration of 0, 1000, 5000, or 30 000 ppm, ad libitum, for 24 months. There was no treatment-related effect on body weight in male and female mice at the lowest or intermediate dose. There was a consistent decrease in body weight in the male and female mice at the highest dose compared with controls. Survival in all dose groups was similar to that of controls. There was a positive trend ($P = 0.016$, trend test; see EPA, 1985b) in the incidence of renal tubule adenoma in dosed male mice: 0/49, 0/49, 1/50 (2%), 3/50 (6%). [The Working Group noted that renal tubule adenoma is a rare tumour in CD-1 mice.] No data on tumours of the kidney

Table 3.1 Studies of carcinogenicity with glyphosate in mice

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Mouse, CD-1 (M, F) 24 mo EPA (1985a, b, 1986, 1991a)	Diet containing glyphosate (technical grade; purity, 99.7%) at concentrations of 0, 1000, 5000, or 30 000 ppm, ad libitum, for 24 mo 50 M and 50 F/group [age, NR]	*Males* Renal tubule adenoma: 0/49, 0/49, 1/50 (2%), 3/50 (6%) *Females* No data provided on the kidney	*P* for trend = 0.016; see Comments	No information was provided on renal tubule adenomas in female mice, or on statistical analyses of tumour data EPA recommended that additional renal sections be cut and evaluated from all control and treated male mice. The pathology report for these additional sections (EPA, 1985b) showed the same incidence of renal tubule adenomas as originally reported, with no significant difference in incidence when comparing control and treated groups; however, the test for linear trend in proportions resulted in *P* = 0.016 EPA (1986) convened a PWG and requested additional pathological and statistical information on kidney tumours observed in male mice treated with glyphosate
		Report from the PWG of the EPA (1986): *Males* Renal tubule adenoma: 1/49 (2%), 0/49, 0/50, 1/50 (2%)	[NS]	
		Renal tubule carcinoma: 0/49, 0/49, 1/50 (2%), 2/50 (4%)	[*P* = 0.037; Cochran–Armitage trend test]	
		Renal tubule adenoma or carcinoma (combined): 1/49 (2%), 0/49, 1/50 (2%), 3/50 (6%)	[*P* = 0.034; Cochran–Armitage trend test]	
Mouse, CD-1 (M, F) 104 wk IMPR (2006)	Diet containing glyphosate (purity, 98.6%) at doses of 0, 100, 300, 1000 mg/kg bw, ad libitum, for 104 wk 50 M and 50 F/group [age, NR]	*Males* Haemangiosarcoma: 0/50, 0/50, 0/50, 4/50 (8%)	[*P* < 0.001; Cochran–Armitage trend test]	
		Histiocytic sarcoma in the lymphoreticular/haemopoietic tissue: 0/50, 2/50 (4%), 0/50, 2/50 (4%)	NS	
		Females Haemangiosarcoma: 0/50, 2/50 (4%), 0/50, 1/50 (2%)	NS	
		Histiocytic sarcoma in the lymphoreticular/haemopoietic tissue: 0/50, 3/50 (6%), 3/50 (6%), 1/50 (2%)	NS	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Mouse, Swiss (M) 32 wk George *et al.* (2010)	Initiation–promotion study Skin application of glyphosate-based formulation (glyphosate, 41%; POEA, ~15%) (referred to as "glyphosate") dissolved in 50% ethanol; DMBA dissolved in 50% ethanol, and TPA dissolved in 50% acetone, used in the groups described below 20 M/group	Skin tumours [called "papillomas" by the authors, following gross examination only]		Short duration of treatment, no solvent controls, and lack of any histopathological evaluation Age at start, NR (mice weighed 12–15 g bw) [The Working Group concluded this was an inadequate study for the evaluation of glyphosate]
	Group I: untreated control (no treatment)	Group I: 0/20		
	Group II: glyphosate only: 25 mg/kg bw topically, 3 × /wk, for 32 wk	Group II: 0/20		
	Group III: single topical application of DMBA, 52 µg/mouse, followed 1 wk later by TPA, 5 µg/mouse, 3 × /wk, for 32 wk	Group III: 20/20*, 7.8 ± 1.1	*$P < 0.05$ vs groups VI and VII	
	Group IV: single topical application of glyphosate, 25 mg/kg bw, followed 1 wk later by TPA, 5 µg/mouse, 3 × /wk, for 32 wk	Group I: 0/20		
	Group V: 3 × /wk topical application of glyphosate, 25 mg/kg bw, for 3 wk, followed 1 wk later by TPA, 5 µg/mouse, 3 × /wk, for 32 wk	Group V: 0/20		
	Group VI: single topical application of DMBA, 52 µg/mouse	Group VI: 0/20		
	Group VII: topical application of TPA, 5 µg/mouse, 3 × /wk, for 32 wk	Group VII: 0/20		
	Group VIII: single topical application of DMBA, 52 µg/mouse, followed 1 wk later by topical treatment with glyphosate, 25 mg/kg bw, 3 × /wk, for 32 wk	Group VIII: 8/20*, 2.8 ± 0.9	*$P < 0.05$ vs group VI	

bw, body weight; DMBA, 7,12-dimethylbenz[*a*]anthracene; EPA, United States Environmental Protection Agency; F, female; M, male; mo, month; NR, not reported; NS, not significant; POEA, polyethoxylated tallowamine; PWG, pathology working group; TPA, 12-*O*-tetradecanoyl-phorbol-13-acetate; vs, versus; wk, week; yr, year

were provided for female mice. No other tumour sites were identified (EPA, 1985a). Subsequent to its initial report (EPA, 1985a), the United States Environmental Protection Agency (EPA) recommended that additional renal sections be cut and evaluated from all male mice in the control and treated groups. The pathology report for these additional sections (EPA, 1985b) indicated the same incidence of renal tubule adenoma as originally reported, with no significant increase in incidence between the control group and treated groups by pairwise comparison. However, as already reported above, the test for linear trend in proportions resulted in a significance of $P = 0.016$. The EPA (1986) also requested that a pathology working group (PWG) be convened to evaluate the tumours of the kidney observed in male mice treated with glyphosate, including the additional renal sections. In this second evaluation, the PWG reported that the incidence of adenoma of the renal tubule was 1/49 (2%), 0/49, 0/50, 1/50 (2%) [not statistically significant]; the incidence of carcinoma of the renal tubule was 0/49, 0/49, 1/50 (2%), 2/50 (4%) [$P = 0.037$, trend test for carcinoma]; and the incidence of adenoma or carcinoma (combined) of the renal tubule was 1/49 (2%), 0/49, 1/50 (2%), 3/50 (6%) [$P = 0.034$, trend test for combined]. [The Working Group considered that this second evaluation indicated a significant increase in the incidence of rare tumours, with a dose-related trend, which could be attributed to glyphosate. Chandra & Frith (1994) reported that only 1 out of 725 [0.14%] CD-1 male mice in their historical database had developed renal cell tumours (one carcinoma).]

[The Working Group noted the differences in histopathological diagnosis between pathologists. Proliferative lesions of the renal tubules are typically categorized according to published criteria as hyperplasia, adenoma, or carcinoma. The difference is not trivial, because focal hyperplasia, a potentially preneoplastic lesion, should be carefully differentiated from the regenerative changes of the tubular epithelium. There is a morphological continuum in the development and progression of renal neoplasia. Thus larger masses may exhibit greater heterogeneity in histological growth pattern, and cytologically more pleomorphism and atypia than smaller lesions (Eustis *et al.*, 1994). Of note, a renal tumour confirmed by the PWG after re-evaluation of the original slides (EPA, 1986), had not been seen in the re-sectioned kidney slides (EPA, 1985b). This may be related to the growth of tumour that – in contrast to tumours in other organs – is not spherical but elliptical because of the potential expansion in tubules. In addition, the concept of tubular expansion without compression of adjacent parenchyma may be at the basis of the discrepancy between the first (EPA, 1985a, b) and second evaluation (EPA, 1986).]

In another study reported to the Joint FAO/WHO Meeting on Pesticide Residues (JMPR), groups of 50 male and 50 female CD-1 mice [age at start not reported] were given diets containing glyphosate (purity, 98.6%) at a concentration that was adjusted weekly for the first 13 weeks and every 4 weeks thereafter to give doses of 0, 100, 300, or 1000 mg/kg bw, ad libitum, for 104 weeks (JMPR, 2006). There was no treatment-related effect on body weight or survival in any of the dosed groups. There was an increase in the incidence of haemangiosarcoma in males – 0/50, 0/50, 0/50, 4/50 (8%) [$P < 0.001$, Cochran–Armitage trend test], and in females – 0/50, 2/50 (4%), 0/50, 1/50 (2%) [not statistically significant], and an increase in the incidence of histiocytic sarcoma in the lymphoreticular/haemopoietic tissue in males – 0/50, 2/50 (4%), 0/50, 2/50 (4%), and in females – 0/50, 3/50 (6%), 3/50 (6%), 1/50 (2%) [not statistically significant for males or females]. [The Working Group considered that this study was adequately reported.]

3.1.2 Initiation–promotion

Groups of 20 male Swiss mice [age at start not reported; body weight, 12–15 g] were given a glyphosate-based formulation (glyphosate, 41%; polyethoxylated tallowamine, ~15%) (referred to as glyphosate in the article) that was dissolved in 50% ethanol and applied onto the shaved back skin (George *et al.*, 2010). Treatment groups were identified as follows:

- Group I – untreated control;
- Group II – glyphosate only (25 mg/kg bw), applied topically three times per week for 32 weeks;
- Group III – single topical application of dimethylbenz[*a*]anthracene (DMBA; in ethanol; 52 µg/mouse), followed 1 week later by 12-*O*-tetradecanoylphorbol-13-acetate (TPA; in acetone; 5 µg/mouse), applied topically three times per week for 32 weeks;
- Group IV – single topical application of glyphosate (25 mg/kg bw) followed 1 week later by TPA (in acetone; 5 µg/mouse), applied topically three times per week for 32 weeks;
- Group V – glyphosate (25 mg/kg bw) applied topically three times per week for 3 weeks (total of nine applications), followed 1 week later by TPA (in acetone; 5 µg/mouse), applied topically three times per week for 32 weeks;
- Group VI – single topical application of DMBA (in ethanol; 52 µg/mouse);
- Group VII –TPA (in acetone; 5 µg/mouse), applied topically three times per week for 32 weeks; and
- Group VIII –single topical application of DMBA (in ethanol; 52 µg/mouse), followed 1 week later by glyphosate (25 mg/kg bw), applied topically three times per week for 32 weeks.

All mice were killed at 32 weeks. Skin tumours were observed only in group III (positive control, DMBA + TPA, 20/20) and group VIII (DMBA + glyphosate, 8/20; $P < 0.05$ versus group VI [DMBA only, 0/20]). No microscopic examination was conducted and tumours were observed "as a minute wart like growth [that the authors called squamous cell papillomas], which progressed during the course of experiment." [The glyphosate formulation tested appeared to be a tumour promoter in this study. The design of the study was poor, with short duration of treatment, no solvent controls, small number of animals, and lack of histopathological examination. The Working Group concluded that this was an inadequate study for the evaluation of glyphosate.]

3.1.3 Review articles

Greim *et al.* (2015) have published a review article containing information on five long-term bioassay feeding studies in mice. Of these studies, one had been submitted for review to the EPA (EPA, 1985a, b, 1986, 1991a), and one to the JMPR (JMPR, 2006); these studies are discussed in Section 3.1.1. The review article reported on an additional three long-term bioassay studies in mice that had not been previously available in the open literature, but had been submitted to various organizations for registration purposes. The review article provided a brief summary of each study and referred to an online data supplement containing the original data on tumour incidence from study reports. The three additional long-term bioassay studies in mice are summarized below. [The Working Group was unable to evaluate these studies, which are not included in Table 3.1 and Section 5.3, because the information provided in the review article and its supplement was insufficient (e.g. information was lacking on statistical methods, choice of doses, body-weight gain, survival data, details of histopathological examination, and/or stability of dosed feed mixture).]

In the first study (identified as Study 12, 1997a), groups of 50 male and 50 female CD-1

mice [age at start not reported] were given diets containing glyphosate (purity, 94–96%) at a concentration of 0, 1600, 8000, or 40 000 ppm for 18 months. The increase in the incidence of bronchiolo-alveolar adenoma and carcinoma, and of lymphoma, was reported to be not statistically significant in males and females receiving glyphosate. [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information.]

In the second study (identified as Study 13, 2001), groups of 50 male and 50 female Swiss albino mice [age at start not reported] were given diets containing glyphosate (purity, > 95%) at a concentration of 0 (control), 100, 1000, or 10 000 ppm for 18 months. The authors reported a statistically significant increase in the incidence of malignant lymphoma (not otherwise specified, NOS) in males at the highest dose: 10/50 (20%), 15/50 (30%), 16/50 (32%), 19/50 (38%; $P < 0.05$; pairwise test); and in females at the highest dose: 18/50 (36%), 20/50 (40%), 19/50 (38%), 25/50 (50%; $P < 0.05$; pairwise test). [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information.]

In the third study (identified as Study 14, 2009a), groups of 51 male and 51 female CD-1 mice [age at start not reported] were given diets containing glyphosate (purity, 94.6–97.6%) at a concentration of 0, 500, 1500, or 5000 ppm for 18 months. Incidences for bronchiolo-alveolar adenoma and carcinoma, malignant lymphoma (NOS), and hepatocellular adenoma and carcinoma in males, and for bronchiolo-alveolar adenoma and carcinoma, malignant lymphoma (NOS) and pituitary adenoma in females, were included in the article. In males, the authors reported that there was a significant positive trend [statistical test not specified] in the incidence of bronchiolo-alveolar carcinoma (5/51, 5/51, 7/51, 11/51) and of malignant lymphoma (0/51, 1/51, 2/51, 5/51). [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information.]

3.2 Rat

See Table 3.2

3.2.1 Drinking-water

Groups of 10 male and 10 female Sprague-Dawley rats (age, 5 weeks) were given drinking-water containing a glyphosate-based formulation at a dose of 0 (control), 1.1×10^{-8}% (5.0×10^{-5} mg/L), 0.09% (400 mg/L) or 0.5% (2.25×10^{3} mg/L), ad libitum, for 24 months (Séralini *et al.*, 2014). [The study reported is a life-long toxicology study on a glyphosate-based formulation and on genetically modified NK603 maize, which the authors stated was designed as a full study of long-term toxicity and not a study of carcinogenicity. No information was provided on the identity or concentration of other chemicals contained in this formulation.] Survival was similar in treated and control rats. [No data on body weight were provided.] In female rats, there was an almost twofold increase in the incidence of tumours of the mammary gland (mainly fibroadenoma and adenocarcinoma) in animals exposed to the glyphosate-based formulation only versus control animals: control, 5/10 (50%); lowest dose, 9/10 (90%); intermediate dose, 10/10 (100%) [$P < 0.05$; Fisher exact test]; highest dose, 9/10 (90%). [The Working Group concluded that this study conducted on a glyphosate-based formulation was inadequate for evaluation because the number of animals per group was small, the histopathological description of tumours was poor, and incidences of tumours for individual animals were not provided.]

In another study with drinking-water, Chruscielska *et al.* (2000) gave groups of 55 male and 55 female Wistar rats (age, 6–7 weeks) drinking-water containing an ammonium salt

of glyphosate as a 13.85% solution [purity of glyphosate, not reported] that was used to make aqueous solutions of 0 (control), 300, 900, and 2700 mg/L, for 24 months [details on the dosing regimen were not reported]. The authors reported that survival and body-weight gain were similar in treated and control animals. No significant increase in tumour incidence was reported in any of the treated groups. [The Working Group noted the limited information provided on dosing regimen, histopathological examination method, and tumour incidences.]

3.2.2 Dietary administration

The JMPR report included information on a 1-year feeding study in which groups of 24 male and 24 female Wistar-Alpk:APfSD rats [age at start not reported] were given diets containing glyphosate (purity, 95.6%) at a concentration of 0, 2000, 8000, or 20 000 ppm, ad libitum, for 1 year (JMPR, 2006). There was a treatment-related decrease in body-weight gain at the two highest doses (significant at 20 000 ppm for both sexes, and at 8000 ppm only in females). There was no treatment-related decrease in survival. No significant increase in tumour incidence was observed in any of the treated groups. [The Working Group noted the short duration of exposure.]

The JMPR report also included information on a 104-week feeding study in which groups of 50 male and 50 female Sprague-Dawley rats [age at start not reported] were given diets containing glyphosate (purity, 98.7–98.9%) at a concentration that was adjusted to provide doses of 0, 10, 100, 300, or 1000 mg/kg bw, ad libitum, for 104 weeks (JMPR, 2006). There was a treatment-related decrease in body-weight gain in males and females at the highest dose. There was no significant treatment-related decrease in survival or increase in tumour incidence in any of the treated groups.

Information was also included in the JMPR report on a 24-month feeding study in which groups of 52 male and 52 female Wistar-Alpk:APfSD rats [age at start not reported] were given diets containing glyphosate (purity, 97.6%) at a concentration of 0, 2000, 6000, or 20 000 ppm, ad libitum, for 24 months (JMPR, 2006). There was a treatment-related decrease in body-weight gain in males and females at the highest dose, and a corresponding significant increase in survival in males. No significant increase in tumour incidence was observed in any of the treated groups.

The EPA (1991a, b, c, d) provided information on a long-term study in which groups of 60 male and 60 female Sprague-Dawley rats (age, 8 weeks) were given diets containing glyphosate (technical grade; purity, 96.5%) at a concentration of 0 ppm, 2000 ppm, 8000 ppm, or 20 000 ppm, ad libitum, for 24 months. Ten animals per group were killed after 12 months. There was no compound-related effect on survival, and no statistically significant decreases in body-weight gain in male rats. In females at the highest dose, body-weight gain was significantly decreased, starting on day 51. In males at the lowest dose, there was a statistically significant increase in the incidence of pancreatic islet cell adenoma compared with controls: 8/57 (14%) versus 1/58 (2%), $P \leq 0.05$ (Fisher exact test). Additional analyses by the EPA (1991a) (using the Cochran–Armitage trend test and Fisher exact test, and excluding rats that died or were killed before week 55) revealed a statistically significant higher incidence of pancreatic islet cell adenoma in males at the lowest and highest doses compared with controls: lowest dose, 8/45 (18%; $P = 0.018$; pairwise test); intermediate dose, 5/49 (10%); highest dose, 7/48 (15%; $P = 0.042$; pairwise test) versus controls, 1/43 (2%). The range for historical controls for pancreatic islet cell adenoma reported in males at this laboratory was 1.8–8.5%. [The Working Group noted that there was no statistically significant positive trend in the incidence of these tumours, and no apparent progression to carcinoma.] There was also a statistically significant positive trend in the incidence of hepatocellular adenoma in

Table 3.2 Studies of carcinogenicity with glyphosate in rats

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat, Sprague-Dawley (M, F) 24 mo Séralini *et al.* (2014)	Drinking-water containing a glyphosate-based formulation at a concentration of 0 (control), 1.1×10^{-8}% (glyphosate, 5.0×10^{-5} mg/L), 0.09% (glyphosate, 400 mg/L) or 0.5% (glyphosate, 2.25×10^{3} mg/L), ad libitum, for 24 mo 10 M and 10 F/group (age, 5 wk)	*Males* No significant increase in tumour incidence observed in any of the treated groups *Females* Mammary tumours (mainly fibroadenomas and adenocarcinomas): 5/10 (50%), 9/10 (90%), 10/10 (100%)*, 9/10 (90%) Pituitary lesions (hypertrophy, hyperplasia, and adenoma): 6/10 (60%), 8/10 (80%), 7/10 (70%), 7/10 (70%)	NS *[$P < 0.05$] [NS]	Data are from an in-depth life-long toxicology study on a glyphosate-based formulation and NK603 genetically modified maize; authors stated that the study was designed as a full chronic toxicity and not a carcinogenicity study. No information provided on the identity or concentration of other chemicals contained in this formulation Histopathology poorly described and tumour incidences for individual animals not discussed in detail. Small number of animals per group [The Working Group concluded this was an inadequate study for the evaluation of glyphosate carcinogenicity]
Rat, Wistar (M, F) 24 mo Chruscielska *et al.* (2000)	Drinking-water containing ammonium salt of glyphosate (13.85% solution) [purity of glyphosate, NR] was used to make aqueous solutions of 0, 300, 900, and 2700 mg/L [Details on dosing regimen, NR] 55 M and 55 F/group (age, 6–7 wk)	No significant increase in tumour incidence observed in any of the treated groups	NS	Limited information on dosing regimen, histopathological examination methods, and tumour incidences
Rat, Wistar-Alpk:APfSD (M, F) 1 yr JMPR (2006)	Diet containing glyphosate (purity, 95.6%) at concentrations of 0, 2000, 8000, or 20 000 ppm, ad libitum, for 1 yr 24 M and 24 F/group [age, NR]	No significant increase in tumour incidence observed in any groups of treated animals	NS	Short duration of exposure
Rat, Sprague-Dawley (M, F) 104 wk JMPR (2006)	Diet containing glyphosate (purity, 98.7–98.9%) at doses of 0, 10, 100, 300, or 1000 mg/kg bw, ad libitum, for 104 wk 50 M and 50 F/group [age, NR]	No significant increase in tumour incidence observed in any groups of treated animals	NS	
Rat, Wistar-Alpk:APfSD (M, F) 24 mo JMPR (2006)	Diet containing glyphosate (purity, 97.6%) at concentrations of 0, 2000, 6000, or 20 000 ppm, ad libitum, for 2 yr 52 M and 52 F/group [age, NR]	No significant increase in tumour incidence observed in any groups of treated animals	NS	

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat Sprague-Dawley (M, F) 24 mo EPA (1991a, b, c, d)	Diet containing glyphosate (technical grade; purity, 96.5%) at concentrations of 0, 2000, 8000, or 20 000 ppm, ad libitum, for 24 mo 60 M and 60 F/group (age, 8 wk) 10 rats/group killed after 12 mo	*Males* *Pancreas (islet cell):* Adenoma: 1/58 (2%), 8/57 (14%)*, 5/60 (8%), 7/59 (12%) Carcinoma: 1/58 (2%), 0/57, 0/60, 0/59 Adenoma or carcinoma (combined): 2/58 (3%), 8/57 (14%), 5/60 (8%), 7/59 (12%) *Liver:* Hepatocellular adenoma: 2/60 (3%), 2/60 (3%), 3/60 (6%), 7/60 (12%) Hepatocellular carcinoma: 3/60 (5%), 2/60 (3%), 1/60 (2%), 2/60 (3%) *Females* *Pancreas (islet cell):* Adenoma: 5/60 (8%), 1/60 (2%), 4/60 (7%), 0/59 Carcinoma: 0/60, 0/60, 0/60, 0/59 Adenoma or carcinoma (combined): 5/60 (8%), 1/60 (2%), 4/60 (7%), 0/59 *Thyroid:* C-cell adenoma: 2/60 (3%), 2/60 (3%), 6/60 (10%), 6/60 (10%) C-cell carcinoma: 0/60, 0/60, 1/60, 0/60	Adenoma, * $P \leq 0.05$ (Fisher exact test with Bonferroni inequality); see comments Adenoma, P for trend = 0.016; see comments NS Adenoma, P for trend = 0.031; see comments	Historical control range for pancreatic islet cell adenoma reported in males at this laboratory, 1.8–8.5% EPA (1991a) performed additional analyses using the Cochran–Armitage trend test and Fisher exact test, and excluding animals that died or were killed before wk 54–55: *Males* *Pancreas (islet cell):* Adenoma: 1/43 (2%), 8/45 (18%; $P = 0.018$), 5/49 (10%), 7/48 (15%; $P = 0.042$) Carcinoma: 1/43 (2%), 0/45 (0%), 0/49 (0%), 0/48 (0%) Adenoma or carcinoma (combined): 2/43 (5%), 8/45 (18%), 5/49 (10%), 7/48 (15%) [There was no statistically significant positive trend in the incidence of pancreatic tumours, and no apparent progression to carcinoma] *Liver:* Hepatocellular adenoma: 2/44 (5%; P for trend = 0.016), 2/45 (4%), 3/49 (6%), 7/48 (15%) Hepatocellular carcinoma: 3/44 (7%); 2/45 (4%), 1/49 (2%), 2/48 (4%) Hepatocellular adenoma or carcinoma (combined): 5/44 (11%), 4/45 (9%), 4/49 (8%), 9/48 (19%) [There was no apparent progression to carcinoma] *Females* *Thyroid:* C-cell adenoma: 2/57 (4%; P for trend = 0.031), 2/60 (3%), 6/59 (10%), 6/55 (11%) C-cell carcinoma: 0/57, 0/60, 1/59 (2%), 0/55 C-cell adenoma or carcinoma (combined): 2/57 (4%), 2/60 (3%), 7/59 (12%), 6/55 (11%) [There was no apparent progression to carcinoma]

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat Sprague-Dawley (M, F) Lifetime (up to 26 mo) EPA (1991a, b, c, d)	Diet containing glyphosate (purity, 98.7%) at concentrations of 0 ppm, 30 ppm (3 mg/kg bw per day), 100 ppm (10 mg/kg bw per day), 300 ppm (31 mg/kg bw per day), ad libitum, up to 26 mo 50 M and 50 F/group [age, NR]	*Males* *Pancreas (islet cell):* Adenoma: 0/50 (0%), 5/49* (10%), 2/50 (4%), 2/50 (4%) Carcinoma: 0/50 (0%), 0/49 (0%), 0/50 (0%), 1/50 (2%) Adenoma or carcinoma (combined): 0/50 (0%), 5/49 (10%), 2/50 (4%), 3/50 (6%) *Females* *Pancreas (islet cell):* Adenoma: 2/50 (4%), 1/50 (2%), 1/50 (2%), 0/50 (0%) Carcinoma: 0/50 (0%), 1/50 (2%), 1/50 (2%), 1/50 (2%) Adenoma or carcinoma (combined): 2/50 (10%), 2/50 (2%), 2/50 (74%), 1/50 (2%)	Adenoma, *[$P < 0.05$; Fisher exact test] NS	[There was no statistically significant positive trend in the incidence of pancreatic tumours, and no apparent progression to carcinoma]

bw, body weight; d, day; F, female; M, male; mo, month; NR, not reported; NS, not significant; wk, week; yr, year

males (P = 0.016) and of thyroid follicular cell adenoma in females (P = 0.031). [The Working Group noted that there was no apparent progression to carcinoma for either tumour type.]

The EPA (1991a, b, c, d) provided information on another long-term study in which groups of 50 male and 50 female Sprague-Dawley rats [age at start not reported] were given diets containing glyphosate (purity, 98.7%) at a concentration of 0, 30 (3 mg/kg bw per day), 100 (10 mg/kg bw per day), or 300 ppm (31 mg/kg bw per day), ad libitum, for life (up to 26 months). No information was provided on body weight or survival of the study animals. An increase in the incidence of pancreatic islet cell adenoma was reported in males at the lowest dose: controls, 0/50 (0%); lowest dose, 5/49 (10%) [P < 0.05; Fisher exact test]; intermediate dose, 2/50 (4%); highest dose, 2/50 (4%). [The Working Group noted that there was no statistically significant positive dose-related trend in the incidence of these tumours, and no apparent progression to carcinoma.]

3.2.3 Review articles

Greim *et al.* (2015) have published a review article containing information on nine long-term bioassay feeding studies in rats. Of these studies, two had been submitted for review to the EPA (1991a, b, c, d), two to the JMPR (JMPR, 2006), and one had been published in the openly available scientific literature (Chruscielska *et al.*, 2000); these studies are discussed earlier in Section 3.2. The review article reported on an additional four long-term bioassay studies in rats that had not been previously published, but had been submitted to various organizations for registration purposes. The review article provided a brief summary of each study and referred to an online data supplement containing the original data on tumour incidence from study reports. The four additional long-term bioassay studies in rats are summarized below. [The Working Group did not evaluate these studies, which are not included in Table 3.2 and Section 5.3, because the information provided in the review article and its supplement was insufficient (e.g. information lacking on statistical methods, choice of doses, body-weight gain, survival data, details on histopathological examination and/or stability of dosed feed mixture).]

In one study (identified as Study 4, 1996), groups of 50 male and 50 female Wistar rats [age at start not reported] were given diets containing glyphosate (purity, 96%) at a concentration of 0, 100, 1000, or 10 000 ppm, ad libitum, for 24 months. It was reported that hepatocellular adenomas and hepatocellular carcinomas were found at non-statistically significant incidences in both males and females. There was no significant increase in tumour incidence in the treated groups. [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information.]

In one study in Sprague-Dawley rats (identified as Study 5, 1997), groups of 50 male and 50 female rats [age at start not reported] were given diets containing glyphosate technical acid [purity not reported] at a concentration of 0, 3000, 15 000, or 25 000 ppm, ad libitum, for 24 months. There was no significant increase in tumour incidence in the treated groups. [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information.]

In a second study in Sprague Dawley rats (identified as Study 6, 1997b), groups of 50 males and 50 females [age at start not reported] were given diets containing glyphosate (purity, 94.6–97.6%) at a concentration of 0, 3000, 10 000, or 30 000 ppm, ad libitum, for 24 months. Non-significant increases in tumour incidences compared with controls were noted for skin keratoacanthoma in males at the highest dose, and for fibroadenoma of the mammary gland in females at the lowest and intermediate doses. [The Working Group was unable to evaluate this

study because of the limited experimental data provided in the review article and supplemental information.]

In another study in male and female Wistar rats (identified as Study 8, 2009b), groups of 51 male and 51 female rats [age at start not reported] were fed diets containing glyphosate (purity, 95.7%) at a concentration of 0, 1500, 5000, or 15 000 ppm, ad libitum, for 24 months. The highest dose was progressively increased to reach 24 000 ppm by week 40. A non-significant increase in tumour incidence was noted for adenocarcinoma of the mammary gland in females at the highest dose (6/51) compared with controls (2/51). [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information. The Working Group noted that tumours of the mammary gland had been observed in other studies in rats reviewed for the present *Monograph*.]

4. Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

4.1.1 Introduction

The herbicidal activity of glyphosate is attributed to interference with the production of essential aromatic amino acids (EPA, 1993b). In plants, glyphosate competitively inhibits the activity of enolpyruvylshikimate phosphate synthase, an enzyme that is not present in mammalian cells. Glyphosate is degraded by soil microbes to aminomethylphosphonic acid (AMPA) (see Fig. 4.1), a metabolite that can accumulate in the environment. In mammals, glyphosate is not metabolized efficiently, and is mainly excreted unchanged into the urine; however, it has been suggested that glyphosate can undergo gut microbial metabolism in humans (Motojyuku *et al.*, 2008) and rodents (Brewster *et al.*, 1991).

4.1.2 Absorption

(a) Humans

Data on the absorption of glyphosate via intake of food and water in humans were not available to the Working Group. Inhalation of glyphosate is considered to be a minor route of exposure in humans, because glyphosate is usually formulated as an isopropylamine salt with a very low vapour pressure (Tomlin, 2000).

In the Farm Family Exposure Study, 60% of farmers had detectable levels of glyphosate in 24-hour composite urine samples taken on the day they had applied a glyphosate-based formulation (Acquavella *et al.*, 2004). Farmers who did not use rubber gloves had higher urinary concentrations of glyphosate than those who did use gloves [indicating that dermal absorption is a relevant route of exposure]. In a separate study, detectable levels of glyphosate were found in urine samples from farm families and non-farm families (Curwin *et al.*, 2007).

In accidental and deliberate intoxication cases involving ingestion of glyphosate-based formulations, glyphosate was readily detectable in the blood (Zouaoui *et al.*, 2013). After deliberate or accidental ingestion, one glyphosate-based formulation was found to be more lethal to humans than another (Sørensen & Gregersen, 1999). [Greater lethality was attributed to the presence of trimethylsulfonium counterion, which might facilitate greater absorption after oral exposure.]

Small amounts of glyphosate can be absorbed after dermal exposures in humans in vitro. For example, when an aqueous solution of 1% glyphosate was applied in an in-vitro human skin model, only 1.4% of the applied dose was absorbed through the skin. Glyphosate is typically formulated as an isopropylamine salt, and is dissolved in a water-based vehicle, while the

stratum corneum is a lipid-rich tissue (Wester *et al.*, 1991). In-vitro studies using human skin showed that percutaneous absorption of a glyphosate-based formulation was no more than 2% of the administered dose over a concentration range of 0.5–154 μg/cm² and a topical volume range of 0.014–0.14 mL/cm². In addition, very little glyphosate (≤ 0.05% of the administered dose) was sequestered in the stratum corneum after dermal application (Wester *et al.*, 1991).

In the human Caco-2 cell line, an in-vitro model of intestinal enterocytes, glyphosate (> 10 mg/mL) was shown to significantly disrupt barrier properties, leading to an increase in paracellular permeability (transport of substances that pass through the intercellular space between the cells) (Vasiluk *et al.*, 2005).

(b) Experimental systems

Three studies have been conducted to investigate the absorption of a single oral dose of glyphosate in rats (Brewster *et al.*, 1991; Chan & Mahler, 1992; EPA, 1993b).

In male Sprague-Dawley rats given [^{14}C]-labelled glyphosate (10 mg/kg bw), the majority of the radiolabel was associated with the gastrointestinal contents and small intestinal tissue 2 hours after administration (Brewster *et al.*, 1991). Approximately 35–40% of the administered dose was found to be absorbed from the gastrointestinal tract. Urinary and faecal routes of elimination were equally important. [The Working Group concluded that glyphosate is incompletely absorbed from the gastrointestinal tract after oral exposure in rats.]

In a study by the United States National Toxicology Programme (NTP) in Fisher 344 rats, 30% of the administered oral dose (5.6 mg/kg bw) was absorbed, as determined by urinary excretion data (Chan & Mahler, 1992). This finding was in accordance with the previously described study of oral exposure in rats (Brewster *et al.*, 1991).

In a study reviewed by the EPA, Sprague-Dawley rats were given an oral dose of glyphosate (10 mg/kg bw); 30% and 36% of the administered dose was absorbed in males and females, respectively (EPA, 1993b). At a dose that was ~10-fold higher (1000 mg/kg bw), oral absorption of glyphosate by the rats was slightly reduced.

In a 14-day feeding study in Wistar rats given glyphosate at dietary concentrations of up to 100 ppm, only ~15% of the administered dose was found to be absorbed (JMPR, 2006). In New Zealand White rabbits or lactating goats given glyphosate as single oral doses (6–9 mg/kg bw), a large percentage of the administered dose was recovered in the faeces [suggesting very poor gastrointestinal absorption of glyphosate in these animal models] (JMPR, 2006).

In monkeys given glyphosate by dermal application, percutaneous absorption was estimated to be between 1% and 2% of the administered dose (Wester *et al.*, 1991). Most of the administered dose was removed by surface washes of the exposed skin.

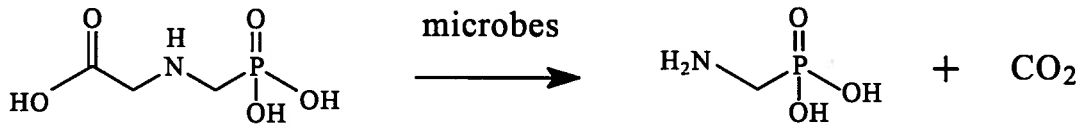
4.1.3 Distribution

(a) Humans

No data in humans on the distribution of glyphosate in systemic tissues other than blood were available to the Working Group. In cases of accidental or deliberate intoxication involving ingestion of glyphosate-based formulations, glyphosate was measured in blood. Mean blood concentrations of glyphosate were 61 mg/L and 4146 mg/L in mild-to-moderate cases of intoxication and in fatal cases, respectively (Zouaoui *et al.*, 2013).

One report, using optical spectroscopy and molecular modelling, indicated that glyphosate could bind to human serum albumin, mainly by hydrogen bonding; however, the fraction of glyphosate that might bind to serum proteins in blood was not actually measured (Yue *et al.*, 2008).

Fig. 4.1 Microbial metabolism of glyphosate to AMPA



Glyphosate is degraded to AMPA by microbial metabolism
Compiled by the Working Group

(b) Experimental systems

In Sprague-Dawley rats given a single oral dose of glyphosate (100 mg/kg bw), glyphosate concentrations in plasma reached peak levels, then declined slowly from day 1 to day 5 (Bernal *et al.*, 2010). The plasma data appeared to fit a one-compartment model with an elimination rate constant of k_{el} = 0.021 hour^{-1}. [The Working Group estimated the elimination half-life of glyphosate to be 33 hours.] Tissue levels of glyphosate were not determined in this study. In a study by Brewster *et al.* (1991), the tissue levels of glyphosate at 2, 6.3, 28, 96, and 168 hours in Sprague-Dawley rats given a single oral dose (10 mg/kg bw) declined rapidly. Tissues with the greatest amounts of detectable radiolabel (> 1% of the administered dose) were the small intestine, colon, kidney, and bone. Peak levels were reached in small intestine tissue and blood by 2 hours, while peak levels in other tissues occurred at 6.3 hours after dosing. After 7 days, the total body burden of [^{14}C]-labelled residues was ~1% of the administered dose, and was primarily associated with the bone (~1 ppm). In every tissue examined after administration of [^{14}C]-labelled glyphosate, essentially 100% of the radiolabel that was present in the tissue was unmetabolized parent glyphosate. Thus, essentially 100% of the body burden was parent compound, with no significant persistence of glyphosate after 7 days (Brewster *et al.*, 1991). In a 14-day feeding study in Wistar rats given diets containing glyphosate at 100 ppm, glyphosate reached steady-state levels in the blood by day 6 (JMPR, 2006). The tissue concentrations of glyphosate had the following rank order: kidneys > spleen > fat > liver. Tissue levels declined rapidly after cessation of exposure to glyphosate. A second study in rats given glyphosate (10 mg/kg bw per day, 14 days) followed by a single oral dose of [^{14}C]-glyphosate (at 10 mg/kg bw) showed that repeated dosing did not alter the tissue distribution of glyphosate (JMPR, 2006).

In rhesus monkeys, tissues harvested 7 days after dermal exposures to [^{14}C]-labelled glyphosate did not contain radiolabel at detectable levels (Wester *et al.*, 1991).

4.1.4 Metabolism and modulation of metabolic enzymes

(a) Metabolism

Glyphosate is degraded in the environment by soil microbes, primarily to AMPA and carbon dioxide (Fig. 4.1; Jacob *et al.*, 1988). A minor pathway for the degradation of glyphosate in bacteria (*Pseudomonas sp.* strain LBr) is via conversion to glycine (Jacob *et al.*, 1988). In a case of deliberate poisoning with a glyphosate-based formulation, small amounts of AMPA (15.1 µg/mL) were detectable in the blood (Motojyuku *et al.*, 2008) [suggesting that this pathway might also operate in humans]. In rats given a single high oral dose of glyphosate (100 mg/kg bw), small amounts of AMPA were detected in the plasma (Bernal *et al.*, 2010). In

male Sprague-Dawley rats given an oral dose of glyphosate (10 mg/kg bw), a very small amount of AMPA (< 0.04% of the administered dose) was detected in the colon 2 hours after dosing; this was attributed to intestinal microbial metabolism (Brewster *et al.*, 1991).

(b) Modulation of metabolic enzymes

(i) Humans

In human hepatic cell lines, treatment with one of four glyphosate-based formulations produced by the same company was shown to enhance CYP3A4 and CYP1A2 levels, while glutathione transferase levels were reduced (Gasnier *et al.*, 2010). [The Working Group noted that it was not clear whether the effects were caused by glyphosate alone or by the adjuvants contained in the formulation.]

(ii) Experimental systems

Exposure of Wistar rats to a glyphosate-based formulation significantly altered some hepatic xenobiotic enzyme activities (Larsen *et al.*, 2014). Liver microsomes obtained from male and female rats treated with the formulation exhibited ~50% reductions in cytochrome P450 (CYP450) content compared with control (untreated) rats. However, opposing effects were observed when assessing 7-ethoxycoumarin *O*-deethylase activity (7-ECOD, a non-specific CYP450 substrate). Female rats treated with the glyphosate-based formulation exhibited a 57% increase in hepatic microsomal 7-ECOD activity compared with controls, while male rats treated with the formulation exhibited a 58% decrease in this activity (Larsen *et al.*, 2014). [The Working Group noted that it was not clear whether the effects were caused by glyphosate alone or by adjuvants contained in the formulation.]

4.1.5 Excretion

(a) Humans

Excretion of glyphosate in humans was documented in several biomonitoring studies. For example, as part of the Farm Family Exposure Study, urinary concentrations of glyphosate were evaluated immediately before, during, and after glyphosate application in 48 farmers and their spouses and children (Acquavella *et al.*, 2004). Dermal contact with glyphosate during mixing, loading, and application was considered to be the main route of exposure in the study. On the day the herbicide was applied, 60% of the farmers had detectable levels of glyphosate in 24-hour composite urine samples, as did 4% of their spouses and 12% of children. For farmers, the geometric mean concentration was 3 µg/L, the maximum value was 233 µg/L, and the highest estimated systemic dose was 0.004 mg/kg bw (Acquavella *et al.*, 2004). In a separate study, detectable levels of glyphosate were excreted in the urine of members of farm families and of non-farm families, with geometric means ranging from 1.2 to 2.7 µg/L (Curwin *et al.*, 2007).

In a study of a rural population living near areas sprayed for drug eradication in Colombia (see Section 1.4.1, Table 1.5), mean urinary glyphosate concentrations were 7.6 µg/L (range, undetectable to 130 µg/L) (Varona *et al.*, 2009). AMPA was detected in 4% of urine samples (arithmetic mean, 1.6 µg/L; range, undetectable to 56 µg/L).

(b) Experimental systems

In an NTP study in Fisher 344 rats given a single oral dose of [^{14}C]-labelled glyphosate (5.6 or 56 mg/kg bw), it was shown that > 90% of the radiolabel was eliminated in the urine and faeces within 72 hours (Chan & Mahler, 1992). In Sprague-Dawley rats given [^{14}C]-labelled glyphosate at an oral dose of 10 or 1000 mg/kg bw, ~60–70% of the administered dose was excreted in the faeces, and the remainder in the urine (EPA,

1993b). By either route, most (98%) of the administered dose was excreted as unchanged parent compound. AMPA was the only metabolite found in the urine (0.2–0.3% of the administered dose) and faeces (0.2–0.4% of the administered dose). [The large amount of glyphosate excreted in the faeces is consistent with its poor oral absorption.] Less than 0.3% of the administered dose was expired as carbon dioxide.

In rhesus monkeys given glyphosate as an intravenous dose (9 or 93 μg), > 95% of the administered dose was excreted in the urine (Wester *et al.*, 1991). Nearly all the administered dose was eliminated within 24 hours. In contrast, in rhesus monkeys given glyphosate by dermal application (5400 μg/20 cm^2), only 2.2% of the administered dose was excreted in the urine within 7 days (Wester *et al.*, 1991).

Overall, systemically absorbed glyphosate is not metabolized efficiently, and is mainly excreted unchanged into the urine.

4.2 Mechanisms of carcinogenesis

4.2.1 Genetic and related effects

Glyphosate has been studied for genotoxic potential in a wide variety of assays. Studies carried out in exposed humans, in human cells in vitro, in other mammals in vivo and in vitro, and in non-mammalian systems in vivo and in vitro, respectively, are summarized in Table 4.1, Table 4.2, Table 4.3, Table 4.4, and Table 4.5. [A review article by Kier & Kirkland (2013) summarized the results of published articles and unpublished reports of studies pertaining to the genotoxicity of glyphosate and glyphosate formulations. A supplement to this report contained information on 66 unpublished regulatory studies. The conclusions and data tables for each individual study were included in the supplement; however, the primary study reports from which these data were extracted were not available to the Working Group. The information provided in the supplement was insufficient regarding topics such as details of statistical methods, choice of the highest dose tested, and verification of the target tissue exposure. The Working Group determined that the information in the supplement to Kier & Kirkland (2013) did not meet the criteria for data inclusion as laid out in the Preamble to the *IARC Monographs*, being neither "reports that have been published or accepted for publication in the openly available scientific literature" nor "data from governmental reports that are publicly available" (IARC, 2006). The review article and supplement were not considered further in the evaluation.]

(a) Humans

(i) Studies in exposed humans

See Table 4.1

In exposed individuals (n = 24) living in northern Ecuador in areas sprayed with a glyphosate-based formulation, a statistically significant increase in DNA damage (DNA strand breaks) was observed in blood cells collected 2 weeks to 2 months after spraying (Paz-y-Miño *et al.*, 2007). The same authors studied blood cells from individuals (n = 92) in 10 communities in Ecuador's northern border, who were sampled 2 years after the last aerial spraying with a herbicide mix containing glyphosate, and showed that their karyotypes were normal compared with those of a control group (Paz-y-Miño *et al.*, 2011).

Bolognesi *et al.* (2009) studied community residents (137 women of reproductive age and their 137 spouses) from five regions in Colombia. In three regions with exposures to glyphosate-based formulations from aerial spraying, blood samples were taken from the same individuals at three time-points (before spraying (baseline), 5 days after spraying and 4 months after spraying) to determine the frequency of micronucleus formation in lymphocytes. The baseline frequency of binucleated cells with micronuclei was significantly higher in subjects

from the three regions where there had been aerial spraying with glyphosate-formulations and in a fourth region with pesticide exposure (but not through aerial spraying), compared with a reference region (without use of pesticide). The frequency of micronucleus formation in peripheral blood lymphocytes was significantly increased, compared with baseline levels in the same individuals, after aerial spraying with glyphosate-based formulations in each of the three regions (see Table 4.1; Bolognesi *et al.*, 2009). Immediately after spraying, subjects who reported direct contact with the glyphosate-based spray showed a higher frequency of binucleated cells with micronuclei. However, the increase in frequency of micronucleus formation observed immediately after spraying was not consistent with the rates of application used in the regions, and there was no association between self-reported direct contact with pesticide sprays and frequency of binucleated cells with micronuclei. In subjects from one but not other regions, the frequency of binucleated cells with micronuclei was significantly decreased 4 months after spraying, compared with immediately after spraying.

(ii) Human cells in vitro

See Table 4.2

Glyphosate induced DNA strand breaks (as measured by the comet assay) in liver Hep-2 cells (Mañas *et al.*, 2009a), lymphocytes (Mladinic *et al.*, 2009b; Alvarez-Moya *et al.*, 2014), GM38 fibroblasts, the HT1080 fibrosarcoma cell line (Monroy *et al.*, 2005), and the TR146 buccal carcinoma line (Koller *et al.*, 2012). DNA strand breaks were induced by AMPA in Hep-2 cells (Mañas *et al.*, 2009b), and by a glyphosate-based formulation in the TR146 buccal carcinoma cell line (Koller *et al.*, 2012).

In human lymphocytes, AMPA (Mañas *et al.*, 2009b), but not glyphosate (Mañas *et al.*, 2009a), produced chromosomal aberrations. Glyphosate did not induce a concentration-related increase in micronucleus formation in human lymphocytes at levels estimated to correspond to occupational and residential exposure (Mladinic *et al.*, 2009a). Sister-chromatid exchange was induced by glyphosate (Bolognesi *et al.*, 1997), and by a glyphosate-based formulation (Vigfusson & Vyse, 1980; Bolognesi *et al.*, 1997) in human lymphocytes exposed in vitro.

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.3

The ability of glyphosate or a glyphosate-based formulation to induce DNA adducts was studied in mice given a single intraperitoneal dose. Glyphosate induced DNA adducts (8-hydroxy deoxyguanosine) in the liver, but not in the kidney, while a glyphosate-based formulation caused a slight increase in DNA adducts in the kidney, but not in the liver (Bolognesi *et al.*, 1997). Peluso *et al.* (1998) showed that a glyphosate-based formulation (glyphosate, 30.4%), but not glyphosate alone, caused DNA adducts (as detected by ^{32}P-DNA post-labelling) in mouse liver and kidney. Glyphosate and a glyphosate-based formulation produced DNA strand breaks in the liver and kidney after a single intraperitoneal dose (Bolognesi *et al.*, 1997).

In mice given a single dose of glyphosate by gavage, no genotoxic effect was observed by the dominant lethal test (EPA, 1980a).

After a single intraperitoneal dose, no chromosomal aberrations were observed in the bone marrow of rats treated with glyphosate (Li & Long 1988), while chromosomal aberrations were increased in the bone marrow of mice given a glyphosate-based formulation (glyphosate isopropylamine salt, ~41%) (Prasad *et al.*, 2009). A single oral dose of a glyphosate-based formulation did not cause chromosomal aberrations in mice (Dimitrov *et al.*, 2006).

In mice treated by intraperitoneal injection, a single dose of glyphosate did not cause

Table 4.1 Genetic and related effects of glyphosate in exposed humans

Tissue	Cell type (if specified)	End-point	Test	Description of exposure and controls	Response[a]/ significance	Comments	Reference
Blood	NR	DNA damage	DNA strand breaks, comet assay	24 exposed individuals in northern Ecuador; areas sprayed with glyphosate-based formulation (sampling 2 weeks to 2 months after spraying); control group was 21 non-exposed individuals	+ $P < 0.001$		Paz-y-Miño *et al.* (2007)
Blood	NR	Chromosomal damage	Chromosomal aberrations	92 individuals in 10 communities, northern border of Ecuador; sampling 2 years after last aerial spraying with herbicide mix containing glyphosate); control group was 90 healthy individuals from several provinces without background of smoking or exposure to genotoxic substances (hydrocarbons, X-rays, or pesticides)	–	182 karyotypes were considered normal [Smoking status, NR]	Paz-y-Miño *et al.* (2011)
Blood	Lymphocytes	Chromosomal damage	Micronucleus formation	55 community residents, Nariño, Colombia; area with aerial glyphosate-based formulation spraying for coca and poppy eradication (glyphosate was tank-mixed with an adjuvant)	+ [$P < 0.001$]	P values for after spraying vs before spraying in the same individuals	Bolognesi *et al.* (2009)
Blood	Lymphocytes	Chromosomal damage	Micronucleus formation	53 community residents, Putumayo, Colombia; area with aerial glyphosate-based formulation spraying for coca and poppy eradication (glyphosate was tank-mixed with an adjuvant)	+ [$P = 0.01$]	P values for after spraying vs before spraying in the same individuals	Bolognesi *et al.* (2009)
Blood	Lymphocytes	Chromosomal damage	Micronucleus formation	27 community residents, Valle del Cauca, Colombia; area where glyphosate-based formulation was applied through aerial spraying for sugar-cane maturation (glyphosate was applied without adjuvant)	+ [$P < 0.001$]	P values for after spraying vs before spraying in the same individuals	Bolognesi *et al.* (2009)

[a] +, positive; –, negative
NR, not reported; vs, versus

micronucleus formation in the bone marrow (Rank *et al.*, 1993), although two daily doses did (Bolognesi *et al.*, 1997; Mañas *et al.*, 2009a). AMPA, the main metabolite of glyphosate, also produced micronucleus formation after two daily intraperitoneal doses (Mañas *et al.*, 2009b). Conflicting results for micronucleus induction were obtained in mice exposed intraperitoneally to a glyphosate-based formulation. A single dose of the formulation at up to 200 mg/kg bw did not induce micronucleus formation in the bone marrow in one study (Rank *et al.* 1993), while it did increase micronucleus formation at 25 mg/kg bw in another study (Prasad *et al.*, 2009). After two daily intraperitoneal doses, a glyphosate-based formulation did not induce micronucleus formation at up to 200 mg/kg bw according to Grisolia (2002), while Bolognesi *et al.* (1997) showed that the formulation did induce micronucleus formation at 450 mg/kg bw. In mice given a single oral dose of a glyphosate-based formulation at 1080 mg/kg bw, no induction of micronuclei was observed (Dimitrov *et al.*, 2006).

(ii) Non-human mammalian cells in vitro

See Table 4.4

Glyphosate did not induce unscheduled DNA synthesis in rat primary hepatocytes, or *Hprt* mutation (with or without metabolic activation) in Chinese hamster ovary cells (Li & Long, 1988).

In bovine lymphocytes, chromosomal aberrations were induced by glyphosate in one study (Lioi *et al.*, 1998), but not by a glyphosate formulation in another study (Siviková & Dianovský, 2006). Roustan *et al.* (2014) demonstrated, in the CHO-K1 ovary cell line, that glyphosate induced micronucleus formation only in the presence of metabolic activation, while AMPA induced micronucleus formation both with and without metabolic activation. Sister-chromatid exchange was observed in bovine lymphocytes exposed to glyphosate (Lioi *et al.*, 1998) or a glyphosate formulation (in the absence but not the presence of metabolic activation) (Siviková & Dianovský, 2006).

(iii) Non-mammalian systems in vivo

See Table 4.5

Fish and other species

In fish, glyphosate produced DNA strand breaks in the comet assay in sábalo (Moreno *et al.*, 2014), European eel (Guilherme *et al.*, 2012b), zebrafish (Lopes *et al.*, 2014), and Nile tilapia (Alvarez-Moya *et al.*, 2014). AMPA also induced DNA strand breaks in the comet assay in European eel (Guilherme *et al.*, 2014b). A glyphosate-based formulation produced DNA strand breaks in numerous fish species, such as European eel (Guilherme *et al.*, 2010, 2012b, 2014a; Marques *et al.*, 2014, 2015), sábalo (Cavalcante *et al.*, 2008; Moreno *et al.*, 2014), guppy (De Souza Filho *et al.*, 2013), bloch (Nwani *et al.*, 2013), neotropical fish *Corydoras paleatus* (de Castilhos Ghisi & Cestari, 2013), carp (Gholami-Seyedkolaei *et al.*, 2013), and goldfish (Cavaş & Könen, 2007).

AMPA, the main metabolite of glyphosate, induced erythrocytic nuclear abnormalities (kidney-shaped and lobed nuclei, binucleate or segmented nuclei and micronuclei) in European eel (Guilherme *et al.*, 2014b). Micronucleus formation was induced by different glyphosate-based formulations in various fish (Grisolia, 2002; Cavaş & Könen, 2007; De Souza Filho *et al.*, 2013; Vera-Candioti *et al.*, 2013).

Glyphosate-based formulations induced DNA strand breaks in other species, including caiman (Poletta *et al.*, 2009), frog (Meza-Joya *et al.*, 2013), tadpoles (Clements *et al.*, 1997), and snail (Mohamed, 2011), but not in oyster (Akcha *et al.*, 2012), clam (dos Santos & Martinez, 2014), and mussel glochidia (Conners & Black, 2004). In earthworms, one glyphosate-based formulation induced DNA strand breaks while two others did not (Piola *et al.*, 2013; Muangphra *et al.*, 2014), highlighting the potential importance of components other than the active ingredient in the formulation.

Table 4.2 Genetic and related effects of glyphosate, AMPA, and glyphosate-based formulations in human cells in vitro

Tissue, cell line	End-point	Test	Results[a]		Dose (LED or HID)	Comments	Reference
			Without metabolic activation	With metabolic activation			
Glyphosate							
Liver Hep-2	DNA damage	DNA strand breaks, comet assay	+	NT	3 mM [507.2 µg/mL]	$P < 0.01$; dose–response relationship ($r \geq 0.90$; $P < 0.05$)	Mañas et al. (2009a)
Lymphocytes	DNA damage	DNA strand breaks, standard and hOGG1 modified comet assay	+	+	3.5 µg/mL	With the hOGG1 modified comet assay, + S9, the increase was significant ($P < 0.01$) only at the highest dose tested (580 µg/mL)	Mladinic *et al.* (2009b)
Lymphocytes	DNA damage	DNA strand breaks, comet assay	+	NT	0.0007 mM [0.12 µg/mL]	$P \leq 0.01$	Alvarez-Moya *et al.* (2014)
Fibroblast GM 38	DNA damage	DNA strand breaks, comet assay	+	NT	4 mM [676 µg/mL]	$P < 0.001$	Monroy *et al.* (2005)
Fibroblast GM 5757	DNA damage	DNA strand breaks, comet assay	(+)	NT	75 mM [12 680 µg/mL]	Glyphosate (ineffective alone, data NR) increased strand breaks induced by H_2O_2 (40 or 50 µM) ($P < 0.004$ vs H_2O_2 alone)	Lueken *et al.* (2004)
Fibrosarcoma HT1080	DNA damage	DNA strand breaks, comet assay	+	NT	4.75 mM [803 µg/mL]	$P < 0.001$	Monroy *et al.* (2005)
Buccal carcinoma TR146	DNA damage	DNA strand breaks, SCGE assay	+	NT	20 µg/mL	Dose-dependent increase ($P \leq 0.05$)	Koller *et al.* (2012)
Lymphocytes	Chromosomal damage	Chromosomal aberrations	–	NT	6 mM [1015 µg/mL]		Mañas *et al.* (2009a)
Lymphocytes	Chromosomal damage	Micronucleus formation	–	(+)	580 µg/mL	$P < 0.01$ at the highest exposure + S9 No concentration-related increase in micronuclei containing the centromere signal (C+)	Mladinic *et al.* (2009a)

Table 4.2 (continued)

Tissue, cell line	End-point	Test	Results[a]		Dose (LED or HID)	Comments	Reference
			Without metabolic activation	With metabolic activation			
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	NT	1000 µg/mL	$P < 0.05$	Bolognesi *et al.* (1997)
AMPA							
Liver Hep-2	DNA damage	DNA strand breaks, comet assay	+	NT	4.5 mM [500 µg/mL]	$P < 0.05$ at 4.5 mM; $P < 0.01$ at up to 7.5 mM Dose–response relationship ($r \geq 0.90$; $P < 0.05$)	Mañas *et al.* (2009b)
Lymphocytes	Chromosomal damage	Chromosomal aberrations	+	NT	1.8 mM [200 µg/mL]	$P < 0.05$	Mañas *et al.* (2009b)
Glyphosate-based formulations							
Liver HepG2	DNA damage	DNA strand breaks, comet assay	(+)	NT	5 ppm	Glyphosate, 400 g/L Dose-dependent increase; greatest increase at 10 ppm Statistical analysis, NR	Gasnier *et al.* (2009)
Buccal carcinoma TR146	DNA damage	DNA strand breaks, SCGE assay	+	NT	20 µg/mL	Glyphosate acid, 450g/L Dose-dependent increase ($P \leq 0.05$)	Koller *et al.* (2012)
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	NT	250 µg/mL	$P < 0.001$ No growth at 25 mg/mL	Vigfusson & Vyse (1980)
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	NT	100 µg/mL	Glyphosate, 30.4% $P < 0.05$	Bolognesi *et al.* (1997)

[a] +, positive; -, negative; (+) or (-) positive/negative in a study with limited quality

AMPA, aminomethyl phosphonic acid; HID, highest ineffective dose; hOGG1, human 8-hydroxyguanosine DNA-glycosylase; LED, lowest effective dose; NR, not reported; NT, not tested; S9, 9000 × *g* supernatant; SCGE, single cell gel electrophoresis; vs, versus

Micronucleus formation was induced by a glyphosate-based formulation (glyphosate, 36%) in earthworms (Muangphra *et al.*, 2014), and by a different glyphosate-based formulation in caiman (Poletta *et al.*, 2009, 2011), and frog (Yadav *et al.*, 2013).

Insects

In standard *Drosophila melanogaster*, glyphosate induced mutation in the test for somatic mutation and recombination, but not in a cross of flies characterized by an increased capacity for CYP450-dependent bioactivation (Kaya *et al.*, 2000). A glyphosate-based formulation also caused sex-linked recessive lethal mutations in *Drosophila* (Kale *et al.*, 1995).

Plants

In plants, glyphosate produced DNA damage in *Tradescantia* in the comet assay (Alvarez-Moya *et al.*, 2011). Chromosomal aberration was induced after exposure to glyphosate in fenugreek (Siddiqui *et al.*, 2012), and in onion in one study (Frescura *et al.*, 2013), but not in another (Rank *et al.*, 1993). A glyphosate-based formulation also induced chromosomal aberration in barley roots (Truta *et al.*, 2011) and onion (Rank *et al.*, 1993), but not in *Crepis capillaris* (hawksbeard) (Dimitrov *et al.*, 2006). Micronucleus formation was not induced by glyphosate in *Vicia faba* bean (De Marco *et al.*, 1992) or by a glyphosate-based formulation in *Crepis capillaris* (Dimitrov *et al.*, 2006).

(iv) Non-mammalian systems in vitro

See Table 4.6

Glyphosate induced DNA strand breaks in erythrocytes of tilapia fish, as demonstrated by comet assay (Alvarez-Moya *et al.*, 2014).

Glyphosate did not induce mutation in *Bacillus subtillis*, *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100, or in *Escherichia coli* WP2, with or without metabolic activation (Li & Long, 1988). However, Rank *et al.* (1993) demonstrated that a glyphosate-based formulation was mutagenic in *S. typhimurium* TA98 in the absence of metabolic activation, and in *S. typhimurium* TA100 in the presence of metabolic activation.

4.2.2 Receptor-mediated mechanisms

(a) Sex-hormone pathway disruption

(i) Humans

Studies in exposed humans

No data were available to the Working Group.

Human cells in vitro

In hormone-dependent T47D breast cancer cells, the proliferative effects of glyphosate (10^{-6} to 1 μM) (see Section 4.2.4) and those of 17β-estradiol (the positive control) were mitigated by the estrogen receptor antagonist, ICI 182780; the proliferative effect of glyphosate was completely abrogated by the antagonist at a concentration of 10 nM (Thongprakaisang *et al.*, 2013). Glyphosate also induced activation of the estrogen response element (ERE) in T47D breast cancer cells that were stably transfected with a triplet ERE-promoter-luciferase reporter gene construct. Incubation with ICI 182780 at 10 nM eliminated the response. When the transfected cells were incubated with both 17β-estradiol and glyphosate, the effect of 17β-estradiol was reduced and glyphosate behaved as an estrogen antagonist. After 6 hours of incubation, glyphosate increased levels of estrogen receptors ERα and ERβ in a dose-dependent manner in T47D cells; after 24 hours, only ERβ levels were increased and only at the highest dose of glyphosate. [These findings suggested that the proliferative effects of glyphosate on T47D cells are mediated by ER.]

In human hepatocarcinoma HepG2 cells, four glyphosate-based formulations produced by the same company had a marked effect on the activity and transcription of aromatase, while glyphosate alone differed from controls, but not significantly so (Gasnier *et al.*, 2009).

Table 4.3 Genetic and related effects of glyphosate, AMPA, and glyphosate-based formulations in non-human mammals in vivo

Species, strain (sex)	Tissue	End-point	Test	Results	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Glyphosate								
Mouse, Swiss CD1 (M)	Liver	DNA damage	DNA adducts, 8-OHdG by LC/UV	+	300 mg/kg bw	i.p.; 1×; sampled after 8 and 24 h	Single dose tested only $P < 0.05$ after 24 h	Bolognesi *et al.* (1997)
Mouse, Swiss CD1 (M)	Kidney	DNA damage	DNA adducts, 8-OHdG by LC/UV	–	300 mg/kg bw	i.p.; 1×; sampled after 8 and 24 h	Single dose tested only	Bolognesi *et al.* (1997)
Mouse, Swiss CD1 (M, F)	Kidney	DNA damage	DNA adducts, ^{32}P-DNA post labelling	–	270 mg/kg bw	i.p.; 1 ×; sampled after 24 h	Glyphosate isopropylammonium salt	Peluso *et al.* (1998)
Mouse, Swiss CD1 (M, F)	Liver	DNA damage	DNA adducts, ^{32}P-DNA post labelling	–	270 mg/kg bw	i.p.; 1 ×; sampled after 24 h	Glyphosate isopropylammonium salt	Peluso *et al.* (1998)
Mouse, Swiss CD1 (M)	Liver	DNA damage	DNA strand breaks, alkaline elution assay	+	300 mg/kg bw	i.p.; 1 ×; sampled after 4 and 24 h	Single dose tested only $P < 0.05$ after 4 h	Bolognesi *et al.* (1997)
Mouse, Swiss CD1 (M)	Kidney	DNA damage	DNA strand breaks, alkaline elution assay	+	300 mg/kg bw	i.p.; 1 ×; sampled after 4 and 24 h	Single dose tested only $P < 0.05$ after 4 h	Bolognesi *et al.* (1997)
Mouse, CD-1 (M)	Uterus after mating	Mutation	Dominant lethal test	–	2000 mg/kg bw	Oral gavage; 1 ×	Proportion of early resorptions evaluated after mating of non-treated females with glyphosate-treated male mice	EPA (1980)
Rat, Sprague-Dawley (M, F)	Bone marrow	Chromosomal damage	Chromosomal aberrations	–	1000 mg/kg bw	i.p.; 1 ×; sampled after 6, 12 and 24 h	Single dose tested only	Li & Long (1988)
Mouse, NMRI-bom (M, F)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	–	200 mg/kg bw	i.p.; 1 ×; sampled after 24 and 48 h	Glyphosate isopropylamine salt	Rank *et al.* (1993)
Mouse, Swiss CD1 (M)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	+	300 mg/kg bw	i.p.; 2 × 150 mg/kg bw with 24 h interval; sampled 6 or 24 h after the last injection	Single dose tested only $P < 0.05$ after 24 h	Bolognesi *et al.* (1997)

Table 4.3 (continued)

Species, strain (sex)	Tissue	End-point	Test	Results	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Mouse, Balb C (M, F)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	+	400 mg/kg bw	i.p.; one injection per 24 h, 2 × 200, sampled 24 h after the last injection	$P < 0.01$ at the highest dose (400 mg/kg bw)	Mañas *et al.* (2009a)
AMPA								
Mouse, Balb C (M, F)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	+	200 mg/kg bw	i.p.; one injection per 24 h, 2 × 100, sampled 24 h after the last injection	$P < 0.01$ at the lowest dose (200 mg/kg bw)	Mañas *et al.* (2009b)
Glyphosate-based formulations								
Mouse, Swiss CD1 (M)	Liver	DNA damage	DNA adducts, 8-OHdG by LC/UV	–	~300 mg/kg bw	i.p.; 1 ×, sampled after 8 and 24 h	Glyphosate, 30.4% Single dose tested only	Bolognesi *et al.* (1997)
Mouse, Swiss CD1 (M)	Kidney	DNA damage	DNA adducts, 8-OHdG by LC/UV	+	~300 mg/kg bw	i.p.; 1 ×, sampled after 8 and 24 h	Glyphosate, 30.4% Single dose tested only $P < 0.05$	Bolognesi *et al.* (1997)
Mouse, Swiss CD1 (M, F)	Kidney	DNA damage	DNA adducts, ^{32}P-DNA post labelling	+	400 mg/kg bw	i.p.; 1 ×; sampled after 24 h	Glyphosate isopropylammonium salt, 30.4%	Peluso *et al.* (1998)
Mouse, Swiss CD1 (M, F)	Liver	DNA damage	DNA adducts, ^{32}P-DNA post labelling	+	400 mg/kg bw	i.p.; 1 ×; sampled after 24 h	Glyphosate isopropylammonium salt, 30.4%	Peluso *et al.* (1998)
Mouse, Swiss CD1 (M)	Liver	DNA damage	DNA strand breaks, alkaline elution assay	+	~300 mg/kg bw	i.p.; 1 ×; sampled after 4 and 24 h	Glyphosate, 30.4% Single dose tested only $P < 0.05$ only after 4 h	Bolognesi *et al.* (1997)
Mouse, Swiss CD1 (M)	Kidney	DNA damage	DNA strand breaks, alkaline elution assay	+	~300 mg/kg bw	i.p.; 1 ×; sampled after 4 and 24 h	Glyphosate, 30.4% Single dose tested only $P < 0.05$ only after 4 h	Bolognesi *et al.* (1997)
Mouse, C57BL (M)	Bone marrow (PCE)	Chromosomal damage	Chromosomal aberrations	–	1080 mg/kg bw	p.o. in distilled water; 1 ×; sampled after 6, 24, 48, 72, 96 and 120 h	Single dose tested only	Dimitrov *et al.* (2006)

Table 4.3 (continued)

Species, strain (sex)	Tissue	End-point	Test	Results	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Mouse, Swiss albino (M)	Bone marrow	Chromosomal damage	Chromosomal aberrations	+	25 mg/kg bw	i.p.; 1 ×; sampled after 24, 48 and 72 h	Glyphosate isopropylamine salt, > 41% The percentage of aberrant cells was increased vs control in a dose- and time-dependent manner ($P < 0.05$)	Prasad *et al.* (2009)
Mouse, NMRI-bom (M, F)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	–	200 mg/kg bw	i.p.; 1 ×; sampled after 24 h	Glyphosate isopropylammonium salt, 480 g/L The percentage of PCE decreased	Rank *et al.* (1993)
Mouse, Swiss (M, F)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	–	200 mg/kg bw	i.p.; 2 × within 24 h interval and sampled 24 h after the last injection	Glyphosate isopropylammonium salt, 480 g/L	Grisolia (2002)
Mouse, Swiss albino (M)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	+	25 mg/kg bw	i.p.; 1 × ; sampled after 24, 48 and 72 h	Glyphosate isopropylamine salt, > 41% Significant induction of micronuclei vs control at both doses and all times ($P < 0.05$)	Prasad *et al.* (2009)
Mouse, Swiss CD1 (M)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	+	450 mg/kg bw	i.p.; 2 × 225 mg/kg with 24 h interval; sampled 6 or 24 h after the last injection	Glyphosate, 30.4% Single dose tested only $P < 0.05$ after 6 h and 24 h	Bolognesi *et al.* (1997)
Mouse, C57BL (M)	Bone marrow	Chromosomal damage	Micronucleus formation	–	1080 mg/kg bw	p.o. in distilled water; 1 × ; sampled after 24, 48, 72, 96 and 120 h	Single dose tested only	Dimitrov *et al.* (2006)

[a] +, positive; -, negative; (+) or (-) positive/negative in a study with limited quality

bw, body weight; F, female; h, hour; HID, highest effective dose; i.p., intraperitoneal; LC, liquid chromatography; LED, lowest effective dose; M, male; PCE, polychromatic erythrocytes; p.o., oral; 8-OHdG, 8-hydroxydeoxyguanosine; UV, ultraviolet

Table 4.4 Genetic and related effects of glyphosate, AMPA, and glyphosate-based formulations in non-human mammalian cells in vitro

Species	Tissue, cell line	End-point	Test	Results[a]		Dose (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Glyphosate								
Rat, Fisher F334	Hepatocytes	DNA damage	Unscheduled DNA synthesis	–	NT	125 µg/mL		Li & Long (1988)
Hamster, Chinese	CHO-K_1BH_4 ovary, cell line	Mutation	*Hprt* mutation	–	–	22 500 µg/mL		Li & Long (1988)
Bovine	Lymphocytes	Chromosomal damage	Chromosomal aberrations	+	NT	17 µM [3 µg/mL]	$P < 0.05$	Lioi *et al.* (1998)
Hamster, Chinese	CHO-K1 ovary cell line	Chromosomal damage	Micronucleus formation	–	+	10 µg/mL	$P \leq 0.001$, in the dark +S9 Negative –S9 in the dark or with light irradiation	Roustan *et al.* (2014)
Bovine	Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	NT	17 µM [3 µg/mL]	$P < 0.05$	Lioi *et al.* (1998)
AMPA								
Hamster, Chinese	CHO-K1 ovary cell line	Chromosomal damage	Micronucleus formation	+	+	0.01 µg/mL	$P \leq 0.05$, in the dark –S9 Highest increase was observed at very low dose (0.0005 µg/mL) –S9 but with light-irradiation ($P < 0.01$)	Roustan *et al.* (2014)
Glyphosate-based formulations								
Bovine	Lymphocytes	Chromosomal damage	Chromosomal aberrations	–	NT	1120 µM [190 µg/mL]	Glyphosate, 62%	Siviková & Dianovský (2006)
Bovine	Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	–	56 µM [9.5 µg/mL]	Glyphosate, 62% Time of exposure, 24 h $P < 0.01$, –S9, at ≥ 56 µM	Siviková & Dianovský (2006)

[a] +, positive; –, negative; (+), weakly positive

AMPA, aminomethyl phosphonic acid; HIC, highest ineffective concentration; *Hprt*, hypoxanthine guanine phosphoribosyl transferase gene; LEC, lowest effective concentration; NT, not tested

Table 4.5 Genetic and related effects of glyphosate, AMPA, and glyphosate-based formulations in non-mammalian systems in vivo

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Glyphosate							
Fish	*Prochilodus lineatus* (sábalo), erythrocytes and gill cells	DNA damage	DNA strand breaks, comet assay	+	0.48 mg/L	Time of exposure 6, 24, and 96 h For erythrocytes, $P = 0.01$ after 6 h, and $P = 0.014$ after 96 h; no significant increase after 24 h For gill cells, $P = 0.02$ only after 6 h at 2.4 mg/L	Moreno *et al.* (2014)
Fish	*Anguilla anguilla* L. (European eel), blood cells	DNA damage	DNA strand breaks, comet assay	+	0.0179 mg/L	Time of exposure 1 and 3 days $P < 0.05$	Guilherme *et al.* (2012b)
Fish	*Danio rerio* (zebrafish), sperm	DNA damage	DNA strand breaks, acridine orange method	+	10 mg/L	After 96 h, DNA integrity was 78.3 ± 3.5%, significantly reduced from control (94.7 ± 0.9%) and 5 mg/L (92.6 ± 1.9%), ($P < 0.05$)	Lopes *et al.* (2014)
Fish	*Oreochromis niloticus* (Nile tilapia) branchial erythrocytes	DNA damage	DNA strand breaks, comet assay	+	7 µM [1.2 mg/L]	Time of exposure, 10 days $P < 0.001$ with concentrations ≥ 7 µM	Alvarez-Moya *et al.* (2014)
Oyster	Oyster spermatozoa	DNA damage	DNA strand breaks, comet assay	–	0.005 mg/L	Time of exposure, 1 h	Akcha *et al.* (2012)
Insect	*Drosophila* standard cross	Mutation	SMART	+	1 mM [0.169 mg/L]	Purity, 96% Increased frequency of small single spots (≥ 1 mM) and total spots (≥ 2 mM) $P = 0.05$	Kaya *et al.* (2000)
Insect	*Drosophila melanogaster*, high bioactivation cross	Mutation	SMART	–	10 mM [1.69 mg/L]	Purity, 96%	Kaya *et al.* (2000)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Plant systems	*Tradescantia* clone 4430 (spiderworts), staminal hair nuclei	DNA damage	DNA strand breaks, comet assay	+	0.0007 mM [0.12 µg/mL]	Glyphosate isopropylamine salt $P < 0.01$ for directly exposed nuclei (dose-dependent increase) and plants	Alvarez-Moya *et al.* (2011)
Plant systems	*Allium cepa* (onion)	Chromosomal damage	Chromosomal aberrations	+	3%	Single dose tested only Partial but significant reversal with distilled water	Frescura *et al.* (2013)
Plant systems	*Allium cepa* (onion)	Chromosomal damage	Chromosomal aberrations	–	2.88 µg/mL	Glyphosate isopropylamine	Rank *et al.* (1993)
Plant systems	*Trigonella foenum-graecum* L. (fenugreek)	Chromosomal damage	Chromosomal aberrations	+	0.2%	$P < 0.001$; positive dose–response relationship	Siddiqui *et al.* (2012)
Plant systems	*Vicia faba* (bean)	Chromosomal damage	Micronucleus formation	–	1400 ppm (1400 µg/g of soil)	Tested with two types of soil, but not without soil	De Marco *et al.* (1992)
AMPA							
Fish	*Anguilla anguilla* L. (European eel)	DNA damage	DNA strand breaks, comet assay	+	0.0118 mg/L	Time of exposure, 1 and 3 days $P < 0.05$ after 1 day of exposure	Guilherme *et al.* (2014b)
Fish	*Anguilla anguilla* L. (European eel)	Chromosomal damage	Other (ENA)	+	0.0236 mg/L	$P < 0.05$ only at highest dose after 3 day exposure (not after 1 day)	Guilherme *et al.* (2014b)
Glyphosate-based formulations							
Fish	*Anguilla anguilla* L. (European eel), blood cells	DNA damage	DNA strand breaks, comet assay	+	0.058 mg/L	$P < 0.05$ Positive dose–response relationship	Guilherme *et al.* (2010)
Fish	*Anguilla anguilla* L. (European eel), blood cells	DNA damage	DNA strand breaks, comet assay improved with the DNA-lesion-specific FPG and Endo III	+	0.058 mg/L	Glyphosate-based formulation, 30.8% Time of exposure, 1 and 3 days With FPG, $P < 0.05$; with comet assay alone, $P < 0.05$ at 116 µg/L	Guilherme *et al.* (2012b)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Fish	*Anguilla anguilla* L. (European eel), blood cells	DNA damage	DNA strand breaks, comet assay improved with the DNA-lesion-specific FPG and Endo III	+	0.116 mg/L	Single dose tested only Time of exposure, 3 days; recovery from non-specific DNA damage, but not oxidative DNA damage, 14 days after exposure $P < 0.05$	Guilherme *et al.* (2014a)
Fish	*Anguilla anguilla* L. (European eel), liver	DNA damage	DNA strand breaks, comet assay improved with the DNA-lesion-specific FPG and Endo III	+	0.058 mg/L	Glyphosate-based formulation, 485 g/L Time of exposure, 3 days $P < 0.05$	Marques *et al.* (2014, 2015)
Fish	*Prochilodus lineatus* (sábalo), erythrocytes and bronchial cells	DNA damage	DNA strand breaks, comet assay	+	10 mg/L	Single dose tested only, for 6, 24, and 96 h $P < 0.05$ for both erythrocytes and bronchial cells	Cavalcante *et al.* (2008)
Fish	*Prochilodus lineatus* (sábalo), erythrocytes and gill cells	DNA damage	DNA strand breaks, comet assay	+	1 mg/L	Glyphosate-based formulation, 480 g/L Time of exposure, 6, 24 and 96 h $P < 0.001$ after 24 and 96 h in erythrocytes and 24 h in gill cells	Moreno *et al.* (2014)
Fish	*Poecilia reticulata* (guppy) gill erythrocytes	DNA damage	DNA strand breaks, comet assay	+	2.83 µL/L [1.833 mg/L]	Glyphosate, 64.8%, m/v (648 g/L) $P < 0.05$	De Souza Filho *et al.* (2013)
Fish	*Channa punctatus* (bloch), blood and gill cells	DNA damage	DNA strand breaks, comet assay	+	3.25 mg/L	Exposure continued for 35 days; blood and gill cells collected on day 1, 7, 14, 21, 28 and 35 $P < 0.01$, for blood and gill cells; DNA damage increased with time and concentration	Nwani *et al.* (2013)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Fish	*Corydoras paleatus* (blue leopard corydoras, mottled corydoras and peppered catfish), blood and hepatic cells	DNA damage	DNA strand breaks, comet assay	+	0.0067 mg/L	Glyphosate, 48% (corresponding to 3.20 µg/L) Single dose tested only, for 3, 6, and 9 days $P < 0.01$, in blood and in liver cells	de Castilhos Ghisi & Cestari (2013)
Fish	*Cyprinus carpio* Linnaeus (carp), erythrocytes	DNA damage	DNA strand breaks, comet assay	+	2 mg/L (10% LC_{50}, 96 h)	Glyphosate, equivalent to 360 g/L Single dose tested only, for 16 days $P < 0.01$	Gholami-Seyedkolaei *et al.* (2013)
Fish	*Carassius auratus* (goldfish), erythrocytes	DNA damage	DNA strand breaks, comet assay	+	5 ppm	Glyphosate equivalent to 360 g/L Time of exposure, 2, 4 and 6 days After 48 h: $P < 0.05$ (5 mg/L) and $P < 0.001$ (10 and 15 mg/L)	Cavaş & Könen (2007)
Fish	*Prochilodus lineatus* (sábalo) erythrocytes	Chromosomal damage	Micronucleus formation	–	10 mg/L	Single dose tested only, for 6, 24, and 96 h Nuclear abnormalities (lobed nuclei, segmented nuclei and kidney-shaped nuclei)	Cavalcante *et al.* (2008)
Fish	*Corydoras paleatus* (blue leopard corydoras, mottled corydoras and peppered catfish), blood and hepatic cells	Chromosomal damage	Micronucleus formation	–	0.0067 mg/L	Glyphosate, 48% (corresponding to 3.20 µg/L) Single dose tested only, for 3, 6 and 9 days	de Castilhos Ghisi & Cestari (2013)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Fish	*Tilapia rendalli* (redbreast tilapia) blood erythrocytes	Chromosomal damage	Micronucleus formation	+	42 mg/kg bw	Glyphosate, 480 g/L Increased frequency of micronucleus formation vs control ($P < 0.05$) in blood samples collected 4 days after a single intra-abdominal injection of 42, 85, or 170 mg/kg bw	Grisolia (2002)
Fish	*Carassius auratus* (goldfish), erythrocytes	Chromosomal damage	Micronucleus formation	+	5 ppm	Glyphosate equivalent to 360 g/L Time of exposure, 2, 4 and 6 days Statistically significant differences: 96 h ($P < 0.05$); 144 h ($P < 0.01$)	Cavaş & Könen (2007)
Fish	*Poecilia reticulata* (guppy) gill erythrocytes	Chromosomal damage	Micronucleus formation, ENA	+	1.41 µL/L [0.914 mg/L]	Glyphosate, 64.8%, m/v (648 g/L) Micronucleus formation, $P < 0.01$ Other nuclear abnormalities, $P < 0.05$ at 1.41 to 5.65 µL/L; concentration-dependent ($r^2 = 0.99$)	De Souza Filho *et al.* (2013)
Fish	*Cnesterodon decemmaculatus* (Jenyns, 1842) peripheral blood erythrocytes	Chromosomal damage	Micronucleus formation	+	3.9 mg/L	Glyphosate, 48% Time of exposure, 48 and 96 h $P < 0.05$, with 3.9 and 7.8 mg/L for 48 and 96 h	Vera-Candioti *et al.* (2013)
Fish	*Cnesterodon decemmaculatus* (Jenyns, 1842) peripheral blood erythrocytes	Chromosomal damage	Micronucleus formation	+	22.9 mg/L	Glyphosate, 48% Time of exposure, 48 and 96 h $P < 0.01$, with 22.9 and 45.9 mg/L, and $P < 0.05$ at 68.8 mg/L, for 96 h	Vera-Candioti *et al.* (2013)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Fish	*Prochilodus lineatus* (sábalo) erythrocytes	Chromosomal damage	Chromosomal aberrations	–	10 mg/L	Single dose tested only, for 6, 24, and 96 h Nuclear abnormalities (lobed nuclei, segmented nuclei and kidney-shaped nuclei)	Cavalcante *et al.* (2008)
Fish	*Anguilla anguilla* L. (European eel), peripheral mature erythrocytes	Chromosomal damage	Other (ENA)	+	0.058 mg/L	Time of exposure, 1 and 3 days Chromosomal breakage and/or chromosomal segregational abnormalities after 3 days of exposure, $P < 0.05$	Guilherme *et al.* (2010)
Caiman	*Caiman latirostris* (broad-snouted caiman), erythrocytes	DNA damage	DNA strand breaks, comet assay	+	0.500 mg/egg	Glyphosate, 66.2% In-ovo exposure; blood sampling at the time of hatching $P < 0.05$ in both experiments (50–1000 µg/egg in experiment 1; 500–1750 µg/egg in experiment 2)	Poletta *et al.* (2009)
Caiman	*Caiman latirostris* (broad-snouted caiman), erythrocytes	DNA damage	DNA strand breaks, comet assay	–	19 800 mg/L	Glyphosate, 66.2% Single dose tested only; in-ovo exposure First spraying exposure at the beginning of incubation period, a second exposure on day 35, then incubation until hatching	Poletta *et al.* (2011)
Caiman	*Caiman latirostris* (broad-snouted caiman), erythrocytes	Chromosomal damage	Micronucleus fomation	+	0.500 mg/egg	Glyphosate, 66.2% In-ovo exposure; blood sampling at the time of hatching $P < 0.05$ in both experiments (50–1000 µg/egg in experiment 1; 500–1750 µg/egg in experiment 2)	Poletta *et al.* (2009)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Caiman	*Caiman latirostris* (broad-snouted caiman), erythrocytes	Chromosomal damage	Micronucleus fomation	+	19.8 g/L	Glyphosate, 66.2% One dose tested; in-ovo exposure First spraying exposure at the beginning of incubation period, a second exposure on day 35, then incubation until hatching. Micronucleus formation, $P < 0.001$ Damage index, $P < 0.001$	Poletta *et al.* (2011)
Frog tadpole	*Rana catesbeiana* (ouaouaron), blood	DNA damage	DNA strand breaks, comet assay	+	1.687 mg/L, p.o.	Time of exposure, 24 h $P < 0.05$, with 6.75 mg/L; and $P < 0.001$ with 27 mg/L (with 108 mg/L, all died within 24 h)	Clements *et al.* (1997)
Frog	*Eleutherodactylus johnstonei* (Antilles coqui), erythrocytes	DNA damage	DNA strand breaks, comet assay	+	0.5 µg a.e./cm²	Glyphosate-based formulation, 480 g/L Exposure to an homogenate mist in a 300 cm² glass terrarium Time of exposure: 0.5, 1, 2, 4, 8 and 24 h $P < 0.05$	Meza-Joya *et al.* (2013)
Frog	*Euflictis cyanophlyctis* (Indian skittering frog), erythrocytes	Chromosomal damage	Micronucleus formation	+	1 mg a.e./L	Glyphosate isopropylamine salt, 41% Time of exposure: 24, 48, 72, and 96 h $P < 0.001$ at 24, 48, 72 and 96 h	Yadav *et al.* (2013)
Snail	*Biomphalaria alexandrina*, haemolymph	DNA damage	DNA strand breaks, comet assay	+	10 mg/L	Glyphosate, 48% Single dose tested only, for 24 h. The percentage of damaged DNA was 21% vs 4% (control) No statistical analysis	Mohamed (2011)
Oyster	Oysters, spermatozoa	DNA damage	DNA strand breaks, comet assay	–	5 µg/L	Glyphosate, 200 µg equivalent/L Time of exposure, 1 h	Akcha *et al.* (2012)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Clam	*Corbicula fluminea* (Asian clam) haemocytes	DNA damage	DNA strand breaks, comet assay	–	10 mg/L	Time of exposure, 96 h Significant increase when atrazine (2 or 10 mg/L) was added to glyphosate ($P < 0.05$) No increase after exposure to atrazine or glyphosate separately	dos Santos & Martinez (2014)
Mussels	*Utterbackia imbecillis* (Bivalvia: Unionidae) glochidia mussels (larvae)	DNA damage	DNA strand breaks, comet assay	–	5 mg/L	Glyphosate, 18% Doses tested: 2.5 and 5 mg/L for 24 h NOEC, 10.04 mg/L	Conners & Black (2004)
Worm	Earthworm, *Eisenia andrei*, coelomocytes	DNA damage	DNA strand breaks, comet assay	–	240 µg a.e./cm²	Monoammonium salt, 85.4%, a.e. Epidermic exposure during 72 h (on filter paper)	Piola *et al.* (2013)
Worm	Earthworm, *Eisenia andrei*, coelomocytes	DNA damage	DNA strand breaks, comet assay	+	15 µg a.e./cm²	Monoammonium salt, 72%, a.e. Epidermic exposure during 72 h (on filter paper) $P < 0.001$	Piola *et al.* (2013)
Worm	Earthworm, *Pheretima peguana*, coelomocytes	DNA damage	DNA strand breaks, comet assay	–	251.50 µg/cm²	Active ingredient, 36% (w/v) Epidermic exposure 48 h on filter paper; LC_{50}, 251.50 µg/cm²	Muangphra *et al.* (2014)
Worm	Earthworm, *Pheretima peguana*, coelomocytes	Chromosomal damage	Micronucleus formation	+	251.50 µg/cm²	Active ingredient, 36% (w/v) Exposure, 48 h on filter paper; LC_{50}, 251.50 µg/cm² filter paper $P < 0.05$, for total micro-, bi-, and trinuclei frequencies at 0.25 µg/cm²; when analysed separately, micro- and trinuclei frequencies significantly differed from controls only at the LC_{50}	Muangphra *et al.* (2014)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Insect	*Drosophila melanogaster*	Mutation	Sex-linked recessive lethal mutations	+	1 ppm	Single dose tested only $P < 0.001$	Kale *et al.* (1995)
Plant systems	*Allium cepa* (onion)	Chromosomal damage	Chromosomal aberrations	+	1.44 µg/mL	Glyphosate-based formulation, 480 g/L The doses of formulation were calculated as glyphosate isopropylamine $P < 0.005$	Rank *et al.* (1993)
Plant systems	*Crepis capillaris* (hawksbeard)	Chromosomal damage	Chromosomal aberrations	–	0.5%	The highest dose tested (1%) was toxic	Dimitrov *et al.* (2006)
Plant systems	*Hordeum vulgare* L. cv. Madalin (barley roots)	Chromosomal damage	Chromosomal aberrations	(+)	360 µg/mL (0.1%)	Reported as "significant"	Truta *et al.* (2011)
Plant systems	*Crepis capillaris* (hawksbeard)	Chromosomal damage	Micronucleus formation	–	0.5%	The highest dose tested (1%) was toxic	Dimitrov *et al.* (2006)

[a] +, positive; –, negative; (+) or (–) positive/negative in a study with limited quality

a.e., acid equivalent; AMPA, aminomethyl phosphonic acid; bw, body weight; ENA, erythrocytic nuclear abnormalities; Endo III, endonuclease III; FPG, formamidopyrimidine glycosylase; h, hour; HID, highest ineffective dose; LC_{50}, median lethal dose; LED, lowest effective dose; NOEC, no-observed effect concentration; p.o., oral; SMART, somatic mutation and recombination test

Table 4.6 Genetic and related effects of glyphosate and glyphosate-based formulations on non-mammalian systems in vitro

Phylogenetic class	Test system (species; strain)	End-point	Test	Results[a]		Concentration (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Glyphosate								
Eukaryote Fish	*Oreochromis niloticus* (Nile tilapia), erythrocytes	DNA damage	DNA strand breaks, comet assay	+	NT	7 µM [1.2 µg/mL]	Glyphosate isopropylamine, 96% $P \leq 0.001$; positive dose–response relationship for doses ≥ 7 µM	Alvarez-Moya *et al.* (2014)
Prokaryote (bacteria)	*Scytonema javanicum* (cyanobacteria)	DNA damage	DNA strand breaks, FADU assay	(+)	NT	10 µM [1.7 µg/mL] (in combination with UVB)	Co-exposure to glyphosate (not tested alone; single dose tested only) enhanced UVB-induced increases	Wang *et al.* (2012)
Prokaryote (bacteria)	*Anabaena spherica* (cyanobacteria)	DNA damage	DNA strand breaks, FADU assay	(+)	NT	10 µM [1.7 µg/mL] (in combination with UVB)	Co-exposure to glyphosate (not tested alone; single dose tested only) enhanced UVB-induced increases	Chen *et al.* (2012)
Prokaryote (bacteria)	*Microcystis viridis* (cyanobacteria)	DNA damage	DNA strand breaks, FADU assay	(+)	NT	10 µM [1.7 µg/mL] (in combination with UVB)	Co-exposure to glyphosate (not tested alone; single dose tested only) enhanced UVB-induced increases	Chen *et al.* (2012)
Prokaryote (bacteria)	*Bacillus B. subtilis*	Differential toxicity	Rec assay	–	NT	2000 µg/disk		Li & Long (1988)
Prokaryote (bacteria)	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA98 and TA100	Mutation	Reverse mutation	–	–	5000 µg/plate		Li & Long (1988)
Prokaryote (bacteria)	*Escherichia coli* WP2	Mutation	Reverse mutation	–	–	5000 µg/plate		Li & Long (1988)

Table 4.6 (continued)

Phylogenetic class	Test system (species; strain)	End-point	Test	Results[a]		Concentration (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Acellular systems	Prophage superhelical PM2 DNA	DNA damage	DNA strand breaks	(–)	NT	75 mM [12.7 mg/mL] (in combination with H_2O_2 (100 µM)	Glyphosate inhibited H_2O_2-induced damage of PM2 DNA at concentrations where synergism was observed in cellular DNA damage (data NR)	Lueken *et al.* (2004)
Glyphosate-based formulations								
Prokaryote (bacteria)	*Salmonella typhimurium* TA98	Mutation	Reverse mutation	+	–	360 µg/plate	Glyphosate isopropylammonium salt, 480 g/L	Rank *et al.* (1993)
Prokaryote (bacteria)	*Salmonella typhimirium* TA100	Mutation	Reverse mutation	–	+	720 µg/plate	Glyphosate isopropylammonium salt, 480 g/L	Rank *et al.* (1993)

[a] +, positive; –, negative; (+) or (–) positive/negative in a study with limited quality

FADU, fluorometric analysis of DNA unwinding; HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported; NT, not tested; UVB, ultraviolet B

Additionally, although all four glyphosate-based formulations dramatically reduced the transcription of ERα and ERβ in ERE-transfected HepG2 cells, glyphosate alone had no significant effect. Glyphosate and all four formulations reduced androgen-receptor transcription in the breast cancer cell line MDA-MB453-kb2, which has a high level of androgen receptor, with the formulations showing greater activity than glyphosate alone.

In a human placental cell line derived from choriocarcinoma (JEG3 cells), 18 hours of exposure to a glyphosate-based formulation (IC_{50} = 0.04%) decreased aromatase activity (Richard *et al.*, 2005). Glyphosate alone was without effect. The concentrations used did not affect cell viability.

Glyphosate, at non-overtly toxic concentrations, decreased aromatase activity in fresh human placental microsomes and transformed human embryonic kidney cells (293) transfected with human aromatase cDNA (Benachour *et al.*, 2007). A glyphosate-based formulation, at non-overtly toxic concentrations, had the same effect. The formulation was more active at equivalent doses than glyphosate alone.

In human androgen receptor and ERα and ERβ reporter gene assays using the Chinese hamster ovary cell line (CHO-K1), glyphosate had neither agonist nor antagonist activity (Kojima *et al.*, 2004, 2010).

(ii) Non-human mammalian experimental systems

In vivo

No data were available to the Working Group.

In vitro

Benachour *et al.* (2007) and Richard *et al.* (2005) reported that glyphosate and a glyphosate-based formulation inhibited aromatase activity in microsomes derived from equine testis. Richard *et al.* (2005) reported an absorbance spectrum consistent with an interaction between a nitrogen atom of glyphosate and the active site of the purified equine aromatase enzyme.

In the mouse MA-10 Leydig cell tumour cell line, a glyphosate-based formulation (glyphosate, 180 mg/L) markedly reduced [$(Bu)_2$] cAMP-stimulated progesterone production (Walsh *et al.*, 2000). The inhibition was dose-dependent, and occurred in the absence of toxicity or parallel reductions in total protein synthesis. In companion studies, the formulation also disrupted steroidogenic acute regulatory protein expression, which is critical for steroid hormone synthesis. Glyphosate alone did not affect steroidogenesis at any dose tested up to 100 µg/L. Forgacs *et al.* (2012) found that glyphosate (300 µM) had no effect on testosterone production in a novel murine Leydig cell line (BLTK1). Glyphosate did not modulate the effect of recombinant human chorionic gonadotropin, which served as the positive control for testosterone production.

(iii) Non-mammalian experimental systems

Gonadal tissue levels of testosterone, 17β-estradiol and total microsomal protein were significantly reduced in adult snails (*Biomphalaria alexandrina*) exposed for 3 weeks to a glyphosate-based formulation (glyphosate, 48%) at the LC_{10} (10% lethal concentration) (Omran & Salama, 2013). These effects persisted after a 2-week recovery period, although the impact on 17β-estradiol was reduced in the recovery animals. The formulation also induced marked degenerative changes in the ovotestis, including absence of almost all the gametogenesis stages. CYP450 1B1, measured by enzyme-linked immunosorbent assay (ELISA), was substantially increased in the treated snails, including after the recovery period.

Glyphosate (0.11 mg/L for 7 days) did not increase plasma vittelogenin levels in juvenile rainbow trout (Xie *et al.*, 2005).

(b) Other pathways

(i) Humans

Studies in exposed humans

No data were available to the Working Group.

Human cells in vitro

Glyphosate did not exhibit agonist activity in an assay for a human pregnane X receptor (PXR) reporter gene in a CHO-K1 cell line (Kojima *et al.*, 2010).

(ii) Non-human mammalian experimental systems

In vivo

In rats, glyphosate (300 mg/kg bw, 5 days per week, for 2 weeks) had no effect on the formation of peroxisomes, or the activity of hepatic carnitine acetyltransferase and catalase, and did not cause hypolipidaemia, suggesting that glyphosate does not have peroxisome proliferator-activated receptor activity (Vainio *et al.*, 1983).

In vitro

Glyphosate was not an agonist for mouse peroxisome proliferator-activated receptors PPARα or PPARγ in reporter gene assays using CV-1 monkey kidney cells in vitro (Kojima *et al.*, 2010). Glyphosate was also not an agonist for the aryl hydrocarbon receptor in mouse hepatoma Hepa1c1c7 cells stably transfected with a reporter plasmid containing copies of dioxin-responsive element (Takeuchi *et al.*, 2008).

(iii) Non-mammalian experimental systems

As a follow-up to experiments in which injection of glyphosate, or incubation with a glyphosate-based formulation (glyphosate, 48%), caused chick and frog (*Xenopus laevis*) cephalic and neural crest terata characteristic of retinoic acid signalling dysfunction, Paganelli *et al.*, (2010) measured retinoic acid activity in tadpoles exposed to a glyphosate-based formulation. Retinoic activity measured by a reporter gene assay was increased by the formulation, and a retinoic acid antagonist blocked the effect. This indicated a possible significant modulation of retinoic acid activity by glyphosate.

4.2.3 Oxidative stress, inflammation, and immunosuppression

(a) Oxidative stress

(i) Humans

Studies in exposed humans

No data were available to the Working Group.

Human cells in vitro

Several studies examined the effects of glyphosate on oxidative stress parameters in the human keratinocyte cell line HaCaT. Gehin *et al.* (2005) found that a glyphosate-based formulation was cytotoxic to HaCaT cells, but that addition of antioxidants reduced cytotoxicity. Elie-Caille *et al.* (2010) showed that incubation of HaCaT cells with glyphosate at 21 mM (the half maximal inhibitory concentration for cytotoxicity, IC_{50}) for 18 hours increased production of hydrogen peroxide (H_2O_2) as shown by dichlorodihydrofluorescein diacetate assay. Similarly, George & Shukla (2013) exposed HaCaT cells to a glyphosate-based formulation (glyphosate, 41%; concentration, up to 0.1 mM) and evaluated oxidative stress using the dichlorodihydrofluorescein diacetate assay. The formulation (0.1 mM) increased maximum oxidant levels by approximately 90% compared with vehicle, an effect similar to that of H_2O_2 (100 mM). Pre-treatment of the cells with the antioxidant *N*-acetylcysteine abrogated generation of oxidants by both the formulation and by H_2O_2. *N*-Acetylcysteine also inhibited cell proliferation induced by the glyphosate-based formulation (0.1 mM). [The Working Group noted the recognized limitations of using dichlorodihydrofluorescein diacetate as a marker of oxidative stress (Bonini *et al.*, 2006; Kalyanaraman *et al.*, 2012),

and that the studies that reported this end-point as the sole evidence for oxidative stress should thus be interpreted with caution.]

Chaufan *et al.* (2014) evaluated the effects of glyphosate, AMPA (the main metabolite of glyphosate), and a glyphosate-based formulation on oxidative stress in HepG2 cells. The formulation, but not glyphosate or AMPA, had adverse effects. Specifically, the formulation increased levels of reactive oxygen species, nitrotyrosine formation, superoxide dismutase activity, and glutathione, but did not have an effect on catalase or glutathione-*S*-transferase activities. Coalova *et al.* (2014) exposed Hep2 cells to a glyphosate-based formulation (glyphosate as isopropylamine salt, 48%) at the LC_{20} (concentration not otherwise specified) and evaluated various parameters of oxidative stress. Exposure to the formulation for 24 hours increased catalase activity and glutathione levels, but did not have an effect on superoxide dismutase or glutathione-*S*-transferase activity.

Using blood samples from non-smoking male donors, Mladinic *et al.* (2009b) examined the effects of in-vitro exposure to glyphosate on oxidative DNA damage in primary lymphocyte cultures and on lipid peroxidation in plasma. Both parameters were significantly elevated at glyphosate concentrations of 580 µg/mL (~3.4 mM), but not at lower concentrations. Kwiatkowska *et al.* (2014) examined the effects of glyphosate, its metabolite AMPA, and *N*-methylglyphosate (among other related compounds) in human erythrocytes isolated from healthy donors. The erythrocytes were exposed at concentrations of 0.01–5 mM for 1, 4, or 24 hours before flow cytometric measurement of the production of reactive oxygen species with dihydrorhodamine 123. Production of reactive oxygen species was increased by glyphosate (≥ 0.25 mM), AMPA (≥ 0.25 mM), and *N*-methylglyphosate (≥ 0.5 mM).

(ii) Non-human mammalian experimental systems

Most of the studies of oxidative stress and glyphosate were conducted in rats and mice, and examined a range of exposure durations, doses, preparations (glyphosate and glyphosate-based formulations), administration routes and tissues. In addition, various end-points were evaluated to determine whether oxidative stress is induced by exposure to glyphosate. Specifically, it was found that glyphosate induces production of free radicals and oxidative stress in mouse and rat tissues through alteration of antioxidant enzyme activity, depletion of glutathione, and increases in lipid peroxidation. Increases in biomarkers of oxidative stress upon exposure to glyphosate in vivo have been observed in blood plasma (Astiz *et al.*, 2009b), liver (Bolognesi *et al.*, 1997; Astiz *et al.*, 2009b), skin (George *et al.*, 2010), kidney (Bolognesi *et al.*, 1997; Astiz *et al.*, 2009b), and brain (Astiz *et al.*, 2009b). Several studies demonstrated similar effects with a glyphosate-based formulation in the liver (Bolognesi *et al.*, 1997; Cavuşoğlu *et al.*, 2011; Jasper *et al.*, 2012), kidney (Bolognesi *et al.*, 1997; Cavuşoğlu *et al.*, 2011) and brain (Cattani *et al.*, 2014), or with a pesticide mixture containing glyphosate in the testes (Astiz *et al.*, 2013). Pre-treatment with antioxidants has been shown to mitigate the induction of oxidative stress by a glyphosate-based formulation (Cavuşoğlu *et al.*, 2011) and by a pesticide mixture containing glyphosate (Astiz *et al.*, 2013).

DNA damage associated with oxidative stress after exposure to glyphosate (e.g. as reported in Bolognesi *et al.*, 1997) is reviewed in Section 4.2.1.

(iii) Non-mammalian experimental systems

Positive associations between exposure to glyphosate and oxidative stress were reported in various tissues in aquatic organisms (reviewed in Slaninova *et al.*, 2009). Glyphosate and various glyphosate-based formulations have been tested in various fish species for effects on a plethora of end-points (e.g. lipid peroxidation, DNA

damage, expression of antioxidant enzymes, levels of glutathione), consistently presenting evidence that glyphosate can cause oxidative stress in fish (Lushchak *et al.*, 2009; Ferreira *et al.*, 2010; Guilherme *et al.*, 2010, 2012a, b, 2014a, b; Modesto & Martinez, 2010a, b; Cattaneo *et al.*, 2011; Glusczak *et al.*, 2011; de Menezes *et al.*, 2011; Ortiz-Ordoñez *et al.*, 2011; Nwani *et al.*, 2013; Marques *et al.*, 2014, 2015; Sinhorin *et al.*, 2014; Uren Webster *et al.*, 2014). Similar effects were observed in bullfrog tadpoles exposed to a glyphosate-based formulation (Costa *et al.*, 2008), and in the Pacific oyster exposed to a pesticide mixture containing glyphosate (Geret *et al.*, 2013).

(b) Inflammation and immunomodulation

(i) Humans

Studies in exposed humans

No data were available to the Working Group.

Human cells in vitro

Nakashima *et al.* (2002) investigated the effects of glyphosate on cytokine production in human peripheral blood mononuclear cells. Glyphosate (1 mM) had a slight inhibitory effect on cell proliferation, and modestly inhibited the production of IFN-gamma and IL-2. The production of TNF-α and IL-1 β was not affected by glyphosate at concentrations that significantly inhibited proliferative activity and T-cell-derived cytokine production.

(ii) Non-human mammalian experimental systems

Kumar *et al.* (2014) studied the pro-inflammatory effects of glyphosate and farm air samples in wildtype C57BL/6 and TLR4$^{-/-}$ mice, evaluating cellular response, humoral response, and lung function. In the bronchoalveolar lavage fluid and lung digests, airway exposure to glyphosate (1 or 100 µg) significantly increased the total cell count, eosinophils, neutrophils, and IgG1 and IgG2a levels. Airway exposure to glyphosate (100 ng, 1 µg, or 100 µg per day for 7 days) also produced substantial pulmonary inflammation, confirmed by histological examination. In addition, glyphosate-rich farm-air samples significantly increased circulating levels of IL-5, IL-10, IL-13 and IL-4 in wildtype and in TLR4$^{-/-}$ mice. Glyphosate was also tested in wildtype mice and significantly increased levels of IL-5, IL-10, IL-13, and IFN-γ (but not IL-4). The glyphosate-induced pro-inflammatory effects were similar to those induced by ovalbumin, and there were no additional or synergistic effects when ovalbumin was co-administered with glyphosate.

Pathological effects of glyphosate on the immune system have been reported in 13-week rat and mouse feeding studies by the NTP (Chan & Mahler, 1992). Relative thymus weight was decreased in male rats exposed for 13 weeks, but increased in male mice. Treatment-related changes in haematological parameters were observed in male rats at 13 weeks and included mild increases in haematocrit [erythrocyte volume fraction] and erythrocytes at 12 500, 25 000, and 50 000 ppm, haemoglobin at 25 000 and 50 000 ppm, and platelets at 50 000 ppm. In female rats, small but significant increases occurred in lymphocyte and platelet counts, leukocytes, mean corpuscular haemoglobin, and mean corpuscular volume at 13 weeks.

Blakley (1997) studied the humoral immune response in female CD-1 mice given drinking-water containing a glyphosate-based formulation at concentrations up to 1.05% for 26 days. The mice were inoculated with sheep erythrocytes to produce a T-lymphocyte, macrophage-dependent antibody response on day 21 of exposure. Antibody production was not affected by the formulation.

(iii) Non-mammalian experimental systems

A positive association between exposure to glyphosate and immunotoxicity in fish has been reported. Kreutz *et al.* (2011) reported alterations

in haematological and immune-system parameters in silver catfish (*Rhamdia quelen*) exposed to sublethal concentrations (10% of the median lethal dose, LC_{50}, at 96 hours) of a glyphosate-based herbicide. Numbers of blood erythrocytes, thrombocytes, lymphocytes, and total leukocytes were significantly reduced after 96 hours of exposure, while the number of immature circulating cells was increased. The phagocytic index, serum bacteria agglutination, and total peroxidase activity were significantly reduced after 24 hours of exposure. Significant decreases in serum bacteria agglutination and lysozyme activity were found after 10 days of exposure. No effect on serum bactericidal and complement natural haemolytic activity was seen after 24 hours or 10 days of exposure to glyphosate.

el-Gendy *et al.* (1998) demonstrated effects of a glyphosate-based formulation (glyphosate, 48%) at 1/1000 of the concentration recommended for field application on humoral and cellular immune response in bolti fish (*Tilapia nilotica*). The mitogenic responses of splenocytes to phytohaemagglutinin, concanavalin A, and lipopolysaccharide in fish exposed to glyphosate for 96 hours were gradually decreased and reached maximum depression after 4 weeks. Glyphosate also produced a concentration-dependent suppression of in-vitro plaque-forming cells in response to sheep erythrocytes.

4.2.4 Cell proliferation and death

(a) Humans

(i) Studies in exposed humans

No data were available to the Working Group.

(ii) Human cells in vitro

Cell proliferation potential was explored in HaCaT keratinocytes exposed to a glyphosate-based formulation (glyphosate, 41%; concentration, up to 0.1 mM) (George & Shukla, 2013). The formulation increased the number of viable cells, as assessed by the MTT assay (based on reduction of the dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) at concentrations up to 0.1 mM, while concentration- and incubation-time-dependent reductions were seen at higher concentrations (up to 1 mM). The formulation (0.01 or 0.1 mM for 72 hours) significantly enhanced cell proliferation (measured by staining for either proliferating cell nuclear antigen or 5-bromo-2′-deoxyuridine); at 0.1 mM, the increases exceeded levels for the positive control, tetradecanoyl-phorbol-13-acetate. The proportion of S-phase cells (assessed using flow cytometry) and the expression of G1/S cell-cycle regulatory proteins (cyclins D1 and E, CDK2, CDK4, and CDK6) increased after exposure to the formulation or the positive control.

Li *et al.* (2013) reported that glyphosate and AMPA inhibited cell growth in eight human cancer cell lines, but not in two immortalized normal prostate cell lines. An ovarian (OVCAR-3) and a prostate (C4–2B) cell line showed the greatest loss in viability, with glyphosate or AMPA at 15–50 mM. Further assays were conducted on AMPA, but not glyphosate, in two prostate cancer cell lines (C4–2B and PC-3), and found cell-cycle arrest (decreased entry of cells into S-phase) and increased apoptosis. [The Working Group noted that the findings from these assays with AMPA are of unclear relevance to the effects of glyphosate.]

Glyphosate (10^{-6} to 1 μM) increased growth by 15–30% relative to controls in hormone-dependent T47D breast cancer cells, but only when endogenous estrogen was minimized in the culture medium (by substitution with 10% dextran-charcoal treated fetal bovine serum). Glyphosate did not affect the growth of hormone-independent MDA-MB231 breast cancer cells cultured in either medium (Thongprakaisang *et al.*, 2013).

Glyphosate (up to 30 μM) did not show cell proliferation potential (5-bromo-2′-deoxyuridine) and did not activate caspase 3 or TP53 in human neuroprogenitor ReN CX cells (Culbreth *et al.*, 2012).

Several studies evaluated the impact of glyphosate or glyphosate-based formulations on apoptotic cell death in the HepG2 human hepatoma cell line. Glyphosate-based formulations induced apoptosis in HepG2 cells, while glyphosate alone was generally without effect or showed effects at considerably higher concentrations (Gasnier *et al.*, 2009, 2010; Mesnage *et al.*, 2013; Chaufan *et al.*, 2014; Coalova *et al.*, 2014). For example, 23.5% of the nuclei of HepG2 cells exposed to a glyphosate-based formulation showed condensed and fragmented chromatin ($P < 0.01$), and caspases 3 and 7 were significantly activated, both effects being indicative of apoptosis (Chaufan *et al.*, 2014). Caspases were unaffected by glyphosate or AMPA alone. Glyphosate and AMPA did not affect cell viability at concentrations up to 1000 mg/L, a concentration that increased rather than decreased cell viability after 48 and 72 hours of incubation. In contrast, cells exposed to glyphosate-based formulation at lower concentrations were not viable. Similarly, Coalova *et al.* (2014) reported that a glyphosate-based formulation (glyphosate, 48%) induced apoptotic cell death in HepG2 cells. Apoptosis was indicated by activation of caspases 3 and 7, and the significant fraction (17.7%) of nuclei with condensed and fragmented chromatin ($P < 0.001$).

In studies with glyphosate and nine different glyphosate-based formulations in three cell lines, glyphosate alone did not increase the activity of adenylate kinase (Mesnage *et al.*, 2013). The activity of caspases 3 and 7 was significantly increased by glyphosate in HepG2 and embryonic kidney HEK293 cells, and elevated (although not significantly) about 1.8 times above control levels in placental choriocarcinoma JEG-3 cells. Two formulations containing an ethoxylated adjuvant induced adenylate kinase activity to a greater extent than caspase activity. All formulations were reported to be more cytotoxic than glyphosate. [In concentration–response curves, glyphosate showed an effect on mitochondrial succinate dehydrogenase activity, a measure of cell viability, that was similar to that shown by one formulation. The calculated 50% lethal concentration in JEG3 cells for mitochondrial succinate dehydrogenase activity was greater for three formulations, although the values appeared inconsistent with the concentration–response curves.]

In HUVEC primary neonate umbilical cord vein cells, and 293 embryonic kidney and JEG3 placental cell lines, Benachour & Séralini (2009) found that glyphosate at relatively high concentrations induced apoptosis, as indicated by induction of caspases 3 and 7, and DNA staining and microscopy. At comparable or lower concentrations, four glyphosate-based formulations all caused primarily necrotic cell death. The umbilical cord HUVEC cells were the most sensitive (by about 100-fold) to the apoptotic effects of glyphosate.

Heu *et al.* (2012) evaluated apoptosis in immortalized human keratinocytes (HaCaT) exposed to glyphosate (5–70 mM). Based on annexin V, propidium iodide and mitochondrial staining, exposures leading to 15% cytotoxicity gave evidence of early apoptosis, while increases in late apoptosis and necrosis were observed at higher levels of cytotoxicity.

(b) Non-human mammalian experimental systems

(i) In vivo

In male Wistar rats, glyphosate (10 mg/kg bw, injected intraperitoneally three times per week for 5 weeks) reduced, but not significantly, the inner mitochondrial membrane integrity of the substantia nigra and cerebral cortex (Astiz *et al.* 2009a). Caspase 3 activity was unaltered in these tissues. Mitochondrial cardiolipin content was significantly reduced, particularly in the substantia nigra, where calpain activity was substantially higher. Glyphosate induced DNA fragmentation in the brain and liver.

(ii) In vitro

In adult Sprague Dawley rat testicular cells exposed in vitro, glyphosate (up to 1%; for 24 or 48 hours) did not provoke cell-membrane alterations (Clair *et al.*, 2012). However, caspase 3 and 7 activity increased with exposure in Sertoli cells alone, and in Sertoli and germ cell mixtures. On the other hand, a glyphosate-based formulation (a 0.1% solution, containing 0.36 g/L of glyphosate) induced membrane alterations and decreased the activity of caspase 3 and 7 in Leydig cells, and in Sertoli and germ cell mixtures. In a separate study, glyphosate increased apoptosis in primary Sertoli cell cultures from mice (Zhao *et al.*, 2013).

Glyphosate (5–40 mM, for 12, 24, 48, or 72 hours) significantly increased cell death in a time- and concentration-dependent manner in differentiated rat pheochromocytoma PC12 (neuronal) cells Gui *et al.* (2012). Apoptotic changes included cell shrinkage, DNA fragmentation, decreased Bcl2 expression, and increased Bax expression. Both autophagy and apoptosis were implicated, as pre-treatment with the pan-caspase inhibitor Z-VAD or the autophagy inhibitor 3-MA inhibited cell loss.

Induction of apoptosis by glyphosate or glyphosate-based formulations was also studied in other cell lines. Glyphosate (10 μM) induced apoptosis in rat heart H9c2 cells, the effect being enhanced when glyphosate was given in combination with the adjuvant TN-20 (5 μM), (Kim *et al.*, 2013). A glyphosate-based formulation induced apoptosis in mouse 3T3-L1 fibroblasts, and inhibited their transformation to adipocytes (Martini *et al.*, 2012). A glyphosate-based formulation (10 mM) did not increase rat hepatoma HTC cell death, but did affect mitochondrial membrane potential (Malatesta *et al.*, 2008).

Glyphosate (up to 30 μM) did not activate caspase 3 or show cell proliferation potential (5-bromo-2′-deoxyuridine) in a mouse neuroprogenitor cell line, but did activate Tp53 at the highest concentration tested (Culbreth *et al.*, 2012).

4.2.5 Other mechanisms

No data on immortalization, epigenetic alterations, altered DNA repair, or genomic instability after exposure to glyphosate were available to the Working Group.

4.3 Data relevant to comparisons across agents and end-points

No data on high-throughput screening or other relevant data were available to the Working Group. Glyphosate was not tested by the Tox21 and ToxCast research programmes of the government of the USA (Kavlock *et al.* 2012; Tice *et al.*, 2013).

4.4 Cancer susceptibility data

No studies that examined genetic, life-stage, or other susceptibility factors with respect to adverse health outcomes that could be associated with exposure to glyphosate were identified by the Working Group.

4.5 Other adverse effects

4.5.1 Humans

In the USA in the past decade, poison-control centres have reported more than 4000 exposures to glyphosate-containing herbicides, of which several hundred were evaluated in a health-care facility, and fatalities were rare (Rumack, 2015). In a pesticide surveillance study carried out by the National Poisons Information Service of the United Kingdom, glyphosate was among the most common pesticide exposure implicated in severe or fatal poisoning cases between 2004 and 2013 (Perry *et al.*, 2014). Deliberate poisonings with glyphosate resulting in toxicity and fatality

have been reported in many countries, including Australia (Stella & Ryan, 2004), Denmark (Mortensen *et al.*, 2000), India (Mahendrakar *et al.*, 2014), Japan (Motojyuku *et al.*, 2008), Republic of Korea (Park *et al.*, 2013), New Zealand (Temple & Smith, 1992), Sri Lanka (Roberts *et al.*, 2010), Taiwan, China (Chen *et al.*, 2009), and Thailand (Sribanditmongkol *et al.*, 2012).

Glyphosate demonstrated no potential for photo-irritation or photo-sensitization in 346 volunteers exposed dermally on normal or abraded skin (Hayes & Laws, 1991). On the other hand, Mariager *et al.* (2013) reported severe burns after prolonged accidental dermal exposure to a glyphosate-based formulation.

4.5.2 Experimental systems

Glyphosate was tested in nine regulatory submissions included in the Toxicity Reference Database (ToxRefDB) and reviewed by the EPA (EPA, 2015). Specifically, study design, treatment group, and treatment-related effect information were captured for four long-term studies and/or carcinogenicity studies, one short-term study, two multigeneration studies of reproductivity, and two studies of developmental toxicity. The NTP also tested glyphosate in a 13-week study in rats and mice (Chan & Mahler, 1992).

In a long-term combined study of toxicity and carcinogenicity in rats given glyphosate at nominal doses of 100, 400, and 1000 mg/kg bw per day, inflammation was observed in the stomach mucosa of females at the intermediate and highest doses (EPA, 1990, 1991b). In males at the highest dose, liver weight, cataracts and lens degeneration in the eyes, and urine specific gravity were increased, while body weight, body-weight gain, and urinary pH were decreased. Pancreatic acinar cell atrophy was observed in males at the highest dose. Pancreatic inflammation was also observed in male rats at the highest dose in a short-term study (nominal doses of 50, 250, and 1000 mg/kg bw per day) (EPA, 1987). In the study by the NTP, cytoplasmic alteration was observed in the parotid and submandibular salivary glands of rats (Chan & Mahler, 1992).

In a study of carcinogenicity in mice given glyphosate at doses of 150, 1500, or 4500 mg/kg bw per day, liver hypertrophy and necrosis were observed in males at the highest dose (EPA, 1983). Other effects in males at the highest dose included increased testes weight, interstitial nephritis, and decreased body weight. In females at the highest dose, ovary weights were increased, proximal tubule epithelial basophilia and hypertrophy was observed, and body weights were decreased. In the study by the NTP, cytoplasmic alteration was observed in the parotid salivary glands in mice (Chan & Mahler, 1992).

Developmental and reproductive toxicity

In a study of developmental toxicity in rats given glyphosate at a dose of 300, 1000, or 3500 mg/kg bw per day, reduced implantation rates and fewer live fetuses were observed in dams at the highest dose (EPA, 1980b). In fetuses at the highest dose, unossified sternebra were observed and fetal weight was reduced.

5. Summary of Data Reported

5.1 Exposure data

Glyphosate is a broad-spectrum herbicide that is effective at killing or suppressing all plant types, including grasses, perennials, and woody plants. The herbicidal activity of glyphosate was discovered in 1970 and since then its use has increased to a point where it is now the most heavily used herbicide in the world, with an annual global production volume in 2012 of more than 700 000 tonnes used in more than 750 different products. Changes in farming practice and the development of genetically modified crops that are resistant to glyphosate have contributed to the increase in use.

There is little information available on occupational or community exposure to glyphosate. Glyphosate can be found in soil, air, surface water and groundwater, as well as in food. It has been detected in air during agricultural herbicide-spraying operations. Glyphosate was detected in urine in two studies of farmers in the USA, in urban populations in Europe, and in a rural population living near areas sprayed for drug eradication in Columbia. However, urinary concentrations were mostly below the limit of detection in several earlier studies of forestry workers who sprayed glyphosate. Exposure of the general population occurs mainly through diet.

5.2 Human carcinogenicity data

In its evaluation of the epidemiological studies reporting on cancer risks associated with exposure to glyphosate, the Working Group identified seven reports from the Agricultural Health Study (AHS) cohort and several reports from case–control studies. The AHS cohort, the pooled analyses of the case–control studies in the midwest USA, and the cross-Canada study were considered key investigations because of their relatively large size. Reports from two or more independent studies were available for non-Hodgkin lymphoma (NHL), multiple myeloma, Hodgkin lymphoma, glioma, and prostate. For the other cancer sites, results from only one study were available for evaluation.

5.2.1 NHL and other haematopoietic cancers

Two large case–control studies of NHL from Canada and the USA, and two case–control studies from Sweden reported statistically significant increased risks of NHL in association with exposure to glyphosate. For the study in Canada, the association was seen among those with more than 2 days/year of exposure, but no adjustment for other pesticides was done. The other three studies reported excesses for NHL associated with exposure to glyphosate, after adjustment for other pesticides (reported odds ratio were 2.1 (95% CI, 1.1–4.0); 1.85 (95% CI, 0.55–6.2); and 1.51 (95% CI, 0.77–2.94). Subtype-specific analyses in a Swedish case–control study indicated positive associations for total NHL, as well as all subtypes, but this association was statistically significant only for the subgroup of lymphocytic lymphoma/chronic lymphocytic leukaemia (OR, 3.35; 95% CI, 1.42–7.89). An elevated risk (OR, 3.1; 95% CI, 0.6–17.1) was also found for B-cell lymphoma in an European study based on few cases. One hospital-based case–control study from France did not find an association between exposure to glyphosate and NHL (OR, 1.0; 95% CI, 0.5–2.2) based on few exposed cases.

A roughly twofold excess of multiple myeloma, a subtype of NHL, was reported in three studies: only among the highest category of glyphosate use (> 2 days/year) in the large Canadian case–control study, in a case–control study from Iowa, USA, and in a French case–control study (all not statistically significant). These three studies did not adjust for the effect of other pesticides. In the AHS, there was no association with NHL (OR, 1.1; 0.7–1.9). For multiple myeloma, relative risk was 1.1 (95% CI, 0.5–2.4) when adjusted for age only; but was 2.6 (95% CI, 0.7–9.4) when adjusted for multiple confounders. No excess in leukaemia was observed in a case–control study in Iowa and Minnesota, USA, or in the AHS.

In summary, case–control studies in the USA, Canada, and Sweden reported increased risks for NHL associated with exposure to glyphosate. The increased risk persisted in the studies that adjusted for exposure to other pesticides. The AHS cohort did not show an excess of NHL. The Working Group noted that there were excesses reported for multiple myeloma in three studies; however, they did not weight this evidence as strongly as that of NHL because of the possibility that chance could not be excluded; none of the

risk estimates were statistically significant nor were they adjusted for other pesticide exposures.

5.2.2. Other cancer sites

No association of glyphosate with cancer of the brain in adults was found in the Upper Midwest Health case–control study. No associations in single case–control studies were found for cancers of the oesophagus and stomach, prostate, and soft-tissue sarcoma. For all other cancer sites (lung, oral cavity, colorectal, pancreas, kidney, bladder, breast, prostate, melanoma) investigated in the large AHS, no association with exposure to glyphosate was found.

5.3 Animal carcinogenicity data

Glyphosate was tested for carcinogenicity in male and female mice by dietary administration in two studies, and in male and female rats by dietary administration in five studies and in drinking-water in one study. A glyphosate-based formulation was also tested in drinking-water in one study in male and female rats, and by skin application in one initiation–promotion study in male mice.

There was a positive trend in the incidence of renal tubule carcinoma and of renal tubule adenoma or carcinoma (combined) in males in one feeding study in CD-1 mice. Renal tubule carcinoma is a rare tumour in this strain of mice. No significant increase in tumour incidence was seen in female mice in this study. In the second feeding study, there was a significant positive trend in the incidence of haemangiosarcoma in male CD-1 mice. No significant increase in tumour incidence was seen in female mice in this study.

For the five feeding studies in rats, two studies in the Sprague-Dawley strain showed a significant increase in the incidence of pancreatic islet cell adenoma in males – one of these two studies also showed a significant positive trend in the incidences of hepatocellular adenoma in males and of thyroid C-cell adenoma in females. Two studies (one in Sprague-Dawley rats, one in Wistar rats) found no significant increase in tumour incidence at any site. One study in Wistar rats was inadequate for the evaluation because of the short duration of exposure.

In the study in Wistar rats given drinking-water containing glyphosate, there was no significant increase in tumour incidence.

A glyphosate-based formulation was found to be a skin-tumour promoter in the initiation–promotion study in male Swiss mice. The study of a glyphosate-based formulation in drinking-water in Sprague-Dawley rats was inadequate for the evaluation because of the small number of animals per group, and the limited information provided on tumour histopathology and incidence in individual animals. These studies of a chemical mixture containing glyphosate were considered inadequate to evaluate the carcinogenicity of glyphosate alone.

5.4. Other relevant data

Direct data on absorption of glyphosate in humans were not available to the Working Group. Glyphosate was detected in the urine of agricultural workers in several studies, and in the blood of poisoning cases, indicative of absorption. Some evidence for absorption through human skin (~2%) was reported in studies in vitro. The minor role of dermal absorption was also shown in a study in non-human primate model in vivo. However, no study examined the rates of absorption in humans. In rodents, several studies showed up to 40% absorption after oral administration of a single or repeated dose.

Glyphosate was measured in human blood. No data on parenchymal tissue distribution for glyphosate in humans were available to the Working Group. In rats given glyphosate by oral administration, concentrations in tissues had the following rank order: kidneys > spleen > fat > liver. Repeated administration had no effect

on the distribution of glyphosate. In a study in rats, the half-life of glyphosate in plasma was estimated to be more than 1 day, indicating that glyphosate is not rapidly eliminated.

In the environment, glyphosate is degraded by soil microbes, primarily to aminomethylphosphonic acid (AMPA) and carbon dioxide. Glyphosate is not efficiently metabolized in humans or other mammals. In rats, small amounts of AMPA were detected in the plasma and in the colon, with the latter being attributed to intestinal microbial metabolism. In humans, small amounts of AMPA are detectable in blood in cases of deliberate glyphosate poisoning. Few studies examined the possible effects of glyphosate-based formulations on metabolizing enzymes, but no firm conclusions could be drawn from these studies.

Studies in rodents showed that systemically absorbed glyphosate is excreted unchanged into the urine, and that the greatest amount is excreted in the faeces, indicating poor absorption. Glyphosate was detected in the urine of humans who were exposed occupationally to glyphosate. AMPA has also been detected in human urine.

Glyphosate is not electrophilic.

A large number of studies examined a wide range of end-points relevant to genotoxicity with glyphosate alone, glyphosate-based formulations, and AMPA.

There is strong evidence that glyphosate causes genotoxicity. The evidence base includes studies that gave largely positive results in human cells in vitro, in mammalian model systems in vivo and in vitro, and studies in other non-mammalian organisms. In-vivo studies in mammals gave generally positive results in the liver, with mixed results for the kidney and bone marrow. The end-points that have been evaluated in these studies comprise biomarkers of DNA adducts and various types of chromosomal damage. Tests in bacterial assays gave consistently negative results.

The evidence for genotoxicity caused by glyphosate-based formulations is strong. There were three studies of genotoxicity end-points in community residents exposed to glyphosate-based formulations, two of which reported positive associations. One of these studies examined chromosomal damage (micronucleus formation) in circulating blood cells before and after aerial spraying with glyphosate-based formulations and found a significant increase in micronucleus formation after exposure in three out of four different geographical areas. Additional evidence came from studies that gave largely positive results in human cells in vitro, in mammalian model systems in vivo and in vitro, and studies in other non-mammalian organisms. The end-points that were evaluated in these studies comprised biomarkers of DNA adducts and various types of chromosomal damage. The pattern of tissue specificity of genotoxicity end-points observed with glyphosate-based formulations is similar to that observed with glyphosate alone. Tests in bacterial assays gave generally negative results.

For AMPA, the evidence for genotoxicity is moderate. While the number of studies that examined the effects of AMPA was not large, all of the studies gave positive results. Specifically, genotoxicity was reported in a study in humans in vitro, a study in mammals in vivo, a study in mammals in vitro, and one study in eels in vivo.

Strong evidence exists that glyphosate, AMPA, and glyphosate-based formulations can induce oxidative stress. Evidence came from studies in many rodent tissues in vivo, and human cells in vitro. In some of these studies, the mechanism was challenged by co-administration of antioxidants and observed amelioration of the effects. Similar findings have been reported in fish and other aquatic species. Various end-points (e.g. lipid peroxidation markers, oxidative DNA adducts, dysregulation of antioxidant enzymes) have been evaluated in numerous studies. This

increased the confidence of the Working Group in the overall database.

There is weak evidence that glyphosate or glyphosate-based formulations induce receptor-mediated effects. In multiple experiments, glyphosate-based formulations affected aromatase activity; glyphosate was active in a few of these studies. Some activity in other nuclear receptor-mediated pathways has been observed for glyphosate or glyphosate-based formulations. In one series of experiments, glyphosate was not found to be a ligand to several receptors and related proteins (aryl hydrocarbon receptor, peroxisome proliferator-activated receptors, pregnane X receptor).

There is weak evidence that glyphosate may affect cell proliferation or death. Several studies in human and rodent cell lines have reported cytotoxicity and cell death, the latter attributed to the apoptosis pathway. Studies that examined the effects of glyphosate alone or a glyphosate-based formulation found that glyphosate alone had no effect, or a weaker effect than the formulation.

There is weak evidence that glyphosate may affect the immune system, both the humoral and cellular response, upon long-term treatment in rodents. Several studies in fish, with glyphosate or its formulations, also reported immunosuppressive effects.

With regard to the other key characteristics of human carcinogens (IARC, 2014), the Working Group considered that the data were too few for an evaluation to be made.

Severe or fatal human poisoning cases have been documented worldwide. In rodents, organ and systemic toxicity from exposures to glyphosate are demonstrated by liver-weight effects and necrosis in animals at high doses. Additionally, effects on the pancreas, testes, kidney and ovaries, as well as reduced implantations and unossified sternebra were seen at similar doses.

No data on cancer-related susceptibility after exposure to glyphosate were available to the Working Group.

Overall, the mechanistic data provide strong evidence for genotoxicity and oxidative stress. There is evidence that these effects can operate in humans.

6. Evaluation

6.1 Cancer in humans

There is *limited evidence* in humans for the carcinogenicity of glyphosate. A positive association has been observed for non-Hodgkin lymphoma.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of glyphosate.

6.3 Overall evaluation

Glyphosate is *probably carcinogenic to humans (Group 2A).*

6.4 Rationale

In making this overall evaluation, the Working Group noted that the mechanistic and other relevant data support the classification of glyphosate in Group 2A.

In addition to limited evidence for the carcinogenicity of glyphosate in humans and sufficient evidence for the carcinogenicity of glyphosate in experimental animals, there is strong evidence that glyphosate can operate through two key characteristics of known human carcinogens, and that these can be operative in humans. Specifically:

- There is strong evidence that exposure to glyphosate or glyphosate-based formulations is genotoxic based on studies in humans in vitro and studies in experimental animals.

One study in several communities in individuals exposed to glyphosate-based formulations also found chromosomal damage in blood cells; in this study, markers of chromosomal damage (micronucleus formation) were significantly greater after exposure than before exposure in the same individuals.

- There is strong evidence that glyphosate, glyphosate-based formulations, and aminomethylphosphonic acid can act to induce oxidative stress based on studies in experimental animals, and in studies in humans in vitro. This mechanism has been challenged experimentally by administering antioxidants, which abrogated the effects of glyphosate on oxidative stress. Studies in aquatic species provide additional evidence for glyphosate-induced oxidative stress.

References

Abraxis (2005). Glyphosate Plate Kit Part No. 500086. Warminster (PA): Abraxis, LLC. Available from: http://www.abraxiskits.com/uploads/products/docfiles/184_PN500086USER.pdf, accessed 28 July 2015.

Acquavella JF, Alexander BH, Mandel JS, Gustin C, Baker B, Chapman P *et al.* (2004). Glyphosate biomonitoring for farmers and their families: results from the Farm Family Exposure Study. *Environ Health Perspect*, 112(3):321–6. doi:10.1289/ehp.6667 PMID:14998747

Akcha F, Spagnol C, Rouxel J (2012). Genotoxicity of diuron and glyphosate in oyster spermatozoa and embryos. *Aquat Toxicol*, 106–107:104–13. doi:10.1016/j.aquatox.2011.10.018 PMID:22115909

Alavanja MC, Samanic C, Dosemeci M, Lubin J, Tarone R, Lynch CF *et al.* (2003). Use of agricultural pesticides and prostate cancer risk in the Agricultural Health Study cohort. *Am J Epidemiol*, 157(9):800–14. doi:10.1093/aje/kwg040 PMID:12727674

Alavanja MC, Sandler DP, McMaster SB, Zahm SH, McDonnell CJ, Lynch CF *et al.* (1996). The Agricultural Health Study. *Environ Health Perspect*, 104(4):362–9. doi:10.1289/ehp.96104362 PMID:8732939

Alvarez-Moya C, Silva MR, Arámbula AR, Sandoval AI, Vasquez HC, González Montes RM (2011). Evaluation of genetic damage induced by glyphosate isopropylamine salt using *Tradescantia* bioassays. *Genet Mol Biol*, 34(1):127–30. doi:10.1590/S1415-47572010005000108 PMID:21637555

Alvarez-Moya C, Silva MR, Ramírez CV, Gallardo DG, Sánchez RL, Aguirre AC *et al.* (2014). Comparison of the *in vivo* and *in vitro* genotoxicity of glyphosate isopropylamine salt in three different organisms. *Genet Mol Biol*, 37(1):105–10. doi:10.1590/S1415-47572014000100016 PMID:24688297

Andreotti G, Freeman LE, Hou L, Coble J, Rusiecki J, Hoppin JA *et al.* (2009). Agricultural pesticide use and pancreatic cancer risk in the Agricultural Health Study Cohort. *Int J Cancer*, 124(10):2495–500. doi:10.1002/ijc.24185 PMID:19142867

Aris A, Leblanc S (2011). Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. *Reprod Toxicol*, 31(4):528–33. doi:10.1016/j.reprotox.2011.02.004 PMID:21338670

Astiz M, de Alaniz MJ, Marra CA (2009a). Effect of pesticides on cell survival in liver and brain rat tissues. *Ecotoxicol Environ Saf*, 72(7):2025–32. doi:10.1016/j.ecoenv.2009.05.001 PMID:19493570

Astiz M, de Alaniz MJ, Marra CA (2009b). Antioxidant defense system in rats simultaneously intoxicated with agrochemicals. *Environ Toxicol Pharmacol*, 28(3):465–73. doi:10.1016/j.etap.2009.07.009 PMID:21784044

Astiz M, Hurtado de Catalfo GE, García MN, Galletti SM, Errecalde AL, de Alaniz MJ *et al.* (2013). Pesticide-induced decrease in rat testicular steroidogenesis is differentially prevented by lipoate and tocopherol. *Ecotoxicol Environ Saf*, 91:129–38. doi:10.1016/j.ecoenv.2013.01.022 PMID:23465731

Band PR, Abanto Z, Bert J, Lang B, Fang R, Gallagher RP *et al.* (2011). Prostate cancer risk and exposure to pesticides in British Columbia farmers. *Prostate*, 71(2):168–83. doi:10.1002/pros.21232 PMID:20799287

Battaglin WA, Kolpin DW, Scribner EA, Kuivila KM, Sandstrom MW (2005). Glyphosate, other herbicides, and transformation products in midwestern streams, 20021. *J Am Water Resour Assoc*, 41(2):323–32. doi:10.1111/j.1752-1688.2005.tb03738.x

Benachour N, Séralini GE (2009). Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. *Chem Res Toxicol*, 22(1):97–105. doi:10.1021/tx800218n PMID:19105591

Benachour N, Sipahutar H, Moslemi S, Gasnier C, Travert C, Séralini GE (2007). Time- and dose-dependent effects of Roundup on human embryonic and placental cells. *Arch Environ Contam Toxicol*, 53(1):126–33. doi:10.1007/s00244-006-0154-8 PMID:17486286

Bernal J, Bernal JL, Martin MT, Nozal MJ, Anadón A, Martínez-Larrañaga MR *et al.* (2010). Development and validation of a liquid chromatography-fluorescence-mass spectrometry method to measure glyphosate and aminomethylphosphonic acid in rat plasma. *J Chromatogr B Analyt Technol Biomed Life*

Sci, 878(31):3290–6. doi:10.1016/j.jchromb.2010.10.013 PMID:21106459

Blair A, Thomas K, Coble J, Sandler DP, Hines CJ, Lynch CF *et al.* (2011). Impact of pesticide exposure misclassification on estimates of relative risks in the Agricultural Health Study. *Occup Environ Med*, 68(7):537–41. doi:10.1136/oem.2010.059469 PMID:21257983

Blakley BR (1997). Effect of Roundup and Tordon 202C herbicides on antibody production in mice. *Vet Hum Toxicol*, 39(4):204–6. PMID:9251167

Bolognesi C, Bonatti S, Degan P, Gallerani E, Peluso M, Rabboni R *et al.* (1997). Genotoxic activity of glyphosate and its technical formulation Roundup. *J Agric Food Chem*, 45(5):1957–62. doi:10.1021/jf9606518

Bolognesi C, Carrasquilla G, Volpi S, Solomon KR, Marshall EJ (2009). Biomonitoring of genotoxic risk in agricultural workers from five Colombian regions: association to occupational exposure to glyphosate. *J Toxicol Environ Health A*, 72(15–16):986–97. doi:10.1080/15287390902929741 PMID:19672767

Bonini MG, Rota C, Tomasi A, Mason RP (2006). The oxidation of 2′,7′-dichlorofluorescein to reactive oxygen species: a self-fulfilling prophesy? *Free Radic Biol Med*, 40(6):968–75. doi:10.1016/j.freeradbiomed.2005.10.042 PMID:16540392

Borggaard OK, Gimsing AL (2008). Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review. *Pest Manag Sci*, 64(4):441–56. doi:10.1002/ps.1512 PMID:18161065

Botero-Coy AM, Ibáñez M, Sancho JV, Hernández F (2013). Improvements in the analytical methodology for the residue determination of the herbicide glyphosate in soils by liquid chromatography coupled to mass spectrometry. *J Chromatogr A*, 1292:132–41. doi:10.1016/j.chroma.2012.12.007 PMID:23332301

Botero-Coy AM, Ibáñez M, Sancho JV, Hernández F (2013b). Direct liquid chromatography-tandem mass spectrometry determination of underivatized glyphosate in rice, maize and soybean. *J Chromatogr A*, 1313:157–65. doi:10.1016/j.chroma.2013.07.037 PMID:23891211

Brewster DW, Warren J, Hopkins WE 2nd (1991). Metabolism of glyphosate in Sprague-Dawley rats: tissue distribution, identification, and quantitation of glyphosate-derived materials following a single oral dose. *Fundam Appl Toxicol*, 17(1):43–51. doi:10.1016/0272-0590(91)90237-X PMID:1916078

Brown LM, Blair A, Gibson R, Everett GD, Cantor KP, Schuman LM *et al.* (1990). Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. *Cancer Res*, 50(20):6585–91. PMID:2208120

Brown LM, Burmeister LF, Everett GD, Blair A (1993). Pesticide exposures and multiple myeloma in Iowa men. *Cancer Causes Control*, 4(2):153–6. doi:10.1007/BF00053156 PMID:8481493

Brüch W, Rosenborg AE, Johler RK, Gudmunsson L, Nielsen CB, Plauborg F, et al. (2013). Monitoring results 1999–2012. The Danish Pesticide Leaching Assessment Programme. Available from: http://pesticidvarsling.dk/publ_result/index.html, accessed 1 December 2014.

Cantor KP, Blair A, Everett G, Gibson R, Burmeister LF, Brown LM *et al.* (1992). Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. *Cancer Res*, 52(9):2447–55. PMID:1568215

Carreón T, Butler MA, Ruder AM, Waters MA, Davis-King KE, Calvert GM *et al.*; Brain Cancer Collaborative Study Group(2005). Gliomas and farm pesticide exposure in women: the Upper Midwest Health Study. *Environ Health Perspect*, 113(5):546–51. doi:10.1289/ehp.7456 PMID:15866761

Cattaneo R, Clasen B, Loro VL, de Menezes CC, Pretto A, Baldisserotto B *et al.* (2011). Toxicological responses of *Cyprinus carpio* exposed to a commercial formulation containing glyphosate. *Bull Environ Contam Toxicol*, 87(6):597–602. doi:10.1007/s00128-011-0396-7 PMID:21931962

Cattani D, de Liz Oliveira Cavalli VL, Heinz Rieg CE, Domingues JT, Dal-Cim T, Tasca CI *et al.* (2014). Mechanisms underlying the neurotoxicity induced by glyphosate-based herbicide in immature rat hippocampus: involvement of glutamate excitotoxicity. *Toxicology*, 320:34–45. doi:10.1016/j.tox.2014.03.001 PMID:24636977

Cavalcante DG, Martinez CB, Sofia SH (2008). Genotoxic effects of Roundup on the fish *Prochiloduslineatus*. *Mutat Res*, 655(1–2):41–6. doi:10.1016/j.mrgentox.2008.06.010 PMID:18638566

Cavaş T, Könen S (2007). Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (*Carassius auratus*) exposed to a glyphosate formulation using the micronucleus test and the comet assay. *Mutagenesis*, 22(4):263–8. doi:10.1093/mutage/gem012 PMID:17426049

Cavuşoğlu K, Yapar K, Oruç E, Yalçın E (2011). Protective effect of *Ginkgo biloba* L. leaf extract against glyphosate toxicity in Swiss albino mice. *J Med Food*, 14(10):1263–72. doi:10.1089/jmf.2010.0202 PMID:21859351

CCM International (2011). Outlook for China glyphosate industry 2012–2016. Available from: http://www.researchandmarkets.com/reports/2101356/outlook_for_china_glyphosate_industry_20122016, accessed 28 July 2015.

Centre de Toxicologie du Québec (1988). Etude de l'exposition professionelle des travailleurs forestiers exposés au glyphosate. Quebec: Le Centre Hospitalier de l'Université Laval. Available from: http://www.santecom.qc.ca/Bibliothequevirtuelle/santecom/35567000039898.pdf, accessed 28 July 2015. [French]

Chan P, Mahler J (1992). NTP technical report on the toxicity studies of glyphosate (CAS No. 1071–83–6)

administered in dosed feed to F344/N rats and B6C3F1 mice. *Toxic Rep Ser*, 16:1–58. PMID:12209170

Chandra M, Frith CH (1994). Spontaneous renal lesions in CD-1 and B6C3F1 mice. *Exp Toxicol Pathol*, 46(3):189–98. doi:10.1016/S0940-2993(11)80080-1 PMID:8000238

Chang FC, Simcik MF, Capel PD (2011). Occurrence and fate of the herbicide glyphosate and its degradate aminomethylphosphonic acid in the atmosphere. *Environ Toxicol Chem*, 30(3):548–55. doi:10.1002/etc.431 PMID:21128261

Chaufan G, Coalova I, Ríos de Molina MC (2014). Glyphosate commercial formulation causes cytotoxicity, oxidative effects, and apoptosis on human cells: differences with its active ingredient. *Int J Toxicol*, 33(1):29–38. doi:10.1177/1091581813517906 PMID:24434723

Chen L, Xie M, Bi Y, Wang G, Deng S, Liu Y (2012). The combined effects of UV-B radiation and herbicides on photosynthesis, antioxidant enzymes and DNA damage in two bloom-forming cyanobacteria. *Ecotoxicol Environ Saf*, 80:224–30. doi:10.1016/j.ecoenv.2012.03.007 PMID:22464588

Chen M-X, Cao Z-Y, Jiang Y, Zhu Z-W (2013). Direct determination of glyphosate and its major metabolite, aminomethylphosphonic acid, in fruits and vegetables by mixed-mode hydrophilic interaction/weak anion-exchange liquid chromatography coupled with electrospray tandem mass spectrometry. *J Chromatogr A*, 1272:90–9. doi:10.1016/j.chroma.2012.11.069 PMID:23261284

Chen YJ, Wu ML, Deng JF, Yang CC (2009). The epidemiology of glyphosate-surfactant herbicide poisoning in Taiwan, 1986–2007: a poison center study. *Clin Toxicol (Phila)*, 47(7):670–7. doi:10.1080/15563650903140399 PMID:19640238

Chruscielska K, Brzezinski J, Kita K, Kalhorn D, Kita I, Graffstein B *et al.* (2000). Glyphosate - Evaluation of chronic activity and possible far-reaching effects. Part 1. Studies on chronic toxicity. *Pestycydy (Warsaw)*, 3–4:11–20.

Clair E, Mesnage R, Travert C, Séralini GÉ (2012). A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells in vitro, and testosterone decrease at lower levels. *Toxicol In Vitro*, 26(2):269–79. doi:10.1016/j.tiv.2011.12.009 PMID:22200534

Clements C, Ralph S, Petras M (1997). Genotoxicity of select herbicides in *Rana catesbeiana* tadpoles using the alkaline single-cell gel DNA electrophoresis (comet) assay. *Environ Mol Mutagen*, 29(3):277–88. doi:10.1002/(SICI)1098-2280(1997)29:3<277::AID-EM8>3.0.CO;2-9 PMID:9142171

Coalova I, Ríos de Molina MC, Chaufan G (2014). Influence of the spray adjuvant on the toxicity effects of a glyphosate formulation. *Toxicol In Vitro*, 28(7):1306–11. doi:10.1016/j.tiv.2014.06.014 PMID:24999230

Cocco P, Satta G, Dubois S, Pili C, Pilleri M, Zucca M *et al.* (2013). Lymphoma risk and occupational exposure to pesticides: results of the Epilymph study. *Occup Environ Med*, 70(2):91–8. doi:10.1136/oemed-2012-100845 PMID:23117219

ColomboPage News Desk (2014). Sri Lanka lifts ban on sale of glyphosate. ColomboPage, Sri Lanka Internet Newspaper [online newspaper]. 13 May, 12:13 am Sri Lanka time. Available from: http://www.colombopage.com/archive_14A/May13_1399920230CH.php, accessed June 2015.

Conners DE, Black MC (2004). Evaluation of lethality and genotoxicity in the freshwater mussel *Utterbackia imbecillis* (Bivalvia: Unionidae) exposed singly and in combination to chemicals used in lawn care. *Arch Environ Contam Toxicol*, 46(3):362–71. doi:10.1007/s00244-003-3003-z PMID:15195808

Costa MJ, Monteiro DA, Oliveira-Neto AL, Rantin FT, Kalinin AL (2008). Oxidative stress biomarkers and heart function in bullfrog tadpoles exposed to Roundup Original. *Ecotoxicology*, 17(3):153–63. doi:10.1007/s10646-007-0178-5 PMID:17987383

Culbreth ME, Harrill JA, Freudenrich TM, Mundy WR, Shafer TJ (2012). Comparison of chemical-induced changes in proliferation and apoptosis in human and mouse neuroprogenitor cells. *Neurotoxicology*, 33(6):1499–510. doi:10.1016/j.neuro.2012.05.012 PMID:22634143

Curwin BD, Hein MJ, Sanderson WT, Nishioka MG, Reynolds SJ, Ward EM *et al.* (2005). Pesticide contamination inside farm and nonfarm homes. *J Occup Environ Hyg*, 2(7):357–67. doi:10.1080/15459620591001606 PMID:16020099

Curwin BD, Hein MJ, Sanderson WT, Striley C, Heederik D, Kromhout H *et al.* (2007). Urinary pesticide concentrations among children, mothers and fathers living in farm and non-farm households in Iowa. *Ann Occup Hyg*, 51(1):53–65. doi:10.1093/annhyg/mel062 PMID:16984946

de Castilhos Ghisi N, Cestari MM (2013). Genotoxic effects of the herbicide Roundup(®) in the fish *Corydoras paleatus* (Jenyns 1842) after short-term, environmentally low concentration exposure. *Environ Monit Assess*, 185(4):3201–7. doi:10.1007/s10661-012-2783-x PMID:22821326

De Marco A, De Simone C, Raglione M, Testa A, Trinca S (1992). Importance of the type of soil for the induction of micronuclei and the growth of primary roots of *Vicia faba* treated with the herbicides atrazine, glyphosate and maleic hydrazide. *Mutat Res*, 279(1):9–13. doi:10.1016/0165-1218(92)90260-7 PMID:1374535

de Menezes CC, da Fonseca MB, Loro VL, Santi A, Cattaneo R, Clasen B *et al.* (2011). Roundup effects on oxidative stress parameters and recovery pattern of *Rhamdia quelen*. *Arch Environ Contam Toxicol*, 60(4):665–71. doi:10.1007/s00244-010-9574-6 PMID:20680259

De Roos AJ, Blair A, Rusiecki JA, Hoppin JA, Svec M, Dosemeci M *et al.* (2005a). Cancer incidence among glyphosate-exposed pesticide applicators in the Agricultural Health Study. *Environ Health Perspect*, 113(1):49–54. doi:10.1289/ehp.7340 PMID:15626647

De Roos AJ, Svec MA, Blair A, Rusiecki JA, Dosemeci M, Alavanja MC *et al.* (2005b). Glyphosate results revisited: De Roos et al. respond. *Environ Health Perspect*, 113(6):A366–7. doi:10.1289/ehp.113-a366

De Roos AJ, Zahm SH, Cantor KP, Weisenburger DD, Holmes FF, Burmeister LF *et al.* (2003). Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. *Occup Environ Med*, 60(9):E11 doi:10.1136/oem.60.9.e11 PMID:12937207

De Souza Filho J, Sousa CC, Da Silva CC, De Sabóia-Morais SM, Grisolia CK (2013). Mutagenicity and genotoxicity in gill erythrocyte cells of *Poecilia reticulata* exposed to a glyphosate formulation. *Bull Environ Contam Toxicol*, 91(5):583–7. doi:10.1007/s00128-013-1103-7 PMID:24042842

Dennis LK, Lynch CF, Sandler DP, Alavanja MC (2010). Pesticide use and cutaneous melanoma in pesticide applicators in the Agricultural Health Study. *Environ Health Perspect*, 118(6):812–7. doi:10.1289/ehp.0901518 PMID:20164001

Dill GM, Sammons RD, Feng PCC, Kohn F, Kretzmer K, Mehrsheikh A *et al.* (2010). Chapter 1: Glyphosate: discovery, development, applications, and properties. In: Nandula VK editor. *Glyphosate resistance in crops and weeds: history, development, and management*. Hoboken (NJ): Wiley; pp. 1–33.

Dimitrov BD, Gadeva PG, Benova DK, Bineva MV (2006). Comparative genotoxicity of the herbicides Roundup, Stomp and Reglone in plant and mammalian test systems. *Mutagenesis*, 21(6):375–82. doi:10.1093/mutage/gel044 PMID:16998229

dos Santos KC, Martinez CB (2014). Genotoxic and biochemical effects of atrazine and Roundup(®), alone and in combination, on the Asian clam *Corbicula fluminea*. *Ecotoxicol Environ Saf*, 100:7–14. doi:10.1016/j.ecoenv.2013.11.014 PMID:24433785

Duke SO, Powles SB (2009). Glyphosate-resistant crops and weeds. Now and in the future. *AgBioForum*, 12(3&4):346–57.

EFSA (2009). 2007 Annual Report on Pesticide Residues according to Article 32 of Regulation (EC) No 396/2005. Parma: European Food Safety Authority. Available from: http://www.efsa.europa.eu/en/efsajournal/pub/305r.htm, accessed 1 November 2014.

el-Gendy KS, Aly NM, el-Sebae AH (1998). Effects of edifenphos and glyphosate on the immune response and protein biosynthesis of bolti fish (*Tilapia nilotica*). *J Environ Sci Health B*, 33(2):135–49. doi:10.1080/03601239809373135 PMID:9536512

Elie-Caille C, Heu C, Guyon C, Nicod L (2010). Morphological damages of a glyphosate-treated human keratinocyte cell line revealed by a micro- to nanoscale microscopic investigation. *Cell Biol Toxicol*, 26(4):331–9. doi:10.1007/s10565-009-9146-6 PMID:20043237

Engel LS, Hill DA, Hoppin JA, Lubin JH, Lynch CF, Pierce J *et al.* (2005). Pesticide use and breast cancer risk among farmers' wives in the Agricultural Health Study. *Am J Epidemiol*, 161(2):121–35. doi:10.1093/aje/kwi022 PMID:15632262

EPA (1980a). Glyphosate; Submission of rat teratology, rabbit teratology, dominant lethal mutagenicity assay in mice. Washington (DC): United States Environmental Protection Agency, Office of Toxic substances. Available from: http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-090.pdf, accessed 10 March 2015.

EPA (1980b). Review of Rodwell DE, Tasker EJ, Blair AM, *et al.* (1980). Teratology study in rats: IRDC No. 401–054. MRID 00046362. Washington (DC): United States Environmental Protection Agency. Available from: http://www.epa.gov/ncct/toxrefdb/, and from http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-090.pdf, accessed 10 March 2015.

EPA (1983). Review of Knezevich A, Hogan G (1983). A chronic feeding study of glyphosate (Roundup Technical) in mice: Project No. 77–2061: Bdn-77- 420. Final Report. MRID 00130406. Washington (DC): United States Environmental Protection Agency. Available from: http://www.epa.gov/ncct/toxrefdb/, accessed 10 March 2015.

EPA (1985a). Glyphosate; EPA Reg.#: 524–308; Mouse oncogenicity study. Document No. 004370. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-183.pdf, accessed 10 March 2015.

EPA (1985b). EPA Reg.#: 524–308; Roundup; glyphosate; pathology report on additional kidney sections. Document No. 004855. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-206.pdf, accessed 10 March 2015.

EPA (1986). Glyphosate; EPA Registration No. 524–308; Roundup; additional histopathological evaluations of kidneys in the chronic feeding study of glyphosate in mice. Document No. 005590. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/

reviews/103601/103601-211.pdf, accessed 10 March 2015.

EPA (1987). Review of Stout L, Johnson C (1987). 90-Day study of glyphosate administered in feed to Sprague-Dawley rats: Proj. ID ML-86–351/EHL 86128. MRID 40559401. Washington (DC): United States Environmental Protection Agency. Available from: http://www.epa.gov/ncct/toxrefdb/, accessed 10 March 2015.

EPA (1990). Review of Stout L, Ruecker F (1990). Chronic study of glyphosate administered in feed to albino rats: Laboratory Project Number: Msl-10495: RD 1014. MRID 41643801. Washington (DC): United States Environmental Protection Agency. Available from: http://www.epa.gov/ncct/toxrefdb/, accessed 10 March 2015.

EPA (1991a). Second peer review of glyphosate. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-265.pdf, accessed 10 March 2015.

EPA (1991b). Glyphosate; 2-year combined chronic toxicity/carcinogenicity study in Sprague-Dawley rats - List A pesticide for reregistration. Document No. 008390. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-263.pdf, accessed June 2015; see also http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-268.pdf, accessed June 2015.

EPA (1991c). Peer review on glyphosate. Document No. 008527. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency.

EPA (1991d). Glyphosate – EPA registration No. 524–308 – 2-year chronic feeding/oncogenicity study in rats with technical glyphosate. Document No. 008897. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-268.pdf, accessed 10 March 2015.

EPA (1992). Determination of glyphosate in drinking water by direct-aqueous-injection HPLC, post column derivatization, and fluorescence detection. In: Methods for the determination of organic compounds in drinking water - Supplement II (EPA/600/R-92–129). Washington (DC): Environmental Monitoring Systems Laboratory, Office of Research and Development, United States Environmental Protection Agency. Available through NTIS (http://www.ntis.gov).

EPA (1993a). Reregistration Eligibility Decision (RED): Glyphosate. EPA 738-R-93–014. Washington (DC): Office of Prevention, Pesticides And Toxic Substances, Office of Pesticide Programs, United States Environmental Protection Agency. Available from: http://www.epa.gov/opp00001/chem_search/reg_actions/reregistration/red_PC-417300_1-Sep-93.pdf, accessed 10 March 2015.

EPA (1993b). RED facts: Glyphosate. EPA-738-F-93–011. Washington (DC): Office of Prevention, Pesticides, and Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/oppsrrd1/reregistration/REDs/factsheets/0178fact.pdf, accessed 4 May 2015.

EPA (1997). Pesticides industry sales and usage –1994 and 1995 market estimates. Washington (DC): Biological and Economic Analysis Division, Office of Pesticide Programs, Office of Prevention, Pesticides And Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/pesticides/pestsales/95pestsales/market_estimates1995.pdf, accessed 10 March 2015.

EPA (2011). Pesticides industry sales and usage – 2006 and 2007 market estimates. Washington (DC): Biological and Economic Analysis Division, Office of Pesticide Programs, Office of Prevention, Pesticides And Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/opp00001/pestsales/07pestsales/market_estimates2007.pdf, accessed 10 March 2015.

EPA (2015). Toxicity Reference Database (ToxRefDB). Computational Toxicology Research Program, United States Environmental Protection Agency. Available from: http://www.epa.gov/ncct/toxrefdb/, accessed 10 March 2015.

Eriksson M, Hardell L, Carlberg M, Akerman M (2008). Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis. *Int J Cancer*, 123(7):1657–63. doi:10.1002/ijc.23589 PMID:18623080

European Commission (2002). Review report for the active substance glyphosate (6511/VI/99-final, 21 January 2002). Brussels: Health and Consumer Protection Directorate-General, European Commission. Available from: http://ec.europa.eu/food/plant/protection/evaluation/existactive/list1_glyphosate_en.pdf, accessed 29 April 2015.

Eustis SL, Hailey JR, Boorman GA, Haseman JK (1994). The utility of multiple-section sampling in the histopathological evaluation of the kidney for carcinogenicity studies. *Toxicol Pathol*, 22(5):457–72. doi:10.1177/019262339402200501 PMID:7899775

FAO (2000). Glyphosate, N-(phosphonomethyl)glycine. Specifications and evaluations for plant protection products. Rome: Food and Agriculture Organization of the United Nations. Available from: http://www.fao.

org/fileadmin/templates/agphome/documents/Pests_Pesticides/Specs/glypho01.pdf, accessed 28 July 2015.

Farm Chemicals International (2015). Glyphosate. In: Crop Protection Database. Willoughby (OH): Meister Media Worldwide. Available from: http://www.farmchemicalsinternational.com/crop-protection-database/#/product/detail/203900/, accessed 2 February 2015.

Ferreira D, da Motta AC, Kreutz LC, Toni C, Loro VL, Barcellos LJ (2010). Assessment of oxidative stress in *Rhamdia quelen* exposed to agrichemicals. *Chemosphere*, 79(9):914–21. doi:10.1016/j.chemosphere.2010.03.024 PMID:20371099

Flower KB, Hoppin JA, Lynch CF, Blair A, Knott C, Shore DL *et al.* (2004). Cancer risk and parental pesticide application in children of Agricultural Health Study participants. *Environ Health Perspect*, 112(5):631–5. doi:10.1289/ehp.6586 PMID:15064173

Forgacs AL, Ding Q, Jaremba RG, Huhtaniemi IT, Rahman NA, Zacharewski TR (2012). BLTK1 murine Leydig cells: a novel steroidogenic model for evaluating the effects of reproductive and developmental toxicants. *Toxicol Sci*, 127(2):391–402. doi:10.1093/toxsci/kfs121 PMID:22461451

Freedonia (2012). World agricultural pesticides: industry study with forecasts for 2016 & 2021. Study #2902, August 2012. Cleveland (OH): The Freedonia Group. Available from: http://www.freedoniagroup.com/brochure/29xx/2902smwe.pdf, accessed 10 March 2015.

Frescura VD, Kuhn AW, Laughinghouse HD 4th, Paranhos JT, Tedesco SB (2013). Post-treatment with plant extracts used in Brazilian folk medicine caused a partial reversal of the antiproliferative effect of glyphosate in the *Allium cepa* test. *Biocell*, 37(2):23–8. PMID:24392578

Gasnier C, Benachour N, Clair E, Travert C, Langlois F, Laurant C *et al.* (2010). Dig1 protects against cell death provoked by glyphosate-based herbicides in human liver cell lines. *J Occup Med Toxicol*, 5(1):29 doi:10.1186/1745-6673-5-29 PMID:20979644

Gasnier C, Dumont C, Benachour N, Clair E, Chagnon MC, Séralini GE (2009). Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. *Toxicology*, 262(3):184–91. doi:10.1016/j.tox.2009.06.006 PMID:19539684

Gehin A, Guillaume YC, Millet J, Guyon C, Nicod L (2005). Vitamins C and E reverse effect of herbicide-induced toxicity on human epidermal cells HaCaT: a biochemometric approach. *Int J Pharm*, 288(2):219–26. doi:10.1016/j.ijpharm.2004.09.024 PMID:15620861

George J, Prasad S, Mahmood Z, Shukla Y (2010). Studies on glyphosate-induced carcinogenicity in mouse skin: a proteomic approach. *J Proteomics*, 73(5):951–64. doi:10.1016/j.jprot.2009.12.008 PMID:20045496

George J, Shukla Y (2013). Emptying of intracellular calcium pool and oxidative stress imbalance are associated with the glyphosate-induced proliferation in human skin keratinocytes HaCaT cells. *ISRN Dermatol*, 2013:825180 doi:10.1155/2013/825180 PMID:24073338

Geret F, Burgeot T, Haure J, Gagnaire B, Renault T, Communal PY *et al.* (2013). Effects of low-dose exposure to pesticide mixture on physiological responses of the Pacific oyster, Crassostrea gigas. *Environ Toxicol*, 28(12):689–99. doi:10.1002/tox.20764 PMID:22012874

Gholami-Seyedkolaei SJ, Mirvaghefi A, Farahmand H, Kosari AA, Gholami-Seyedkolaei SJ, Gholami-Seyedkolaei SJ (2013). Optimization of recovery patterns in common carp exposed to Roundup using response surface methodology: evaluation of neurotoxicity and genotoxicity effects and biochemical parameters. *Ecotoxicol Environ Saf*, 98:152–61. doi:10.1016/j.ecoenv.2013.09.009 PMID:24094415

Glusczak L, Loro VL, Pretto A, Moraes BS, Raabe A, Duarte MF *et al.* (2011). Acute exposure to glyphosate herbicide affects oxidative parameters in piava (*Leporinus obtusidens*). *Arch Environ Contam Toxicol*, 61(4):624–30. doi:10.1007/s00244-011-9652-4 PMID:21465245

Glyphosate Task Force (2014). How is glyphosate used? Glyphosate facts. Updated 10 March 2014. Darmstadt: Industry Task Force on Glyphosate. Available from: http://www.glyphosate.eu/how-glyphosate-used, accessed 21 April 2015.

Granby K, Vahl M (2001). Investigation of the herbicide glyphosate and the plant growth regulators chlormequat and mepiquat in cereals produced in Denmark. *Food Addit Contam*, 18(10):898–905. doi:10.1080/02652030119594 PMID:11569770

Greim H, Saltmiras D, Mostert V, Strupp C (2015). Evaluation of carcinogenic potential of the herbicide glyphosate, drawing on tumor incidence data from fourteen chronic/carcinogenicity rodent studies. *Crit Rev Toxicol*, 45(3):185–208. doi:10.3109/10408444.2014.1003423 PMID:25716480

Grisolia CK (2002). A comparison between mouse and fish micronucleus test using cyclophosphamide, mitomycin C and various pesticides. *Mutat Res*, 518(2):145–50. doi:10.1016/S1383-5718(02)00086-4 PMID:12113765

Guha N, Ward MH, Gunier R, Colt JS, Lea CS, Buffler PA *et al.* (2013). Characterization of residential pesticide use and chemical formulations through self-report and household inventory: the Northern California Childhood Leukemia study. *Environ Health Perspect*, 121(2):276–82. PMID:23110983

Gui YX, Fan XN, Wang HM, Wang G, Chen SD (2012). Glyphosate induced cell death through apoptotic and autophagic mechanisms. *Neurotoxicol Teratol*, 34(3):344–9. doi:10.1016/j.ntt.2012.03.005 PMID:22504123

Guilherme S, Gaivão I, Santos MA, Pacheco M (2010). European eel (*Anguilla anguilla*) genotoxic and

pro-oxidant responses following short-term exposure to Roundup–a glyphosate-based herbicide. *Mutagenesis*, 25(5):523–30. doi:10.1093/mutage/geq038 PMID:20643706

Guilherme S, Gaivão I, Santos MA, Pacheco M (2012a). DNA damage in fish (*Anguilla anguilla*) exposed to a glyphosate-based herbicide – elucidation of organ-specificity and the role of oxidative stress. *Mutat Res*, 743(1–2):1–9. doi:10.1016/j.mrgentox.2011.10.017 PMID:22266476

Guilherme S, Santos MA, Barroso C, Gaivão I, Pacheco M (2012b). Differential genotoxicity of Roundup(®) formulation and its constituents in blood cells of fish (*Anguilla anguilla*): considerations on chemical interactions and DNA damaging mechanisms. *Ecotoxicology*, 21(5):1381–90. doi:10.1007/s10646-012-0892-5 PMID:22526921

Guilherme S, Santos MA, Gaivão I, Pacheco M (2014a). Are DNA-damaging effects induced by herbicide formulations (Roundup® and Garlon®) in fish transient and reversible upon cessation of exposure? *Aquat Toxicol*, 155:213–21. doi:10.1016/j.aquatox.2014.06.007 PMID:25058560

Guilherme S, Santos MA, Gaivão I, Pacheco M (2014b). DNA and chromosomal damage induced in fish (*Anguilla anguilla* L.) by aminomethylphosphonic acid (AMPA)–the major environmental breakdown product of glyphosate. *Environ Sci Pollut Res Int*, 21(14):8730–9. doi:10.1007/s11356-014-2803-1 PMID:24696215

Hardell L, Eriksson M (1999). A case-control study of non-Hodgkin lymphoma and exposure to pesticides. *Cancer*, 85(6):1353–60. doi:10.1002/(SICI)1097-0142(19990315)85:6<1353::AID-CNCR19>3.0.CO;2-1 PMID:10189142

Hardell L, Eriksson M, Nordstrom M (2002). Exposure to pesticides as risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: pooled analysis of two Swedish case-control studies. *Leuk Lymphoma*, 43(5):1043–9. PMID:12148884

Hayes WJ Jr, Laws ER Jr editors. (1991). *Classes of pesticides*. Handbook of Pesticide Toxicology. Volume 3:. New York (NY): Academic Press, Inc.; p. 1340.

Heu C, Elie-Caille C, Mougey V, Launay S, Nicod L (2012). A step further toward glyphosate-induced epidermal cell death: involvement of mitochondrial and oxidative mechanisms. *Environ Toxicol Pharmacol*, 34(2):144–53. doi:10.1016/j.etap.2012.02.010 PMID:22522424

Hidalgo C, Rios C, Hidalgo M, Salvadó V, Sancho JV, Hernández F (2004). Improved coupled-column liquid chromatographic method for the determination of glyphosate and aminomethylphosphonic acid residues in environmental waters. *J Chromatogr A*, 1035(1):153–7. doi:10.1016/j.chroma.2004.02.044 PMID:15117086

Hilton CW (2012). Monsanto & the global glyphosate market: case study. The Wiglaf Journal. June 2012. Available from: http://www.wiglafjournal.com/pricing/2012/06/monsanto-the-global-glyphosate-market-case-study/, accessed 28 July 2015.

Hoar SK, Blair A, Holmes FF, Boysen CD, Robel RJ, Hoover R *et al.* (1986). Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. *JAMA*, 256(9):1141–7. doi:10.1001/jama.1986.03380090081023 PMID:3801091

Humphries D, Byrtus G, Anderson AM (2005). Glyphosate residues in Alberta's atmospheric deposition, soils and surface waters. Alberta: Water Research Users Group, Alberta Environment. Available from: http://environment.gov.ab.ca/info/library/6444.pdf, accessed 13 November 2014.

IARC (2006). Data for the Monographs. In: Preamble to the IARC Monographs (amended January 2006). Lyon: International Agency for Research on Cancer. Available from: http://monographs.iarc.fr/ENG/Preamble/index.php, accessed 28 July 2015.

IARC (2014). Table 1. Key characteristics of carcinogens. In: Instructions for authors. Lyon: International Agency for Research on Cancer. Available from: http://monographs.iarc.fr/ENG/Preamble/previous/Instructions_to_Authors_S4.pdf, accessed 28 July 2015.

IPCS (1994). Glyphosate. Environmental Health Criteria 159. Geneva: International Programme on Chemical Safety, World Health Organization. Available from: http://www.inchem.org/documents/ehc/ehc/ehc159.htm, accessed 28 July 2015.

IPCS (1996). Glyphosate. WHO/FAO Data Sheets on Pesticides, No. 91 (WHO/PCS/DS/96.91). Geneva: International Programme on Chemical Safety, World Health Organization. Available from: http://apps.who.int/iris/handle/10665/63290.

IPCS (2005). Glyphosate. International Chemical Safety Card (ICSC 0160). Geneva: International Programme on Chemical Safety, World Health Organization. Available from: http://www.inchem.org/documents/icsc/icsc/eics0160.htm, accessed 2 February 2015.

Jacob GS, Garbow JR, Hallas LE, Kimack NM, Kishore GM, Schaefer J (1988). Metabolism of glyphosate in Pseudomonas sp. strain LBr. *Appl Environ Microbiol*, 54(12):2953–8. PMID:3223761

Jan MR, Shah J, Muhammad M, Ara B (2009). Glyphosate herbicide residue determination in samples of environmental importance using spectrophotometric method. *J Hazard Mater*, 169(1–3):742–5. doi:10.1016/j.jhazmat.2009.04.003 PMID:19411135

Jasper R, Locatelli GO, Pilati C, Locatelli C (2012). Evaluation of biochemical, hematological and oxidative parameters in mice exposed to the herbicide glyphosate-Roundup(®). *Interdiscip Toxicol*, 5(3):133–40. doi:10.2478/v10102-012-0022-5 PMID:23554553

Jauhiainen A, Räsänen K, Sarantila R, Nuutinen J, Kangas J (1991). Occupational exposure of forest workers to glyphosate during brush saw spraying work. *Am Ind Hyg*

Assoc J, 52(2):61–4. doi:10.1080/15298669191364334 PMID:2011980

JMPR (2006). Glyphosate. In: Joint FAO/WHO Meeting on Pesticide Residues. Pesticide residues in food – 2004: toxicological evaluations. Report No. WHO/PCS/06.1. Geneva: World Health Organization; pp. 95–169. Available from: http://whqlibdoc.who.int/publications/2006/9241665203_eng.pdf?ua=1, accessed 6 March 2015.

Johnson PD, Rimmer DA, Garrod AN, Helps JE, Mawdsley C (2005). Operator exposure when applying amenity herbicides by all-terrain vehicles and controlled droplet applicators. *Ann Occup Hyg*, 49(1):25–32. PMID:15596423

Kachuri L, Demers PA, Blair A, Spinelli JJ, Pahwa M, McLaughlin JR *et al.* (2013). Multiple pesticide exposures and the risk of multiple myeloma in Canadian men. *Int J Cancer*, 133(8):1846–58. doi:10.1002/ijc.28191 PMID:23564249

Kale PG, Petty BT Jr, Walker S, Ford JB, Dehkordi N, Tarasia S *et al.* (1995). Mutagenicity testing of nine herbicides and pesticides currently used in agriculture. *Environ Mol Mutagen*, 25(2):148–53. doi:10.1002/em.2850250208 PMID:7698107

Kalyanaraman B, Darley-Usmar V, Davies KJ, Dennery PA, Forman HJ, Grisham MB *et al.* (2012). Measuring reactive oxygen and nitrogen species with fluorescent probes: challenges and limitations. *Free Radic Biol Med*, 52(1):1–6. doi:10.1016/j.freeradbiomed.2011.09.030 PMID:22027063

Karunanayake CP, Spinelli JJ, McLaughlin JR, Dosman JA, Pahwa P, McDuffie HH (2012). Hodgkin lymphoma and pesticides exposure in men: a Canadian case-control study. *J Agromed*, 17(1):30–9. doi:10.1080/1059924X.2012.632726 PMID:22191501

Kavlock R, Chandler K, Houck K, Hunter S, Judson R, Kleinstreuer N *et al.* (2012). Update on EPA's ToxCast program: providing high throughput decision support tools for chemical risk management. *Chem Res Toxicol*, 25(7):1287–302. doi:10.1021/tx3000939 PMID:22519603

Kaya B, Creus A, Yanikoğlu A, Cabré O, Marcos R (2000). Use of the Drosophila wing spot test in the genotoxicity testing of different herbicides. *Environ Mol Mutagen*, 36(1):40–6. doi:10.1002/1098-2280(2000)36:1<40::AID-EM6>3.0.CO;2-K PMID:10918358

Kier LD, Kirkland DJ (2013). Review of genotoxicity studies of glyphosate and glyphosate-based formulations. *Crit Rev Toxicol*, 43(4):283–315. doi:10.3109/10408444.2013.770820 PMID:23480780

Kim YH, Hong JR, Gil HW, Song HY, Hong SY (2013). Mixtures of glyphosate and surfactant TN20 accelerate cell death via mitochondrial damage-induced apoptosis and necrosis. *Toxicol In Vitro*, 27(1):191–7. doi:10.1016/j.tiv.2012.09.021 PMID:23099315

Kojima H, Katsura E, Takeuchi S, Niiyama K, Kobayashi K (2004). Screening for estrogen and androgen receptor activities in 200 pesticides by in vitro reporter gene assays using Chinese hamster ovary cells. *Environ Health Perspect*, 112(5):524–31. doi:10.1289/ehp.6649 PMID:15064155

Kojima H, Takeuchi S, Nagai T (2010). Endocrine-disrupting potential of pesticides via nuclear receptors and aryl hydrocarbon receptor *J Health Sci*, 56(4):374–86. doi:10.1248/jhs.56.374

Koller VJ, Fürhacker M, Nersesyan A, Mišík M, Eisenbauer M, Knasmueller S (2012). Cytotoxic and DNA-damaging properties of glyphosate and Roundup in human-derived buccal epithelial cells. *Arch Toxicol*, 86(5):805–13. doi:10.1007/s00204-012-0804-8 PMID:22331240

Kolpin DW, Thurman EM, Lee EA, Meyer MT, Furlong ET, Glassmeyer ST (2006). Urban contributions of glyphosate and its degradate AMPA to streams in the United States. *Sci Total Environ*, 354(2–3):191–7. doi:10.1016/j.scitotenv.2005.01.028 PMID:16398995

Kreutz LC, Gil Barcellos LJ, de Faria Valle S, de Oliveira Silva T, Anziliero D, Davi dos Santos E *et al.* (2011). Altered hematological and immunological parameters in silver catfish (*Rhamdia quelen*) following short term exposure to sublethal concentration of glyphosate. *Fish Shellfish Immunol*, 30(1):51–7. doi:10.1016/j.fsi.2010.09.012 PMID:20883798

Kuang H, Wang L, Xu C (2011). Overview of analytical techniques for herbicides in foods. In: Soloneski S, Larramendy ML, editors. Herbicides, theory and applications. Available from: http://www.intechopen.com/books/herbicides-theory-and-applications, accessed 28 July 2015.

Kumar S, Khodoun M, Kettleson EM, McKnight C, Reponen T, Grinshpun SA *et al.* (2014). Glyphosate-rich air samples induce IL-33, TSLP and generate IL-13 dependent airway inflammation. *Toxicology*, 325:42–51. doi:10.1016/j.tox.2014.08.008 PMID:25172162

Kwiatkowska M, Huras B, Bukowska B (2014). The effect of metabolites and impurities of glyphosate on human erythrocytes (in vitro). *Pestic Biochem Physiol*, 109:34–43. doi:10.1016/j.pestbp.2014.01.003 PMID:24581382

Landgren O, Kyle RA, Hoppin JA, Beane Freeman LE, Cerhan JR, Katzmann JA *et al.* (2009). Pesticide exposure and risk of monoclonal gammopathy of undetermined significance in the Agricultural Health Study. *Blood*, 113(25):6386–91. doi:10.1182/blood-2009-02-203471 PMID:19387005

Larsen K, Najle R, Lifschitz A, Maté ML, Lanusse C, Virkel GL (2014). Effects of sublethal exposure to a glyphosate-based herbicide formulation on metabolic activities of different xenobiotic-metabolizing enzymes in rats. *Int J Toxicol*, 33(4):307–18. doi:10.1177/1091581814540481 PMID:24985121

Lavy TL, Cowell JE, Steinmetz JR, Massey JH (1992). Conifer seedling nursery worker exposure to

glyphosate. *Arch Environ Contam Toxicol*, 22(1):6–13. doi:10.1007/BF00213295 PMID:1554254

Lee EA, Strahan AP, Thurman EM (2001). Methods of analysis by the U.S. Geological Survey Organic Geochemistry Research Group — determination of glyphosate, aminomethylphophonic acid, and glufosinate in water using online solid-phase extraction and high-performance liquid chromatography/mass spectrometry. Open-File Report 01–454. Lawrence (KS): United States Geological Survey. Available from: http://ks.water.usgs.gov/pubs/reports/ofr.01-454.pdf, accessed 28 July 2015.

Lee WJ, Cantor KP, Berzofsky JA, Zahm SH, Blair A (2004a). Non-Hodgkin's lymphoma among asthmatics exposed to pesticides. *Int J Cancer*, 111(2):298–302. doi:10.1002/ijc.20273 PMID:15197786

Lee WJ, Colt JS, Heineman EF, McComb R, Weisenburger DD, Lijinsky W *et al.* (2005). Agricultural pesticide use and risk of glioma in Nebraska, United States. *Occup Environ Med*, 62(11):786–92. doi:10.1136/oem.2005.020230 PMID:16234405

Lee WJ, Lijinsky W, Heineman EF, Markin RS, Weisenburger DD, Ward MH (2004b). Agricultural pesticide use and adenocarcinomas of the stomach and oesophagus. *Occup Environ Med*, 61(9):743–9. doi:10.1136/oem.2003.011858 PMID:15317914

Lee WJ, Sandler DP, Blair A, Samanic C, Cross AJ, Alavanja MC (2007). Pesticide use and colorectal cancer risk in the Agricultural Health Study. *Int J Cancer*, 121(2):339–46. doi:10.1002/ijc.22635 PMID:17390374

Li AP, Long TJ (1988). An evaluation of the genotoxic potential of glyphosate. *Fundam Appl Toxicol*, 10(3):537–46. doi:10.1016/0272-0590(88)90300-4 PMID:3286348

Li Q, Lambrechts MJ, Zhang Q, Liu S, Ge D, Yin R *et al.* (2013). Glyphosate and AMPA inhibit cancer cell growth through inhibiting intracellular glycine synthesis. *Drug Des Dev Ther*, 7:635–43. PMID:23983455

Lioi MB, Scarfì MR, Santoro A, Barbieri R, Zeni O, Di Berardino D *et al.* (1998). Genotoxicity and oxidative stress induced by pesticide exposure in bovine lymphocyte cultures in vitro. *Mutat Res*, 403(1–2):13–20. doi:10.1016/S0027-5107(98)00010-4 PMID:9726001

Lopes FM, Varela Junior AS, Corcini CD, da Silva AC, Guazzelli VG, Tavares G *et al.* (2014). Effect of glyphosate on the sperm quality of zebrafish *Danio rerio*. *Aquat Toxicol*, 155:322–6. doi:10.1016/j.aquatox.2014.07.006 PMID:25089920

Lubick N (2009). Environmental impact of cocaine strategy assessed [News]*Nature*, Published online 12 November, doi doi:10.1038/news.2009.1080

Lueken A, Juhl-Strauss U, Krieger G, Witte I (2004). Synergistic DNA damage by oxidative stress (induced by H_2O_2) and nongenotoxic environmental chemicals in human fibroblasts. *Toxicol Lett*, 147(1):35–43. doi:10.1016/j.toxlet.2003.10.020 PMID:14700526

Lushchak OV, Kubrak OI, Storey JM, Storey KB, Lushchak VI (2009). Low toxic herbicide Roundup induces mild oxidative stress in goldfish tissues. *Chemosphere*, 76(7):932–7. doi:10.1016/j.chemosphere.2009.04.045 PMID:19450865

Mahendrakar K, Venkategowda PM, Rao SM, Mutkule DP (2014). Glyphosate surfactant herbicide poisoning and management. *Indian J Crit Care Med*, 18(5):328–30. doi:10.4103/0972-5229.132508 PMID:24914265

Malatesta M, Perdoni F, Santin G, Battistelli S, Muller S, Biggiogera M (2008). Hepatoma tissue culture (HTC) cells as a model for investigating the effects of low concentrations of herbicide on cell structure and function. *Toxicol In Vitro*, 22(8):1853–60. doi:10.1016/j.tiv.2008.09.006 PMID:18835430

Mañas F, Peralta L, Raviolo J, García Ovando H, Weyers A, Ugnia L *et al.* (2009b). Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetic tests. *Ecotoxicol Environ Saf*, 72(3):834–7. doi:10.1016/j.ecoenv.2008.09.019 PMID:19013644

Mañas F, Peralta L, Raviolo J, Ovando HG, Weyers A, Ugnia L *et al.* (2009a). Genotoxicity of glyphosate assessed by the comet assay and cytogenetic tests. *Environ Toxicol Pharmacol*, 28(1):37–41. doi:10.1016/j.etap.2009.02.001 PMID:21783980

Mance D 3rd (2012). The great glyphosate debate. *Northern Woodlands* [online magazine]. 8 March. Available from: http://northernwoodlands.org/articles/article/the-great-glyphosate-debate, accessed 28 July 2015.

Mariager TP, Madsen PV, Ebbehøj NE, Schmidt B, Juhl A (2013). Severe adverse effects related to dermal exposure to a glyphosate-surfactant herbicide. *Clin Toxicol (Phila)*, 51(2):111–3. doi:10.3109/15563650.2013.763951 PMID:23360343

Marques A, Guilherme S, Gaivão I, Santos MA, Pacheco M (2014). Progression of DNA damage induced by a glyphosate-based herbicide in fish (*Anguilla anguilla*) upon exposure and post-exposure periods–insights into the mechanisms of genotoxicity and DNA repair. *Comp Biochem Physiol C Toxicol Pharmacol*, 166:126–33. doi:10.1016/j.cbpc.2014.07.009 PMID:25110831

Marques A, Guilherme S, Gaivão I, Santos MA, Pacheco M (2015). Erratum to: "Progression of DNA damage induced by a glyphosate-based herbicide in fish (*Anguilla anguilla*) upon exposure and post-exposure periods - Insights into the mechanisms of genotoxicity and DNA repair" [Comp. Biochem. Physiol. C 166 (2014) 126–133]. *Comp Biochem Physiol C Toxicol Pharmacol*, 168C:1 doi:10.1016/j.cbpc.2014.10.008 PMID:25521452

Martini CN, Gabrielli M, Vila MC (2012). A commercial formulation of glyphosate inhibits proliferation and differentiation to adipocytes and induces apoptosis in 3T3-L1 fibroblasts. *Toxicol In Vitro*, 26(6):1007–13. doi:10.1016/j.tiv.2012.04.017 PMID:22546541

McDuffie HH, Pahwa P, McLaughlin JR, Spinelli JJ, Fincham S, Dosman JA *et al.* (2001). Non-Hodgkin's lymphoma and specific pesticide exposures in men: cross-Canada study of pesticides and health. *Cancer Epidemiol Biomarkers Prev*, 10(11):1155–63. PMID:11700263

McQueen H, Callan AC, Hinwood AL (2012). Estimating maternal and prenatal exposure to glyphosate in the community setting. *Int J Hyg Environ Health*, 215(6):570–6. doi:10.1016/j.ijheh.2011.12.002 PMID:22261298

Mesnage R, Bernay B, Séralini GE (2013). Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. *Toxicology*, 313(2–3):122–8. doi:10.1016/j.tox.2012.09.006 PMID:23000283

Meza-Joya FL, Ramírez-Pinilla MP, Fuentes-Lorenzo JL (2013). Toxic, cytotoxic, and genotoxic effects of a glyphosate formulation (Roundup®SL-Cosmoflux®411F) in the direct-developing frog *Eleutherodactylus johnstonei*. *Environ Mol Mutagen*, 54(5):362–73. doi:10.1002/em.21775 PMID:23625742

Ministry of Chemicals & Fertilizers (2008). Performance of chemical & petrochemical industry at a glance (2001–2007). New Delhi: Monitoring and Evaluation Division, Department of Chemicals and Petrochemicals, Government of India. Available from: http://chemicals.nic.in/stat0107.pdf, accessed February 2015.

Mladinic M, Berend S, Vrdoljak AL, Kopjar N, Radic B, Zeljezic D (2009b). Evaluation of genome damage and its relation to oxidative stress induced by glyphosate in human lymphocytes in vitro. *Environ Mol Mutagen*, 50(9):800–7. doi:10.1002/em.20495 PMID:19402152

Mladinic M, Perkovic P, Zeljezic D (2009a). Characterization of chromatin instabilities induced by glyphosate, terbuthylazine and carbofuran using cytome FISH assay. *Toxicol Lett*, 189(2):130–7. doi:10.1016/j.toxlet.2009.05.012 PMID:19477249

MLHB (2013). Determination of glyphosate residues in human urine samples from 18 European countries. Bremen: Medical Laboratory of Bremen. Available from: https://www.foeeurope.org/sites/default/files/glyphosate_studyresults_june12.pdf, accessed 24 November 2014.

Modesto KA, Martinez CB (2010a). Effects of Roundup Transorb on fish: hematology, antioxidant defenses and acetylcholinesterase activity. *Chemosphere*, 81(6):781–7. doi:10.1016/j.chemosphere.2010.07.005 PMID:20684975

Modesto KA, Martinez CB (2010b). Roundup causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish *Prochilodus lineatus*. *Chemosphere*, 78(3):294–9. doi:10.1016/j.chemosphere.2009.10.047 PMID:19910015

Mohamed AH (2011). Sublethal toxicity of Roundup to immunological and molecular aspects of *Biomphalaria alexandrina* to *Schistosoma mansoni* infection. *Ecotoxicol Environ Saf*, 74(4):754–60. doi:10.1016/j.ecoenv.2010.10.037 PMID:21126764

Monge P, Wesseling C, Guardado J, Lundberg I, Ahlbom A, Cantor KP *et al.* (2007). Parental occupational exposure to pesticides and the risk of childhood leukemia in Costa Rica. *Scand J Work Environ Health*, 33(4):293–303. doi:10.5271/sjweh.1146 PMID:17717622

Monroy CM, Cortés AC, Sicard DM, de Restrepo HG (2005). [Cytotoxicity and genotoxicity of human cells exposed in vitro to glyphosate] *Biomedica*, 25(3):335–45. doi:10.7705/biomedica.v25i3.1358 PMID:16276681

Moreno NC, Sofia SH, Martinez CB (2014). Genotoxic effects of the herbicide Roundup Transorb and its active ingredient glyphosate on the fish *Prochilodus lineatus*. *Environ Toxicol Pharmacol*, 37(1):448–54. doi:10.1016/j.etap.2013.12.012 PMID:24448465

Mortensen OS, Sørensen FW, Gregersen M, Jensen K (2000). [Poisonings with the herbicides glyphosate and glyphosate-trimesium] [in Danish] *Ugeskr Laeger*, 162(35):4656–9. PMID:10986892

Motojyuku M, Saito T, Akieda K, Otsuka H, Yamamoto I, Inokuchi S (2008). Determination of glyphosate, glyphosate metabolites, and glufosinate in human serum by gas chromatography-mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci*, 875(2):509–14. doi:10.1016/j.jchromb.2008.10.003 PMID:18945648

Muangphra P, Kwankua W, Gooneratne R (2014). Genotoxic effects of glyphosate or paraquat on earthworm coelomocytes. *Environ Toxicol*, 29(6):612–20. doi:10.1002/tox.21787 PMID:22644885

Nakashima K, Yoshimura T, Mori H, Kawaguchi M, Adachi S, Nakao T *et al.* (2002). [Effects of pesticides on cytokine production by human peripheral blood mononuclear cells–fenitrothion and glyphosate] *Chudoku Kenkyu*, 15(2):159–65. PMID:12108020

NCBI (2015). Glyphosate. Compound summary for CID 3496. PubChem Open Chemistry Database. Bethesda (MD): National Center for Biotechnology Information, United States National Library of Medicine. Available from: http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=3496, accessed 5 March 2015.

Nedelkoska TV, Low GKC (2004). High-performance liquid chromatographic determination of glyphosate in water and plant material after pre-column derivatisation with 9-fluorenylmethyl chloroformate. *Anal Chim Acta*, 511(1):145–53. doi:10.1016/j.aca.2004.01.027

NIH (2015). Questionnaires and study data. Agricultural Health Study. National Institutes of Health. Available from: http://aghealth.nih.gov/collaboration/questionnaires.html, accessed 12 June 2015.

Nordström M, Hardell L, Magnuson A, Hagberg H, Rask-Andersen A (1998). Occupational exposures, animal exposure and smoking as risk factors for hairy cell leukaemia evaluated in a case-control study. *Br*

J Cancer, 77(11):2048–52. doi:10.1038/bjc.1998.341 PMID:9667691

NPIC (2010). Glyphosate. General fact sheet. Oregon State University: National Pesticide Information Center. Available from: http://npic.orst.edu/factsheets/glyphogen.pdf, accessed June 2015.

Nwani CD, Nagpure NS, Kumar R, Kushwaha B, Lakra WS (2013). DNA damage and oxidative stress modulatory effects of glyphosate-based herbicide in freshwater fish, *Channa punctatus*. *Environ Toxicol Pharmacol*, 36(2):539–47. doi:10.1016/j.etap.2013.06.001 PMID:23816461

Omran NE, Salama WM (2013). The endocrine disrupter effect of atrazine and glyphosate on *Biomphalaria alexandrina* snails. *Toxicol Ind Health*, doi:10.1177/0748233713506959 PMID:24215068

Orsi L, Delabre L, Monnereau A, Delval P, Berthou C, Fenaux P *et al.* (2009). Occupational exposure to pesticides and lymphoid neoplasms among men: results of a French case-control study. *Occup Environ Med*, 66(5):291–8. doi:10.1136/oem.2008.040972 PMID:19017688

Ortiz-Ordoñez E, Uría-Galicia E, Ruiz-Picos RA, Duran AG, Trejo YH, Sedeño-Díaz JE *et al.* (2011). Effect of Yerbimat herbicide on lipid peroxidation, catalase activity, and histological damage in gills and liver of the freshwater fish *Goodea atripinnis*. *Arch Environ Contam Toxicol*, 61(3):443–52. doi:10.1007/s00244-011-9648-0 PMID:21305274

Paganelli A, Gnazzo V, Acosta H, López SL, Carrasco AE (2010). Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signalling. *Chem Res Toxicol*, 23(10):1586–95. doi:10.1021/tx1001749 PMID:20695457

Pahwa P, Karunanayake CP, Dosman JA, Spinelli JJ, McLaughlin JR; Cross-Canada Group(2011). Soft-tissue sarcoma and pesticides exposure in men: results of a Canadian case-control study. *J Occup Environ Med*, 53(11):1279–86. doi:10.1097/JOM.0b013e3182307845 PMID:22068131

Park JS, Kwak SJ, Gil HW, Kim SY, Hong SY (2013). Glufosinate herbicide intoxication causing unconsciousness, convulsion, and 6th cranial nerve palsy. *J Korean Med Sci*, 28(11):1687–9. doi:10.3346/jkms.2013.28.11.1687 PMID:24265537

Paz-y-Miño C, Muñoz MJ, Maldonado A, Valladares C, Cumbal N, Herrera C *et al.* (2011). Baseline determination in social, health, and genetic areas in communities affected by glyphosate aerial spraying on the northeastern Ecuadorian border. *Rev Environ Health*, 26(1):45–51. doi:10.1515/reveh.2011.007 PMID:21714381

Paz-y-Miño C, Sánchez ME, Aréval M, Muñoz MJ, Witte T, De-la-Carrera GO *et al.* (2007). Evaluation of DNA damage in an Ecuadorian population exposed to glyphosate. *Genet Mol Biol*, 30(2):456–60. doi:10.1590/S1415-47572007000300026

Peluso M, Munnia A, Bolognesi C, Parodi S (1998). ^{32}P-postlabeling detection of DNA adducts in mice treated with the herbicide Roundup. *Environ Mol Mutagen*, 31(1):55–9. doi:10.1002/(SICI)1098-2280(1998)31:1<55::AID-EM8>3.0.CO;2-A PMID:9464316

Perry L, Adams RD, Bennett AR, Lupton DJ, Jackson G, Good AM *et al.* (2014). National toxicovigilance for pesticide exposures resulting in health care contact - An example from the UK's National Poisons Information Service. *Clin Toxicol (Phila)*, 52(5):549–55. doi:10.3109/15563650.2014.908203 PMID:24735003

Pesticide Residues Committee (2007). Pesticide residues monitoring report. Fourth quarter report 2006. York: Pesticide Residues Committee. Available from: http://www.pesticides.gov.uk/guidance/industries/pesticides/advisory-groups/PRiF/PRC-Pesticides-Residues-Commitee/PRC_Results_and_Reports/PRC_Reports_by_Year/pesticide-residue-committee-prc-2006, accessed 2 November 2014.

Pesticide Residues Committee (2008). Pesticide residues monitoring report. Fourth quarter report 2007. York: Pesticide Residues Committee. Available from: http://www.pesticides.gov.uk/guidance/industries/pesticides/advisory-groups/PRiF/PRC-Pesticides-Residues-Commitee/PRC_Results_and_Reports/PRC_Reports_by_Year/pesticides-residues-committee-prc-reports-2007, accessed 2 November 2014.

Pesticide Residues Committee (2009). Pesticide residues monitoring report. Fourth quarter report 2008. York: Pesticide Residues Committee. Available from: http://www.pesticides.gov.uk/guidance/industries/pesticides/advisory-groups/PRiF/PRC-Pesticides-Residues-Commitee/PRC_Results_and_Reports/PRC_Reports_by_Year/pesticide-residues-committee-prc-reports-2009.htm?wbc_purpose=Ba, accessed 2 November 2014.

Pesticide Residues Committee (2010). Pesticide residues monitoring report. Fourth quarter report 2009. York: Pesticide Residues Committee. Available from: http://www.pesticides.gov.uk/guidance/industries/pesticides/advisory-groups/PRiF/PRC-Pesticides-Residues-Commitee/PRC_Results_and_Reports/PRC_Reports_by_Year/pesticide-residues-committee-prc-reports-2010, accessed 2 November 2014.

Piola L, Fuchs J, Oneto ML, Basack S, Kesten E, Casabé N (2013). Comparative toxicity of two glyphosate-based formulations to *Eisenia andrei* under laboratory conditions. *Chemosphere*, 91(4):545–51. doi:10.1016/j.chemosphere.2012.12.036 PMID:23332878

Poletta GL, Kleinsorge E, Paonessa A, Mudry MD, Larriera A, Siroski PA (2011). Genetic, enzymatic

and developmental alterations observed in *Caiman latirostris* exposed in ovo to pesticide formulations and mixtures in an experiment simulating environmental exposure. *Ecotoxicol Environ Saf*, 74(4):852–9. doi:10.1016/j.ecoenv.2010.12.005 PMID:21185601

Poletta GL, Larriera A, Kleinsorge E, Mudry MD (2009). Genotoxicity of the herbicide formulation Roundup (glyphosate) in broad-snouted caiman (*Caiman latirostris*) evidenced by the Comet assay and the Micronucleus test. *Mutat Res*, 672(2):95–102. doi:10.1016/j.mrgentox.2008.10.007 PMID:19022394

Prasad S, Srivastava S, Singh M, Shukla Y (2009). Clastogenic effects of glyphosate in bone marrow cells of swiss albino mice. *J Toxicol*, 2009:308985 doi:10.1155/2009/308985 PMID:20107585

Rank J, Jensen AG, Skov B, Pedersen LH, Jensen K (1993). Genotoxicity testing of the herbicide Roundup and its active ingredient glyphosate isopropylamine using the mouse bone marrow micronucleus test, *Salmonella* mutagenicity test, and *Allium* anaphase-telophase test. *Mutat Res*, 300(1):29–36. doi:10.1016/0165-1218(93)90136-2 PMID:7683765

República de El Salvador (2013). Asamblea Legislativa aprueba reformas que prohíben pesticidas que dañan la salud, 5 September 2013. Available from: http://www.asamblea.gob.sv/noticias/archivo-de-noticias/asamblea-legislativa-aprueba-reformas-que-prohiben-pesticidas-que-danan-la-salud, accessed 28 April 2015. [Spanish]

Richard S, Moslemi S, Sipahutar H, Benachour N, Seralini GE (2005). Differential effects of glyphosate and Roundup on human placental cells and aromatase. *Environ Health Perspect*, 113(6):716–20. doi:10.1289/ehp.7728 PMID:15929894

Roberts DM, Buckley NA, Mohamed F, Eddleston M, Goldstein DA, Mehrsheikh A *et al.* (2010). A prospective observational study of the clinical toxicology of glyphosate-containing herbicides in adults with acute self-poisoning. *Clin Toxicol (Phila)*, 48(2):129–36. doi:10.3109/15563650903476491 PMID:20136481

Roustan A, Aye M, De Meo M, Di Giorgio C (2014). Genotoxicity of mixtures of glyphosate and atrazine and their environmental transformation products before and after photoactivation. *Chemosphere*, 108:93–100. doi:10.1016/j.chemosphere.2014.02.079 PMID:24875917

Ruder AM, Waters MA, Butler MA, Carreón T, Calvert GM, Davis-King KE *et al.*; Brain Cancer Collaborative Study Group(2004). Gliomas and farm pesticide exposure in men: the Upper Midwest Health Study. *Arch Environ Health*, 59(12):650–7. doi:10.1080/00039890409602949 PMID:16789473

Rueppel ML, Brightwell BB, Schaefer J, Marvel JT (1977). Metabolism and degradation of glyphosphate in soil and water. *J Agric Food Chem*, 25(3):517–28. doi:10.1021/jf60211a018 PMID:858844

Rumack BH (2015). Emergency medical treatment. Glyphosate isopropylamine salt. POISINDEX(R) Information System. CCIS Volume 164, edition expires May, 2015. Available from: http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~M2Dk5e:2.

Sanchís J, Kantiani L, Llorca M, Rubio F, Ginebreda A, Fraile J *et al.* (2012). Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry. *Anal Bioanal Chem*, 402(7):2335–45. doi:10.1007/s00216-011-5541-y PMID:22101424

Schinasi L, Leon ME (2014). Non-Hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: a systematic review and meta-analysis. *Int J Environ Res Public Health*, 11(4):4449–527. doi:10.3390/ijerph110404449 PMID:24762670

Séralini GE, Clair E, Mesnage R, Gress S, Defarge N, Manuela Malatesta M *et al.* (2014). Republished study:long-term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. *Environmental Sciences Europe*, 26(1):1–14. doi:10.1186/s12302-014-0014-5

Siddiqui S, Meghvansi MK, Khan SS (2012). Glyphosate, alachor and maleic hydrazide have genotoxic effect on *Trigonella foenum-graecum* L. *Bull Environ Contam Toxicol*, 88(5):659–65. doi:10.1007/s00128-012-0570-6 PMID:22392005

Simonsen L, Fomsgaard IS, Svensmark B, Spliid NH (2008). Fate and availability of glyphosate and AMPA in agricultural soil. *J Environ Sci Health B*, 43(5):365–75. doi:10.1080/03601230802062000 PMID:18576216

Sinhorin VD, Sinhorin AP, Teixeira JM, Miléski KM, Hansen PC, Moreira PS *et al.* (2014). Effects of the acute exposition to glyphosate-based herbicide on oxidative stress parameters and antioxidant responses in a hybrid Amazon fish surubim (*Pseudoplatystoma* sp). *Ecotoxicol Environ Saf*, 106:181–7. doi:10.1016/j.ecoenv.2014.04.040 PMID:24840881

Siviková K, Dianovský J (2006). Cytogenetic effect of technical glyphosate on cultivated bovine peripheral lymphocytes *Int J Hyg Environ Health*, 209(1):15–20. doi:10.1016/j.ijheh.2005.07.005 PMID:16373198

Slaninova A, Smutna M, Modra H, Svobodova Z (2009). A review: oxidative stress in fish induced by pesticides. *Neuro Endocrinol Lett*, 30:Suppl 1: 2–12. PMID:20027135

Solomon KR, Anadón A, Carrasquilla G, Cerdeira AL, Marshall J, Sanin LH (2007). Coca and poppy eradication in Colombia: environmental and human health assessment of aerially applied glyphosate. *Rev Environ Contam Toxicol*, 190:43–125. doi:10.1007/978-0-387-36903-7_2 PMID:17432331

Sorahan T (2015). Multiple myeloma and glyphosate use: a re-analysis of US Agricultural Health Study (AHS) data. *Int J Environ Res Public Health*, 12(2):1548–59. doi:10.3390/ijerph120201548 PMID:25635915

Sørensen FW, Gregersen M (1999). Rapid lethal intoxication caused by the herbicide glyphosate-trimesium (Touchdown). *Hum Exp Toxicol*, 18(12):735–7. doi:10.1191/096032799678839590 PMID:10627661

Sribanditmongkol P, Jutavijittum P, Pongraveevongsa P, Wunnapuk K, Durongkadech P (2012). Pathological and toxicological findings in glyphosate-surfactant herbicide fatality: a case report. *Am J Forensic Med Pathol*, 33(3):234–7. doi:10.1097/PAF.0b013e31824b936c PMID:22835958

Stella J, Ryan M (2004). Glyphosate herbicide formulation: a potentially lethal ingestion. *Emerg Med Australas*, 16(3):235–9. doi:10.1111/j.1742-6723.2004.00593.x PMID:15228468

Székács A, Darvas B (2012). Forty years with glyphosate. In: Hasaneen MNAE-G, editor. Herbicides – properties, synthesis and control of weeds. Croatia: InTech, pp. 247–84. Available from: http://cdn.intechweb.org/pdfs/25624.pdf, accessed 28 July 2015.

Takeuchi S, Iida M, Yabushita H, Matsuda T, Kojima H (2008). In vitro screening for aryl hydrocarbon receptor agonistic activity in 200 pesticides using a highly sensitive reporter cell line, DR-EcoScreen cells, and in vivo mouse liver cytochrome P450–1A induction by propanil, diuron and linuron. *Chemosphere*, 74(1):155–65. doi:10.1016/j.chemosphere.2008.08.015 PMID:18835618

Temple WA, Smith NA (1992). Glyphosate herbicide poisoning experience in New Zealand. *N Z Med J*, 105(933):173–4. PMID:1589162

Thongprakaisang S, Thiantanawat A, Rangkadilok N, Suriyo T, Satayavivad J (2013). Glyphosate induces human breast cancer cells growth via estrogen receptors. *Food Chem Toxicol*, 59:129–36. doi:10.1016/j.fct.2013.05.057 PMID:23756170

Tian J, Shi H, Li X, Yin Y, Chen L (2012). Coupling mass balance analysis and multi-criteria ranking to assess the commercial-scale synthetic alternatives: a case study on glyphosate. *Green Chem*, 14:1990–2000.

Tice RR, Austin CP, Kavlock RJ, Bucher JR (2013). Improving the human hazard characterization of chemicals: a Tox21 update. *Environ Health Perspect*, 121(7):756–65. doi:10.1289/ehp.1205784 PMID:23603828

Tomlin CDS, editor (2000). The pesticide manual: a world compendium. 12th ed. Croydon: British Crop Protection Council. Available from: http://trove.nla.gov.au/work/6273016, accessed 28 July 2015.

Transparency Market Research (2014). Global glyphosate market expected to reach US$8.79 billion in 2019. New York: Transparency Market Research. Posted on 9 December 2014. Available from: http://www.transparencymarketresearch.com/pressrelease/glyphosate-market.htm, accessed 21 April 2015.

Truta E, Vochita G, Rosu CM, Zamfirache MM, Olteanu Z (2011). Evaluation of Roundup-induced toxicity on genetic material and on length growth of barley seedlings. *Acta Biol Hung*, 62(3):290–301. doi:10.1556/ABiol.62.2011.3.8 PMID:21840831

Tu M, Hurd C, Randall JM (2001). Weed control methods handbook: tools & techniques for use in natural areas. Version April 2001. Arlington (VA): Wildland Invasive Species Team, The Nature Conservancy. Available from: http://www.invasive.org/gist/products/handbook/01.TitleContents.pdf, accessed 28 July 2015.

Uren Webster TM, Laing LV, Florance H, Santos EM (2014). Effects of glyphosate and its formulation, Roundup, on reproduction in zebrafish (*Danio rerio*). *Environ Sci Technol*, 48(2):1271–9. doi:10.1021/es404258h PMID:24364672

Vainio H, Linnainmaa K, Kähönen M, Nickels J, Hietanen E, Marniemi J *et al.* (1983). Hypolipidemia and peroxisome proliferation induced by phenoxyacetic acid herbicides in rats. *Biochem Pharmacol*, 32(18):2775–9. doi:10.1016/0006-2952(83)90091-6 PMID:6626247

Varona M, Henao GL, Díaz S, Lancheros A, Murcia A, Rodríguez N *et al.* (2009). Evaluación de los efectos del glifosato y otros plaguicidas en la salud humana en zonas objeto del programa de erradicación de cultivos ilícitos. [Effects of aerial applications of the herbicide glyphosate and insecticides on human health] *Biomedica*, 29(3):456–75. [Spanish]. doi:10.7705/biomedica.v29i3.16 PMID:20436997

Vasiluk L, Pinto LJ, Moore MM (2005). Oral bioavailability of glyphosate: studies using two intestinal cell lines. *Environ Toxicol Chem*, 24(1):153–60. doi:10.1897/04-088R.1 PMID:15683179

Vera-Candioti J, Soloneski S, Larramendy ML (2013). Evaluation of the genotoxic and cytotoxic effects of glyphosate-based herbicides in the ten spotted livebearer fish *Cnesterodon decemmaculatus* (Jenyns, 1842). *Ecotoxicol Environ Saf*, 89:166–73. doi:10.1016/j.ecoenv.2012.11.028 PMID:23273868

Vigfusson NV, Vyse ER (1980). The effect of the pesticides, Dexon, Captan and Roundup, on sister-chromatid exchanges in human lymphocytes in vitro. *Mutat Res*, 79(1):53–7. doi:10.1016/0165-1218(80)90147-0 PMID:7432366

Waddell BL, Zahm SH, Baris D, Weisenburger DD, Holmes F, Burmeister LF *et al.* (2001). Agricultural use of organophosphate pesticides and the risk of non-Hodgkin's lymphoma among male farmers (United States). *Cancer Causes Control*, 12(6):509–17. doi:10.1023/A:1011293208949 PMID:11519759

Walsh LP, McCormick C, Martin C, Stocco DM (2000). Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression.

Environ Health Perspect, 108(8):769–76. doi:10.1289/ehp.00108769 PMID:10964798

Wang G, Deng S, Li C, Liu Y, Chen L, Hu C (2012). Damage to DNA caused by UV-B radiation in the desert cyanobacterium *Scytonema javanicum* and the effects of exogenous chemicals on the process. *Chemosphere*, 88(4):413–7. doi:10.1016/j.chemosphere.2012.02.056 PMID:22436589

Wester RC, Melendres J, Sarason R, McMaster J, Maibach HI (1991). Glyphosate skin binding, absorption, residual tissue distribution, and skin decontamination. *Fundam Appl Toxicol*, 16(4):725–32. doi:10.1016/0272-0590(91)90158-Z PMID:1884912

Xie L, Thrippleton K, Irwin MA, Siemering GS, Mekebri A, Crane D *et al.* (2005). Evaluation of estrogenic activities of aquatic herbicides and surfactants using an rainbow trout vitellogenin assay. *Toxicol Sci*, 87(2):391–8. doi:10.1093/toxsci/kfi249 PMID:16049272

Yadav SS, Giri S, Singha U, Boro F, Giri A (2013). Toxic and genotoxic effects of Roundup on tadpoles of the Indian skittering frog (*Euflictis cyanophlyctis*) in the presence and absence of predator stress. *Aquat Toxicol*, 132–133:1–8. doi:10.1016/j.aquatox.2013.01.016 PMID:23454306

Yin G (2011). Glyphosate: There is no substitute. Farm Chemicals International. 3 March 2011. Willoughby (OH): Meister Media Worldwide. Available from: http://www.farmchemicalsinternational.com/crop-inputs/herbicides/glyphosate-there-is-no-substitute/, accessed June 2015.

Yoshioka N, Asano M, Kuse A, Mitsuhashi T, Nagasaki Y, Ueno Y (2011). Rapid determination of glyphosate, glufosinate, bialaphos, and their major metabolites in serum by liquid chromatography-tandem mass spectrometry using hydrophilic interaction chromatography. *J Chromatogr A*, 1218(23):3675–80. doi:10.1016/j.chroma.2011.04.021 PMID:21530973

Yue Y, Zhang Y, Zhou L, Qin J, Chen X (2008). In vitro study on the binding of herbicide glyphosate to human serum albumin by optical spectroscopy and molecular modeling. *J Photochem Photobiol B*, 90(1):26–32. doi:10.1016/j.jphotobiol.2007.10.003 PMID:18035550

Zahm SH, Weisenburger DD, Babbitt PA, Saal RC, Vaught JB, Cantor KP *et al.* (1990). A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. *Epidemiology*, 1(5):349–56. doi:10.1097/00001648-199009000-00004 PMID:2078610

Zhao W, Yu H, Zhang J, Shu L (2013). [Effects of glyphosate on apoptosis and expressions of androgen-binding protein and vimentin mRNA in mouse Sertoli cells] *Nan Fang Yi Ke Da Xue Xue Bao*, 33(11):1709–13. PMID:24273285

Zouaoui K, Dulaurent S, Gaulier JM, Moesch C, Lachâtre G (2013). Determination of glyphosate and AMPA in blood and urine from humans: about 13 cases of acute intoxication. *Forensic Sci Int*, 226(1–3):e20–5. doi:10.1016/j.forsciint.2012.12.010 PMID:23291146

Comment Letter I-Fra3 Fraser, Mary

Response 1

Comment noted on the commenter's displeasure with the time for review and the provision of CDs to 29 public libraries. Ms. Fraser's request for extension of time to 120 days was not granted. Two requests for limited extensions of review time were granted to NMWD for receipt of comments on October 6, 2015, and to CDFW for receipt of comments on October 19, 2015. The California Department of Parks and Recreation missed the comment deadline by 14 days, and the District decided to include their brief comments into the PEIR, The NOA stated that the PEIR was available on the District's website, so the public could access the document from their homes. Ms. Fraser was also given a CD at the public hearing on September 15. A hard copy of the entire document was available at District offices for review by appointment to facilitate its review by anyone who did not have access to a computer.

The distribution and availability of the Draft PEIR follows CEQA requirements and common practices of providing appropriate opportunities for public review and comment. The standard 45-day review period was extended by 5 days to allow for additional review time by interested parties on the District's mailing list (356 addresses). Each hard copy cost approximately $500 to produce, and the District is not obligated to provide hard copies without charge. The District is also not required to place large, expensive display advertisements in the newspapers. Only the legal notices are required along with posting of the NOA at the County Clerks' offices. The District's mailing list was originally developed by the Marin County Planning Department for use in notifying the public of the availability of CEQA documents. It included 81 nongovernmental organizations and special interest groups/associations.

Although Ms. Fraser states she has not had time to adequately review the entire PEIR, she did provide seven separate written comments, participated in the group represented by the Law Offices of Stephan C. Volker, and provided oral comments at two of the three public hearings.

The PEIR was organized to have specific chapters on ecological health (Chapter 6) and on human health (Chapter 7) to help those who were only interested in these two subjects to focus quickly. Chapter 1 listed the environmental topics and concerns covered under each chapter (Section 1.5 Environmental Concerns). Most PEIR reviewers only read the Summary followed by Chapters 1 and 2 and the specific resource chapters of concern to them. Very few people read an entire EIR (any EIR) cover to cover.

Response 2

Concerning the comment on the public hearing presentation, the District uses several biorational formulations of mosquito larvicides that contain three bacterial active ingredients that are found in nature. Certain formulations containing these active ingredients are labeled for use with organic crops by the Organic Materials Review Institute and the USEPA. Examples of bacteria pathogenic to mosquitoes are *Bacillus sphaericus* (Bs), the several strains of *Bacillus thuringiensis israelensis* (Bti), and *Saccharopolyspora spinosa*. Two bacteria, Bs and Bti, produce proteins that are toxic to most mosquito larvae, while *Saccharopolyspora spinosa* produces compounds known as spinosyns, which effectively control all larval mosquitoes. Bs can reproduce in natural settings for some time following release. The Bti materials the District applies do not contain live organisms but only spores made up of specific protein molecules. All three bacteria are naturally occurring soil organisms that are also commercially produced for use as mosquito larvicides. These are the only three active ingredients approved for use in controlling larval mosquitoes when organic production is in progress.

None of the other natural or synthetic materials involved in vector control were cited in the presentation as being consistent with organic farming. Glyphosate is a synthetic chemical and is not approved for use on organic farms. The PEIR preparers reviewed numerous studies on glyphosate, the WHO report, and scientific reviews of the World Health Organization (WHO) report in determining that potential use by the

District poses a less-than-significant impact on human health. The WHO report is the result of a "panel discussion and vote" by the International Agency for Research on Cancer (IARC) about the potential for selected chemicals and products that have achieved some level of public interest and concern but may or may not be supported by the data and information available. The panel is comprised of several European scientists and government organizations reporting to the WHO (a scientifically conservative advocacy agency) sponsored by the UN. This group is known to generally follow the "precautionary principle" that is used by some members of the public to argue against chemical use. The precautionary principle is a hypothesis generally rejected by the scientific community that "unless one can prove there is or can be no adverse impacts of a substance it should be considered hazardous." To those with scientific training, this suggests that one must "prove a negative" which is essentially impossible in any statistical sense of a scientific study.

In fact, the IARC has been challenged by dozens of technical experts who evaluated the process used by the panel to list glyphosate as a probable carcinogen. It has been demonstrated that IARC rejected the 800 studies / 3,000 documents that gave glyphosate a positive safety result, basing their decision of "probably carcinogenic" on only eight studies, of which three actually included results that were themselves arguably insignificant. After the WHO publication listing glyphosate as a probable carcinogen, dozens of practicing scientists in the mainstream scientific community (including European Food Safety Administration, the German Federal Institute for Risk Assessment (*BfR*) and the lead author of one of the studies used by IARC to draw their conclusions) have criticized and disputed the results of the IARC for using a poor methodology and inadequate research. The conclusions drawn by the IARC about the potential adverse effects of glyphosate were based on studies that are not relevant to actual, potential exposures and on studies that were based on high exposures to petri dish cells and in vitro laboratory conditions.

Glyphosate exposure was not associated with cancer incidence overall or with most of the cancer subtypes studied by de Roos et al (2005). Given the widespread use of glyphosate, and the paucity of information indicating significant and relevant causality, the nonscientific claims that glyphosate exhibits numerous low-level or sublethal adverse effects (Seneff nd.) are insufficient to refute the weight of the evidence that the District's use of glyphosate would not have significant impacts. Relevant to this conclusion is the fact that many of the publications cited as demonstrating adverse impacts from glyphosate, particularly those suggesting sublethal adverse effects of glyphosate by Seneff et al, have been discounted and the reports retracted after pressure from the active research community. The reports submitted by Seneff and many of her co-authors have been retracted due to pressure from editorial boards and practicing scientists. Some of the reports she has submitted incorrectly and unscientifically relate correlation to causality. This method produces two general statements, for example, that cannot be linked statistically or even practically. She has been ostracized for this approach, and some of her papers have had to be retracted. An example of this un-scientific comparison is to relate national health data with national pesticide use and suggest that they are clearly linked, while, in fact, there is no connection to actual exposures. For example, the following study had to be retracted after pressure from the editors: One of the papers that was critically reviewed and retracted is "Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize" (Séralini et al. 2012).

To be retracted in science journals is a harsh critique of a submitted work and means that the editorial board and a number of recognized scientists in the field have demonstrated a fatal or a series of fatal flaws in the logic, assumptions, and conclusions. Seneff is a computer modeler who uses meta-analysis techniques and not a practicing researcher. She fails to evaluate the risk/benefit analyses that are utilized by USEPA and other regulators. The District's PEIR scientists have reviewed each of the seven articles submitted directly by Ms. Fraser.

The senior toxicologist on the consultant team who prepared the District's PEIR, Dr. Bill Williams, concluded that there have been no demonstrated significant adverse health effects (even for pesticide applicators). The studies reporting potential human health effects are associated with extreme exposures

to applicators during misuse scenarios and spills and/or working in the preparation of the commercial products (Mink et al. 2012). These conditions and potential exposure conditions are neither typical nor likely in the use and applications by trained District staff. All application directions include detailed procedures to deal with a spill. Glyphosate remains a reliable and environmentally compatible product for use in the numerous situations where control of vegetation is needed for habitat management (for vector control or for invasive species control). Importantly, it has been demonstrated that herbicides are a different class of chemicals than those classified as insecticides that have specific, demonstrated autonomic effects. The media reports about the hazards of glyphosate and its several commercial products have not been clearly associated with human health. The numerous reports about "possible" connections to metabolic processes and subtle effects also include confounding factors that make scientifically defensible claims impossible. Where there are reports of adverse subtle effects, they are usually based on laboratory studies of changes to cells after immersion exposures, which are exposures far above any possible actual human (or animal) exposure. The implication and correlation of such exposures to actual potential exposures in humans or animals are not realistic.

USEPA continually reviews the available scientific data and other relevant information in support of the registration of glyphosate (i.e., commercial product Roundup for weed control) and has indicated that there are sufficient data to assess the hazards of and to make a determination on aggregate exposure for glyphosate including exposure resulting from the tolerances established by this action. USEPA's assessment of exposures and risks associated with glyphosate are clearly indicated in the numerous studies used to develop the guidance for use. Using these data, the USEPA has set maximum safe exposure levels for both humans and animals (tolerances) of pesticide residues for crops based on the huge number of scientific studies and complex risk assessment approaches provided in support of the active ingredient in the products. These tolerances are hundreds of times higher than estimated toxic values using total exposure values to pesticides (including safety levels to protect children and others who may be vulnerable). The USDA tests crops each year to make sure they do not exceed the tolerances. Very few pesticides are found above the tolerance levels (despite some unsubstantiated media reports). The exposures that were used in the WHO evaluation and studies were not reasonable examples of the exposures that might be encountered by humans. US Environmental Protection Agency (USEPA) 1993 and National Pesticide Information Center, Oregon State University 2011. There are occasionally media reports of studies linking glyphosate to cancers of various types, but these are generally results from cultured cells in the laboratory. Extrapolation of these very high dose laboratory studies to animals and humans are not reliable indicators of potential adverse effects from the Program use, because they would require direct exposure or even ingestion, neither of which is reasonably foreseeable under the Program. See Response O-VOL-22.

Response 3

The commenter suggests that the PEIR's information on glyphosate is inaccurate and relies on old information. The fact that a study was done in 1993 does not make it invalid. Moreover, the body of information for the registration of glyphosate has been submitted to the USEPA in dozens of studies, first by the manufacturer and its university and contractor scientists. The suite of studies required for approval include dozens and dozens of potential acute and chronic tests to detect possible effects to mammals (as surrogates for humans as well as for wildlife), birds, invertebrates, and bees. The research test data submitted to the USEPA is an ongoing process required for its Re-registration Eligibility Decision (RED) in which label changes and use patterns are reviewed and updated. See also Response 2 above on EPA continual reviews of the available studies.

The links considered in this comment are to the following studies:

> Mesnage et al., 2014, Major pesticides are more toxic to human cells than their declared active principles, BioMed Res International Volume 2014

> Mesnage et al, 2012, Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity,*Toxicology* 313 (2-3) 2013

> Samsel and Seneff, 2015, Glyphosate, pathways to modern diseases III: Manganese, neurological diseases, and associated pathologies, *Surg Neurol Int* 6

> Stephanie Seneff Home Page with selected abstracts and references of 25

These studies are addressed above in Response 2 and do not result in a change to any of the conclusions reached in the PEIR.

Response 4

The commenter is concerned about other ingredients combined with glyphosate that enhance the toxicity of glyphosate. Ms. Fraser cites the following studies: Mesnage et al (2014) and selected abstracts in PowerPoint from her Home Page (Seneff at Mit).

There are numerous pesticide products that include inert and/or chemically different additives to enhance the spray characteristics, adhesion properties, and efficacy. Many of those products have been specially tested for toxicity and registered with the USEPA for specific vector control purposes (National Park Service 2016). Although some of these mixture products have been associated with increased toxicity, numerous studies have demonstrated that the increase in toxicity may be due to a surfactant additive. In most instances, these special formulations of pesticide products are intended to reduce the potential for adverse effects or to specifically be used for aquatic environments, e.g. a glyphosate product, Accord, which has been shown to be safer to aquatic wildlife than some of the other formulations of glyphosate (Brodman et al. 2010).

All chemicals can cause adverse effects or even become be toxic at levels exceeding individual species “tolerance” levels. However, the sensitivity and tolerance levels are determined by the USEPA and other regulatory agencies using laboratory tests with numerous species of concern that are estimated to be potentially exposed to an application. The results of these tests on each chemical are published in numerous publically available USEPA documents summarizing the testing results with metrics such as the LD50, LC50 and maximum estimated tolerance levels. For the pesticides used by the District for vector control, these metrics are indicated in detail in Appendix B of the PEIR, with information on a current species of interest (honeybee).

Studies submitted to, reviewed by, and accepted by the USEPA scientists in support of the registration of glyphosate indicate that both technical and formulated glyphosate are practically nontoxic to honeybees with a contact LD50 value greater than 100 µg/bee applied directly to the thorax with a saturated Q-tip (Frasier and Jenkins 1972, the contract scientists for the manufacturer, who submitted the studies for inclusion in the toxicity evaluation). This technique, designed to simulate a worst case exposure to the bee, results in considerably greater exposure than likely under natural conditions in the environment where applications could occur. Over the past decades, to update and support the original data submitted by Frasier and Jenkins, several studies on glyphosate have been conducted to confirm and validate the toxicity estimates first submitted to USEPA for registration. These studies have been submitted to the USEPA for inclusion in the Integrated Risk Information System (IRIS, USEPA). The recent data submissions and reports continue to support the finding that glyphosate is “relatively” nontoxic to honey bees (Porterfield 2015; Zhu et al. 2015; Giesy et al. 2000; see also Table 6-1 in Appendix B of the PEIR).

While it has been reported that the addition of some surfactants to the base chemical glyphosate may make the products more toxic to some biota, the primary concern is toxicity based on studies using high, continuous exposures in laboratory tests. The exposures in the laboratory studies are clearly not representative to realistic field conditions or exposures in field applications because the laboratory studies involve captive test species, unable to choose uncontaminated food or habitat. Many laboratory tests are designed and conducted to determine the ‘worst-case” exposure to a chemical and then to lower the test

concentrations slowly until a test concentration shows no adverse effect to the test animals (USEPA 2012; Williams et al. 1994). In this way, the concentrations that produce exposures with little or no adverse response can be documented and used to define the applications that should not be hazardous to the animals and environment. As in all relevant laboratory toxicity studies, the exposures in laboratory conditions are essentially 100 percent with no ability to choose areas of lessor concentrations, and use non-representative exposures. The best available evidence indicates that glyphosate toxicity would not occur as a result of the District's method of use of the chemical under the Program.

See also Response O-VOL-17.

Response 5

The commenter is referring to Section 4.6.2.3 in Appendix B which was prepared in June 2013. Since then, additional studies have been reviewed including the WHO report, which was published in 2015. The WHO report is the result of a "panel discussion" by the International Agency for Research on Cancer (IARC) about the potential for selected chemicals and products that have achieved some level of public interest and concern but may or may not be supported by the data and information available. In fact, the IARC has been challenged by dozens of technical experts who evaluated the process used by the panel to list glyphosate as a probable carcinogen. It has been demonstrated that IARC rejected the 800 studies / 3,000 documents that gave glyphosate a positive safety result, basing their decision of "probably carcinogenic" on only eight studies, of which three actually included results, which results were arguably insignificant. See Response 2 above.

The commenter suggested that glyphosate degrades slowly and is not subject to biodegration. The product Roundup (glyphosate active ingredient) is degraded over time by soil microbes into breakdown products and naturally-occurring substances. Giesy et al. (2000) in field studies concluded the following: "Field studies indicate that glyphosate typically dissipates rapidly from both simple ecosystems, such as agricultural, and more complex ecosystems, such as forestry, regardless of the diverse edaphic [soil] and climatic conditions." The authors also concluded that field studies conducted in agricultural and forest soils (13 studies, five countries, 47 different sites) indicated an average half-life of 32 days. In 2002, the European Commission completed an assessment of fate and behavior of glyphosate in environment (European Commission 2002). Under a wide range of climatic conditions found in the US, Canada, and Europe, the mean half- life for glyphosate degradation in field soil was reported to be 30 days, with a range from 1 to 130 days, while the half-life of glyphosate (the time required for half of the compound to dissipate or degrade) varies, depending on conditions. The variability in rates of glyphosate degradation is believed to be due to the varying microbial activity and extent of soil-binding at the different study sites (Giesy et al. 2000).

The comment on the lawsuit against Monsanto for false advertising is not relevant to the PEIR discussion on glyphosate products for vegetation management as part of the District's IVMP. No further response is required.

Response 6

The attachments provided by the commenter by email are provided following her comment include the following seven studies:

> Anthony Samsel and Stephanie Seneff, Glyphosate's Suppression of Cytochrome P450 Enzymes and Amino Acid Biosynthesis by the Gut Microbiome: Pathways to Modern Diseases (Samsel and Seneff 2013a)

> Anthony Samsel and Stephanie Seneff. Glyphosate, pathways to modern diseases II: Celiac sprue and gluten intolerance (Samsel and Seneff 2013b)

> Anthony Samsel and Stephanie Seneff. Glyphosate, pathways to modern diseases III: Manganese, neurological diseases, and associated pathologies (Samsel and Seneff 2015)

> Judy Hoy, Nancy Swanson, and Stephanie Seneff. The High Cost of Pesticides: Human and Animal Diseases (Hoy et al. 2015)

> Chen I-wan, Glyphosate, Roundup, Glyphosate-Tolerance GM Soybeans, Chemical Extracted Soybean Food Oil/Soybean Powder Cause Serious Harm to Health of American/Chinese People. Reference Information (Chen I-wan 2014)

> Robin Mesnage, Nicolas Defarge, Joël Spiroux de Vendômois, and Gilles-Eric Séralini. Major Pesticides Are More Toxic to Human Cells Than Their Declared Active Principles (Mesnage et al. 2014)

> R. Mesnagea, B. Bernayc, G. E. Séralini. Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity (Mesnage et al. 2012)

These studies provided by Mary Frasier have been reviewed for their content and applicability to exposure to glyphosate and ethoxylated adjuvants that may be correlated with diseases and health. They are addressed and considered for the Final PEIR as follows:

> All three Samsel et al, publications (2013a, 2013b, and 2015) are discussions of the possible impacts of glyphosate on metabolic and gastric functions, even gluten intolerance. Although there are extensive illustrations and graphics, the relation between the diseases listed and actual pesticide exposures are not conclusive. The papers read like monographs covering dozens of potential adverse impacts of glyphosate. One of the co-authors (Seneff) has had issues with the credibility of the assumptions and conclusions in her papers, and these papers do not clearly equate the linkages to metabolic impacts. The conclusions made cannot be easily evaluated or supported as the illustrations all present summary data from other sources. The presentations are not relevant to the PEIR as presented.

> Hoy et al. (2015) discusses the linkage of human and wildlife health and pesticide use in Montana, suggesting a relationship to glyphosate. The focus on diseases at the state level vs pesticide uses at the state level does not provide an adequate link to a causal correlation. The presentations are not relevant to the PEIR as presented.

> Mesnagea et al. (2012 and 2014), both discuss possible linkage of glyphosate products and ethoxylated adjuvants in the onset of sublethal or chronic adverse effects. They provide information on all three classes of pesticides: herbicides, fungicides, and insecticides. The papers contain summaries of possible impacts of these pesticides and focus on the formulations rather than the active ingredient of each pesticide. The information is interesting and organized, but the information is not specific or relevant to the PEIR.

> Chen I-wan (2014) is an extensive discussion of cancer incidence in several areas of China that the author suggests are linked to food products. He provides extensive and comprehensive details of the demographics of cancer incidence in China. The report provides a level of information generally attributed to a monograph about the cancers in China. The information is interesting and impressive, but the information is not relevant to the PEIR.

Response 7

The commenter asks that glyphosate not be used and that no chemicals be used; she supports the No Chemical Program. This comment will be considered by the District's Board of Trustees in its consideration whether to approve the Program as proposed, or with modifications.

Additional References

Brodman, R., W.D. Newman, K. Laurie, S. Osterfeld, and N. Lenzo. 2010. Interaction of an aquatic herbicide and predatory salamander density on wetland communities. Journal of Herpetology 44(1):69-82

Chen, I-wan. 2014. Glyphosate, Roundup, Glyphosate-Tolerance GM Soybeans, Chemical Extracted Soybean Food Oil/Soybean Powder Cause Serious Harm to Health of American/Chinese People. Reference Information. June 18.

De Roos, A.J., A. Blair, J.A. Rusiecki, J.A. Hoppin, M. Svec, M. Dosemeci, D.P. Sandler, and M.C. Alavanja. 2005. Cancer incidence among glyphosate-exposed pesticide applicators in the Agricultural Health Study. Environmental Health Perspectives 113 (1): 49–54. January.

European Commission. 2002. Report for the Active Substance Glyphosate, Directive 6511/VI/99, January 21. Available online at http://europa.eu.int/comm/food/fs/ph_ps/pro/eva/existing/list1_glyphosate_en.pdf.

Frasier, W.D., G. Jenkins. 1972. The acute contact and oral toxicities of CP67573 and MON2139 to worker honey bees. Report 4G1444 submitted to US Environmental Protection Agency by Monsanto Company and prepared by Huntingdon Research.

Giesy J.P., S. Dobson, and K.R. Solomon. 2000. Ecotoxicological risk assessment for Roundup herbicide. Reviews of Environmental Contamination and Toxicology 167: 35-120.

Hoy, J., N. Swanson, and S. Seneff, 2015. The High Cost of Pesticides: Human and Animal Diseases. Poult Fish Wildl Sci 3:1.

Mesnage, R., B. Bernay, and G.-E. Séralini. 2012. Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. Elsevier Ireland Ltd.

Mesnage, R., N. DeFarge, J. Spiroux de Vendômois, and G.-E. Séralini. 2014. Major pesticides are more toxic to human cells than their declared active principles. Article ID 179691. Hindawi Publishing Corporation, BioMed Research International,

Mink, P.J., J.S. Mandel, B.D. Sceurman, and J.I. Lundin. 2012. Epidemiologic studies of glyphosate and cancer: a review. Regul Toxicol Pharmacol 63 (3):440-452.

National Park Service. 2016. Invasive Plant Management Plan for Yosemite National Park ESA.

National Pesticide Information Center, Oregon State University. 2011. Glyphosate Technical Fact Sheet. npic@ace.orst.edu.

Porterfield, A, 2015. Study finds glyphosate and acetamiprid to have relative low toxicity for honey bees. Entomology Today, October 13,

Séralini, G-E, E. Clair, R. Mesnage, S. Gress, N. Defarge, M. Malatesta, D. Hennequin, and J. Spiroux de Vendômois. Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. Food and Chemical Toxicology 50 (11) 2012.

Samsel, A., and S. Seneff. 2013a. Glyphosate's suppression of cytochrome P450 enzymes and amino acid biosynthesis by the gut microbiome: pathways to modern diseases. Entropy 15: 1416-1463.

Samsel, A., and S. Seneff. 2013b. Glyphosate, pathways to modern diseases II: celiac sprue and gluten intolerance. Interdisciplinary Toxicology 6(4): 159–184.

Samsel, A., and S. Seneff. 2015. Glyphosate, pathways to modern diseases III: Manganese, neurological diseases, and associated pathologies. Surg Neurol Int 6:45.

US Environmental Protection Agency (USEPA). 2012. Test Guidelines for Pesticides and Toxic Substances. Series 850 under FIFRA, TSCA, and FFDCA. June. Available online at http://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-850-ecological-effects-test-guidelines

Williams. B. et al., eds. 1994. Assessing Pesticide Impacts on Birds. Final Report of the Avian Effects Dialogue Group, 1988-1993. RESOLVE, Center for Environmental Dispute Resolution.

World Health Organization (WHO). 2015. Evaluation of five organophosphate insecticides and herbicides. Includes rebuttal discussions. IARC Monographs 112.

Zhu,YC, J. Adamczyk, T. Rinderer, J. Yao, R. Danka, R. Luttrell, and J. Gore. 2015. Spray toxicity and risk potential of 42 commonly used formulation of row crop pesticides to adult honey bees. Journal of Economic Entomology 108 (6): 2640-2647.

I-Fra4

Comment 4 from Mary Fraser

Dear District Board of Directors and Staff,
I urge you to choose the No Chemical option from the Draft PEIR. Many other places are understanding how detrimental to the environment and human health the use of pesticides are. Attached is a list of many other places that have either banned or restricted pesticides. Here is the text of a speech that I gave about the other places that have banned pesticides and their reasons for doing so.

1

Board of Supervisors and Ladies and Gentlemen,

My name is Mary Fraser. I am a resident of Marin Co.

I am here today to urge you to ban pesticides in Marin County. Today I want to talk about other places that have banned pesticides and why they chose to do so. Each place is a little different but they all chose public health over the use of pesticides.

As you may know, the Marin Municipal Water District recently chose to exclude the use of pesticides from the 26,000 acres they manage. This is a continuation of a 9 yr. hiatus in the use of pesticides. No one wants pesticides in our water supply.

2

Richmond CA has banned pesticides. This effort was led by 2 doctors, one of whom is the retired chief of cardiology for Kaiser Permanente. Dr. Jeff Ritterman wrote and published an extensive article outlining the public health reasons for banning pesticides. I had the privilege to hear Dr. Ritterman speak recently. He started his talk by saying that the compelling evidence against pesticide use was the birth defects that are showing up in South America. We are talking about babies born with no skull. Babies born with no arms AND no legs. Babies born with one big cyclops eye. Defects nearly identical to the ones that happened in VietNam after we defoliated the country with Agent Orange. And we did not ban the entire formula of Agent Orange. Only 2, 4 T. We still use 2, 4 D on our agriculture.

So in Argentina, because of the health consequences, 30,000 doctors have called for a ban on pesticides. 30,000 doctors.

In El Salvador, 20,000 men have died in the last 5 years because they started using pesticides in the sugar cane fields. In El Salvador they've banned 32 different pesticides.

I-Fra4

Sri Lanka has the same issue. Thousands are dying from kidney disease. One of the reasons that they have such terrible kidney diseases is because they have serpentine soil. We have serpentine soil in Marin. Sri Lanka has banned pesticides.

France has banned them. The Netherlands has banned non commercial use. German ministers are calling for a ban in the entire European Union. All of these countries operate on the precautionary principle, where chemicals have to be proven safe before they can be used. In America, we have the opposite policy. Chemicals undergo minimal testing by the manufacturer and then the public has to prove that a chemical is unsafe before it can be forced off of the market. This can take decades and have untold consequences.

2

So I'm asking the Board of Supervisors to be our heroes and ban pesticides on all of the property that the County of Marin owns, leases and manages, including Open Space.

Places where the use of glyphosate and/or pesticides are restricted or banned.

Marin County:

Marin Municipal Water District, Marin County, CA. 22,000 acres of watershed- Pesticides banned. Vote taken 7/7/15 http://www.marinij.com/environment-and-nature/20150708/marin-water-district-herbicides-wont-be-used-on-mount-tam

City of Fairfax, CA- Pesticides banned on commons.

City of Belvedere, CA- Pesticides banned on commons.

City of Sausalito, CA-Pesticides under review

Reed School District, Tiburon, CA- Pesticides not used on school district grounds.

I-Fra4

Larkspur-Corte Madera School District, Corte Madera, CA-Pesticides not used on school grounds.

Mill Valley School District, Mill Valley, CA- Pesticides not used on school grounds.

States:

Connecticut: Pesticides banned on municipal playgrounds:

http://www.beyondpesticides.org/dailynewsblog/2015/07/connecticut-bans-toxic-lawn-pesticides-in-municipal-playgrounds-statewide

New York: Pesticides banned on school grounds

-

Countries:

Country of the Netherlands- Glyphosate banned for non-commercial use.
http://sustainablepulse.com/2014/04/04/dutch-parliament-bans-glyphosate-herbicides-non-commercial-use/#.VZ2Wca5Vikp

Country of Sri Lanka-Glyphosate banned
http://www.publicintegrity.org/2014/03/13/14418/sri-lanka-bans-monsanto-herbicide-citing-potential-link-deadly-kidney-disease.

County of El Salvador- Glyphosate banned by legislature.

I-Fra4

Country of Bermuda- Glyphosate imports suspended.
http://www.todayinbermuda.com/news/health/item/1471-health-minister-importation-of-roundup-weed-spray-suspended

-

Country of Brazil. Chief prosecutor wants glyphosate banned.
http://www.globalresearch.ca/brazils-public-prosecutor-wants-to-ban-monsantos-chemicals-following-recent-glyphosate-cancer-link/5449440

Country of Germany and the European Union (EU). EU ban proposed by ministers.
http://www.globalresearch.ca/german-ministers-call-for-eu-wide-ban-on-monsantos-deadly-glyphosate-herbicide-roundup/5451831

1. Country of Argentina. 30,000 medical doctors call for ban on glyphosate.
   http://www.globalresearch.ca/30000-doctors-in-argentina-demand-that-glyphosate-be-banned/5445542

   -

   Country of Columbia. Glyphosate banned for use on illicit crops.
   http://www.bbc.com/news/world-latin-america-32677411

   -

   Country of France. Sale of glyphosate banned.
   http://www.naturalnews.com/050248_french_legislation_glyphosate_ban_Monsanto_GMOs.html

   -

   States:

   Connecticut: Pesticides banned on municipal playgrounds:

http://www.beyondpesticides.org/dailynewsblog/2015/07/connecticut-bans-toxic-lawn-pesticides-in-municipal-playgrounds-statewide

New York: Pesticides banned on school grounds

I-Fra4

-

Cities:

City of Richmond, CA- Moratorium on organophosphate pesticides

Chicago, IL,

Takoma Park, MD

Laguna Hills, CA

Durham, CT

Paris, France

University of Vermont Law School and Emory University campuses have banned all pesticides

Santa Barbara, CA

Carrboro, NC

Plainville, CT

I-Fra4

Santa Fe, NM

The township of Marblehead, Massachusetts instituted one of the earliest ordinances calling for the use of Organic Land Care on township lands and has successfully implemented fully organic techniques for turf management.

Between 1996 and 2005 San Francisco municipal agencies achieved an 85% reduction in gallons of liquid pesticide, a 55% decrease in pounds of solid pesticides, and a 90% decrease in the use of Roundup®, one of the herbicides most widely used by municipalities. San Francisco has been so successful that in 2005 New York City passed a similar local law based largely on San Francisco's ordinance.

Mary Fraser
[redacted]

Comment Letter I-Fra4 Fraser, Mary

Response 1

The commenter asks that no chemicals be used; she supports the No Chemical Program. This comment will be considered by the District's Board of Trustees in its consideration whether to approve the Program as proposed, or with modifications.

Response 2

The commenter provides a speech to the Marin County Board of Supervisors that is against the use of various pesticides followed by lists of places where glyphosate or other pesticides are restricted.

The Draft PEIR listed the communities in the Program Area that had adopted IPM policies or restrictions on pesticide use in Section 3.1.3.3. Several of these local policies and ordinances are applicable only to government (city-owned) property and have exceptions for protection of public health. As stated in this section:

> "Typically, policies and programs related directly to pesticide use are outside the purview of local planning and zoning regulation. However, some cities and counties have enacted regulations on pesticide use as part of their municipal code. Local governing bodies may pass ordinances that regulate or restrict pesticide use in their own operations. However, these restrictions do not apply to state operations and would not be applicable to treatments the District proposes under the Program because California state law preempts local regulation and restriction of pesticide use. The District is a regulatory agency formed pursuant to California Health and Safety Code Section 2000 et seq. State law charges the District with the authority and responsibility to take all necessary or proper steps for the control of mosquitoes and other vectors in the District (see Section 1.1.3)." (page 3-4)

See the transcript from the public hearing held in Santa Rosa, California, on September 17, 2015 (T-Santa Rosa) for additional comments related to the other locations with regulations on pesticide use. Regulations in other cities and countries on pesticide use are outside the scope of the District's PEIR.

For additional responses to comments on glyphosate, please see responses to comments I-Fra3.

This Page Intentionally Left Blank

This Page Intentionally Left Blank

I-Fra5

Marin/Sonoma Mosquito and Vector Control District's Integrated Vector Management Program Draft PEIR

WRITTEN COMMENT

Name/Affiliation: Mary Fraser Date: 9/12/15

Address: [redacted]

City: Mill Valley Zip Code: 94941 Telephone [redacted]

Please provide comments and concerns on the content of the Draft PEIR and the potential environmental effects of the Proposed Program.

Please see the attached document - "A Generation in Jeopardy - How Pesticides are Undermining our Children's Health and Intelligence -

Please add this document to your PEIR.

1

peir@msmosquito.com

Please use backside of page for additional comments, if needed. This comment form may be handed in at the public hearing to District staff or mailed to the attention of Philip Smith, to arrive on or prior to **October 2, 2015** at the Marin/Sonoma Mosquito and Vector Control District, 595 Helman Lane, Cotati CA 94931.

I-Fra5

A Generation in Jeopardy

How pesticides are undermining our children's health & intelligence

PESTICIDE ACTION NETWORK NORTH AMERICA

I-Fra5

Pesticide Action Network North America

Pesticide Action Network North America (PAN North America) works to replace the use of hazardous pesticides with ecologically sound and socially just alternatives. As one of five PAN Regional Centers worldwide, we link local and international consumer, labor, health, environment and agriculture groups into an international citizens' action network. This network challenges the global proliferation of pesticides, defends basic rights to health and environmental quality, and works to ensure the transition to a just and viable society.

Acknowledgements

This report would not have been possible without the dedicated and careful work of hundreds of scientists at academic institutions in the U.S. and around the world. The contribution of these researchers to our collective understanding of the links between pesticide exposure and children's health is truly invaluable.

A Generation in Jeopardy also reflects the efforts and expertise of many individuals both within Pesticide Action Network and among our partner organizations and institutions. Susan Kegley, Heather Pilatic, Linda Wells and Kathryn Gilje provided useful comments and direction as the report was being developed and finalized. Several academic reviewers representing expertise in neurodevelopmental and carcinogenic impacts of pesticides on children's health provided substantive comments. Laura Cossette, Kristen Parks and Maria Reyna provided valuable research assistance.

Thanks also go to Brenda J. Willoughby who formatted the report for publication, Sara Knight who tracked down images, created figures and otherwise assisted immeasurably with production, Mateo Rutherford and Roy Rojas of Berkeley Interpretation, Translation and Transcription Services (BITTS) who translated the Executive Summary into Spanish, and Janet Stephens and Kathryn Gilje for final proofing and copy editing.

Funding for this report was provided by The A&A Fund, the Arntz Family Foundation, the Bellwether Foundation Inc., The California Endowment, the Cedar Tree Foundation, the Ceres Foundation, the Ceres Trust, the David B. Gold Foundation, the Fred Gellert Family Foundation, the Kresge Foundation, the McKnight Foundation, the Roy A. Hunt Foundation and the Wallace Genetic Foundation, as well as PAN's supporting members.

The authors bear responsibility for any factual errors. Recommendations and views expressed are those of Pesticide Action Network North America, and do not necessarily represent the views of our funders and supporters.

Special thanks to our colleagues at Californians for Pesticide Reform (CPR), Tracey Brieger and Sarah Aird, for strategic thinking and input as the report was being conceived and drafted, as well as assistance with the report's release and dissemination. PAN North America is a member of CPR, and is releasing this report in partnership with the coalition. The CPR coalition includes over 185 public interest organizations committed to improving and protecting public health, sustainable agriculture, and environmental quality by building a movement across California to change statewide pesticide policies and practices. See www.PesticideReform.org or call 510-788-9025 for more information about CPR's statewide work.

1611 Telegraph Ave, Suite 1200
Oakland CA 94612-2130
Tel 510.788.9020 • Fax 415.981.1991
www.panna.org • www.pesticideinfo.org

I-Fra5

A Generation in Jeopardy

How pesticides are undermining our children's health & intelligence

Kristin S. Schafer, MA
Emily C. Marquez, PhD

with
Medha Chandra, PhD
Kendra Hutchens, PhD Candidate
Margaret Reeves, PhD
Meriel Watts, PhD, PAN Asia-Pacific

October 2012
Pesticide Action Network North America

I-Fra5

Table of Contents

I-Fra5

A Generation in Jeopardy

Executive Summary

Children today are sicker than they were a generation ago. From childhood cancers to autism, birth defects and asthma, a wide range of childhood diseases and disorders are on the rise. Our assessment of the latest science leaves little room for doubt: pesticides are one key driver of this sobering trend.

As the recent President's Cancer Panel reports, we have been "grossly underestimating" the contribution of environmental contamination to disease, and the policies meant to protect us have fallen far short. Nearly 20 years ago, scientists at the National Research Council called for swift action to protect young and growing bodies from pesticides.[1] Yet today, U.S. children continue to be exposed to pesticides that are known to be harmful in places they live, learn and play.

This report reviews dozens of recent studies that examine the impact of pesticides on children's health. Our analysis reveals the following:

- **Compelling evidence now links pesticide exposures with harms to the structure and functioning of the brain and nervous system.** Neurotoxic pesticides are clearly implicated as contributors to the rising rates of attention deficit/hyperactivity disorder, autism, widespread declines in IQ and other measures of cognitive function.
- **Pesticide exposure contributes to a number of increasingly common health outcomes for children, including cancer, birth defects and early puberty.** Evidence of links to certain childhood cancers is particularly strong.
- **Emerging science suggests that pesticides may be important contributors to the current epidemic of childhood asthma, obesity and diabetes.**
- **Extremely low levels of pesticide exposure can cause significant health harms,** particularly during pregnancy and early childhood.

Children's developing bodies are particularly vulnerable to the health harms of pesticides.

Prioritizing children's health requires real change

As a nation, we value the wellbeing of our children. In addition to our natural urge to protect what we love, we know that at a societal level their successful development is key to a vibrant, secure future. Poll after poll shows more than 80 percent of Americans consider healthy children a top priority. We must line up our practice and policies with these values.

Many communities across the country have stepped up to create local or state policies to protect children from pesticide exposure. From pesticide-free schools, parks and playgrounds to protective buffer zones in agricultural areas, locally-driven actions are leading the way to healthier childhood environments.

But to ensure protection of all children from the harms of pesticides, we must dramatically reduce the use of these chemicals nationwide. An estimated 1.1 billion

pounds of pesticides are used in the U.S. every year, with more than 20,000 products on the market. This volume of use is undermining the health of the next generation and, as the science demonstrates, derailing development of our children's potential.

Scientists have understood for decades that children are particularly vulnerable to the harms of pesticide exposure. Quickly growing bodies take in more of everything; they eat, breathe and drink more, pound for pound, than adults. As physiological systems undergo rapid changes from the womb through adolescence, interference from pesticides and industrial chemicals—even at very low levels—can derail the process in ways that lead to significant health harms.

Reducing overall pesticide use would not only limit children's exposure during their most vulnerable years, it would also lower pesticide levels in the bodies of men and women of childbearing age—protecting current and future generations in one fell swoop. Those pesticides most harmful to children should be first on the list.

I-Fra5

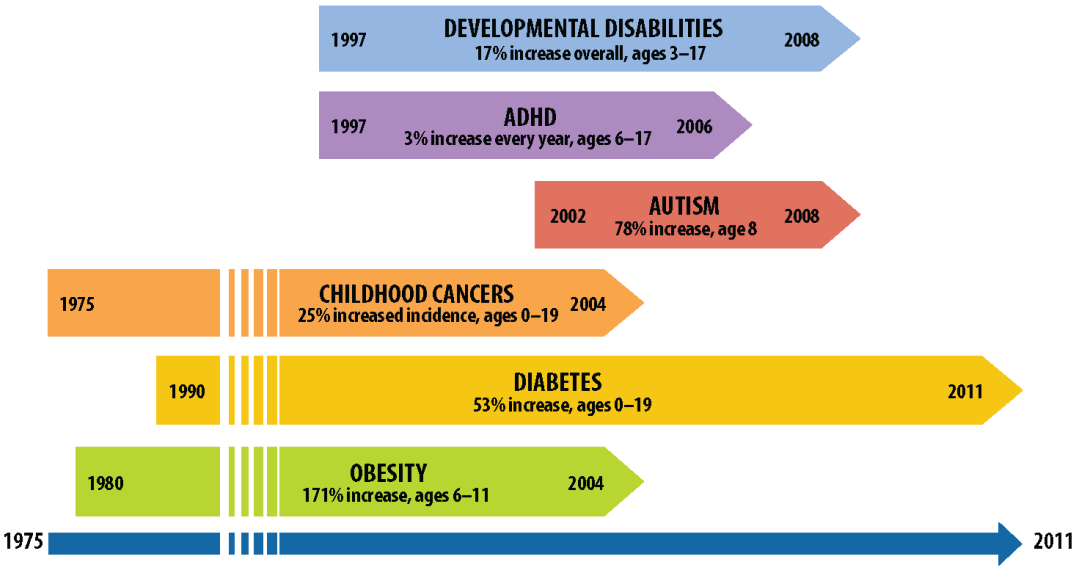
While we must each do what we can with food choices and decisions about home pest control, we cannot accomplish this goal at an individual household level. Policy change is required.

Effective policies urgently needed

To protect children from the health harms of pesticides, policymakers need much more effective tools. We believe change is most urgently needed in the way decisions are made about these three questions:

- Which pesticides are used in agriculture?
- Which pesticides are used in places children live, learn and play?
- How are farmers supported as they reduce reliance on pesticides?

Figure 1: Children's Health Harms on the Rise, 1975–2011*



Statistics show steady increases in many childhood diseases and disorders over the past 30 years. Those highlighted here are just some of the health harms on the rise.
Sources: see endnotes 4, 13,24, 52 and 94.

* With the exception of cancer, all other data are prevalence data, i.e., representing the U.S. population or based on data at several sites within the U.S. Prevalence is total number of cases in a population at a given time, while incidence is a measure of the number of new cases per year. The autism data are from 14 sites in the Autism and Developmental Disabilities Monitoring Network and are not considered fully representative of the U.S. population. The 1990 diabetes data are for type 1 only (type 2 being extremely rare among children at that time), while 2011 data include both type 1 and 2. Prevalence of type 2 diabetes among children is difficult to determine for various reasons, including difficulty of diagnosis.

I-Fra5

We recommend the following policy changes in each of these arenas:

1. Prevent the pesticide industry from selling agricultural products that can harm children's health

- *Take swift action on existing pesticides:* If studies find a pesticide to be a neurodevelopmental or reproductive toxicant, endocrine disruptor or human carcinogen—*and* it has been measured in humans, in schools or homes, or as residues on food or in drinking water—EPA should target the pesticide for rapid phaseout, triggering USDA resources to assist rapid farmer transitions to safer pest control methods.
- *Block harmful new pesticides:* EPA should not approve any new pesticide that scientific studies suggest is a neurodevelopmental or reproductive toxicant, endocrine disruptor or human carcinogen—including short-term "conditional" registrations.
- *Prevent harmful low-level exposures:* EPA should act on existing evidence that exposures to endocrine disrupting pesticides pose a particular danger to developing children; the long-delayed endocrine disruptor screening program (EDSP) should be swiftly implemented.

2. Protect children where they live, learn & play

- *Kid-safe homes, daycares & schools:* EPA should withdraw approval of existing pesticide products and not approve new pesticides for use in homes, daycare centers or schools when scientific evidence indicates the chemicals are possible neurodevelopment or reproductive toxicants, endocrine disruptors or human carcinogens.
- *Safer parks & playgrounds:* State and local officials should enact policies requiring that all public playgrounds, playing fields and parks be managed without using pesticides that studies show are harmful to children's health.

Table 1: Pesticides & Childhood Health Harms

		Childhood Health Harms*					
		Brain & nervous system impacts	Childhood cancers	Birth defects	Reproductive & developmental harms	Metabolic effects (e.g., obesity, diabetes)	Immune disorders, asthma
Pesticides	**Herbicides** 442 million lbs† *e.g., atrazine, glyphosate, 2,4-D*	✓	✓	✓	✓		✓
	Insecticides 65 million lbs *e.g., chlorpyrifos, malathion, permethrin*	✓	✓		✓	✓	✓
	Fungicides 44 million lbs *e.g., mancozeb, chlorothalonil*	✓	✓	✓	✓		✓
	Fumigants 108 million lbs *e.g., metam sodium, methyl bromide, chloropicrin*	✓	✓		✓		

Researchers have linked exposure to various pesticides with a range of childhood health harms. A ✓ indicates that links to the health harm are particularly well supported by scientific evidence.

* See Appendix A and www.pesticideinfo.org

† 2007 use estimates, refers to "active ingredient." From *Pesticide Industry Sales & Usage, 2006 and 2007 Market Estimates*, U.S. EPA, Washington, DC, Feb 2011. See www.epa.gov/opp00001/pestsales/07pestsales/market_estimates2007.pdf. Table 3.4.

I-Fra5

3. Invest in farmers stepping off the pesticide treadmill

- *Corral resources for farmers:* Federal and state officials should mobilize and coordinate existing resources to help farmers adopt well-known, effective pest management strategies that reduce reliance on pesticides.
- *Increase investment in innovative farming:* Congress should authorize significant funding for programs supporting farmers' adoption of sustainable practices that reduce use of harmful pesticides.
- *Set use reduction goals:* EPA and USDA should set specific and aggressive national pesticide use reduction goals, focusing first on pesticides that studies show to be harmful to children. To track progress toward this goal, farmers should work with applicators and pest control advisors to report their pesticide use to a nationally searchable database.
- *Source for children's health:* Food distributors should require that their suppliers limit use of pesticides that harm children's health.

These proposals are all common-sense measures in the face of clear evidence that our children's wellbeing is at risk. It's time to muster the political will to prioritize the health of our children, grandchildren and future generations.

Even at very low levels, pesticide exposure can derail development and undermine the ability to learn.

I-Fra5

1 Brainpower at Risk

New studies find pesticides can compromise intelligence

Knowledge of environmental causes of neurodevelopmental disorders is critically important because they are potentially preventable. — Dr. Philip Landrigan

The process of establishing the architecture of the human brain begins in the womb and continues into early adulthood. During this long window of development, many complex processes take place, involving tens of billions of nerve cells making trillions of connections. Cells migrate from one section of the brain to another, and nerve tracts are laid as the final structure of the brain is created.

Many of the processes that occur during brain development are vulnerable to disruption from pesticides. Exposure to neurotoxic pesticides during critical moments of fetal development, even at very low levels, has been shown to fundamentally alter brain architecture.[2] Pesticides that disrupt the hormone system—and particular those affecting the functioning of the thyroid, which plays a key role in brain development—can cause lasting damage. The impacts of exposures are often irreversible because unlike other organs, the brain cannot repair damaged cells (see sidebar).

Children whose brain infrastructure or nervous system fails to develop normally may be disabled for the rest of their lives. Developmental disabilities include autism spectrum disorders, attention deficit disorders, hearing loss, intellectual impairment and vision loss. People with developmental disabilities are often challenged by everyday life activities such as language, mobility, learning and independent living. Reduced cognitive abilities can also lead to behavioral problems, from aggression and social alienation to increased risk of drug abuse.[3]

A "Silent Pandemic"

Some 15 percent of all U.S. children have one or more developmental disabilities—representing a 17 percent increase in the past decade. For some disorders, the numbers are rising even more rapidly.[4] Overall, researchers estimate that between

Mechanisms of Harm

Misfiring neurons & altered brain architecture

Pesticides can interfere with brain function and development in several ways; we describe three of the most common and best understood mechanisms of harm here:

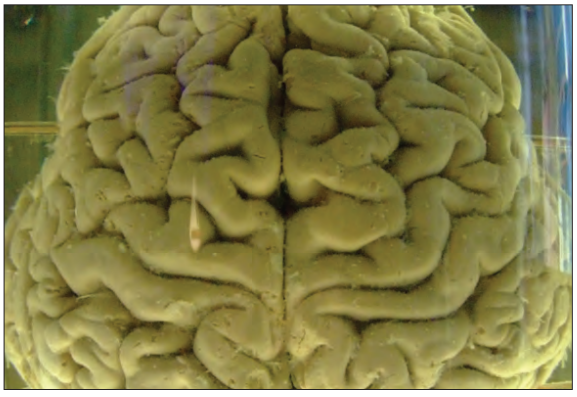
Neurotransmitter control: Organophosphate pesticides can block the normal functioning of acetylcholinesterase, an enzyme that degrades—and thus controls—a neurotransmitter called acetylcholine. When the functioning of the enzyme is blocked, acetylcholine is not degraded and neurons continue firing instead of shutting down after they've accomplished their mission. This can cause serious problems in the normal functioning of the nervous system.

Developing brain cells: To date, EPA assessments have relied on acetylcholinesterase levels as a marker of organophosphate exposure risk, yet studies now show adverse effects can occur at much lower doses than those that block acetylcholinesterase. For example, chlorpyrifos has been shown to interfere with neural cell replication, differentiation and survival. As the brain structure is developing—particularly at key stages *in utero*—chlorpyrifos can disrupt the process in ways that permanently alter the architecture of the brain.*

Sodium flow into nerve cells: Pyrethroid insecticides act on neurons by perturbing voltage-sensitive sodium channels. These sodium "gates" are what allow sodium to flow into a nerve cell, controlling how a neuron fires and transmits signals along a nerve. Pyrethroids cause these gates to open and close more slowly, changing how the nerve cell normally responds—either inducing repetitive firing or causing the nerve cell not to fire at all.†

* Rauh, V. A., F. P. Perera, M. K. Horton, R. M. Whyatt, R. Bansal, X. Hao, et al. "Brain Anomalies in Children Exposed Prenatally to a Common Organophosphate Pesticide." *Proceedings of the National Academy of Sciences.* May 2012 109 (20): 7871-6. See http://www.pnas.org/cgi/doi/10.1073/pnas.1203396109.

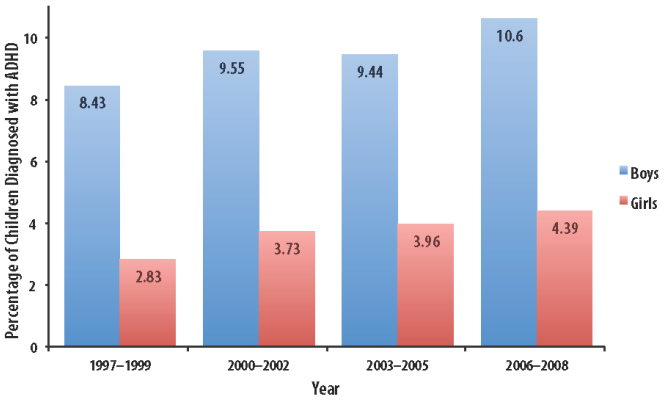
† Shafer T.J., D.A. Meyer and K.M. Crofton. "Developmental neurotoxicity of pyrethroid insecticides: critical review and future research needs." *Environ Health Persp.* Feb 2005 113(2):123-36. See http://www.ncbi.nlm.nih.gov/pubmed/15687048.



Pesticides can interfere with brain function in several ways, from altering architecture during fetal development to interfering with neurostransmitter control. Gaetan Lee

I-Fra5

Figure 2: ADHD Prevalence among Children Ages 3 to 17, from 1997–2008



The number of children diagnosed with ADHD increased an average of 3 percent every year from 1997 to 2008. Boys are much more likely to be affected. Source: C. Boyle et al., "Trends in the Prevalence of Developmental Disabilities in U.S. Children, 1997– 2008."

400,000 and 600,000 of the four million U.S. children born each year are affected by a neurodevelopmental disorder.[5]

Public health experts from Harvard and Mt. Sinai Hospital have called the damage that chemicals are causing children's developing minds a "silent pandemic,"[6] and scientists now point to a combination of genetic and environmental factors to explain this rapid rise of developmental, learning and behavioral disabilities.[7]

Some children, for example, may have a genetic susceptibility to attention deficit/hyperactivity disorder (ADHD) or autism, but it may only develop if the child is exposed to a triggering chemical during a certain period of development. Other children may be genetically programmed to produce less of a common detoxifying enzyme, rendering their brain and nervous system more susceptible to lasting harm when they are exposed to neurotoxic pesticides (see sidebar, p. 25).[8]

Genetic mutations that occur in parents (both men and women) in response to chemical exposures over the course of their lifetime can also, according to recent research, raise the risk of neurodevelopmental disorders for their children.[9, 10]

The National Academy of Sciences now estimates that about one third of all neurobehavioral disorders (such as autism and ADHD) are caused either directly by pesticides and other chemicals or by interaction between environmental exposures and genetics.[11] Some experts say this estimate is likely to be low, as the health profession is just beginning to fully recognize the contributions of environmental factors to disease formation.*

Whatever the mechanism of harm, recent studies leave little doubt that exposures to pesticides during fetal development, infancy and childhood may contribute significantly to decline in the cognitive abilities of our children. A recent comprehensive review of the science on health effects of pesticides by the Ontario College of Family Physicians found exposure to pesticides in the womb to be "consistently associated with measurable deficits in child neurodevelopment."[12]

We look here at three areas where the evidence is particularly strong: ADHD, autism and falling IQs. A few of the key studies are highlighted below, and more detailed descriptions—along with additional studies—are provided in Appendix A.

ADHD rates continue to rise

ADHD is quite clearly on the rise, and though changes in diagnosis play a role, this cannot fully explain the trend. The number of children diagnosed with ADHD increased an average of three percent every year from 1997 to 2006, and an average 5.5 percent per year from 2003 to 2007 (see Figure 2).[13, †]

The Centers for Disease Control and Prevention (CDC) estimates that ADHD now affects three to seven percent of all school children in the U.S.; one independent study puts the figure at 14 percent.[14] Boys are much more likely to be diagnosed with ADHD, although the American Psychological Association notes that girls are more likely to suffer from the "attention deficit" part of the disorder, and their symptoms are often overlooked.[15]

A variety of brain functions are compromised in children exhibiting ADHD. Learning is often impaired, and those with the disorder may exhibit impulsive behavior and hyperactivity, and lack the ability to sustain attention.

As with other neurodevelopmental disorders, the social impacts can be immense. Parents report that children with ADHD have almost three times as many problems interacting with peers as children without. Diagnosed children are almost 10 times as likely to have difficulties that interfere with friendships, including experiencing exclusion from peer groups.[16]

The Science

Researchers estimate that from 20 to 40 percent of ADHD cases are caused by something other than genetics.[17] Studies have found links to a variety of environmental contaminants, including exposure to organophosphate and pyrethroid insecticides during pregnancy and throughout childhood.

* See for example the 2010 President's Cancer Panel report "Reducing Environmental Cancer Risk: What we can do now" http://deainfo.nci.nih.gov/advisory/pcp/annualReports/index.htm.

† The CDC outlines diagnostic criteria here: http://www.cdc.gov/ncbddd/adhd/diagnosis.html, specifying that children must display at least six characteristic behaviors within six months, and that some symptoms must be present before the age of seven. CDC explains shifts in diagnostic criteria here: http://www.cdc.gov/mmwr/preview/mmwrhtml/ss5810a1.htm.

I-Fra5

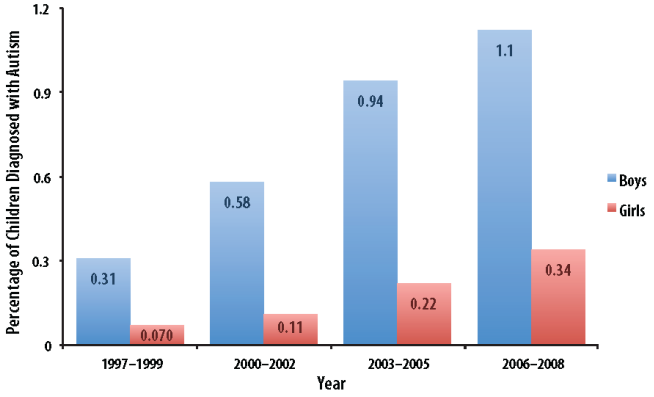
- Children with higher levels of organophosphate breakdown products in their urine were more likely to have ADHD. Researchers found that 94 percent of the 1000+ children tested by CDC had detectable levels of these metabolites, and those with levels above the median were twice as likely to be diagnosed with ADHD as those with no metabolites found.[18]
- Organophosphate metabolites at levels commonly found in the bodies of U.S. children are linked to increased likelihood of ADHD. Every 10-fold increase in levels of organophosphate metabolites in the urine of children aged eight to 15 years was associated with a 55 to 72 percent increased likelihood of the disorder.[19]
- Prenatal organophosphate exposure has been linked to attention problems. Each ten-fold increase in a pregnant mother's urinary concentration of organophosphate metabolites led to a five-fold increased risk that her child would be diagnosed with ADHD by age five.[20]
- Children with low birth-weight are more likely to have ADHD,[21] and there is considerable evidence linking reduced birth-weight with prenatal exposure to organophosphate pesticides.[22]
- Mouse pups were hyperactive after being exposed to the pyrethroid insecticides pyrethrin or cypermethrin, and adult mice injected with permethrin or deltamethrin had long-term elevation of the dopamine transporter, a marker that has been linked to ADHD.[23]

Table 2:
Chemicals Contributing to Autism

- Lead
- Methylmercury
- Polychlorinated biphenyls
- **Organophosphate pesticides**
- **Organochlorine pesticides**
- **Endocrine disruptors**
- Automotive exhaust
- Polycyclic aromatic hydrocarbons
- Brominated flame retardants
- Perfluorinated compounds

This list from public health experts includes both commonly used organophosphate pesticides and long lasting organochlorine pesticides, as well as other chemicals commonly found in consumer products. Source: Landrigan, et al., 2012

Figure 3: Autism Prevalence among Children Ages 3 to 17, from 1997–2008



Rates of autism have risen dramatically in the past decade. While overall prevalence is higher among boys, the rate of increase is higher among girls. Source: C. Boyle et al, "Trends in the Prevalence of Developmental Disabilities in U.S. Children, 1997–2008."

Autism rates jump 250% in one decade

The autism spectrum includes classic autism, Asperger's Syndrome and atypical autism. Incidence rates have risen rapidly in recent years; in its 2012 report, CDC estimated—based on 2008 data on eight-year-olds from 14 states—that 1.1 percent of U.S. children, or one in every 88, are now on the autism spectrum. Boys are more likely to have the disorder, with one in 54 affected.

Data from the National Health Interview Surveys reveal a dramatic rate of increase. Between 1997 and 2008, autism prevalence among boys ages three to 17 years increased 261%. Prevalence among girls, while much lower than boys overall, rose even more quickly, showing an increase of more than 385% over the same period (see Figure 3).[24]

In California, the number of children with autism who are enrolled in statewide programs rose from 3,864 in 1987 to 11,995 in 1998, an increase of more than 210 percent in 11 years.[25] Other states saw similar rates of increase between 2002 and 2006.[26] Though shifts in diagnosis account for some of this dramatic rise, public health experts have determined that diagnostic changes do not fully explain the trend.

Researchers believe autism spectrum disorders reflect changes in brain structure occurring during critical windows of development in the womb. These shifts in brain architecture may be caused by genetics, environmental insults such as chemical exposure, or an interaction between the two.[27, 28]

In 2012, a group of researchers led by Dr. Philip Landrigan of Mt. Sinai Medical Center released a list of ten types of chemicals most likely to be linked to the development of autism (see Table 2), and laid out an urgent strategy for research into the role of these contaminants and how children can be better protected from them. The list includes both commonly used organophosphate pesticides and longlasting organochlorine

pesticides, as well as other chemicals commonly found in consumer products.[29]

The Science

Studies examining the links between pesticide exposure and autism suggest prenatal exposures are particularly damaging.

- One study in California's Central Valley found that when mothers were exposed early in pregnancy to the organochlorine pesticides endosulfan and dicofol, the risk of autism among their children increased sharply. Children whose mothers lived within 500 feet of fields being sprayed were six times more likely to be on the autism spectrum.[30]
- Mothers in California's central coast region who had higher levels of organophosphate metabolites in their urine during pregnancy were much more likely to have children with pervasive developmental disorder—which can include or be an indicator of autism. The risk more than doubled each time metabolite concentrations went up by a factor of 10.[31]
- A study in New York City found that infants most exposed to chlorpyrifos *in utero* were significantly more likely to have pervasive developmental disorders—including autism—by the time they were three years old.[32]
- A trio of U.S. studies examined links between environmental exposures among parents (including, but not limited to, pesticides) and incidence of autism among their children.[33] Among other findings, the scientists reported that older fathers are more likely to transmit tiny, spontaneous gene mutations—that occur over a lifetime in response to environmental stressors—to their offspring, that in turn increase the risk of autism. Recent research in Iceland confirmed these findings.[34]
- Minnesota researchers explored the interaction of exposure to organophosphate pesticides, gene expression and dietary factors as potential contributors to autism.[35] Among other things, they found that mineral deficiencies linked to high fructose corn syrup consumption* make developing minds more susceptible to the neurotoxic effects of pesticides.

These various recent studies show how complex the path to our current autism epidemic has been. But evidence suggests that pesticide exposure—particularly during pregnancy—is implicated in a number of ways.

Derailed brain development means falling IQs

The societal implications of reduced cognitive abilities across an entire generation are nothing short of staggering and have been a concern among public health specialists since the IQ effects of lead exposure became clear in the 1970s. As Dr. Ted Schettler observed back in 2000:

> A loss of five points in IQ is of minimal significance in a person with an average IQ. However a shift of five IQ points in the average IQ of a population of 260 million increases the number of functionally disabled by over 50 percent (from 6.0 to 9.4 million), and decreases the number of gifted by over 50 percent (from 6.0 to 2.6 million).[36]

* High fructose corn syrup is found in a wide range of processed foods and beverages.

I-Fra5

Twelve years later, Dr. David Bellinger echoed this observation. He pointed out that cognitive effects, often dismissed as "clinically unimportant" at the individual level, become very significant across a whole society in terms of declining intellectual capacity, lost economic productivity and increased costs for education and health care.

Bellinger reviewed published data linking organophosphates and cognitive effects, and concluded that overall, exposure to organophosphate insecticides may be responsible for lowering U.S. children's IQ level† by 17 million points—not much less than the 23 million point loss attributed to lead poisoning.[37]

Bellinger argues that because the potential impacts of organophosphates are so widespread and significant to society, "a risk assessment that focuses solely on individual risk, and fails to consider the problem in a public health context" is misleading and will not lead policymakers to sound and protective decisions.

The Science

Pesticide exposure during pregnancy can have dramatic effects on cognitive development. From a wide range of animal research to studies tracking the intellectual development of children over time, the evidence points squarely at prenatal pesticide exposures as significantly harming the development and functioning of the brain. These harms can then lead to both lower IQ levels and neurodevelopmental delays.

- A particularly compelling study used Magnetic Resonance Imaging (MRI) technology to observe the developing brains of infants who had been exposed to chlorpyrifos during pregnancy. Researchers observed significant structural changes, including abnormal areas of thinning and enlargement. Areas of the brain related to attention, language, reward systems, emotions and control were affected.[38]
- Three cohort studies‡ released in 2011 document cognitive impairment caused by exposure to organophosphates in the womb.§ The first study found that higher metabolite levels in a mothers' urine late in pregnancy increased the likelihood of reduced cognitive development in their children.[39] The second study linked prenatal exposure to a seven-point reduction in IQ by age seven.[40] The third study found that even very low levels of chlorpyrifos residues in cord blood resulted in lower IQ and reduced working memory.[41]
- Pregnant mothers exposed to chlorpyrifos through household use (before this use was withdrawn)¶ had infants with lower birth weight and reduced head circumference, both indicators of impaired cognitive ability later in childhood.[42]

† The accuracy of Intelligence Quotient (IQ) testing to measure intellectual capacity has long been a source of contention, but IQ is currently the best index for measuring cognitive abilities across a population.

‡ See sidebar in Appendix A for a description of the various types of scientific studies highlighted in this report.

§ See this editorial in *Environmental Health Perspectives* for a discussion of the importance of these three studies: "Strength in Numbers: Three Separate Studies Link In Utero Organophosphate Pesticide Exposure and Cognitive Development," available online at: http://ehp03.niehs.nih.gov/article/fetchArticle.action?articleURI=info%3Adoi%2F10.1289%2Fehp.1104137

¶ Chlorpyrifos was withdrawn from home use in 2001, but remains widely used in agricultural settings where farm, farmworker and rural community mothers and children still face exposure. Children also continue to be exposed from residue on fruits and vegetables.

- Exposure to the organophosphate pesticides diazinon and parathion during early childhood may reduce cognitive function, according to results from animal studies. Low-dose exposures caused changes in the developing brains of rats known to correspond to reduced ability to learn.[43] Other animal studies indicate that *in utero* and neonatal exposure to organophosphates increases the risk of developmental delays.[44]
- Children at three months of age who were most highly exposed to the pyrethroid pesticide synergist piperonyl butoxide,* as assessed by personal air monitors, scored 3.9 points lower on the Bayley Mental Developmental Index. These scores are predictive of school readiness, and the authors described their results as modest, yet "worrisome."[45]
- Prenatal exposure to the DDT† breakdown product DDE is also associated with neurodevelopmental delays in children, especially the "psychomotor" skills linking movement or muscular activity with mental processes.[46] And exposure *in utero* to DDT itself has been associated with reduced cognitive functioning, memory and verbal skills among preschoolers.[47]

Strong emerging evidence links childhood pesticide exposure to other, adult-onset neurological effects such as Parkinson's and Alzheimer's diseases; these studies are not examined here.[48]

The combined, society-wide impact of the various syndromes, disorders and deficits resulting from damage to children's brains and nervous systems early in life is immense. Health professionals and educators across the country have indicated concern that our current policies don't adequately protect our children as their nervous systems develop.[49] Something must be done to address this gap, as the results of such exposures have profound consequences for individuals, families and society as a whole.

I-Fra5

Exposure of a developing fetus, infant or child to neurotoxic pesticides can lead to greater risk of learning disabilities and significant drops in IQ.

* Piperonyl butoxide, or PBO, is commonly included in formulations of pyrethroid pesticide products to increase the potency of the active ingredient.

† Agricultural uses of DDT were banned in the U.S. in 1972, but because of its persistence, DDT and its breakdown products continue to appear in human blood samples. DDT use continues in some countries for malaria control programs.

I-Fra5

2 Cancer, Birth Defects & Early Puberty

Latest science links many childhood health harms to pesticide exposure

If we are going to live so intimately with these chemicals—eating and drinking them, taking them into the very marrow of our bones—we had better know something about their nature and their power. —Rachel Carson

Our children face a range of health challenges that were not encountered by past generations. Public health experts are concerned, and are increasingly focusing on the contributing role of environmental factors such as pesticides and other chemicals.

The President's Cancer Panel's 2010 report, for example, concluded that the role environmental contaminants play in contributing to cancer has been "grossly underestimated" and called for urgent action to reduce the current widespread exposure to carcinogens. The Panel's chair, Dr. LaSalle Leffall, urged preventative measures to protect public health—even in the face of some uncertainty.*

> The increasing number of known or suspected environmental carcinogens compels us to action, even though we may currently lack irrefutable proof of harm.[50]

Meanwhile, evidence continues to mount linking chemical exposure to a range of children's health harms. Below we present a summary of some of the growing body of recent findings on pesticides and childhood cancer, birth defects and early puberty. More detailed descriptions and additional studies are included in Appendix A.

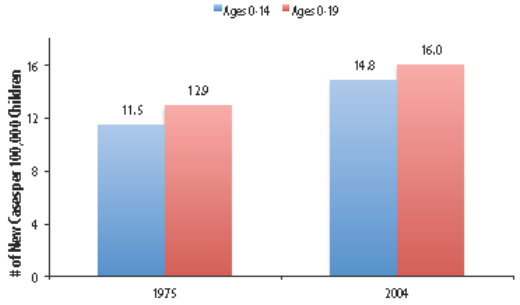
Some childhood cancers linked to pesticides

Cancer is the second most common cause of death among U.S. children one to 14 years old.† Over the past 30 years, the number of children diagnosed with all forms of invasive cancer has increased 29 percent, from 11.5 cases to 14.8 cases per 100,000 children per year (see Figure 4).[51]

There are many types of childhood cancer, and incidence rates vary widely. Leukemia and childhood brain cancers are now the most common cancers among children, with rates for these two cancers rising 40 to 50 percent since 1975: leukemia from 3.3 to 4.9 per 100,000 children, and brain cancers from 2.3 to 3.2 (see Table 3).[52]

Survival rates have also risen. Improved cancer treatments have led to dramatic increases in survival of all types of childhood cancer, particularly leukemia (from 50 percent survival in 1975 to more than 80 percent in 2004) and non-Hodgkins lymphoma (from 43 to 87 percent survival over the same time period.) For all types of childhood cancers, African-American children have a lower survival rate than do white children (73 vs. 81 percent).[53]

Figure 4: Incidence of Cancer among Children, 1975 & 2004



Over the past 30 years, the number of children diagnosed with all forms of cancer has increased from 11.5 to 14.8 cases per 100,000 children per year.
Source: SEER, 2004

Table 3: Top 5 Childhood Cancers

- Leukemia
- Brain and other nervous system tumors
- Neuroblastoma
- Wilms' tumor
- Lymphoma

The types of cancers that occur most often in children are different from those seen in adults.
Source: American Cancer Society

For some cancers, genetics is a powerful predictor. But as outlined by the President's Cancer Panel, cancers can have multiple and often interacting causes. In some cases genetic factors make an individual more susceptible, and exposure to environmental carcinogens may trigger cancer development.

The Science

A large number of recent studies link pesticide exposure to childhood leukemia, brain tumors and neuroblastoma. Some evidence suggests pesticide exposure may also be associated with other types of children's cancer, such as non-Hodgkin's lymphoma, Wilms' tumor and Ewing's sarcoma. Many studies

* This call for action in the face of some uncertainty is an example of the "Precautionary Principle," an approach to decision making that has been adopted by many local governments in the U.S. and in countries around the world. For a definition and more information, see the Science and Environmental Health Network's FAQ: http://www.sehn.org/ppfaqs.html

† Lethal accidents are the most common cause of death.

find *in utero* exposure during key windows of fetal development or parental exposure before conception to be particularly important.

- Home insecticide use during pregnancy can increase risk of childhood leukemia, according to a review of 15 studies over the past two decades. Timing of exposure appears to be particularly important.[54]
- The risk of a child developing acute lymphocytic leukemia—the most common type of childhood leukemia—is higher when the mother is exposed to home insecticides during pregnancy. Risk increased with the frequency of the mother's exposure; the highest risk was associated with use of household insecticides more than five times over the course of gestation.[55]
- Mothers who have a particular genetic variant of an enzyme involved with the metabolic processing of wastes and toxins (including carcinogens)* are more likely to have a child with leukemia when they use pesticide products during pregnancy.[56]
- Several case-control studies link exposure to herbicides and household insecticides during pregnancy to an increased risk of childhood brain cancer.[57]
- Higher risk of neuroblastoma, the most common cancer among infants, was observed in children whose parents reported garden and home pesticide use.[58] An older case-control study of U.S. and Canadian children indicated increased risk of neuroblastoma among children whose fathers were landscapers and groundskeepers.[59]
- In a national case-control study in Australia, increased risk of Ewing's sarcoma tumors among children was linked to occupational exposures of mothers and fathers who worked on farms around the time of conception.[60]
- Children who lived in areas of high agricultural activity in the U.S from birth to age 15 experienced significantly increased risk of childhood cancers.[61] And a study in Norway of agricultural census data found that of 323,359 children under 14, those who grew up on a farm—combined with a high level of pesticides purchased by the family—were nearly twice as likely have brain tumors.[62]

A number of studies—not reviewed here—explore potential links between prenatal or childhood pesticide exposures and incidence of cancers later in life. For example, according to the President's Cancer Panel, girls who were exposed to DDT before they reach puberty are five times more likely to develop breast cancer in middle age.[63]

In general, the association between pesticide exposures and childhood cancer outcomes may be underestimated, as data are somewhat limited and studies focus on certain cancers more than others. In addition, common methodological problems—such as occupational exposures being identified only through self-reporting or job title, considerations of other routes of exposure, small sample sizes, and relying on recall to estimate exposures—may contribute to skewed findings.[64]

* The CYP1A1 gene codes for the expression and activity level of an enzyme that helps clear the body of potentially harmful compounds.

I-Fra5

Birth defects rise with seasonal or occupational exposures

Birth defects are the leading cause of infant mortality in the U.S., accounting for 19 percent of the 29,138 infant deaths in 2007. And the overall incidence of birth defects is rising.[65] According to CDC data, about one in every 33 babies born today has some kind of birth defect.[66] Birth defects can affect almost any part of the body; some are mild and impact appearance only, others affect the functioning of organs and can be life threatening, although overall survival rates have increased significantly since 1979.[67]

Incidence trends vary by specific birth defect. Cleft lip/palate is the most common birth defect reported, and incidence has declined slightly over the last decade. Rates of Down Syndrome, gastroschisis (an abdominal wall defect resulting in protrusion of the intestines) and anencephaly (absence of portions of the brain, skull and scalp) have all increased since 1999.[68]

Like many children's health outcomes, a combination of genetic and environmental factors is often at play. CDC's research on environmental factors has focused primarily on smoking, alcohol intake, obesity and diabetes.[69] Other scientists, however, have examined the role of parental exposure to pesticides and other chemicals before conception, and of mothers' exposure to environmental contaminants during pregnancy (see sidebar, p. 17).

The Science

Parents exposed to pesticides occupationally, from exposures in their community or by in-home pesticide use may increase the risk of birth defects in their newborn. Studies indicate that exposure of both mothers and fathers, particularly during the period of conception, can influence birth defect outcomes. Several studies in agricultural areas have correlated conception during peak pesticide spray season with increased birth defect risk.

A mother's exposure during pregnancy can also play a key role, with specific timing once again emerging as a critically important variable.

Children whose mothers were exposed to herbicides and household insecticides during pregnancy have an increased risk of developing brain cancer.

Farmworker Families & Pesticides

As a community organizer and health educator in North Carolina, Ana Duncan Pardo works with many communities directly affected by pesticides.

When we spoke with Ana about her experience working with farmworkers, she described a particular instance—when she was setting up for a presentation to farmworker parents—that awoke her to the health harms faced by many of these families:

> Within five minutes I had noted multiple cleft palates and several children with apparent Down Syndrome.... It was shocking and disturbing to walk into a room with a group of parents and children that easily represented three to four times the national average for birth defects.

Farmworkers and their families face unique risks, as the harmful chemicals applied in the field follow workers home on their skin, shoes and clothing, and may also drift into their homes from the nearby fields. And, like all families, the food they eat every day may contain pesticide residues.

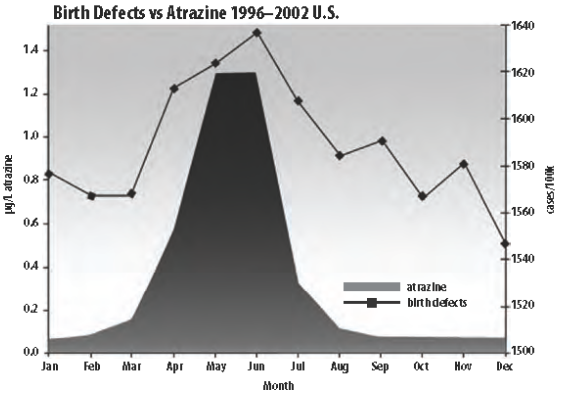
Ana Duncan Pardo is the farmworker organizer & communications coordinator for Toxic Free North Carolina, and a member of PAN's board.

- A multi-year, national review of USGS water data and CDC birth defect records found a strong seasonal association between birth defects and the presence of the herbicide atrazine in surface water. Infants conceived between April and July, when elevated concentrations of the herbicide are found, have a significantly higher birth defect risk (see Figure 5).[70]
- In Washington state, a seasonal analysis of the risk of the abdominal wall defect gastroschisis showed prevalence peaking when conception occurred between March and May. The birth defect occurred most frequently among infants whose mothers lived within 50 kilometers of a site with high surface water concentration of atrazine.[71]
- Male pesticide applicators in Minnesota had a significantly higher number of children with birth defects, in a study examining 4,935 births to pesticide applicator fathers over three years. The birth defects were more common among boy offspring than girls.[72] Egyptian fathers exposed to pesticides at work also had a greater risk of having children with congenital malformations.[73]
- Increased risk of boys' urogenital malformations such as hypospadia, micropenis and cryptorchidism* has been linked in many studies to prenatal exposure to environmental contaminants. One recent meta-analysis of studies from seven countries (Canada, Denmark, Italy, Netherlands, Norway, Spain and the U.S.) indicated a 36 percent increased risk of hypospadia when mothers were exposed to pesticides at work, and a 19 percent increased risk with fathers' occupational exposure to pesticides.[74]

* Hypospadia is a defect in which the urethral opening develops in the wrong location along the shaft of the penis. Micropenis is a defect where boys have severely reduced penile size, and cryptorchidism is a defect where the testes descend improperly, or not at all.

I-Fra5

Figure 5: Atrazine Seasonal Exposure & Birth Defects



Seasonal exposure to pesticides during pregnancy has been linked to increased risk of birth defects. Source: Winchester, P.D., J. Huskins and J. Ying. "Agrichemicals in surface water and birth defects in the United States." Acta Paediatrica. 2009 98: 664–669.

- The risk of having a child with neural tube defects, which are birth defects of the brain and spinal cord, has also been linked to pesticide exposure. Studies indicate a higher risk of this birth defect if insecticide bombs or foggers are used in the home during the period of conception. Risk is also higher if women live within a quarter mile of a cultivated field where pesticides are sprayed.[75]
- Mothers exposed to pesticides at work during a particular period of pregnancy have a significantly greater risk of having a child with anencephaly (a rare defect involving absence of a large part of the brain and skull).[76] A meta-analysis of studies examining fathers' exposure to Agent Orange (containing the herbicides 2,4-D and 2,4,5-T) found the risk of having offspring with spina bifida, a "split spine" defect caused by incomplete formation of the neural tube, was twice as high among those fathers who were exposed.[77] †

Many epidemiological studies over the years have found no association between pesticide exposure and birth defects. It must be considered, however, that these studies may not have taken timing of exposure into account, a variable that is proving to be a critical factor in birth defect outcomes. And as with cancer studies, results may be skewed by use of inappropriate surrogates for pesticide exposure (e.g. job title) or inaccurate subject recall.

Changes in puberty timing linked to low-level exposures

Young girls in the U.S. are moving from childhood to adolescence at an ever-younger age. Changes in the timing of sexual development over the past two decades have been so widespread that the age of "normal" puberty onset has been redefined by health professionals.[78]

† Agent Orange was widely used as a defoliant during the Vietnam War and was often contaminated with dioxins which have also been linked to birth defects. One of the herbicide ingredients, 2,4-D, is still in use in the U.S., and a proposal is currently under consideration for a genetically engineered variety of corn designed to allow increased 2,4-D application.

I-Fra5

Dr. Herman-Giddens and her colleagues first documented this acceleration in 1996, in a study finding that the number of girls having some sign of puberty onset before the age of eight was "substantially higher" than previously found.[79]

These initial findings of early puberty were corroborated in 2010 by researchers who found that by age seven, 10 percent of white girls, 23 percent of black non-Hispanic girls, and 15 percent of Hispanic girls had begun the process of breast development, also known as thelarche.[80] Some changes in pubertal development in boys have also been documented.

Changes in puberty timing are concerning for several reasons. For both boys and girls, self-esteem and body image issues can sometimes lead to self-destructive behaviors and poor performance in school. Additionally for girls, both early puberty and obesity (a contributing factor for early puberty) have been linked to health impacts later in life, increasing the risk for breast cancer and later reproductive health issues such as polycystic ovary syndrome.[81, 82]

These changes cannot be fully explained by ethnic, geographic, or socioeconomic factors, and thus a growing body of research has turned to examining the role of endocrine-disrupting chemicals in accelerating puberty in children.[83]

The Science

Although the number of studies is relatively small, researchers have found some associations between pesticide exposure—either during fetal development or early childhood—and effects on puberty.

Most studies focus on *in utero* exposures to pesticides with endocrine-disrupting effects that can interfere with the healthy development of the reproductive system—particularly if exposure occurs at certain times in the process (see sidebar).[84] The majority of studies focus on precocious puberty in girls, but a few studies have also found links between pesticide exposure and changes in the timing of puberty among boys.

Much of the research to date examines impacts of long-lasting organochlorine pesticides. Some of these are chemicals that have already been banned in the U.S. (e.g., DDT, hexachlorobenzene); others are in the process of being phased out (e.g., lindane, endosulfan); but all are still present in our food supply, environment, and in our bodies.[85, *] Though few studies have yet examined the connections, pesticides currently in use are also implicated in some studies.

- Prenatal exposure to the herbicide atrazine was linked to delayed pubertal development in both male and female rats in a recently released animal study.[86]
- Danish greenhouse workers exposed to a range of pesticides during pregnancy were more likely to have daughters showing breast development from 6–11 years old.[87] Increased likelihood of early puberty in girls in Jerusalem was found to coincide with seasons of intensified pesticide usage.[88]
- Daughters in Michigan were more likely to reach puberty at a younger age if their mothers had higher blood levels of the DDT breakdown product, DDE. Participants in this study included women who regularly consumed fish from the Great Lakes, which for years have been heavily contaminated with industrial pollutants such as PCBs and DDT.[89]
- Higher blood levels of hexachlorobenzene and DDE were associated with early puberty among Flemish boys.[90] Two recent studies of boys in India and Russia linked exposure to the pesticide endosulfan and the industrial by-product dioxin to delayed puberty among boys.[91]
- The pyrethroid insecticide esfenvalerate[†] has shown endocrine-disrupting effects related to puberty timing in female rats. Rats exposed to low levels (half of EPA's "no observable effect" level) for seven days showed significant delays in onset of puberty.[92]

As evidence mounts that developmental exposures to pesticides can have an effect on puberty timing, additional studies are now focusing on such endocrine-disrupting effects of pesticides currently in use.

Mechanisms of Harm

Endocrine disruption = development derailed

The term "endocrine" refers to systems in the body that are controlled by hormones, such as brain development, growth, reproduction and puberty. Hormones are chemicals synthesized in the body that bind to receptors to trigger actions at the cellular level resulting in physiological changes. Once their job is done they are released and free to act again.

Some pesticides act as "endocrine disruptors" that mimic hormones and can interfere with systems normally controlled by hormonal action. If such disruption occurs at times during development known as "windows of vulnerability,"—such as when the reproductive system is coalescing, brain or nervous systems are developing, immune system is forming or puberty is getting underway—the process can be derailed in significant ways, sometimes with life-long effects.

Because hormones themselves act at extremely low levels, biological processes controlled by hormones are tremendously sensitive. This means there often is no "threshold" or "safe" dose when it comes to endocrine disrupting compounds.*

* Zoeller, R.T., T. R. Brown, L. L. Doan, A. C. Gore, N. E. Skakkebaek, A. M. Sotp et al. "Endocrine-Disrupting Chemicals and Public Health Protection: A Statement of Principles from The Endocrine Society." *Endocrinology* June 2012. See http://endo.endojournals.org/content/early/2012/06/21/en.2012-1422.abstract.

Vandenberg, L., T. Colborn, T. Hayes, J. Heindel, D. Jacobs, D.H. Lee, et al. "Hormones and Endocrine-Disrupting Chemicals: Low-Dose Effects and Nonmonotonic Responses." *Endocrine Reviews.* March 2012 33(3): 378-455.

* CDC sampling from 1999–2000, for example, found DDT's breakdown product in blood samples of 99 percent of U.S. population. See http://www.cdc.gov/exposurereport/.

† Esfenvalerate is listed for Tier 1 screening under EPA's Endocrine Disruptor Screening Program. See http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2009-0634-0001.

I-Fra5

3 Emerging Science
Obesity, diabetes & asthma

Chemicals that disrupt hormone messages have the power to rob us of rich possibilities that have been the legacy of our species and, indeed, the essence of our humanity. —Theo Colburn

Many of the health challenges facing children today have strong genetic and/or behavioral components. The rise in childhood obesity, for example, in part reflects the increasingly sedentary habits of many U.S. children.* But it's becoming increasingly clear that personal lifestyle choices do not tell the whole story.

The speed and scope of the society-wide rise in childhood health problems suggest a complex interaction of genetic, behavioral and environmental variables. Researchers are beginning to tease apart these interactions to more fully understand how exposure to environmental contaminants are involved.

We examine here the rapidly emerging science exploring how pesticides may contribute to the recent rise in childhood obesity, diabetes and asthma. Additional studies are included and described in Appendix A.

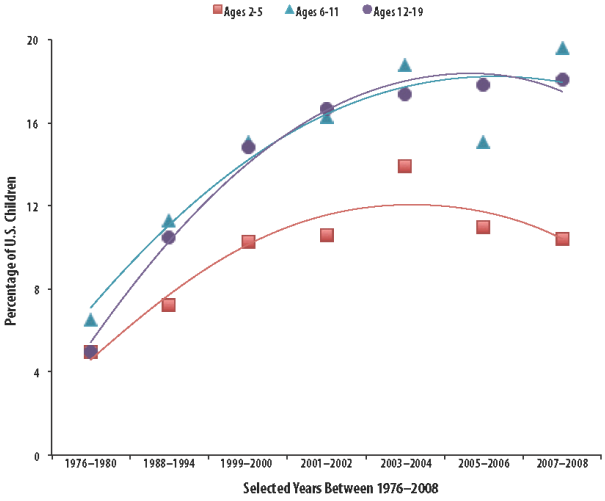
Childhood obesity, diabetes & disrupted metabolism

The recent dramatic rise in childhood obesity in the U.S. has the focused attention of health specialists and the public. The number of clinically obese children has more than tripled in the past 30 years, with obese children ages six to 11 jumping from seven percent of the total in 1980 to nearly 20 percent in 2008. The percentage of obese adolescents (12–19 years old) increased from five to 18 percent over the same period (see Figure 6).[93, †]

Obesity is closely linked to childhood diabetes, which is also on the rise. According to the National Institutes of Health, about 215,000 Americans under the age of 20 had diabetes in 2010—up from roughly 123,000 in 1990.[94, 95]

In addition to increasing related health risks, both obesity and diabetes can have a negative effect on quality of life in terms of ability to engage in physical activities, societal acceptance and self-image.

Figure 6: Prevalence of Obesity among Children Ages 2 to 19 between 1976–2008



Prevalence of obese U.S. children ages 6 -11 jumped from 7 percent in 1980 to 20 percent in 2008, while the percentage of obese adolescents increased from 5 to 18 percent. Source: Center for Disease Control, "Prevalence of Obesity Among Children and Adolescents: United States, Trends 1963-1965 Through 2007-2008."

The Science

So much new science exists around the links between obesity and environmental contaminants that a new term, "obesogen" (like carcinogen) has emerged in the literature.[‡] Findings increasingly suggest that exposures to pesticides and other chemicals play a role by altering developmental programming in ways that raise the likelihood of obesity and related metabolic effects such as diabetes.[96]

In 2002, Baillie-Hamilton reviewed data suggesting that the obesity epidemic coincided with the marked increase in usage of industrial chemicals, including pesticides, over the past 40 years (see Figure 7). The author suggested that pesticides and other industrial chemicals potentially cause weight gain by affecting the hormones that control weight, altering sensitivity

* CDC points to estimates that U.S. children spend an average 4.5 hours a day watching television and 7.5 hours using entertainment media (TV, computers, video games, cell phones and movies) as a contributing factor to childhood obesity. See http://www.cdc.gov/obesity/childhood/problem.html

† See CDC's "History of State Obesity Prevalence" showing trends in adult obesity by state from 2000-2010, at the bottom of this page: http://www.cdc.gov/obesity/data/adult.html

‡ See Wendy Holtcamp's review article, "Obesogens: An Environmental Link to Obesity" *(Environmental Health Perspectives,* Feb. 2012) for an overview of the current literature. Available online at http://ehp03.niehs.nih.gov/article/info%3Adoi%2F10.1289%2Fehp.120-a62#r13.

I-Fra5

to neurotransmitters, or altering the activity of the sympathetic nervous system.[97]

In the 10 years since this review, many studies have linked exposure to endocrine-disrupting chemicals with increased incidence of obesity and diabetes.[98] The National Institutes of Health is offering grants to study "the role of environmental chemical exposures in the development of obesity, type 2 diabetes and metabolic syndrome,"[99] and the National Children's Study, an ongoing 21-year prospective study of 100,000 U.S. children, is now exploring the hypothesis that prenatal exposures to endocrine disruptors are linked to obesity.[100]

- In one animal study, rats exposed to low-level doses of the organophosphate pesticide chlorpyrifos early in life developed metabolic dysfunction resembling pre-diabetes.[101]
- In Denmark, children exposed prenatally to pesticides through their mothers' work in greenhouses had significantly higher BMI (body mass index) scores than greenhouse worker mothers who were not occupationally exposed, with highly exposed children also having larger skin folds and higher body fat percentages.[102]
- Exposure to the pesticide lindane* during childhood has been linked with increased abdominal fat, increased waist circumference, higher BMI and fat mass percentage in adults.[103]
- Organochlorine pesticide exposure† can be a predictor of developing type 2 diabetes later in life, particularly among obese individuals. Serum concentrations of organochlorines were strongly associated with type 2 diabetes, and the association was stronger among obese persons than non-obese persons.[104]
- Obese children are more likely to have higher concentrations of 2,5-DCP in their urine, a metabolite of the pesticide found in mothballs (p-dichlorobenzene). This correlation was observed in data from the National Health and Nutrition Examination Survey (NHANES).[105]

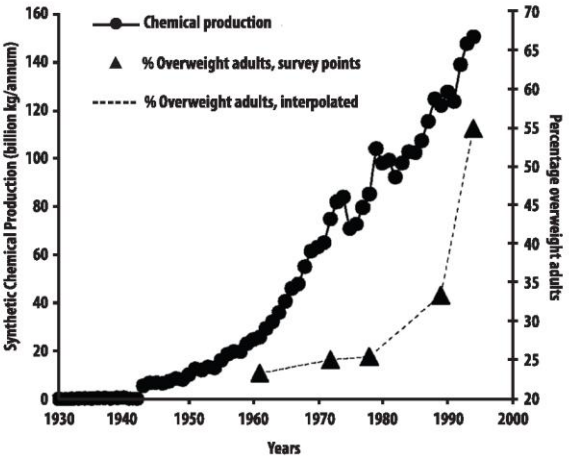
A number of specific genes have been identified as contributing to obesity, with several thought to specifically contribute to obesity in children. Such genes may play a role in regulating metabolic hormones.[106]

Scientists are now investigating the role of environmental factors (such as exposure to pesticides) in influencing the expression of such genes. Such "epigenetic" changes can include the expression of genes that are typically "silent," or inactivation of a gene that is normally active. Researchers are finding that some of these changes can be passed from one generation to the next (see sidebar).[107]

* Lindane, an organochlorine insecticide, is slated for global phaseout under the Stockholm Convention on Persistent Organic Pollutants. Agricultural uses were phased out in the U.S. in 2006; pharmaceutical uses (lice shampoos and scabies treatments) were phased out in California in 2001, but are still allowed in other states.

† Most organochlorine pesticides are now banned in the U.S., and many have been targeted for international phaseout under the Stockholm Convention. Rapid implementation of this treaty will reduce further exposure to these long lasting chemicals that continue to travel the globe on air and water currents.

Figure 7. Chemical Production & the Percentage of Overweight Adults in the U.S.



Researchers note that the obesity epidemic coincides with the increase in use of industrial chemicals, including pesticides, over the past 40 years. Source: Baillie-Hamilton, P.F. "Chemical toxins: a hypothesis to explain the global obesity epidemic." *J Altern Complement Med.* 2002 8: 185–192.

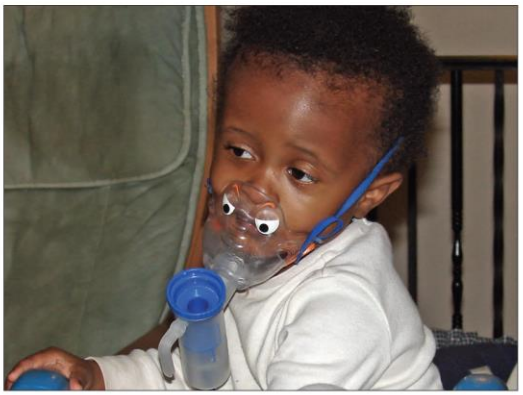
Mechanisms of Harm

Changing gene signals

Many environmental pollutants can strip or add chemical tags to DNA, locking the expression of genes on or off and changing how they function. These changes are called "epigenetic tags," and have been linked to various health effects including early puberty, disrupted ovarian function, death of sperm-forming cells and changes in metabolic rate.

Recent studies suggest that some chemicals can even override the genetic "reset button" that usually protects a developing fetus from such changes being passed from one generation to the next.

I-Fra5



Today, more than seven million children have asthma, up from just over two million 30 years ago.

Asthma epidemic affects more than seven million children

Asthma is a chronic disease of the pulmonary system that causes wheezing, breathlessness, chest tightness and coughing. The number of U.S. children with asthma today is much higher than it was 30 years ago, rising from 2.1 million in 1980 to 7.1 million in 2009.[108] Today, it is the most common chronic childhood disease in the U.S. (see Figure 8).

Asthma is the leading cause of hospital admission among urban children, with over 200,000 hospitalizations every year. Asthma is also the top cause of days lost from school, with more than 10.1 million school days missed every year.[109] Missed school days in turn negatively impact academic performance, such that children with severe asthma symptoms are more likely to suffer academically than children with milder symptoms.[110]

Asthma disproportionately affects people of color. Data from 2009 show that roughly one in six (17 percent) non-Hispanic black children had asthma in 2009, the highest rate among any racial/ethnic group. Overall, boys are more likely than girls to suffer from asthma (11.3 vs 7.9 percent) from birth through adolescence. As adults, women are more likely to be asthmatic than men.[111,*]

The Science

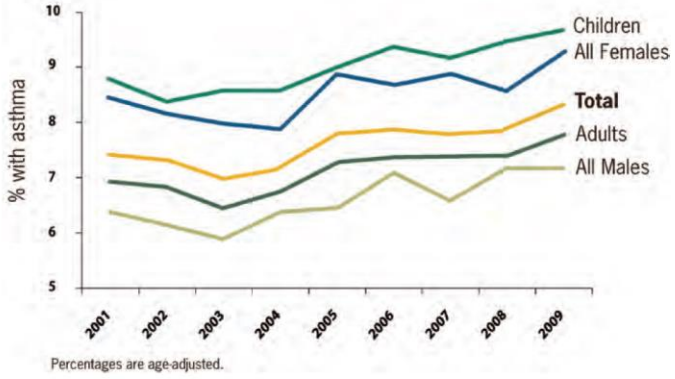
Many studies have explored the relative importance of common "respiratory irritants" in the home environment to triggering the onset of asthma, including cockroaches, dust mites, molds and air pollutants. Many pesticides are considered respiratory irritants,[†] and studies suggest that pesticide exposures may play a role in triggering asthma attacks, exacerbating symptoms, or heightening the overall risk of developing asthma.[112]

Pesticides may also play a role in increasing asthma incidence by affecting the body's immune system, triggering either hypersensitivity or suppression of the body's immune response. Allergic responses, for example, are a hypersensitivity of the immune system to an allergen in the environment.[113]

Numerous studies have documented the association of pesticides and asthma incidence for adults, and more recent studies have examined potential links to both asthma incidence and triggering or exacerbation of wheezing episodes among children.

- In a study of over 4,000 children from 12 southern California communities, exposure to pesticides in the first year of life significantly increased the risk of being diagnosed with asthma by age five.[114]
- A cross-sectional study of 3,291 Lebanese school children found a potential association between childhood asthma and parental occupational exposure to a range of current use pesticides.[115]
- In Spain, children diagnosed with asthma at age six had higher levels of cord serum DDE at birth than children without asthma. And in a study of 343 German children aged 7–10 years who had the DDT breakdown product

Figure 8: Asthma Prevelence by Age and Sex in U.S., 2001–2009



Source Centers for Disease Control and Prevention, Vital Signs: Asthma in the U.S. See http://www.cdc.gov/VitalSigns/Asthma/index.html, viewed May 2012.

* In May 2012, the President's Task Force on Environmental Health and Safety Risks to Children released the *Coordinated Federal Action Plan to Reduce Racial and Ethnic Asthma Disparities*. The effort lays out a plan to address this crucial public health challenge during the next three to five years. See http://www.epa.gov/asthma/childrenstaskforce.

† See the *Recognition and Management of Pesticide Poisonings* page of EPA's National Pesticide Information Center site: http://npic.orst.edu/health/child.html

I-Fra5

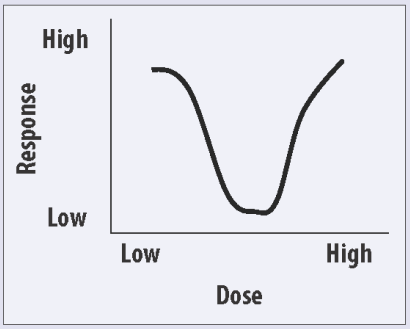
Rethinking "Safe"

Why the dose does not make the poison

Traditional toxicology relied for years on the mantra "the dose makes the poison." We now know that this statement is, in many cases, simply inaccurate. It assumes that the level of harm always increases as the level of exposure goes up (i.e., that every "dose response curve" follows a linear pattern). Assuming a higher dose is always more dangerous, policymakers often base regulations on a level below which no health risks is expected—a "safe" threshold. The reality, as scientists now understand, is quite different.

For some pesticides, the linkage between exposure and effect actually follows a U-shaped curve. In this scenario, a very low dose elicits a high level of "response" or health harm. At a higher dose that is along the bottom of the U, this same chemical elicits little or no response. Then at the highest doses, the effects increase again. For other pesticides, an inverted U–shaped curve can occur, where intermediate doses cause the greatest response, and testing at high doses can completely miss the effect.

Given these complex dose-response patterns, picking a threshold dose—below which exposure can always be considered "safe"—is simply not possible. Throw into the mix the dramatic differences in how sensitive individuals may be to chemical exposures, plus the vulnerabilities of children at particular times during development, and it quickly becomes clear that it is much more than the "dose" that determines how much harm a pesticide will cause.*



* Vandenberg, L., T. Colborn, T. Hayes, J. Heindel, D. Jacobs, D.H. Lee, et al. "Hormones and Endocrine-Disrupting Chemicals: Low-Dose Effects and Nonmonotonic Responses." *Endocrine Reviews*. March 2012 33(3): 378–455.

DDE present in their blood, the risk of having asthma was significantly higher.[116, *]

- Childhood exposure to organophosphate, carbamate and pyrethroid insecticides may trigger or exacerbate asthma symptoms among children by promoting bronchial constriction.[117]

Recognizing the rising prevalence of asthma among U.S. children, Dr. David Schwartz recently called on fellow researchers to focus more attention on the potential links between exposure to air pollutants and environmental contaminants like pesticides and childhood asthma.[118]

* These measurements were taken from blood serum and were thought to represent early life or prenatal exposures, but the actual route of exposure was not known.

I-Fra5

4 Critical Junctures

Children exposed just as they are most vulnerable

Children cannot make choices about their environment; it is up to adults to make the right decisions to ensure that they are protected. — Dr. Lynn R. Goldman

Environments we would like to consider "safe" often bring children into contact with pesticides and other chemicals that have been linked to health harms. Many chemicals pass across the placenta into the womb, where they become part of the first environment of a developing fetus. In the months after birth, infants begin to explore their new world, often testing new sights and smells by touching and bringing objects to their mouths. When harmful chemicals are present, they are often taken in.

The environments of toddlers and school-age children expand to include daycare centers, classrooms, playing fields and parks, all of which may offer risk of pesticide exposure. Residues on and in food—from breastmilk to the highchair to the school lunch tray—are also an important source of pesticides throughout childhood.

Many pesticides can pass across the placenta into the womb, where they become part of the first environment of a developing fetus.

Physiological systems undergo rapid development at various stages of childhood, in finely tuned processes often triggered and orchestrated by hormones. During this same period, children take in more food, water and air than adults pound-for-pound, and their biological systems are less able to process harmful contaminants than adults.

In short, the multiple pathways of pesticide exposure mean that in a given day, a child may absorb a wide range of potentially harmful chemicals just as their young bodies are at their most vulnerable.

Fetal pesticide exposures can have life-long effects

Exposure to pesticides has been clearly documented during one of a human organism's most vulnerable stages: fetal development.

Pesticides that have accumulated for years in an expectant mother's body—stored in blood and fatty tissues—can be mobilized during pregnancy and cross the placental barrier. A mother's exposures to pesticides during pregnancy add to this chemical mixture in the womb.[119]

Many studies have documented the pesticide load newborns bring with them into the world. Researchers in New York documented pesticides and their breakdown products in umbilical cord blood of more than 80 percent of newborn infants tested.[120] One 2001 study found metabolites of organophosphate pesticides in 100 percent of the cord blood samples taken.[121] A pilot study of amniotic fluid also found organophosphate metabolites, providing further evidence of fetal exposure.[122]

Pesticide residues from the food mothers eat during pregnancy have also been found in infants. A recent Canadian study showed that when pregnant women consumed soybeans, corn and potatoes that had been genetically modified for use with particular herbicides, metabolites of one of the herbicides showed up in cord blood of 100 percent of their babies.[123] *

Fetal development is almost entirely controlled by the expectant mother's hormones, acting at very low levels to trigger and control growth of the various systems of the body. Some chemicals—including many pesticides—mimic hormones and so interfere with natural developmental processes. This disruption of hormone function can lead to irreversible life-long effects including birth defects or learning disabilities in childhood, or adult onset cancer or infertility later in life (see sidebar, p. 17).[124]

Pesticide exposures common at home, daycare & school

Pesticides tend to be especially persistent in the indoor environment where sunlight, rain, soil microorganisms and high temperatures cannot degrade them, which means longer windows of exposure.

At home & in daycare facilities

Infants and toddlers have busy hands that often reach their mouths, and they commonly play on or near the floor—so

* The women in the study were in urban environments, and had no contact with the herbicides beyond residues on or in their food.

I-Fra5

Children as Farmworkers

Some children are exposed to pesticides as they work in agricultural fields. Specific rules vary from state to state, but federal law allows children under 12 to do field work outside of school hours on farms where their parents are employed.*

Age restrictions for hazardous work such as applying pesticides are more lenient in the agriculture sector, and age restrictions simply do not apply for children working on farms owned or operated by a parent or guardian.

Documenting the exact number of child workers in U.S. agriculture is difficult, and estimates vary widely. A Human Rights Watch report published in 2000 put the number somewhere between 300,000 and 800,000.† The nonprofit group Toxic Free North Carolina recently documented the experience and voices of young farmworkers facing pesticide exposure in the field; the stories can be viewed at www.panna.org/youngfarmworkers.

* U.S. Dept. of Labor. "Child Labor Requirements in Agricultural Occupations Under the Fair Labor Standards Act." June 2007. See http://www.dol.gov/whd/regs/compliance/childlabor102.htm.

† Human Rights Watch. *Fingers to the Bone: United States Failure to Protect Child Farmworkers.* Washington: Human Rights Watch, 2000.

National Center for Farmworker Health. *Child Labor.* Buda, Texas. 2009. See www.ncfh.org/docs/fs-Child%20Labor.pdf

Davis, S. and J.B. Leonard, *The Ones the Law Forgot: Children Working in Agriculture,* Farmworker Justice, Washington DC. 2000.

when pesticides are used in homes or daycare facilities, exposure is a near certainty. Inhaling spray droplets, vapors or pesticide-contaminated dust from indoor use of pesticide products is one of the primary routes of exposure for many U.S. children. Pesticides used to control ticks and fleas on pets are another important source of children's exposure.[125]

One Massachusetts study found residues of DDT in house dust many decades after use of the chemical had been discontinued.[126] Even pesticides that are relatively short-lived in the environment are more persistent indoors; one study found the semi-volatile insecticide chlorpyrifos to be longer lasting than expected in closed apartments, detectable for more than two weeks on rugs, furniture, soft toys and pillows.[127] Pesticide vapors often settle after application indoors, so levels tend to be highest in the infant breathing zone.[128]

Exposure from home lawns and gardens or outdoor play areas at daycare centers can also be significant. Children often roll and play on lawns and sit or lie on bare soil, and toddlers are known to put dirt directly into their mouths.[129] If pesticides have been used in these areas, the likelihood of ingestion or inhalation is high.

In rural communities, the risk may be compounded by drift from nearby agricultural fields. A study conducted in Washington State found residues of several agricultural pesticides—including chlorpyrifos and ethyl parathion—in outdoor play areas.[130] Air monitoring studies using PAN's Drift Catcher in

Evidence shows that when pesticides are used at home, on pets or in daycare centers, children's exposure is a near certainty.

California and Minnesota have documented a range of agricultural pesticides in backyards and play areas as well.[131, 132]

Rural infants and toddlers also face potential exposure from drift directly into their homes, and from pesticide contamination of water supplies. Water sampling results from Illinois, Nebraska, Iowa and Minnesota detected the common herbicide atrazine at levels above those linked to low birth weight.[133] Young children in farmworker families face additional exposure from residues carried into the home on the bodies and work clothes of working family members.[134]

At school & on playgrounds

Pesticides used in school buildings can settle on desks, books, counters and walls. When children touch contaminated surfaces, they may absorb chemical residues that can remain in the school environment for days. Herbicides used to keep playing fields free of weeds may be picked up on children's hands, bodies, clothes and tennis shoes, or drift into classrooms after application.

According to one recent national review, of the 40 pesticides most commonly used in schools, 28 are probable or possible carcinogens, 26 have been shown to cause reproductive effects, 26 damage the nervous system, and 13 have been linked to birth defects.[135]

In rural areas, pesticides often drift into schoolyards during and after spraying on nearby fields. Community air monitoring studies across the country using the Drift Catcher device have documented pesticides in or near school grounds in agricultural communities,[136] and incidents of pesticide poisonings in schools are not uncommon. For example:

- In Florida, high school students used a Drift Catcher to measure the pesticides endosulfan, diazinon and trifluralin* drifting into the school from nearby cabbage fields.[137]

* Endosulfan is currently being phased out in the U.S., and also globally under the Stockholm Convention on Persistent Organic Pollutants. See http://www.epa.gov/oppsrrd1/reregistration/endosulfan/endosulfan-cancl-fs.html.

- Schoolchildren in Strathmore, CA were exposed to pesticides sprayed in a neighboring field, feeling dizzy and falling sick in November, 2007.[138]
- Seven children were hospitalized and a total of 11 people sickened in Kahuku, Hawaii, in 2007, when fumes from an organophosphate insecticide drifted over the school from a nearby sod farm.[139]

Pesticide use on playing fields has raised concerns among families and environmental health advocates nationwide. The National Coalition for Pesticide-Free Lawns notes that "the common, everyday practices used to maintain our children's playing fields are unintentionally and unnecessarily exposing them to carcinogens, asthmagens, and developmental toxins," and calls for a shift to organic turf management on playing fields across the country.[140]

Pesticide residues, from breastmilk to the school lunch tray

Pesticide residues in food and drink are a key source of constant, low-level exposure to mixtures of pesticides throughout childhood.

Nature's Finest, Compromised

Pesticides in breastmilk

Human breastmilk is without doubt the best source of nutrition for infants, offering the perfect combination of fats, carbohydrates and proteins for developing babies. It also offers protection from infection, increases resistance to chronic disease and contributes to the emotional wellbeing of both infant and mother.

But decades of breastmilk sampling also leaves no doubt that around the world, nature's perfect food for infants is compromised by pesticides and other toxic chemicals. Today there is no corner of the planet where human breastmilk remains pure. The chemicals found in a mother's milk represent a combination of long-lasting pesticides and industrial pollutants that have accumulated over a lifetime (many of which the body tends to store in fatty tissues), and shorter-lived chemicals that a woman is exposed to during pregnancy and breastfeeding.

This chemical burden is transferred to nursing infants just as their bodies are most vulnerable to chemical harms. The good news is that analysis of decades of banked breastmilk in Sweden shows that bans on specific chemicals can result in rapid and dramatic decreases in the levels of some of those compounds in human milk.*

* Norén K., D. Meironyté. "Certain organochlorine and organobromine contaminants in Swedish human milk in perspective of past 20-30 years." *Chemosphere*. May-Jun 2000;40(9-11):1111-23. See http://www.ncbi.nlm.nih.gov/pubmed/10739053.

Natural Resources Defense Council. "Healthy Milk, Healthy Baby: Chemical Pollution and Mother's Milk." See www.nrdc.org/breastmilk.

I-Fra5

Children take in more food, water and air than adults pound-for-pound, just as their bodies are less able to process harmful contaminants.

Studies from around the world have documented pesticides in human breastmilk, though experts agree it remains the best source of nutrition for infants (see sidebar). Baby foods and fruit juices consumed by infants and toddlers tend to be highly processed, which can sometimes concentrate pesticide residues existing on the fresh produce.[141] U.S. researchers measuring pesticides in baby foods found low-level residues of many pesticides, including eight known to be toxic to the nervous system, five that disrupt hormones and eight that are potential carcinogens.[142]

Food consumed by school-age children can also contain pesticide residues. Researchers examining the diets of urban children found that 14 percent of the foods sampled contained at least one organophosphate pesticide. In total, 11 different organophosphates and three pyrethroids were found.[143] USDA residue sampling of produce commonly eaten by children—such as carrots, apples and peaches—found metabolites of dozens of different pesticides in each of these foods over the course of their testing (26 found in carrots, 42 in apples and 62 in peaches).*

Pesticides directly measured in children's bodies also tell a story about the importance of dietary exposure. Researchers compared levels of organophosphate metabolites in the urine of children who were eating organic fruit, vegetables and juice with children eating conventionally farmed produce. They found that those with more organic diets had metabolite levels six times lower than those with conventional diets.[144] Other studies show that when families switched to organic fruits and vegetables, metabolites of the insecticides chlorpyrifos and malathion fell quickly to undetectable levels.[145]

The widespread presence of pesticide metabolites in children's bodies,[146] combined with studies showing that changes in these levels are linked to changes in dietary exposure, make a very clear case that pesticide residues in food are a consistent source of children's daily intake of a mixture of pesticides.

* These numbers do not necessarily reflect residues on a single sample. See USDA data at www.whatsonmyfood.org.

I-Fra5

Why children are particularly vulnerable

So what do all of these well-documented pesticide exposure pathways mean for children's health?

In their first six months of life, children take in roughly 15 times more water than the average adult per pound of body weight.[147] Children also inhale more air. Up to around age 12, a child's breathing rate is roughly twice that of an adult, which means a child will inhale roughly double the dose of a pesticide in the air from spray drift or household use.[148]

Exposure to pesticides occurs largely through touching, inhaling or ingesting. For each of these routes, children are much more likely to absorb what they come into contact with than adults. The skin of infants and young children, for example, is particularly permeable, and the skin surface area relative to body weight is much greater in children than adults.[149] The lung surface area relative to rate of breathing is also higher among children,[150] and absorption levels in the gastrointestinal tract are also greater (especially for alkaline pesticides), as adult levels of gastric acid are not reached until a child is about two years old.[151]

As noted above, the brain and nervous system are especially vulnerable during fetal development and for the first six months of life. During this period the blood-brain barrier,* which provides the adult nervous system some protection from toxic substances, is not yet fully developed.[152]

Finally, young bodies are less equipped to process and excrete harmful chemicals as the liver and kidneys—the body's primary detoxifying organs—are not yet fully developed. Levels of enzymes that help the body process chemicals are also not yet at full strength (see sidebar). Genetic variations lead to tremendous range in the production of these protective enzymes—with some newborns as much as 164 times more vulnerable to chlorpyrifos than less sensitive adults.[153]

According to researchers, this finding alone means that most, if not all infants and toddlers—as well as a subpopulation of adults—are much more likely to have adverse health effects from organophosphate exposure. Policies that don't account for this variability fail to protect the most vulnerable, leaving many children in harm's way.

* The blood-brain barrier is made up of high-density cells that protect the brain from potentially harmful substances circulating in the bloodstream.

Mechanisms of Harm

When enzymes don't detoxify

Enzymes are proteins that catalyze reactions on a molecular level, and there are many that occur naturally in the human body. Without enzymes to catalyze reactions, some of the chemical reactions that make up the normal functioning of our body could take much longer, or not happen at all.

One key human enzyme, known as paraoxonase 1 (or "PON1"), catalyzes the metabolic process that renders organophosphate pesticides and other compounds less harmful to our systems. Researchers say infants have very low levels of this enzyme up to age two, and children don't reach adult PON1 levels until about age seven.* This suggests that children are less protected from harmful contaminants by enzyme activity, and newborns may be especially vulnerable.

There is also tremendous natural variability in the level and effectiveness of the PON1 enzyme, which means some individuals are much more susceptible to health harms of organophosphate pesticides and other contaminants.†

* Huen K., K. Harley, A. Bradman, B. Eskenazi, N. Holland. "Longitudinal changes in PON1 enzymatic activities in Mexican-American mothers and children with different genotypes and haplotypes." *Toxicol Appl Pharmacol.* 2010. 244(2):181-9. See http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2846980/?tool=pubmed

† Holland, N., C. Furlong, M. Bastaki, R. Richter, A. Bradman, K. Huen, et al. "Paraoxonase Polymorphisms, Haplotypes, and Enzyme Activity in Latino Mothers and Newborns." *Environ Health Persp.* July 2006 114 (7): 985–991. See http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1513322/.

The human body undergoes rapid growth and development throughout childhood, with many processes vulnerable to disruption from pesticides and other chemicals.

I-Fra5

5 Case Studies
Communities win protections for children

What we love we must protect. — Sandra Steingraber

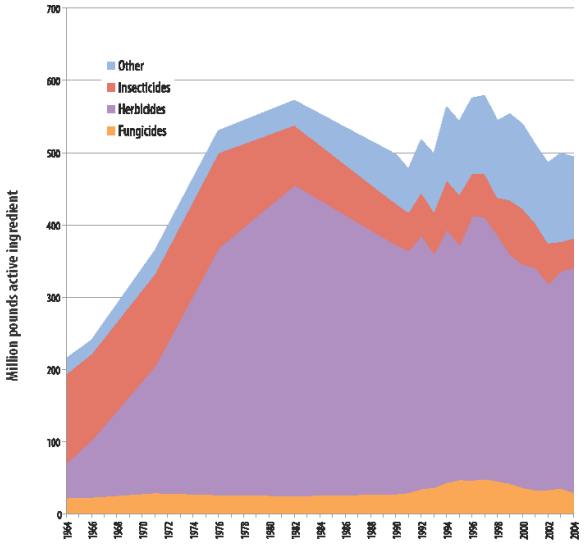
Since the middle of the last century, the overall increase in pesticide use in this country has been steady and dramatic. As documented above, these pesticides are a critical contributor to many of the chronic diseases and disorders now affecting our children.

To address the unique vulnerability of children, concerned communities, public health officials and advocates are beginning to put policies in place at the state and local level that reduce the use of harmful pesticides. In this chapter we provide a brief overview of U.S. pesticide use patterns and trends, and highlight on-the-ground stories of successful efforts to protect children from exposure in their early environments.

Pesticide use now 1.1 billion pounds yearly

Since 1945, use of herbicides, insecticides and other pesticides has grown from less than 200 million to more than 1.1 billion pounds per year, with well over 1,000 chemicals registered and formulated into more than 20,000 pesticide products (see Figure 9). This does not include pesticides used as wood preservatives or specialty biocides (in plastics and paints, for example). If these products are included, the number jumps to more than five billion pounds annually.[154, 155, 156]

Figure 9: Pesticide Use on Major Crops, 1964–2004



Source: "Land and Farm Resources: AREI, 2006 Edition," USDA Economic Research Service

Pesticide use in agriculture

The majority of pesticides are used in agricultural fields, with weed-killing herbicides being the highest by volume. Soil fumigants, which are injected as a gas into soil before planting to kill weeds, insects and fungi, are used at particularly high volumes and have a tendency to drift after application. Use of organophosphate insecticides, which gained widespread use in the 1980s as replacement chemicals for long-lasting organochlorine pesticides (such as DDT, chlordane and aldrin) has gradually declined in recent years.

In part to address growing concerns about organophosphate toxicity, a group of insecticides called pyrethroids were marketed as "safer" and gained widespread use in the 1990s, and use has grown rapidly. According to the American Chemical Society, use of pyrethroids in California (agricultural, structural and landscape maintenance applications) almost tripled from 1992 to 2006.[157] Recent research suggests that pyrethroids may be more harmful to humans than originally believed, acting as developmental neurotoxicants, endocrine disruptors and carcinogens.[158, *]

Another class of pesticides now in widespread and rapidly rising use is neonicotinoids. Most neonicotinoids show much lower toxicity in mammals than insects, but emerging science demonstrates that many may also have neurodevelopmental effects, and some are considered likely carcinogens by EPA.[159] These pesticides are considered 'systemic,' which means they are often applied at the root (as seed coating or drench) and are then taken up through the plant's vascular system. Systemic pesticides on food cannot be washed off.

Neonicotinoid pesticides have been linked with honey bee colony collapse disorder and bee kills, and several products have been banned in European countries for this reason. One neonicotinoid, imidacloprid, is now one of the most widely used insecticides in the world.[160]

* Ten years' worth of adverse-reaction reports (filed by manufacturers) show that pyrethrins and pyrethroids together accounted for more than 26 percent of all fatal, "major," and "moderate" human pesticide poisoning incidents in the U.S. in 2007, up from 15 percent in 1998. See http://apps.cdpr.ca.gov/calpiq/calpiq_input.cfm to see the primary data; for data analysis, see http://www.iwatchnews.org/environment/health-and-safety/perils-new-pesticides.

I-Fra5

Pesticide use at home

While 80 percent of all pesticides are applied in agricultural fields, use in homes, gardens, playgrounds, schools, hospitals and other buildings is also significant—and as noted above, such uses pose a particular risk to children's health.

In 2007, an estimated 78 million pounds of pesticides (measured by active ingredient) were applied in homes and gardens across the country, with the herbicides 2,4-D and glyphosate (RoundUp) topping the list.[161] The household pesticide product industry has an estimated annual net worth of $1.4 billion; according to EPA, more than 78 million households—roughly 74 percent of all households in the U.S.—report using pesticides at home (see Table 5).[162]

Many home-use insecticides contain pyrethroids, and the chemicals are used extensively in homes where the potential for exposure to children is very high. Researchers from Emory University and the CDC found that even children fed an exclusively organic diet had pyrethroid metabolites in their systems after their parents had used pyrethroid insecticides in their homes.[163]

Neonicotinoid products are widely used in pet products to control fleas and ticks—another use which poses particularly high exposure risks for children.[164]

Safer pest control at schools & daycare centers

More than 3,000 pesticide products are currently approved for use in schools;[165] yet current national pesticide rules do not address the use of pesticides in and around schools or daycare centers. The federal School Environmental Protection Act (SEPA) was first introduced in November 1999 in an attempt to address this oversight—and it continues to be debated in Congress today.

In the non-profit sector, the national Children's Environmental Health Network (CEHN) moved to fill this gap by creating the Eco-Healthy Child Care (EHCC) program to provide tools that facilities need to create environmentally healthy spaces for children. Today, the program endorses over 1600 "Eco-Healthy" daycare facilities across the country and provides this list to parents online.*

To protect children's health, several states have put policies in place prohibiting the use of pesticides on playing fields and playgrounds.

Meanwhile, several states are moving forward with policies designed to protect children from pesticides in these early environments.

- In 2005 Connecticut lawmakers prohibited use of pesticides on K–8 lawns and playing fields; in 2009, the law was extended to daycare center grounds. Through this policy, schools have successfully implemented organic turf programs in various municipalities.[166]
- New York followed suit in 2010, signing the Child Safe Playing Fields Act into law to ban the cosmetic use of pesticides on playgrounds and sports fields at schools (including high schools) and daycare centers.[167]

Table 4: Pesticide Usage in All Market Sectors, 2007

Pesticide Class	Active Ingredient
Herbicides	531 million lbs
Insecticides	93 million lbs
Fungicides	70 million lbs
Fumigants/Nematicides	133 million lbs
Other	30 million lbs
Total	*857 million lbs*

Herbicides are the most commonly used type of pesticide in the U.S., with 531 million pounds of active ingredient applied in 2007. Source: *Pesticide Industry Sales & Usage, 2006 and 2007 Market Estimates*, U.S. EPA, Washington, DC Feb 2011. See www.epa.gov/opp00001/pestsales/07pestsales/market_estimates2007.pdf.

Table 5: Households Using Pesticides

Pesticide Type	# Households
Insecticides	59 million
Fungicides	14 million
Herbicides	41 million
Repellents	53 million
Disinfectants	59 million
Any pesticides	78 million

According to EPA, more than 78 million households—roughly 74 percent of all households in the U.S.—use pesticides at home. Source: EPA estimates based on the 1992 EPA National Home and Garden Survey and 2000 U.S. Census Bureau population estimates (www.quickfacts.census.gov/qfd/states).

* See http://www.cehn.org/ehcc for more information about this program.

I-Fra5

At What Cost?

Economic impacts of health harms

The impact on families of caring for—and sometimes losing—a child in ill health cannot be reflected in monetary terms. Nor can the incalculable costs of lowered IQ, lost opportunities and social alienation that can accompany developmental effects. But actual costs of providing medical care for a child with a chronic condition or illness can be calculated, and according to public health officials, health care costs for childhood diseases are significant. Here are some examples:

ADHD: Researchers estimate annual ADHD health care costs in the U.S. to be between $36 and $52 billion (in 2005 dollars).*

Autism: One analyst at the Harvard School of Public Health estimates that it costs $3.2 million to care for an autistic person over their lifetime.†

Cancer: The total costs per case of childhood cancer—from treatment, to laboratory costs to lost parental wages—is an estimated $623,000 per year.‡ This translates into a society-wide cost of roughly $6.5 billion annually for the 10,400 newly diagnosed cases each year.

Asthma: Families nationwide pay a combined total of $14.7 billion dollars a year on medical care costs of asthma.§, ¶ The combined direct and indirect costs of asthma to the U.S. economy were an estimated $19.7 billion in 2007.**

Society-wide costs also include higher educational costs for public school systems to meet the needs of children with neurodevelopmental disorders, missed school days (and thus less well-educated students) caused by asthma, and the general productivity losses due to time parents and caregivers take off from work to care for an ill child.

The numbers above do not take into consideration the loss to individuals, families and society as a whole of children not reaching their full physical or intellectual potential. The overall impact of lost creativity, productivity, problem-solving skills and civic engagement, along with higher rates of social alienation and disruption, cannot be overstated.

* Pelham W., E.M. Foster and J.A Robb. "The Economic Impact of Attention Deficit/Hyperactivity Disorder in Children and Adolescents" *Journal of Pediatric Psychology*. 2007. See http://jpepsy.oxfordjournals.org/content/32/6/711.full.pdf+html.

Centers for Disease Control and Prevention. Attention-Deficity/Hyperactivity Disorder (ADHD): Data and Statistics in the United States. See http://www.cdc.gov/ncbddd/adhd/data.html.

† Ganz, Michael "The Costs of Autism," in *Understanding Autism: From Basic Neuroscience to Treatment* (CRC Press, 2006). See http://www.hsph.harvard.edu/news/press-releases/2006-releases/press04252006.html

‡ Landrigan, P.J., C.B. Schechter, J.M. Lipton, M.C. Fahs and J. Schwartz. "Environmental pollutants and disease in American children: estimates of morbidity, mortality, and costs for lead poisoning, asthma, cancer, and developmental disabilities." *Environ. Health Perspect.* 2002; 110, 721–728.

§ EPA, Children's Heath Protection. "Fast Facts on Children's Health." See http://yosemite.epa.gov/ochp/ochpweb.nsf/content/fastfacts.htm. Viewed June 2012.

¶ Centers for Disease Control and Prevention. *Vital Signs: Asthma in the U.S.* See http://www.cdc.gov/VitalSigns/Asthma/Index.html. Viewed May 2012.

** EPA, Children's Heath Protection. "Fast Facts on Children's Health." See http://yosemite.epa.gov/ochp/ochpweb.nsf/content/fastfacts.htm. Viewed June 2012.

- Many school districts in California have significantly reduced pesticide use after a 2000 state law required pesticide reporting and provided incentives for adoption of IPM. School districts in Los Angeles, San Francisco, Santa Barbara and Palo Alto have made particular progress.[168]
- In 2001, California legislators passed a law (AB 947) allowing county agricultural commissioners to restrict pesticide spraying near sensitive sites, including schools and daycare facilities. Under this provision, communities in Tulare County won new rules in 2008 requiring a quarter mile buffer zone banning the aerial application of restricted-use pesticides around schools when they are in session or due to be in session within 24 hours, occupied farm labor camps and residential areas.[169] Kern, Stanislaus, Merced and Fresno counties enacted similar rules in subsequent years.

Pesticide-free school lunches

Currently, neither state nor national policies are in place to reduce pesticide residues in school lunches. But many communities across the country are leading the way to provide children with nutritious school lunches including fresh (sometimes locally produced) fruits and vegetables free from pesticides.

- In Washington state, the Olympia School District has implemented an Organic Choices Salad Bar (25 percent of the produce is purchased directly from local farms and 50 percent of the salad bar is organic), and the Orcas Island Farm-to-Cafeteria Program integrates produce from local, organic farmers and a school garden, and hosts student chef competitions.
- In Minnesota, the White Earth Land Recovery Project added a farm-to-school component in the 2007–2008 school year to their Mino-miijim (Good Food) Program to help reach their goal of food sovereignty on the reservation and promote access to fresh, local and organic ingredients.[170]
- Berkeley, California's Edible Schoolyard (ESY) Project began as a one-acre "interactive classroom" providing primarily organic, fresh fruits and vegetables for student's meals at King Middle School. It has grown into an online initiative building and sharing a food curriculum, and it has inspired similar programs across the country.[171]

Many of these programs are part of the National Farm to School Network (NFSN), which connects K–12 schools across the country with local farms in an attempt to serve healthy meals at school lunch tables while supporting local, often organic, farmers.[172]

Parks & playgrounds without pesticides

Communities across the country are choosing to manage public parks and playgrounds without harmful pesticides. In the Pacific Northwest, 17 cities are phasing out pesticide use with the creation of 85 pesticide-free parks and playgrounds, building momentum for strong policies at the local level despite legislative hurdles (see sidebar on following page).[173]

I-Fra5

Farm-to-school programs across the country are providing children with fresh, pesticide-free fruits and vegetables in school cafeterias.

Seattle in particular has emerged as a pioneer of pesticide-free cities, dramatically reducing its pesticide use in parks by an estimated 80 percent since the 1970s. In 1999, they adopted a pesticide reduction strategy for all city departments and designated 14 pesticide-free parks.[174] The program is now expanding to 22 parks and 50 acres distributed throughout the city.[175]

On the other side of the country, New Jersey legislators unanimously voted in 2011 to pass "The Child Safe Playing Field Act" prohibiting pesticide use on all municipal, county and state playgrounds and playing fields, as well as daycare and school grounds.[176]

Many other communities across the country are following this trend. From a pilot program in Lawrence, Kansas to innovative communities throughout Oregon, California and Colorado, cities are creating pesticide-free parks and playgrounds for children to safely enjoy.

The Pre-emption Law Hurdle & Canada's Local Pesticide Bans

As of 2010, 40 states had pre-emption laws specifically prohibiting municipalities from passing local pesticide ordinances that are stricter than state policy. These laws, which are strongly supported by the pesticide industry, limit the ability of city or county governments to ban or restrict pesticide use.

Such pre-emption laws do not exist in Canada. Over the past 20 years, dozens of Canadian cities have used their local authority to outlaw the application of home and garden pesticides for "cosmetic" purposes such as lawn care.

In 1991, the municipal council of Hudson, Canada, enacted the first ban on cosmetic uses. Similar local bans were adopted across the country, and today more than 170 Canadian cities and towns have passed full or partial bans on pesticide use, and the provinces of Quebec, Nova Scotia and Ontario have enacted comprehensive cosmetic pesticide bans. According to Canadian community activists, more than 22 million Canadians (65% of the population) are now protected from exposure to cosmetic pesticides.*

* Pesticide Free B.C. "Pesticide Bylaw Communities Across Canada." See http://www.pesticidefreebc.org/index.php?option=com_content&view=category&layout=blog&id=53&Itemid=72. Viewed July 2012.

A Generation in Jeopardy • Pesticide Action Network North America 25

I-Fra5

6 Investing in a Healthier Future

A solid start for our children must be a national priority

Those who argue that societies cannot afford to make immediate investments in reducing environmental pollution fail to appreciate that there are some forms of harm that cannot be repaired. — Deborah Axelrod, Devra Lee Davis & Lovell A. Jones

As a nation, we value the wellbeing of our children. In addition to our natural urge to protect what we love, we know that at a societal level their success is key to a vibrant, secure future. Poll after poll shows more than 80 percent of Americans consider healthy children a top priority.[177] We must line up our practice and policies with these values.

Our current use of over a billion pounds of pesticides every year puts their wellbeing at risk and, as the science demonstrates, can derail brain and body development and rob them of their full potential.

If there were no other way to control pests, it would be one kind of choice: weighing one set of needed benefits against known and evolving harms. But given the fact that there are many proven ways to control pests without use of harmful chemicals, the choice is quite clear. It is time to have policies in place that better protect our children (see sidebar).

The National Research Council recommended swift action to protect children from pesticides nearly 20 years ago, and it has been 50 years since Rachel Carson sounded the initial alarm about the health harms pesticides can cause. What is standing in the way?

Pesticide industry well served by current policies

Our current system of industrial agriculture and pest control relies on chemical inputs sold by a handful of corporations. These multinational corporations wield tremendous control over the system, from setting research agendas[178] to financing, crop selection and inputs throughout the production and distribution chain.

Not surprisingly, these same corporations also hold significant sway in the policy arena, investing millions of dollars every year to influence voters, lawmakers and regulators at both the state and federal level to protect the market for pesticides.[179]

The result is agriculture, food and pest control systems that serve the interests of these corporations well. It does not, however, serve farmers, who have lost day-to-day control of their operations and are putting themselves and their families in harm's way. Farmworker interests are not served, as workers are continuously exposed to chemicals known to harm human health.

And the health of children across the country is compromised by exposure to pesticides used to control pests in agriculture and where they live, learn and play.

In short, the system is broken.

Prioritizing children's health requires real change

The best way to protect children from the harms of pesticides is to dramatically reduce the volume used nationwide. This would not only limit children's exposure during their most vulnerable years, it would also lower pesticide levels in the bodies of men and women of childbearing age—protecting current and future generations in one fell swoop. Those pesticides most harmful to children should be first on the list.

This is not a small change, and not a recommendation made lightly. Yet the science tells us the problem is serious and urgent, and that viable and safer alternatives are available. If we stay on our current path, our children will not reach their full potential as we continue to compromise their health.

U.S. Pesticide Rules

Overdue for overhaul?

A little over 100 years ago, Congress enacted our first national pesticide law. The 1910 Insecticide Act put labeling guidelines in place to protect farmers from "hucksters" selling ineffective, misbranded or adulterated pesticide products.

To this day, we control pesticides through a system of registration and labeling. The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), passed by Congress in 1947, is our primary national pesticide law. It has been updated several times in the last 65 years as the health and environmental effects of pesticides came into light, most significantly in 1972 and again in 1996.

It remains, however, a system of registration and labeling, and as such has significant shortcomings. Our current pesticide rules:

- Do not allow for quick response to emerging science;
- Do not assess risk based on real-world exposures;
- Rely heavily on corporate safety data that is not peer-reviewed; and
- Do not encourage the safest form of pest control.

In addition, enforcement of any guidelines or restrictions specified on product labels is relegated to state governments that rarely have adequate resources for the job. Overall, our current rules do not provide adequate tools to protect children from the harms of pesticide exposure.

Informed household food choices can help protect families and grow the market for food that is produced without harmful pesticides—encouraging more farmers to make this shift. And reducing household use of pesticides can provide immediate and long lasting benefits to children's health.* But the burden of protecting children from dangerous chemicals cannot rest solely with individual families. Policy change is required.

Recommendations: Effective policies urgently needed

To protect our children from the health harms of pesticides, policymakers must have much more effective tools. We believe such tools are most urgently needed as decisions are made about these three questions:

- Which pesticides are used in agriculture?
- Which pesticides are used in places children live, learn and play?
- How are farmers supported as they reduce reliance on pesticides?

We recommend the following policy changes in these three arenas:

1. Prevent the pesticide industry from selling agricultural products that can harm children's health

Given the wide-ranging susceptibility of children to pesticide exposures, plus the potential impacts on children from extremely low doses of toxic chemicals, the current approach to assessing and controlling risks of agricultural pesticides does not adequately protect our children.

Decisionmakers must have tools to remove an agricultural pesticide from the market quickly or deny a newly proposed pesticide market access when science suggests it can harm children's developing minds or bodies and there is evidence that children are likely to be exposed. Specifically, we recommend that rulemakers should:

- *Take swift action on existing pesticides:* If studies find a pesticide to be a neurodevelopmental or reproductive toxicant, endocrine disruptor or human carcinogen—*and* it has been measured in humans, in schools or homes, or as residues on food or in drinking water—EPA should target the pesticide for rapid phaseout, triggering USDA resources to assist rapid farmer transitions to safer pest control methods.†
- *Block harmful new pesticides:* EPA should not approve any new pesticide that scientific studies suggest is a neurodevelopmental or reproductive toxicant, endocrine disruptor or human carcinogen—including short-term "conditional" registrations.
- *Prevent harmful low-level exposures:* EPA should act on existing evidence that exposures to endocrine disrupting pesticides pose a particular danger to developing children;

* In addition to choosing non-toxic approaches to pest control (see PAN's Homes, Pets & Gardens online resource at http://www.panna.org/your-health/home-pets-garden), see also the National Pesticide Information Center's page on Pesticides and Children for suggestions on reducing children's exposure in the home: http://npic.orst.edu/health/child.html.

† See, for example, criteria and process for developing the "chemicals of high concern" list in Maine. http://www.maine.gov/dep/safechem/highconcern/chemicals.htm

I-Fra5

The best way to protect children from the harms of pesticides is to dramatically reduce the volume used nationwide.

the long-delayed endocrine disruptor screening program (EDSP) should be swiftly implemented. At the current rate, it will be 2017 before the first set of *only 58 chemicals* are screened.

The insecticide chlorpyrifos provides a clear example of the startling flaws in our regulatory system. Over 10 million pounds of the pesticide are still applied in agricultural

When Is There Enough Evidence to Act?

Scientific studies often identify a "link" or "association" between exposure to a particular pesticide and a specific health harm—but individual studies rarely demonstrate definitive causation. Epidemiological studies often lack statistical power, and case control and animal studies may miss key variables such as exposure timing.

A "weight of the evidence" approach recognizes that a body of scientific work will contain conflicting studies, but holds that when a number of well designed, robust studies come to similar conclusions, the findings should be considered valid.*

When such findings involve widespread, significant and irreversible health harms to our children, the bar for taking action should not be high. When credible evidence of harm emerges, a pesticide product should immediately be taken off the market until its manufacturer can prove its safety. Put simply, it is time the burden of proof shifted to the pesticide corporations, rather than regulators—and the public—as it currently stands.

* Basketter, D., B. Nicholas, S. Cagen, J. Carrillo, H. Certa, D. Eigler et al. "Application of a Weight of Evidence Approach to Assessing Discordant Sensitisation Datasets: Implications for REACH." *Regulatory Toxicology and Pharmacology* 55, no. 1. Oct 2009; 90–96.

Hill, A B. "The Environment and Disease: Association or Causation?" *Proceedings of the Royal Society of Medicine* 58. May 1965; 295–300.

Vandenberg, L., T. Colborn, T. Hayes, J. Heindel, D. Jacobs, D.H. Lee, et al. "Hormones and Endocrine-Disrupting Chemicals: Low-Dose Effects and Nonmonotonic Responses." *Endocrine Reviews.* March 2012 33(3): 378-455.

I-Fra5

Investing in farmers who grow food without relying on chemicals that harm children's health must be a national priority.

fields every year, more than a decade after household uses were withdrawn because of *clear dangers to children's developing brains.** Yet children across the country continue to be exposed—in rural schools and communities, and by eating foods that have been treated with the neurotoxic chemical.

2. Protect children where they live, learn & play

Policymakers need strong tools to protect children from exposure to pesticides where they live, learn and play. Such protections will help keep developing bodies and minds healthy during the years they are most vulnerable to harm from chemical exposures.

We recommend rapid implementation of the following measures:

- *Kid-safe homes, daycares & schools:* EPA should withdraw approval of existing pesticide products and not approve new pesticides for use in homes, daycare centers or schools when scientific evidence indicates the chemicals are possible neurodevelopment or reproductive toxicants, endocrine disruptors or human carcinogens.
- *Safer parks & playgrounds:* State and local officials should enact policies requiring that all public playgrounds, playing fields and parks be managed without using pesticides that studies show are harmful to children's health.
- *Protective buffer zones:* State legislators should establish—or give local governments authority to establish—protective pesticide-free buffer zones around schools, daycare centers and residential neighborhoods in agricultural areas.
- *Healthier school lunches:* Local school districts, state agencies and USDA's Farm-to-school program should provide schools with incentives to procure fresh, local fruits and vegetables that have been grown without pesticides that studies show are harmful to children's health.

3. Invest in farmers stepping off the pesticide treadmill

Investing in farmers who grow food without relying on chemicals that harm children's health must be a national priority. Specifically:

- *Corral resources for farmers:* Federal and state officials should mobilize and coordinate existing resources to help farmers adopt well-known, effective pest management strategies that reduce reliance on pesticides. USDA, EPA and many state agencies and universities have important programs—research, outreach and education—with this stated aim that could be ramped up in complementary ways.
- *Increase investment in innovative farming:* Congress should authorize significant funding for programs supporting farmers' adoption of sustainable practices that reduce use of harmful pesticides. Existing programs receive a small fraction of the funding supplied to programs serving conventional growers.
- *Set use reduction goals:* EPA and USDA should set specific and aggressive national pesticide use reduction goals, focusing first on pesticides studies show to be harmful to children.† To track progress toward this goal, farmers should work with applicators and pest control advisors to report their pesticide use to a nationally searchable database.‡
- *Source for children's health:* Food distributors should require that their suppliers limit use of pesticides that harm children's health.

Effective agroecological methods exist for production of all major crops—but these approaches are often knowledge-intensive, requiring significant training as well as real changes in farm operation.§ Growers need direct support to make the shift away from pesticide reliance, including provision of hands-on field training and technical advice from independent experts as well as incentives to invest in agroecological practices.

These proposals are all commonsense measures in the face of clear evidence that our children's wellbeing is at risk. It's time to muster the political will and prioritize the health of our children, grandchildren and future generations.

* Chlorpyrifos was phased out for household use after studies clearly indicated that exposed children had smaller head circumference, a known indicator of reduced cognitive function.

† See Appendix B.

‡ Pesticide use reporting is already in place in California; lessons learned from implementation of this program (established in 1990) should inform and enable rapid adoption of a federal use reporting system.

§ Agroecological practices are based on the application of intricate place-based knowledge of soil/plant/animal interactions designed to prevent or minimize pest problems. Farmers are successfully using such practices in virtually every crop now grown in the U.S.

I-Fra5

Notes

1 National Research Council. *Pesticides in the Diets of Infants and Children*. Washington, DC. National Academy Press.1993. See http://www.nap.edu/openbook.php?isbn=0309048753.

2 Selevan, S.G., C.A. Kimmel and P. Mendola. "Identifying critical windows of exposure for children's health." *Environ Health Perspect*. June 2000 108(Suppl 3): 451–455. See http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1637810/.

Rauh, V. A., F. P. Perera, M. K. Horton, R. M. Whyatt, R. Bansal, X. Hao, et al. "Brain Anomalies in Children Exposed Prenatally to a Common Organophosphate Pesticide." *Proceedings of the National Academy of Sciences*. May 2012 109 (20): 7871-6. See http://www.pnas.org/cgi/doi/10.1073/pnas.1203396109.

Horton, M.K., L.G. Kahn, F. Perera, D.B. Barr and V. Rauh. "Does the Home Environment and the Sex of the Child Modify the Adverse Effects of Prenatal Exposure to Chlorpyrifos on Child Working Memory?" *Neurotoxicology and Teratology*. July 2012. http://linkinghub.elsevier.com/retrieve/pii/S0892036212001389.

3 Duncan, D., J.L. Matson, J.W. Bamburg, K.E. Cherry and T. Buckley. "The relationship of self-injurious behavior and aggression to social skills in persons with severe and profound learning disability," *Research in Developmental Disabilities*. Vol 20, Issue 6, Nov/Dec 1999: 441–448. See http://dx.doi.org/10.1016/S0891-4222(99)00024-4.

4 Boyle et al. "Trends in the Prevalence of Developmental Disabilities in US Children, 1997— 2008." *Pediatrics*. 2011. See http://pediatrics.aappublications.org/content/early/2011/05/19/peds.2010-2989.full.pdf+html.

5 Landrigan P.J., L. Lambertini and L.S. Birnbaum. "A Research Strategy to Discover the Environmental Causes of Autism and Neurodevelopmental Disabilities." *Environ Health Perspect*. April 2012 120: a258-a260. http://dx.doi.org/10.1289/ehp.1104285.

6 Grandjean and Landrigan. "Developmental Neurotoxicity of Industrial Chemicals," *The Lancet*. Nov. 2006, Vol. 368. See http://www.hsph.harvard.edu/news/press-releases/2006-releases/press11072006.html.

7 Schettler, T., J. Stein, F. Reich and M. Valenti. *In Harm's Way: Toxic threats to child development*. A report by Greater Boston Physicians for Social Responsibility. 2000. See http://www.sehn.org/ecomedpublications.html.

Szpir M. "Tracing the Origins of Autism: A Spectrum of New Studies." *Environ Health Perspect*. July 2006 114: A412-A418. See http://dx.doi.org/10.1289/ehp.114-a412.

Landrigan P.J., L. Lambertini L, L.S. Birnbaum. "A Research Strategy to Discover the Environmental Causes of Autism and Neurodevelopmental Disabilities." *Environ Health Persp*. April 2012 120: a258-a260. http://dx.doi.org/10.1289/ehp.1104285.

8 Eskenazi B., K. Huen, A. Marks, K.G.Harley, A. Bradman, D.B. Barr, et al. "PON1 and Neurodevelopment in Children from the CHAMACOS Study Exposed to Organophosphate Pesticides *in Utero*." *Environ Health Perspect*. Aug 2010 118: 1775-1781. See http://dx.doi.org/10.1289/ehp.1002234.

Holland, N., C. Furlong, M. Bastaki, R. Richter, A. Bradman, K. Huen, et al. "Paraoxonase Polymorphisms, Haplotypes, and Enzyme Activity in Latino Mothers and Newborns." *Environ Health Perspect*. July 2006 114 (7): 985–991. See http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1513322/.

9 Insel, T. *The New Genetics of Autism: Why Environment Matters*. National Institute of Mental Health. April 2012. See http://www.nimh.nih.gov/about/director/2012/the-new-genetics-of-autism-why-environment-matters.shtml.

10 Kong A., M.L. Frigge, G. Masson, S. Besenbacher, P. Sulem, G. Magnusson, et al. "Rate of *de novo* mutations and the importance of father's age to disease risk," *Nature*. Aug 2012; 488 (7412): 471-5. See http://www.ncbi.nlm.nih.gov/pubmed/22914163.

11 National Research Council 2000. *Scientific Frontiers in Developmental Toxicology and Risk Assessment*. Washington, DC: National Academy Press: pg 21. See http://www.nap.edu/catalog.php?record_id=9871.

12 Ontario College of Family Physicians. *Systematic Review of Pesticide Health Effects*. 2012. See http://www.ocfp.on.ca/docs/pesticides-paper/2012-systematic-review-of-pesticide.pdf?sfvrsn=6.

13 Pastor P.N. and C.A. Reuben. "Diagnosed attention deficit hyperactivity disorder and learning disability: United States, 2004–2006." National Center for Health Statistics. *Vital Health Stat* 10 (237). 2008. See also *Attention Deficit Hyperactivity Disorder (ADHD/ADD) Fact Sheet*, Attention Deficit Disorder Association, http://www.add.org/?page=ADHD_Fact_Sheet, viewed Aug 2012.

14 Landrigan et al. 2012, *op.cit.*

15 Crawford, N. "ADHD, A Women's Issue," *Monitor on Psychology*. 34(2) Feb 2003. See http://www.apa.org/monitor/feb03/adhd.aspx.

16 Centers for Disease Control and Prevention. *Attention-deficit/Hyperactivity Disorder (ADHD)*. http://www.cdc.gov/ncbddd/adhd/data.html. viewed July 2012.

17 See Developmental Pyrethroid Exposure and ADHD, grant proposal from Rutgers University. http://www.labome.org/grant/r21/es/developmental/pyrethroid/developmental-pyrethroid-exposure-and-adhd-7278327.html,

18 Bouchard M., et al. "Attention-deficit/hyperactivity disorder and urinary metabolites of organophosphate pesticides," *Pediatrics*. 2010 125 (6): 1270-1277. DOI: 10.1542/peds.2009-3058.

19 Kuehn, B. "Increased Risk of ADHD Associated With Early Exposure to Pesticides, PCBs," *JAMA*. July 2010 304(1): 27-28. See http://jama.jamanetwork.com/article.aspx?articleid=186163.

20 Marks, A.R., K. Harley, A. Bradman, K. Kogut, D.B. Barr, C. Johnson, et al. "Organophosphate Pesticide Exposure and Attention in Young Mexican-American Children: The CHAMACOS Study." *Environ Health Persp*. Dec 2010 118,(12): 1768–1774.

21 Pastor et al. 2008, *op. cit.*

22 Sathyanarayana S., O. Basso, C.J. Karr, P. Lozano, M. Alavanja, D.P. Sandler, et al. "Maternal pesticide use and birth weight in the agricultural health study" *J Agromedicine*. April 2010 15 (2): 127-36. See http://www.ncbi.nlm.nih.gov/pubmed/20407994.

Fenster L., B. Eskenazi, M. Anderson, A. Bradman, K. Harley, H. Hernandez, et al. "Association of in utero organochlorine pesticide exposure and fetal growth and length of gestation in an agricultural population." *Environ Health Persp*. April 2006 114 (4): 597-602. See http://www.ncbi.nlm.nih.gov/pubmed/16581552.

23 Elwan, M.A, J.R. Richardson, T.S. Guillot, W.M. Caudle and G.W. Miller. "Pyrethroid Pesticide-induced Alterations in Dopamine Transporter Function." *Toxicology and Applied Pharmacology*. March 2006 211(3),: 188–197.

Nasuti, C., R. Gabbianelli, M.L. Falcioni, A.D. Stefano, P. Sozio and F. Cantalamessa. "Dopaminergic System Modulation, Behavioral Changes, and Oxidative Stress After Neonatal Administration of Pyrethroids." *Toxicology*. Jan 2007 229 (3): 194–205.

Faraone, S.V. and S.A. Khan. "Candidate Gene Studies of Attention-deficit/hyperactivity Disorder." *The Journal of Clinical Psychiatry*. 2008 67 Suppl 8: 13–20. http://www.ncbi.nlm.nih.gov/pubmed/16961425.

24 Boyle et al., 2011, op cit..

Baio, Jon. *Prevalence of Autism Spectrum Disorders—Autism and Developmental Disabilities Monitoring Network, 14 Sites, United States, 2008*. Autism and Developmental Disabilities Monitoring Network Surveillance Year 2008 Principal Investigators. Morbidity and Mortality Weekly Report, March 30, 2012. http://www.cdc.gov/mmwr/preview/mmwrhtml/ss6103a1.htm.

25 Goldman, L.R. and S. Koduru. *Chemicals in the Environment and Developmental Toxicity to Children: A Public Health and Policy Perspective*. School of Hygiene and Public Health Johns Hopkins University, Baltimore, MD. June 2000.

26 Dufault, R., W.J. Lukiw, R. Crider, R. Schnoll, D. Wallinga and R. Deth. "A macroepigenetic approach to identify factors responsible for the autism epidemic in the United States," *Clinical Epigenetics*. 2012 4:6 http://www.clinicalepigeneticsjournal.com/content/4/1/6/abstract.

CDC press release "CDC estimates 1 in 88 children in United States has been identified as having an autism spectrum disorder." http://www.cdc.gov/media/releases/2012/p0329_autism_disorder.html, April 2012.

27 Roberts, E.M., P.B. English, J.K. Grether, G.C. Windham, L. Somberg and C. Wolff. "Maternal Residence Near Agricultural Pesticide Applications and Autism Spectrum Disorders Among Children in the California Central Valley." *Environ Health Persp*. 2007 115 (10): 1482-9. See http://ehp.niehs.nih.gov/docs/2007/10168/abstract.html.

28 Shelton, J.F., I. Hertz-Picciotto and I.N. Pessah. "Tipping the Balance of Autism Risk: Potential Mechanisms Linking Pesticides and Autism." *Environ Health Persp*. April 2012 120 (7): 944–951.

29 Landrigan et al. 2012, *op. cit.*

30 Roberts et al. 2007, *op. cit.*

31 Eskenazi B., A.R. Marks, A. Bradman, K. Harley, D.B. Barr, C. Johnson, et al. "Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children," *Environ Health Persp*. May 2007 115(5): 792–8. See http://www.ncbi.nlm.nih.gov/pubmed/17520070.

32 Rauh, V.A., R. Garfinkel, F.P. Perera, H.F. Andrews, L. Hoepner, D.B. Barr,et al. "Impact of Prenatal Chlorpyrifos Exposure on Neurodevelopment in the First 3 Years of Life Among Inner-City Children." *Pediatrics*. Dec 2006 118 (6): e1845–e1859.

33 Sanders S.J., M.T. Murtha, A.R. Gupta, J.D. Murdoch, M.J. Raubeson, A.J. Willsey, et al. "De novo mutations revealed by whole-exome sequencing are strongly associated with autism." *Nature*. April 2012 485(7397): 237-41. See http://www.ncbi.nlm.nih.gov/pubmed/22495306.

O'Roak B.J., L. Vives, S. Girirajan, E. Karakoc, N. Krumm, B.P. Coe, et al. "Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations," *Nature*. Apr 2012 485 (7397): 246-50. See http://www.ncbi.nlm.nih.gov/pubmed/22495309.

Neale B.M., Y. Kou, L. Liu, A. Ma'ayan, K.E. Samocha, A. Sabo, et al. "Patterns and rates of exonic de novo mutations in autism spectrum disorders," *Nature*. Apr 2012 485 (7397): 242-5. See http://www.ncbi.nlm.nih.gov/pubmed/22495311.

34 Kong et al. 2012 *op. cit.*

35 Dufault R., W.J. Lukiw, R. Crider, R. Schnoll, D. Wallinga, R. Deth, "A macroepigenetic approach to identify factors responsible for the autism epidemic in the United States," *Clin Epigenetics*. Apr 2012 4(1):6. See http://www.ncbi.nlm.nih.gov/pubmed/22490277.

36 Schettler et al, 2000 *op. cit.*

Needleman, H.L., C. Gunnoe, A Leviton, R. Reed, H. Peresie, C. Maher et al. "Deficits in Psychologic and Classroom Performance of Children with Elevated Dentine Lead Levels." *N Engl J Med* 1979; 300:689-695.

37 Bellinger, D.C. "A Strategy for Comparing the Contributions of Environmental Chemicals and Other Risk Factors to Neurodevelopment of Children." *Environ Health Persp*. 120, no. 4 Apr 2012: 501–507.

38 Rauh et al, 2012 *op. cit.*

39 Engel, S.M., J. Wetmur, J. Chen, C. Zhu, D.B. Barr, R.L. Canfield, et al. "Prenatal Exposure to Organophosphates, Paraoxonase 1, and Cognitive Development in Childhood." *Environ Health Persp*. April 2011 119 (8):: 1182–1188.

40 Bouchard, M.F., J. Chevrier, K.G. Harley, K. Kogut, M. Vedar, N. Calderon, et al. "Prenatal Exposure to Organophosphate Pesticides and IQ in 7-Year-Old Children." *Environ Health Persp*. April 2011 119 (8): 1189–1195.

41 Rauh, V., S. Arunajadai, M. Horton, F. Perera, L. Hoepner, D.B. Barr et al. "Seven-Year Neurodevelopmental Scores and Prenatal Exposure to Chlorpyrifos, a Common Agricultural Pesticide." *Environ Health Persp*. April 2011 119 (8): 1196–1201.

See http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3237355/.

42 Whyatt, R.M. and D.B. Barr. "Measurement of Organophosphate Metabolites in Postpartum Meconium as a Potential Biomarker of Prenatal Exposure: a Validation Study." *Environ Health Persp*. April 2001 109 (4): 417–420.

Whyatt, R.M., V. Rauh, D.B. Barr, D.E. Camann, H.F. Andrews, R. Garfinkel, et al. "Prenatal Insecticide Exposures and Birth Weight and Length Among an Urban Minority Cohort." *Environ Health Persp*.. Mar 2004 112 (10): 1125–1132.

I-Fra5

Berkowitz, G.S., J.G. Wetmur, E. Birman-Deych, J. Obel, R.H. Lapinski, J.H. Godbold, et al. "In Utero Pesticide Exposure, Maternal Paraoxonase Activity, and Head Circumference." *Environ Health Persp.* Nov 2003 112 (3): 388–391.

43 Slotkin, T.A., B.E. Bodwell, E.D. Levin and F.J. Seidler. "Neonatal Exposure to Low Doses of Diazinon: Long-Term Effects on Neural Cell Development and Acetylcholine Systems," *Env Health Persp.* Mar 2008 116(3): 340-8. See http://ehp03.niehs.nih.gov/article/fetchArticle.action?articleURI=info%3Adoi%2F10.1289%2Fehp.11005.

44 Eskenazi, B., A. Bradman and R. Castorina. "Exposures of Children to Organophosphate Pesticides and Their Potential Adverse Health Effects." *Environ Health Persp.* June 1999 107 Suppl 3: 409–419.

Eskenazi et al, 2007, *op. cit.*

45 Horton, M.K., A. Rundle, D.E. Camann, D.B. Barr, V.A. Rauh and R.M. Whyatt. "Impact of Prenatal Exposure to Piperonyl Butoxide and Permethrin on 36-Month Neurodevelopment." *Pediatrics.* Feb 2011 127,(3): e699–e706.

46 Eskenazi, B. "In Utero Exposure to Dichlorodiphenyltrichloroethane (DDT) and Dichlorodiphenyldichloroethylene (DDE) and Neurodevelopment Among Mexican American Children." *Pediatrics.* July 2006 118 (1): 233–241.

Torres-Sánchez, L., S.J. Rothenberg, L. Schnaas, M.E. Cebrián, E. Osorio, M. del Carmen Hernández, et al. "In Utero p,p'-DDE Exposure and Infant Neurodevelopment: A Perinatal Cohort in Mexico." *Environ Health Persp.* Jan 2007 115 (3): 435–439.

47 Morales, E. J. Sunyer, F. Castro-Giner, X. Estivill, J. Julvez, N. Ribas-Fitó, et al. "Influence of Glutathione S-Transferase Polymorphisms on Cognitive Functioning Effects Induced by p,p'-DDT among Preschoolers," *Environ Health Persp.* Nov 2008 116 (11): 1581–1585; see http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2592282/.

Eskenazi et al. 2006, *op. cit.*

48 Landrigan, P.J., L. Claudio, S.B. Markowitz, G.S. Berkowitz, B.L. Brenner, H. Romero, et al. "Pesticides and Inner-city Children: Exposures, Risks, and Prevention." *Environ Health Persp.* June 1999 107 Suppl 3.: 431–437.

Eskenazi et al 2010, *op.cit.*

Richfield EK, Barlow BK, Brooks AI. "Developmental pesticide exposures and the Parkinson's disease phenotype," Birth Defects Res A Clin Mol Teratol. Mar 2005: 73(3):136-9. See http://www.ncbi.nlm.nih.gov/pubmed/15751039..

Suk, W.A., K. Murray and M.D. Avakian. "Environmental Hazards to Children's Health in the Modern World." *Mutation Research.* Nov 2003 544 (2–3): 235–242.

49 PAN press release: "Toxic Brain Chemical Must Be Banned: Health Professionals Demand EPA Take Action," Oct 2011. See http://www.panna.org/press-release/toxic-brain-chemical-must-be-banned-health-professionals-demand-epa-take-action.

50 Leffall, L.D. and M.L. Kripke. *Reducing Environmental Cancer Risk: What We Can Do Now.* Annual Report. President's Cancer Panel. U.S. Department of Health and Human Services, National Institutes of Health, National Cancer Institute, 2010.

51 Cancer in children, Centers for Disease Control and Prevention. See http:/www.cdc.gov/Features/dsCancerInChildren/, viewed July 2012.

52 Ries L.A.G., D. Melbert, M. Krapcho, A. Mariotto, B.A. Miller, E.J. Feuer, et al. eds. *Surveillance Epidemiology and End Results (SEER) Cancer Statistics Review, 1975-2004,* Childhood Cancers. National Cancer Institute. See http://seer.cancer.gov/csr/1975_2004/; Table XXVIII-6. For more resources, visit http://www.cancer.gov/cancertopics/factsheet/Sites-Types/childhood-Diabetes

53 *Ibid.*

54 Metayer, C. and P.A. Buffler. "Residential exposures to pesticides and childhood leukaemia." *Radiation Protection Dosimetry.* 2008 132: 212–219.

55 Infante-Rivard, C. and S. Weichenthal. "Pesticides and Childhood Cancer: An Update of Zahm and Ward's 1998 Review." *Journal of Toxicology and Environmental Health, Part B.* 2007 10: 81–99.

Metayer, C. and P. A. Buffler. "Residential Exposures to Pesticides and Childhood Leukaemia." *Radiation Protection Dosimetry.* Oct 2008 132(2): 212–219.

Soldin, O.P., H.Nsouly-Maktabi, J.M. Genkinger, C.A. Loffredo, J.A. Ortega-Garcia, D. Colantino, et al. "Pediatric Acute Lymphoblastic Leukemia and Exposure to Pesticides." *Therapeutic Drug Monitoring.* Aug 2009 31(4): 495–501.

56 Infante-Rivard, C., D. Labuda, M. Krajinovic and D. Sinnett. "Risk of childhood leukemia associated with exposure to pesticides and with gene polymorphisms." *Epidemiology.* 1999 10: 481–487.

57 van Wijngaarden, E., P.A. Stewart, A.F. Olshan, D.A. Savitz and G.R. Bunin. "Parental occupational exposure to pesticides and childhood brain cancer." *Am. J. Epidemiol.* 2003 157: 989–997.

Schüz, J., U. Kaletsch, P. Kaatsch, R. Meinert and J. Michaelis. "Risk factors for pediatric tumors of the central nervous system: results from a German population-based case-control study." *Med Pediatr Oncol.* 2001 36: 274–282.

58 Daniels,J., A. Olshan, K. Teschke, I. Hertz-Picciotto., D. Savitz, J. Blatt, et al.. "Residential Pesticide Exposure and Neuroblastoma," *Epidemiology.* Jan 2001 12 (1): 20-27. See http://journals.lww.com/epidem/Abstract/2001/01000/Residential_Pesticide_Exposure_and_Neuroblastoma.5.aspx.

59 Olshan, A.F., A.J. De Roos, K. Teschke,, J.P. Neglia, D. Stram, B. Pollock et al. "Neuroblastoma and Parental Occupation." *Cancer Causes & Control: CCC.* Dec 1999 10(6): 539–549.

van Wijngaarden, E., P. Stewart, A. Olshan, D. Savitz and G. Bunin. "Parental Occupational Exposure to Pesticides and Childhood Brain Cancer." *American Journal of Epidemiology.* June 2003 157 (11): 989–997.

60 Valery, P., W. McWhirter and A. Sleigh. "Farm Exposures, Parental Occupation, and Risk of Ewing's Sarcoma in Australia: A National Case-Control Study." *Cancer Causes and Contro.* 2002 13(3): 263-270. See https://researchers.anu.edu.au/publications/14364.

61 Carozza Li, B., K. Elgethun and R. Whitworth. "Risk of Childhood Cancers Associated with Residence in Agriculturally Intense Areas in the United States." *Environ Health Persp.* Jan 2008 116(4): 559–565.

62 Kristensen, P., A. Andersen, L.M. Irgens, A.S. Bye and L. Sundheim. "Cancer in Offspring of Parents Engaged in Agricultural Activities in Norway: Incidence and Risk Factors in the Farm Environment." *International Journal of Cancer. Journal International Du Cancer.* Jan 1996 65 (1): 39–50.

63 Cohn B.A., M.A. Wolff, P.M. Cirillo and R.I. Sholtz. "DDT and breast cancer in young women: New data on the significance of age at exposure." *Environ Health Persp.* 2007 115(10): 1406-1414. See http://www.ehponline.org/docs/2007/10260/abstract.html.

64 Zahm, S.H. and M.H. Ward. "Pesticides and childhood cancer." *Environ. Health Perspect.* 1998 106 (3): 893–908.

Infante-Rivard, C. and S. Weichenthal. "Pesticides and Childhood Cancer: An Update of Zahm and Ward's 1998 Review." *Journal of Toxicology and Environmental Health, Part B.* 2007 10: 81–99.

Jurewicz, J. and W. Hanke. "Exposure to pesticides and childhood cancer risk: has there been any progress in epidemiological studies?" *Int J Occup Med Environ Health.* 2006 19: 152–169.

65 *Infant, neonatal, and postneonatal deaths, percent of total deaths, and mortality rates for the 15 leading causes of infant death by race and sex: United States, 1999–2005.* (National Vital Statistics System 2002/2003). See http://www.cdc.gov/nchs/datawh/statab/unpubd/mortabs/lcwk7_10.htm.

66 Update on overall prevalence of major birth defects—Atlanta, Georgia, 1978-2005.MMWR Morb Mortal Wkly Rep 2008 57:1-5.

67 EPA Report on the Environment: Birth Defects Prevalence and Mortality. See http://cfpub.epa.gov/eroe/index.cfm?fuseaction=detail.viewInd&lv=list.listbyalpha&r=239796&subtop=381; viewed June 2012.

68 *Ibid.*

69 Centers for Disease Control and Prevention: Birth Defects Research and Tracking. See http://www.cdc.gov/ncbddd/birthdefects/research.html; viewed June 2012.

70 Winchester, P.D., J. Huskins and J. Ying. "Agrichemicals in surface water and birth defects in the United States." *Acta Paediatrica.* 2009 98: 664–669.

71 Waller, S.A., K. Paul, S.E. Peterson and J.E. Hitti. "Agricultural-related Chemical Exposures, Season of Conception, and Risk of Gastroschisis in Washington State." *American Journal of Obstetrics and Gynecology.* March 2010 202(3): 241.e1–241.e6.

72 Garry, V.F., D. Schreinemachers, M.E. Harkins and J. Griffith. "Pesticide Appliers, Biocides, and Birth Defects in Rural Minnesota." *Environ Health Persp.* 1996 104(4): 394–399.

73 El-Helaly, M., K. Abdel-Elah, A. Hausseln and H. Shalaby. "Paternal occupational exposures and the risk of congenital malformations — A case-control study." *Int Journal of Occ Med and Environ Health.* 2011 24(2): 218–227.

74 Rocheleau, C.M., P.A. Romitti and L.K. Dennis. "Pesticides and Hypospadias: a Meta-analysis." *Journal of Pediatric Urology.* Feb 2009 5(1): 17–24.

75 Brender, J.D., M. Felkner, L. Suarez, M.A. Canfield and J.P. Henry. "Maternal Pesticide Exposure and Neural Tube Defects in Mexican Americans." *Annals of Epidemiology.* 2010 20(1): 16–22.

76 Lacasana, M. "Maternal and paternal occupational exposure to agricultural work and the risk of anencephaly." *Occupational and Environmental Medicine.* 2006 63(10): 649–656.

77 Ngo, A.D., R. Taylor and C.L. Roberts. "Paternal exposure to Agent Orange and spina bifida: a meta-analysis." *European Journal of Epidemiology.* 2009 25(1): 37–44.

78 Weil, E. "Puberty Before Age 10: A New 'Normal?'" *New York Times Magazine.* March 2012. See http://www.nytimes.com/2012/04/01/magazine/puberty-before-age-10-a-new-normal.html?_r=4&seid=auto&smid=tw-nytmag&pagewanted=all.

79 Herman-Giddens, M., E. Slora, R. Wasserman, C. Bourdony, M. Bhapkar, G. Koch et al. "Secondary Sexual Characteristics and Menses in Young Girls Seen in Office Practice," *Pediatrics.* 1997 99(4): 505-12. See http://www.pediatricsdigest.mobi/content/99/4/505.short

80 Biro F.M., M.P. Galvez, L.C. Greenspan, P.A. Succop, N. Vangeepuram, S.M. Pinney, et al. "Pubertal assessment method and baseline characteristics in a mixed longitudinal study of girls." *Pediatrics.* Sep 2010 126(3):e583-90. See http://www.ncbi.nlm.nih.gov/pubmed/20696727.

81 Steingraber, S. *The Falling Age of Puberty in U.S. Girls: What We Know, What We Need to Know.* The Breast Cancer Fund, August 2007.

82 Walvoord, E.C. "The Timing of Puberty: Is It Changing? Does It Matter?" *Journal of Adolescent Health.* 2010 47(5): 433–439.

83 Parent, A., G. Rasier, A. Gerard, S. Heger, C. Roth, C. Mastronardi, et al. "Early Onset of Puberty: Tracking Genetic and Environmental Factors." *Hormone Research.* 2005 64(2): 41–47.

84 Biro F.M., L.C. Greenspan and M.P. Galvez. "Puberty in girls in the 21st Century." *J Pediatr Adolesc Gynecol.* July 2012. See http://www.ncbi.nlm.nih.gov/pubmed/22841372.

85 *Second National Report on Human Exposure to Environmental Chemicals,* Centers for Disease Control and Prevention, 2003. http://www.cdc.gov/exposurereport/.

Schafer, K., M. Reeves, S. Spitzer and S. Kegley. *Chemical Trespass: Pesticides in our bodies and corporate accountability.* Pesticide Action Network North America, San Francisco, CA. 2004. See http://www.panna.org/issues/publication/chemical-tresspass-english.

86 Mantovani, A. "Endocrine Disruptors and Puberty Disorders from Mice to Men (and Women)." *Endocrine Disruptors and Puberty,* 2012: 119–137. See http://www.springerlink.com/index/10.1007/978-1-60761-561-3_4.

87 Wohlfahrt-Veje, C., K. Main, I. Schmidt, M. Boas, T. Jensen, P. Grandjean, et al. "Lower birth weight and increased body fat at school age in children prenatally exposed to modern pesticides: a prospective study." *Environ Health.* 2011 10: 79.

88 Boneh, A., H. Landau and N. Friedlander. "Age and seasonal factors in the incidence of premature sexual development in girls in the Jerusalem area." *Clin Invest Med.* 1989 12: 172–174.

89 Vasiliu, O. "In utero exposure to organochlorines and age at menarche." *Human Reproduction.* 2004 19 (7): 1506–1512.

90 Den Hond, E., W. Dhooge, L. Bruckers, G. Schoeters, V. Nelen, E. van de Mieroop, et al. "Internal exposure to pollutants and sexual maturation in Flemish adolescents." *J Expo Sci Environ Epidemiol.* 2011 21(3): 224–233.

91 Korrick, S.A., M. Lee, P. Williams, O. Sergeyev, J. Burns, D. Patterson, et al. "Dioxin Exposure and Age of Pubertal Onset

I-Fra5

among Russian Boys." *Environmental Health Perspectives*. 2011 119 (9):1339–1344.

Saiyed, H., A. Dewan, V. Bhatnagar, Shenoy, Udyavar, R. Shenoy, et al. "Effect of Endosulfan on Male Reproductive Development." *Environ Health Persp*. 2003 111 (16): 1958–1962.

92 Pine, M.D., J.K. Hiney, B. Lee and W. Les Dees. "The Pyrethroid Pesticide Esfenvalerate Suppresses the Afternoon Rise of Luteinizing Hormone and Delays Puberty in Female Rats." *Environ Health Persp*. May 2008 116(9): 1243–1247.

93 Centers for Disease Control and Prevention: Childhood Obesity Facts. See http://www.cdc.gov/healthyyouth/obesity/facts.htm, viewed June 2012.

94 Aubert, R. *Diabetes in America*, 2nd edition. National Diabetes Data Group of the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD. 1995. See http://diabetes.niddk.nih.gov/dm/pubs/america/index.aspx.

95 *National Diabetes Information Clearinghouse*, US Dept of Health & Human Services. See http://diabetes.niddk.nih.gov/statistics/index.aspx, viewed July 2012.

96 Ribas-Fitó, N., E. Cardo, M. Sala, M. Eulália de Muga, C. Mazón, et al. "Breastfeeding, exposure to organochlorine compounds, and neurodevelopment in infants." *Pediatrics*. 2003 111(5 Pt 1): e580–585.

Baillie-Hamilton, P.F. "Chemical toxins: a hypothesis to explain the global obesity epidemic." *J Altern Complement Med*. 2002 8: 185–192.

97 Baillie-Hamilton, 2002, *op cit.*

98 Holtcamp, W. "Obesogens: An Environmental Link to Obesity." *Environ Health Persp*. Feb 2012. 120:a62-a68. See http://dx.doi.org/10.1289/ehp.120-a62.

Janesick, A., and B. Blumberg. "Endocrine Disrupting Chemicals and the Developmental Programming of Adipogenesis and Obesity." *Birth Defects Research Part C: Embryo Today: Reviews 93*, no. 1. March 2011: 34–50.

Lee, D.H., M. Steffes, A. Sjödin, R. Jones, L. Needham, D. Jacobs et al. "Low Dose Organochlorine Pesticides and Polychlorinated Biphenyls Predict Obesity, Dyslipidemia, and Insulin Resistance among People Free of Diabetes." *PLoS ONE*. 2011 6: e15977.

Lee, D.H., I. Lee, K. Song, M. Steffes, W. Toscano, B. Baker et al. "A strong dose-response relation between serum concentrations of persistent organic pollutants and diabetes: results from the National Health and Examination Survey 1999-2002." *Diabetes Care*. 2006 29(7): 1638–1644.

99 NIH. Role of Environmental Chemical Exposures in the Development of Obesity, Type 2 Diabetes and Metabolic Syndrome (R01). National Institutes of Health Grants [website]. Bethesda, MD: National Institutes of Health, Department of Health and Human Services, 2011. See http://grants.nih.gov/grants/guide/pa-files/PAR-11-170.html.

100 Trasande, L., C. Cronk, M. Durkin, M. Weiss, D. Schoeller, E. Gall, et al. "Environment and Obesity in the National Children's Study." *Environ Health Persp*. 2008.117(2): 159-166. doi:10.1289/ehp.11839.

Dirinck, E., P. Jorens, A. Covaci, T. Geens, L. Roosens, H. Neels, et al. " Obesity and Persistent Organic Pollutants: Possible Obesogenic Effect of Organochlorine Pesticides and Polychlorinated Biphenyls." *Obesity*. 2010 19: 709–714.

101 Slotkin, T.A. "Does early-life exposure to organophosphate insecticides lead to prediabetes and obesity?" *Reproductive Toxicology*. 2011 31: 297–301.

102 Wohlfahrt-Veje 2011, *op. cit.*.

103 Dirinck, E., P. Jorens, A. Covaci, T. Geens, L. Roosens, H. Neels, et al. " Obesity and Persistent Organic Pollutants: Possible Obesogenic Effect of Organochlorine Pesticides and Polychlorinated Biphenyls." *Obesity*. 2010 19: 709–714.

104 Lee, D.H. et al., 2011, op cit.

Lee, D.H. et al., 2006, op cit.

105 Twum, C. and Y. Wei. "The association between urinary concentrations of dichlorophenol pesticides and obesity in children." *Reviews on Environ Health*. 2011 26(3): 215–219.

106 Rhee, K.E.,S. Phelan and J. McCaffery. "Early Determinants of Obesity: Genetic, Epigenetic, and *in Utero* Influences." *Int Journal of Pediatrics*. 2012: 1–9.

107 *Ibid.*

108 Centers for Disease Control and Prevention, *Vital Signs: Asthma in the U.S.* See http://www.cdc.gov/VitalSigns/Asthma/index.html, viewed May 2012.

Akinbami, L.J., J.E. Moorman and X Lui. "Asthma prevalence, health care use, and mortality: United States, 2005-2009." *Natl Health Stat Report*. 2011: 1–14.

Schwartz, D.A. "Gene-Environment Interactions and Airway Disease in Children." *Pediatrics*. 2009 123: S151–S159.

Akinbami, L.J., J. Moorman, C. Bailey, H. Zahran, M. King, C. Johnson et al. "Trends in asthma prevalence, health care use, and mortality in the United States, 2001-2010." *NCHS Data Brief*. 2012 94: 1–8.

109 Landrigan, P.J., C.B. Schechter, J.M. Lipton, M.C. Fahs and J. Schwartz. "Environmental Pollutants and Disease in American Children: Estimates of Morbidity, Mortality, and Costs for Lead Poisoning, Asthma, Cancer, and Developmental Disabilities." *Environ Health Persp*. July 2002 110(7): 721–728.

110 Diette, G.B., L. Markson, E. Skinner, T. Nguyen, P. Algatt-Bergstrom and A Wu. "Nocturnal asthma in children affects school attendance, school performance, and parents' work attendance." *Arch Pediatr Adolesc Med* 2000 154,(9): 923–928.

111 *Vital Signs: Asthma in the U.S., op. cit.*

112 Hernández, A.F., T. Parrón and R. Alarcón. "Pesticides and asthma." *Current Opinion in Allergy and Clinical Immunology*. 2011 11: 90–96.

Vital Signs: Asthma in the U.S., op. cit

113 Hernández et al. 2011, *op. cit.*

114 Salam, M.T., Y.F. Li, B. Langholz and F.D. Gilliland. "Early-Life Environmental Risk Factors for Asthma: Findings from the Children's Health Study." *Environ Health Persp*. 2003 112: 760–765.

115 Salameh, P.R., I. Baldi, P. Brochard, C. Raherison, B. Abi Saleh and R. Salamon. "Respiratory symptoms in children and exposure to pesticides." *European Respiratory Journal*. 2003 22(3): 507–512.

116 Sunyer, J., M. Torrent, R. Garcia-Esteban, N. Ribas-Fitó, D. Carrizo, I. Romieu, et al. "Early exposure to dichlorodiphenyldichloroethylene, breastfeeding and asthma at age six." *Clin. Exp. Allergy*. 2006 36,(10): 1236–1241.

Karmaus, W., J. Kuehr and H. Kruse. "Infections and atopic disorders in childhood and organochlorine exposure." *Arch Environ Health*. 2001 56(6): 485–492.

117 Hernández, A.F., 2011, *op. cit.*

Hoppin, J.A., D.M. Umbach, S.J. London, M.C.R. Alavanja and D.P. Sandler. "Chemical predictors of wheeze among farmer pesticide applicators in the Agricultural Health Study." *Am J Respir Crit Care Med*. 2002 165(5): 683–689.

Eskenazi, B., A. Bradman and R. Castorina. "Exposures of children to organophosphate pesticides and their potential adverse health effects." *Environ. Health Perspect*. 1999 107 Suppl 3: 409–419.

Newton, J.G. and A.B. Breslin. "Asthmatic reactions to a commonly used aerosol insect killer." *Med J Aust*. 1983 1: 378–380.

118 Schwartz, D.A. "Gene-Environment Interactions and Airway Disease in Children." *Pediatrics*. March 2009 123, Supplement: S151–S159.

119 Daston, G., E. Faustman, G. Ginsberg, P. Fenner-Crisp, S. Olin, B. Sonawane, et al. "A Framework for Assessing Risks to Children from Exposure to Environmental Agents." *Environ Health Persp*. Feb 2004 112 (2): 238–256.

120 Whyatt, R.M., D. Barr, D. Camann, P. Kinney, J. Barr, H. Andrews, et al. "Contemporary-use Pesticides in Personal Air Samples During Pregnancy and Blood Samples at Delivery Among Urban Minority Mothers and Newborns." *Environ Health Persp*. May 2003 111(5): 749–756.

121 Whyatt, R.M. and D.B. Barr. "Measurement of Organophosphate Metabolites in Postpartum Meconium as a Potential Biomarker of Prenatal Exposure: a Validation Study." *Environ Health Persp*. April 2001 109(4): 417–420.

122 Bradman A., D.B. Barr, B.G.C. Henn, T. Drumheller, C. Curry and B. Eskenazi. "Measurement of Pesticides and Other Toxicants in Amniotic Fluid as a Potential Biomarker of Prenatal Exposure: A Validation Study." *Environ Health Persp*. 2003 111:1779-1782. See http://dx.doi.org/10.1289/ehp.6259.

123 Aris, A. and S. Leblanc. "Maternal and Fetal Exposure to Pesticides Associated to Genetically Modified Foods in Eastern Townships of Quebec, Canada." *Reproductive Toxicology*. May 2011 31(4): 528–533.

124 Vandenberg, L., T. Colborn, T. Hayes, J. Heindel, D. Jacobs, D.H. Lee, et al. "Hormones and Endocrine-Disrupting Chemicals: Low-Dose Effects and Nonmonotonic Responses." *Endocrine Reviews*. March 2012 33(3): 378–455.

125 Landrigan, P.J., L. Claudio, S.B. Markowitz, G.S. Berkowitz, B.L. Brenner, H. Romero, et al. "Pesticides and Inner-city Children: Exposures, Risks, and Prevention." *Environ Health Persp*. June 1999 107 (3): 431–437.

CPCHE. *Child Health and the Environment – a Primer*. Canadian Partnership for Child Health and the Environment. Toronto. 2005. See http://www.healthyenvironmentforkids.ca/sites/healthyenvironmentforkids.ca/files/cpche-resources/Primer.pdf.

Pest Management and Pesticide Use in California Child Care Centers; Prepared for the California Department of Pesticide Regulation by the Center for Children's Environmental Health Research, UC Berkeley. June 2010. See http://cerch.org/research-programs/child-care/pest-management-and-pesticide-use-in-california-child-care-centers/.

126 Charlier, C., A. Albert, P. Herman, E. Hamoir, U. Gaspard, M. Meurisse et al. "Breast cancer and serum organochlorine residues." *Occ and Environ Medicine*. 2003 60(5): 348-51. See http://sciencereview.silentspring.org/epid_detail.cfm?id=248.

127 Gurunathan, S., M. Robson, N. Freeman, B. Buckley, A. Roy, R. Meyer, et al. "Accumulation of chlorpyrifos on residential surfaces and toys accessible to children." *Environ Health Perspect*. Jan 1998 106(1): 9–16. See http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1532945/.

128 Fenske R.A., K. Black, K. Elkner, L. Chorng-Li, M.M. Methner and R. Soto. "Potential exposure and health risks of infants following indoor residential pesticide applications." *Am J Pub Health*. 1990 80(6): 689-93.

129 *Pesticides in the Diet of Infants and Children*. National Research Council. National Academy Press, Washington D.C. 1993.

130 Simcox N.J., R.A. Fenske, S.A. Wolz. I.C. Lee and D.A. Kalman. "Pesticides in household dust and soil: exposure pathways for children of agricultural families." *Environ Health Perspect*. 1995 103(12):1126-34.

131 Fenske, R.A., C. Lu, D.Barr and L. Needham. "Children's Exposure to Chlorpyrifos and Parathion in an Agricultural Community in Central Washington State." *Environ Health Perspect*. May 2002 11(5):: 549–553.

132 *Air Monitoring for Chlorpyrifos in Lindsay, California*. Pesticide Action Network. San Francisco, CA, USA. 2006.

Pesticide Drift Monitoring in Minnesota: Technical Report. Pesticide Action Network, 2012. Both studies available at http://www.panna.org/science/drift/stories-from-the-field

133 PAN 2012, *op. cit.*

134 Curl C.L., R.A. Fenske, J.C. Kissel, J.H. Shirai, T.F. Moate, W. Griffith, et al. "Evaluation of take-home organophosphorus pesticide exposure among agricultural workers and their children." *Environ Health Perspect*. 2002 110(12): A787–A792.

Bradman, A., D. Whitakerb, L. Quiro ⊠ Sa, R. Castorinaa, B.C. Hennc, M. Nishiokad, et al. "Pesticides and their Metabolites in the Homes and Urine of Farmworker Children Living in the Salinas Valley, CA." *Journal of Exposure Science and Environ Epidemiology*. 2007 17: 331–349

135 Owens, K. *Schooling of State Pesticide Laws*, Beyond Pesticides, Washington, DC 2009. See http://www.beyondpesticides.org/schools/index.php.

136 PAN 2006, *op. cit.*, PAN 2012, *op. cit.*

137 *Air Monitoring in Hastings, Florida, December 2006*. Pesticide Action Network, San Francisco, CA. April 2007.See http://www.panna.org/science/drift/stories-from-the-field.

138 *Pesticides may be making kids sick at school*, Associated Press, May 2007. See http://www.msnbc.msn.com/id/18681428/#.UASd6XD4SF4

139 Uyeno, K. "School Samples Test Positive for Pesticides," *Hawaii News Now*. See http://www.hawaiinewsnow.com/Global/story.asp?S=6567673.

I-Fra5

140 Gunn, E. and C. Osborne. *Pesticides and playing fields: Are we unintentionally harming our children?* Beyond Pesticides, Washington D.C. 1997.

141 Balinova A.M., R.I. Mladenova and D.D. Shtereva. "Effects of processing on pesticide residues in peaches intended for baby food." *Food Addit Contam*. Sept 2006 23(9): 895-901.

142 Landrigan et al 1999, *op. cit.*

143 Chensheng, L., F.J. Schenck, M.A. Pearson and J.W. Wong. "Assessing Children's Dietary Pesticide Exposure: Direct Measurement of Pesticide Residues in 24-hr Duplicate Food Samples." *Environ Health Persp*. Nov 2010 118(11): 1625–1630.

144 Curl, C.L., R.A. Fenske and K. Elgethun. "Organophosphorus Pesticide Exposure of Urban and Suburban Preschool Children with Organic and Conventional Diets." *Environ Health Persp*. March 2003 111(3): 377–382.

145 Lu, C., K. Toepel, R. Irish, R.A. Fenske, D.B. Barr and R. Bravo. "Organic Diets Significantly Lower Children's Dietary Exposure to Organophosphorus Pesticides." *Environ Health Persp*. 2006 114.: 260–263.

Chensheng, L., D.B. Barr, M.A. Pearson and L.A. Waller. "Dietary Intake and Its Contribution to Longitudinal Organophosphorus Pesticide Exposure in Urban/suburban Children." *Environ Health Persp*. April 2008 116(4): 537–542.

146 Centers for Disease Control and Prevention, *The Fourth National Report on Human Exposure to Environmental Chemicals, 2009*. See http://www.cdc.gov/exposurereport/.

147 Landrigan et al. 1999, *op. cit.*

148 Miller, M.D, M.A. Marty, A. Arcus, J. Brown, D. Morry and M. Sandy. "Differences Between Children and Adults: Implications for Risk Assessment at California EPA." *International Journal of Toxicology*. October 2002 21(5): 403–418.

149 *Ibid.*

150 Bennett, W.D and K.L. Zeman. "Effect of Body Size on Breathing Pattern and Fine-particle Deposition in Children." *Journal of Applied Physiology*. Sept 2004 97(3): 821–826.

151 Louis, G.B., United Nations Environment Programme, International Labour Organisation, World Health Organization, Inter-Organization Programme for the Sound Management of Chemicals, and International Program on Chemical Safety. "Principles for evaluating health risks in children associated with exposure to chemicals." 2006. See http://site.ebrary.com/id/10214527.

152 Schwenk, M., U. Gundert-Remy, G. Heinemeyer, K. Olejniczak, R. Stahlmann, W. Kaufmann, et al. "Children as a Sensitive Subgroup and Their Role in Regulatory Toxicology: DGPT Workshop Report." *Archives of Toxicology*. Jan 2003 77(1): 2–6.

Louis et al. 2006, *op. cit.*

153 Furlong, CE, N. Holland, R. Richter, A. Bradman, A. Ho and B. Eskenazi. "PON1 Status of Farmworker Mothers and Children as a Predictor of Organophosphate Sensitivity." *Pharmacogenetics and Genomics*. March 2006 16(3): 183–190.

154 *Pesticide Industry Sales & Usage, 2006 and 2007 Market Estimates*, US EPA, Washington, DC Feb 2011. See www.epa.gov/opp00001/pestsales/07pestsales/market_estimates2007.pdf.

155 "Pesticide Usage in the United States: Trends in the 20th Century." *CIPM Technical Bulletin*. 2003

156 Goldman, L and S. Koduru. *Chemicals in the Environment and Developmental Toxicity to Children: A Public Health and Policy Perspective*. School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD. June 2000.

157 Gan, J., et al. Synthetic Pyrethroids; ACS Symposium Series; American Chemical Society: Washington, DC, 2008. See http://pubs.acs.org/doi/abs/10.1021/bk-2008-0991.ch001.

158 Shafer, T.J., D.A. Meyer, and K.M. Crofton. "Developmental Neurotoxicity of Pyrethroid Insecticides: Critical Review and Future Research Needs." *Environ Health Persp* 113, no. 2. Oct 2004: 123–136.

See also Permethrin: Technical Summary, The Endocrine Disruption Exchange, http://www.endocrinedisruption.com/pesticides.permethrin.summary.php.

159 For an overview of health effects with multiple references provided, see PAN AP Fact Sheet "Highly Hazardous Pesticides: Neonicotinoids", PAN Asia Pacific, 2012. See http://www.panap.net/en/p/page/pesticides-campaigns-hhps/185.

Chao, S.L. and J.E. Casida.. "Interaction of Imidacloprid Metabolites and Analogs with the Nicotinic Acetylcholine Receptor of Mouse Brain in Relation to Toxicity". *Pesticide Biochemistry and Physiology*. 1997 58: 77–88. DOI:10.1006/pest.1997.2284. See http://www.sciencedirect.com/science/article/pii/S0048357597922847.

Imidacloprid - Human Health and Ecological Risk Assessment – Final Report. USDA Forest Service. December 2005.

160 Yamamoto, I. "Nicotine to Nicotinoids: 1962 to 1997", in *Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor*, eds. Yamamoto, I. and Casida, J. Springer-Verlag, Tokyo, 1999 pp. 3–27.

161 Pesticide Use Trends in the U.S.: Pesticides for Home and Garden Uses. Univ of Florida Extension, EDIS – "This document is PI-140, one of a series of the Pesticide Information Office, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Published January 2007. Revised February 2011. See http://edis.ifas.ufl.edu/pi177.

162 *Pesticide Industry Sales & Usage, 2006 and 2007 Market Estimates*, U.S. EPA, Washington, DC Feb 2011. See www.epa.gov/opp00001/pestsales/07pestsales/market_estimates2007.pdf.

163 Lu C., D.B. Barr, M. Pearson, S. Bartell and R. Bravo. "A Longitudinal Approach to Assessing Urban and Suburban Children's Exposure to Pyrethroid Pesticides." *Environ Health Perspect*. 2006 114:1419-1423.

164 See Pest Management and Pesticide use in California Child Care Centers, UC Berkeley, 2012. Available at http://cerch.org/information-for/childcare-providers/.

165 *Use, Effects and Alternatives to Pesticides in Schools*, Report to the Ranking Minority Member, Committee on Governmental Affairs, U.S. Senate. United States General Accounting Office 1999. See www.gao.gov/new.items/rc00017.pdf.

166 Owens, K. "Schooling of State Pesticide Laws 2010 Update." *Pesticides and You*. Fall 2009 29(3): 9–20.

167 "Child Safe Playing Field Act Signed into Law by New York Governor" Beyond Pesticides Daily News Blog, May 2010. See http://www.beyondpesticides.org/dailynewsblog/?p=3637.

168 *Green Schools Within Reach: Moving Beyond the Healthy Schools Act of 2000*. Californians for Pesticide Reform. See http://pesticidereform.org/article.php?id=385.

169 "Tulare County Residents Win Greater Protection from Dangerous Pesticides: New rules announced for pesticide applications around schools, homes and labor camps," Press Release, Californians for Pesticide Reform. Feb 2008. See www.panna.org/sites/default/files/imported/files/CPR20080220.pdf.

170 White Earth Land Recovery Project, Farm to School Program. See http://nativeharvest.com/node/255 , viewed July 2012.

171 Rauzon, S., M. Wang, N. Studer and P. Crawford. An Evaluation of the School Lunch Initiative. A report by the Dr. Robert C and Veronica Atkins Center for Weight and Health, University of California at Berkeley, Sept 2010. See edibleschoolyard.org/sites/default/files/file/Final%20Report_9-22-10v4_LoRes.pdf.

172 The Olympia School Districts' Organic Choices Program, National Farm to School Network. See http://www.farmtoschool.org/state-programs.php?action=detail&id=8&pid=58, viewed June 2012.

173 *Action Alert: Help protect Ashland Parks, Schools and Waters from Pesticides*. Klamath Siskiyou Wildlands Center. See http://kswild.org/get-involved/ActionAlerts/help-reduce-or-eliminate-pesticides-at-ashland-parks-and-schools, viewed June 2012.

174 *Pesticide-free parks: It's time!* Northwest Coalition for Alternatives to Pesticides, Eugene OR. 2005. See www.pesticide.org/get-the-facts/ncap-publications-and-reports/pesticide-free-parks/pfptime.pdf.

175 "Horticulture: Pesticide reduction." Seattle Parks and Recreation. See http://www.seattle.gov/parks/horticulture/pesticide.htm, viewed June 2012.

176 NJ Senate Environment Committee Passes Nation's Strongest Pesticide Bill, Press Release, Clean Water Action, Jan 2011. See http://www.cleanwater.org/press/nj-senate-environment-committee-passes-nation%E2%80%99s-strongest-pesticide-bill.

177 New Polling Data Indicates Overwhelming Public Support for Chemicals Regulation. Safer Chemicals Healthy Families: Resources. Sept 2010. See http://www.saferchemicals.org/resources/opinion-2010.html

178 Philpott, T. "How Your College Is Selling Out to Big Ag," *Mother Jones*, May 2012. See http://www.motherjones.com/tom-philpott/2012/05/how-agribusiness-dominates-public-ag-research

179 Agribusiness. OpenSecrets.org, Center for Responsive Politics. See http://www.opensecrets.org/industries/indus.php?ind=A, viewed August 2012. See also Undue Influence, Pesticide Action Network at http://www.panna.org/issues/pesticides-profit/undue-influence.

I-Fra5

Appendix A

More Science: Key study descriptions

Our intention in undertaking this review was not to conduct a comprehensive evaluation of the evidence. The body of scientific literature exploring how pesticides affect children's health is wide, deep and decades long.

Our goal is to provide a snapshot of recent findings, coming fast and furious in the just the past few years, that—taken together—provide compelling reason for concern about the impact of pesticides on our children's health.

In the report itself we highlight a few of the key findings for each health effect, focusing on studies that were particularly compelling, and/or represented other studies we reviewed with similar findings. We simplified descriptions of each study to provide a basic sense of how the research was conducted and what researchers found. Here in Appendix A we provide a bit more detail on some of the key studies described above, as well as additional studies. Study descriptions are organized by health effect, and alphabetically by author within each category.

Brain & nervous system harms (reduced cognitive function, autism, ADHD)

Bouchard M.F., D.C. Bellinger, R.O. Wright and M.G. Weisskopf."Attention-deficit/ hyperactivity disorder and urinary metabolites of organophosphate pesticides." *Pediatrics* 2010. 125(6): e1270–e1277.

This study examines the association between urinary concentrations of organophosphate metabolites and ADHD in children eight to 15 years of age. Researchers analyzed cross-sectional data from the National Health and Nutrition Examination Survey for 1139 children representative of the U.S. population. Urinary DMAP metabolite levels (which are an indicator of exposure to OP pesticides), an ADHD assessment, and household surveys were used in the analysis. The data support the hypothesis that organophosphate exposure, at levels common among U.S. children, may contribute to ADHD prevalence.

Eskenazi B., K. Huen, A. Marks, K.G.Harley, A. Bradman, D.B. Barr, et al. "PON1 and Neurodevelopment in Children from the CHAMACOS Study Exposed to Organophosphate Pesticides in Utero." *Environ Health Perspect*. Aug 2010 118: 1775-1781. See http://dx.doi.org/10.1289/ehp.1002234.

The enzyme paraoxonase 1 (PON1) detoxifies metabolites of some organophosphate (OP) pesticides, andPON1 genetic polymorphisms influence enzyme activity and quantity. The study authors investigated whether PON1 genotypes and enzyme activity levels in mothers and their children were linked to neurodevelopmental changes, and whether PON1 levels and genotypes had an effect on the association of *in utero* exposure to OP pesticides (as assessed by maternal urinary concentrations of dialkyl phosphate metabolites, a marker of OP pesticide exposure) and neurodevelopment and behavior. The researchers found that of the 353 two-year-olds assessed, children with a certain variation of PON1 (the $PON1_{-108T}$ allele) scored more poorly on the Mental Development Index and somewhat lower on the Psychomotor Development Index. The authors concluded that while the variations of PON1 were associated with outcomes in child neurobehavioral development, additional research is needed to confirm whether it modifies the relation with *in utero* exposure to OP pesticides.

Pessah I.N., P.J. Lein. "Evidence for environmental susceptibility in autism" in: *Autism*, (Zimmerman AW, ed). Totowa, NJ: Humana Press 2008 409–428.

The authors aim to illustrate how research into the pathophysiology and genetics of autism may inform the identification of environmental susceptibility factors that promote adverse outcomes in brain development. They highlight three examples of gene-environment interactions that are likely to contribute to autism risk, including: (1) pesticides that interfere with the neurotransmitter acetylcholine; (2) pesticides that interfere with γ-aminobutyric acid (GABA) neurotransmission; and (3) persistent organic pollutants that directly

A Study by Any Other Name…

Epidemiological study: A study of distribution or patterns in health trends or characteristics and their causes or influences in specific populations. Includes both case-control and all types of cohort studies.

Case-control study: Compares a "case" group (e.g., U.S. children ages 0–14 with cancer) with a group serving as a control (e.g., cancer-free U.S. children ages 0–14).

Cohort study: Profiles a specific population where shared exposure may be assumed, such as occupational exposure to pesticides among farmworkers.

Prospective cohort study: Follows a group that is slightly different in some respects. (i.e., studying a cohort of pesticide applicators who use varying protective methods while working with pesticides.)

Longitudinal cohort study: Tracks a specific group over time. For example, a UC Berkeley study on the central California coast has followed a specific group of children from conception through adolescence.

Meta-analysis: Pulls together several studies on the same topic and does further statistical analysis on the basic findings.

Review: Examines the "state of the science" and often provides evaluation of conflicting pieces of data. Review authors give their view on what is currently happening in the field.

I-Fra5

alter calcium ion (Ca^{2+}) signaling pathways and Ca^{2+}-dependent effectors. If both genetic factors and environmental ones converge to interrupt the same neurotransmitter or signaling systems at critical times during development, adverse effects can be amplified.

Rauh V.A., F.P. Perera, M.K. Horton, R.M. Whyatt, R. Bansal, X. Hao X, et al. "Brain anomalies in children exposed prenatally to a common organophosphate pesticide." *Proc Natl Acad Sci* 2012 109(20):7871-6.

This study investigated associations between prenatal exposure to chlorpyrifos and brain morphology (examining brain structure). With a sample of 40 children—who experienced low prenatal exposure to tobacco smoke and polycyclic aromatic hydrocarbons—20 subjects with high chlorpyrifos exposure were compared to 20 low-exposure subjects. The data revealed a significant association between prenatal exposure to chlorpyrifos, at standard use levels, and structural changes in the developing human brain. High exposure was associated with the enlargement of several areas of the brain and in preliminary analyses, the reversal of sex differences or a lack of expected sex differences.

Shafer, T.J., D.A. Meyer and K.M. Crofton. "Developmental Neurotoxicity of Pyrethroid Insecticides: Critical Review and Future Research Needs." *Environmental Health Perspectives* 113, no. 2 Oct 2004: 123–136 .

A review of pyrethroid insecticides and the data related to potential developmental neurotoxic effects of pyrethroids, with recommendations for improving study design and statistical analyses. The review discusses the various effects on voltage-sensitive sodium channels, which are a primary target of pyrethroids.

Childhood cancers

Carozza S.E., B. Li, K. Elgethun and R. Whitworth."Risk of childhood cancers associated with residence in agriculturally intense areas in the United States." *Environ Health Persp* 2008 116(4): 559–565.

Researchers from the U.S. evaluated whether children under the age of 15 who live in a county associated with greater agriculture production—and hence, exposure to pesticide drift—experienced different risk rates for developing cancer. Using incidence data for U.S. children provided by the North American Association of Central Cancer Registries, researchers were able to compare county-level, sex- and age-specific rates of childhood cancer with agricultural census data containing county acreage, percent cropland, and percent acres for specific crops. The data revealed statistically significant increase in risk for many types of childhood cancers for residents living in those counties with a moderate to high level of agricultural activity. Risk for different cancers varied by type of crop; for example, there was increased risk of non-Hodgkin lymphoma and thyroid cancer associated with residence at diagnosis in counties that produced corn or oats.

Infante-Rivard C, S. Weichenthal. Pesticides and childhood cancer: an update of Zahm and Ward's 1998 review. *J Toxicol Environ Health B Crit Rev* 2007 10(1): 81–99.

Infante-Rivard and Weichenthal reviewed the epidemiological and ecological studies published since the 1998 Zahm and Ward review. The authors found that15 case-control studies, four cohort studies, and two ecological studies have been published since this review, and 15 of these 21 studies reported a statistically significant increase in risk of childhood cancer among children whose parents were experienced occupational pesticide exposure. These studies found that the risk of all childhood cancers increased with the frequency of maternal exposure to herbicides and plant insecticides. Furthermore, maternal and paternal exposure to insecticides and herbicides up to five years before having a child increased risk of all childhood brain tumors, astroglial tumors, non-Hodgkin's lymphoma, primitive neuroectodermal tumors, and other glial tumors. Parental occupation in agriculture is also associated with an increased risk of Ewing's sarcoma. The authors conclude that evidence supports an association between at least some pesticide exposure and childhood cancer.

Kristensen, P., A. Andersen, L.M. Irgens, A.S. Bye and L. Sundheim. "Cancer in Offspring of Parents Engaged in Agricultural Activities in Norway: Incidence and Risk Factors in the Farm Environment." *International Journal of Cancer. Journal International Du Cancer.* Jan 1996 65 (1): 39–50.

A cohort study in Norway of 323,359 children born between 1952–1991 reported that children 0-14 years had a nearly doubled risk for brain tumors and a more than tripled risk for neuroepithelial tumors except for astrocytomas associated with pesticide purchase. These associations were stronger when sub-groups, such as growing up on the farm, were considered. Offspring born April–June showed a clustering of neuroepithelial brain tumors, suggesting that paternal exposure during periods of increased pesticide application, from 0–3 months before conception, may have been a factor.

Meinert, R., J. Schuz, U. Kaletsch and J. Michaelis. "Leukemia and Non-Hodgkins Lymphona in Childhood and Exposure to Pesticides: Results of a Register-based Case-Control Study in Germany." *Am Journal of Epidemiology* 2000. 151 (7): 639-646.

A case-control study conducted in Germany from 1993-1997 found parental occupational exposure to be related to childhood cancer regardless of period of exposure and type of cancer, which the authors point out might be due to different recall of past exposures between parents of cases and parents of controls. Residential insecticide use was associated with childhood lymphoma, both professional exterminator and parental usage were significantly associated with increased risk.

Nielsen S.S., R. McKean-Cowdin, F.M. Farin, E.A. Holly, S. Preston-Martin and B.A. Mueller. "Childhood brain tumors, residential insecticide exposure, and pesticide metabolism genes." *Environ Health Persp* 2009 118(1): 144-149.

Researchers in California and Washington found evidence of increased risk of childhood brain tumors (CBT) associated with certain genetic polymorphisms when kids were exposed to insecticides. Strong interactions between genotype and insecticide exposure during childhood was observed. Among exposed children, CBT risk increased per $PON1_{-108T}$ allele, whereas among children never exposed, CBT was not increased. Nielsen et al. concluded childhood exposure to organophosphorus pesticides coupled with a reduced ability to detoxify these pesticides, may be associated with CBT.

I-Fra5

van Wijngaarden E, P.A. Stewart, A.F. Olshan, D.A. Savitz and G.R. Bunin. "Parental occupational exposure to pesticides and childhood brain cancer." *Am J Epidemiol* 2003. 157(11): 989–997.

Researchers from the U.S. evaluated parental exposure to pesticides at home or on the job in relation to the occurrence of brain cancer in children. The sample consisted of children diagnosed with cancer and matching controls from four U.S. states. Interviews were performed with the biological mothers of the subjects to assess the residential and occupational exposure to pesticides in the two years before the child was born. The data revealed a significant risk of astrocytoma associated with residential use and exposure to herbicides. Combining parental exposures to herbicides form both residential and occupational sources, the elevated risk remained significant.

Birth defects

Brender, J.D., M. Felkner, L. Suarez, M.A. Canfield and J.P. Henry. "Maternal Pesticide Exposure and Neural Tube Defects in Mexican Americans." *Annals of Epidemiology*. 2010 20(1): 16–22.

Researchers investigated the relationship between maternal pesticide exposures and neural tube defects (NTDs) in offspring comparing to groups of Mexican American women (184 in case group, 225 for comparison). After adjusting for differences in maternal education levels, smoking, and folate intake during pregnancy, women who reported using pesticides in their homes or yards were twice as likely to have children with NTDs than women not reporting exposures (95% confidence interval [CI], 1.2–3.1) Case-women were also more likely to live within ¼ mile of agricultural fields. As possible sources of pesticide exposure increased, risk of NTDs also increased. Associations were stronger for risk of anencephaly than for spina bifida.

Garry V.F., M.E. Harkins, L.L. Erickson, L.K. Long-Simpson, S.E. Holland and B.L. Burroughs. "Birth defects, season of conception, and sex of children born to pesticide applicators living in the Red River Valley of Minnesota, USA." *Environ Health Persp* 2002. 110(3): 441–449.

A cross-sectional study performed in the Red River Valley of Minnesota examined the reproductive health outcomes in 695 farm families (analyzed data from 1,532 children) from parent-reported birth defects. Researchers determined conceptions in the spring time led to significantly more children born with birth defects, compared to children conceived in any other season. Their data suggests environmental agents present in the spring, like herbicides, have an adverse effect on the birth defect rate. Furthermore, the data revealed an association between fungicide exposure and the determination of child sex—affecting the survival rate of the male fetus (female to male birth ration is 1.25 to 1).

Gaspari L., F. Paris, C. Jandel, N. Kalfa, M. Orsini, J.P. Daures and C. Sultan. "Prenatal environmental risk factors for genital malformations in a population of 1442 french male newborns: a nested case-control study." *Hum Reprod* 2011. 26(11): 3155–3162.

Researchers from France analyzed a physician's examinations and parental interviews for 1442 full-term newborn males in southern France to identify risk factors for male external genital malformations, with a focus on parental occupational exposure to endocrine disrupting chemicals, such as organochlorine pesticides. Infants were examined for cryptochidism, hypospadias, and micropenis, while a questionnaire asked parents about the pregnancy, personal characteristics, lifestyle, and occupational exposure to EDCs. In total, 39 cases of genital malformation were reported (2.70%). A significant relationship was observed between newborn cryptochidism, hypospadias or micropenis and parental occupational exposure to pesticides with the odds of genital malformation increasing 4.41-fold. These data supports the hypothesis that prenatal contamination by pesticides may be a potential risk factor for newborn male external genital malformation.

Rocheleau, C.M, P.A. Romitti and L.K. Dennis. "Pesticides and Hypospadias: a Meta-analysis." *Journal of Pediatric Urology*. Feb 2009 5(1): 17–24.

A meta-analysis of studies done in 7 different countries (Canada, Denmark, Italy, Netherlands, Norway, Spain, US) indicated a 36% increased risk of hypospadia with maternal occupational exposure and a 19% increased risk of hypospadias with paternal occupational exposure.

Winchester PD, Huskins J, Ying J. 2009. Agrichemicals in surface water and birth defects in the United States. *Acta Paediatr* 98(4): 664–669.

Researchers from Indiana and Ohio compared water data from the USGS National Water Quality Assessment (NAWQA)—measuring the levels of nitrates, atrazine, and other pesticides in surface water—and Centers for Disease Control data detailing monthly pregnancy and birth outcome outcomes. The data reveal that between 1996 and 2002 women in the US were significantly more likely to give birth to a child with birth defects if conception had occurred in the months of April through July. NAWQA surface water samples indicate that concentrations of atrazine, nitrates, and other pesticides were also higher in the months of April through July. This correlation was statistically significant, demonstrating elevated concentrations of agrichemicals in surface water coincided with a higher risk of birth defects among live births for children conceived between April and July.

Early puberty

Aksglaede L., K. Sorensen, J.H. Petersen, N.E. Skakkebaek and A. Juul. "Recent decline in age at breast development: the Copenhagen puberty study." *Pediatrics* 2009. 123(5): e932-939.

Researchers from Denmark collected data from 2095 females aged 5.6 to 20 years in two Copenhagen cohorts (1991–1993 and 2006–2008) to examine differences in breast development. Using the most accurate method of palpation, Aksglaede et al. found the onset of puberty—defined as the mean estimated age at the attainment of glandular breast tissue—occurred significantly earlier in the 2006 cohort. The ages at which menarche and pubic hair development occurred also slightly decreased in the 2006 cohort. As a result of these timing changes in early and later markers of puberty, the length of puberty appears to have increased. The authors interpreted these observations as indicative of gonadotropin-independent estrogenic actions at the level of breast development, rather than an earlier activation of the pituitary-gonadal axis. These changes in timing could not be explained by alterations in reproductive hormones and BMI, suggesting other factors involved need to be explored.

Gladen B., N. Ragan and W. Rogan. "Pubertal growth and development and prenatal and lactational exposure to polychlorinated biphenyls and Dichlorodiphenyl Dichloroethene." ***Pediatrics*** **2000. 136(4): 490-496.**

Researchers from the National Institute of Environmental Health Sciences explored the relationship between prenatal and early-life exposure to PCBs and DDE on children. This is one of a very few studies examining environmental contaminants and male puberty onset. Using 594 children from the North Carolina Infant Feeding Study cohort, they found no effect on the ages at which puberty began. However, the height and weight (adjusted for height) of boys at puberty increased with transplacental exposure to DDE.

Massart F., P. Seppia, D. Pardi, S. Lucchesi, C. Meossi, L. Gagliardi et al. "High incidence of central precocious puberty in a bounded geographic area of northwest Tuscany: an estrogen disrupter epidemic?" ***Gynecol Endocrinol*** **2005. 20(2): 92–98.**

Researchers in Italy preformed an analysis of central precocious puberty (CPP) distribution in northwest Tuscany (NWT). The overall incidence rate of sexual precocity is estimated at 10–20 per 100, a rate similar to that found in four of the cities in the NWT sample; however 47 percent of the CPP cases found in NWT were in the Viareggio area, a rate of 161 per 100,000. This area hosts a high density of navy yards and greenhouses—consequently it is at higher risk of chemical estrogen pollution. As this population represented only 13.73 percent of the total population of NWT, living in this area significantly increased the risk of CPP. The definite geographic distribution of CPP in this suggests that environmental involvement/pollution may be a major determinant of CPP development.

Nebesio T and O. Hirsh Pescovitz. " Historical perspectives." ***Endocrinologist*** **2005. 15(1):44-48.**

Nebesio and Pescovitz reviewed reports alleging endocrine disruptors blamed for altering the age of normal puberty, including an examination of studies implicating pesticides and accidental environmental exposures. Studies reviewed include two seminal studies on early puberty in girls: Vasiliu et al.'s (2004) examination of the Michigan anglers cohort daughters and Krzstevska-Konstantinova et al.'s (2001) examination of precocious puberty in native and non-native Belgian girls. Nebesio and Hirsch Pescovitz (2005) also review Boneh et al. (1989), who examined cases of girls with precocious sexual development from Jerusalem over a 10-year time period and found strong evidence for a seasonal increase in incidences of early sex development observed (from April–June). Seasonal pesticide usage was a potential cause, but the reasons for this were unknown.

Steingraber S. 2007. ***The falling age of puberty in U.S. girls: what we know, what we need to know.*** **The Breast Cancer Fund.**

In this report Steingraber suggests that pubertal onset and menarche are two sexual maturation processes that appear to be becoming uncoupled, therefore increasing the length of puberty in girls. The author cites environmental contaminants as the cause in light of recent evidence suggesting even minimal exposure to an endocrine disruptor on sex hormones can have a profound consequence in childhood.

I-Fra5

Obesity & diabetes

Baillie-Hamilton, P.F. "Chemical toxins: a hypothesis to explain the global obesity epidemic." ***J Altern Complement Med*** **2002 8(2): 185–192.**

Hamilton puts forth a new hypothesis to explain the global obesity epidemic: chemical toxins. Overeating and inactivity do not fully explain the current trend in obesity. Baillie-Hamilton calls for an examination of environmental causes rather than genetic factors. The sympathetic nervous system is perhaps the key weight-controlling system, and is targeted by many of the commonest synthetic chemicals. Numerous widely used synthetic chemicals induce weight gain, including pesticides (specifically organochlorines and organophosphates). They do so by disrupting major weight controlling hormones, altering levels and sensitivity to neurotransmitters, interfering with metabolic processes, and causing widespread damage to body tissues. These interferences change appetite, food efficiency, and the metabolism of fats, proteins, and carbohydrates.

Janesick, A. and B. Blumberg. "Endocrine Disrupting Chemicals and the Developmental Programming of Adipogenesis and Obesity." ***Birth Defects Research Part C: Embryo Today*** **Reviews 2011. 93, no. 1: 34–50.**

This review article explores possible explanations for the variation in individual propensity to gain weight and accrue body mass, even at identical levels of caloric input. The authors review evidence from clinical, epidemiological, and biological studies showing that obesity is largely programmed early in life, including prenatally. They examine the environmental obesogen hypothesis, which holds that "prenatal or early life exposure to certain endocrine disrupting chemicals can predispose exposed individuals to increased fat mass and obesity. Obesogen exposure can alter the epigenome of multipotent stromal stem cells, biasing them toward the adipocyte lineage at the expense of bone." Individuals exposed to obesogens early in life or prenatally might thus experience changes in their stem cell compartment, which in turn influences adipogenic fate

Lee D.H., I.K. Lee, K. Song, M. Steffes, W. Toscano, B.A. Baker and D.R. Jacobs."A strong dose-response relation between serum concentrations of persistent organic pollutants and diabetes: results from the National Health and Examination Survey 1999-2002." ***Diabetes Care*** **2006 29(7): 1638–1644.**

Researchers performed a cross-sectional examination of the association between serum concentrations of six POPs (selected because they were detectable in greater than 80 percent of participants) and diabetes prevalence. After adjustments were made for confounding variables (age, sex, race and ethnicity, poverty income ratio, BMI and waist circumference) diabetes prevalence was strongly positively associated with lipid adjustment serum concentrations of all six POPs tested for in the sample of 2,016 adult participants from the National Health and Nutrition Examination Survey 1999–2002. Furthermore, the association between POPs and diabetes was much stronger among obese subjects compared to lean subjects.

Lee, D.H., M.W. Steffes, A. Sjödin, R.S. Jones, L.L. Needham, D.R. Jacobs. "Low dose organochlorine pesticides and polychlorinated biphenyls predict obesity,

I-Fra5

dyslipidemia, and insulin resistance among people free of diabetes." *PLoS One* 2011 6(1): e15977.

In a follow up study to their 2010 study of low-dose persistent organic pollutant (POP) exposure and prediction of type 2 diabetes, Lee et al. conducted a nested case-control study to explore the relationship between serum concentrations of POPs and adiposity, dyslipidemia, and insulin resistance among people confirmed to be diabetes free (assessing study subjects on 5 occasions over 20 years). Researchers concluded that simultaneous exposure to various OC pesticides and PCBs in the general population may contribute to the development of obesity, dyslipidemia, and insulin resistance—common precursors of type 2 diabetes and cardiovascular diseases—among those without diabetes. POPs exposure may also contribute to excess adiposity and other dysmetabolic conditions. Ten POPs were found to predict future higher triglycerides and 14 POPs predicted lower HDL-cholesterol. Among organochorine pesticides, p,p'-DDE most consistently predicted higher BMI, triglycerides and HOMA-IR, as well as a lower HDL-cholesterol at year 20.

Newbold R.R., E. Padilla-Banks, R.J. Snyder, T.M. Phillips and W.M. Jefferson. "Developmental exposure to endocrine disruptors and the obesity epidemic." Reprod Toxicol 2007. 23(3): 290–296.

Research from the US has shown an association between exposure to environmental endocrine disrupting chemicals with the development of obesity. Researchers utilize an animal model of developmental exposure to diethylstilbestrol (DES)—a potent perinatal endocrine disruptor with estrogenic activity—to study the mechanisms involved in programming an organism for obesity. Their data supports the idea that brief exposure early in life to environmental endocrine disrupting chemicals, especially those with estrogenic activity, like DES. These chemicals may contribute to overweight and obesity as well as other obesity-associated diseases (type 2 diabetes and cardiovascular disease). This research complicates the current understanding of obesity and necessitates a consideration of more complex factors, including environmental chemicals.

Asthma

Hernández A.F., T. Parrón and R. Alarcón. "Pesticides and asthma." *Curr Opin Allergy Clin Immunol* 2011 11(2): 90–96.

Hernández et al. performed a review of clinical and epidemiological studies that link exposure to pesticides, asthma attacks, and an increased risk of developing asthma. These authors concluded that while many pesticides are sensitizers or irritants, their potential to sensitize is limited. However, more importantly, pesticides may increase the risk of developing asthma, exacerbate a previous asthmatic condition or even trigger asthma attacks by increasing bronchial hyper-responsiveness.

Salam MT, Y.F. Li, B. Langholz, F.D. Gilliland. "Early-life environmental risk factors for asthma: findings from the Children's Health Study." *Environ Health Perspect* 2003 112(6): 760–765.

Researchers from the University of Southern California selected 4,244 subjects from the Children's Health Study conducted in 12 southern California communities to measure the relationship between childhood environmental exposures and asthma risk. Matching those subjects diagnosed with asthma before age five with asthma-free counterparts that acted as controls (matched for age, sex, community of residence, and in utero exposure to maternal smoking), the authors concluded that environmental exposures during the first year of life are associated with an increase in the risk for early-onset persistent asthma, a subtype of asthma associated with long-term morbidity. Compared to never-exposed children, children exposed to herbicides within the first year of life had a 4.6-fold increased risk of asthma and children exposed to pesticides had a 2.4-fold increase in risk—considered together children exposed to any pesticide or herbicide in the first year of life experience a 2.53-fold higher risk of asthma compared to children who were never exposed to either of those.

Salameh P.R., I. Baldim, P. Brochard, C. Raherison, B.A. Saleh and R. Salamon. "Respiratory symptoms in children and exposure to pesticides." *Eur Respir J* 2003 22(3): 507–512.

Public health researchers from Lebanese University in Lebanon and Victor Segalen Bordeaux II University in France conducted a cross-sectional study to evaluate if exposure to pesticides resulted in chronic effects on the respiratory health of Lebanese children. From 19 public schools, 3,291 randomly selected school children—aged five to 16 years—revealed exposure (residential, paraoccupational, and domestic) to pesticides was significantly associated with respiratory disease (1.82-fold higher) and chronic respiratory symptoms such as chronic phlegm, chronic wheezing, and wheezing at any point (the only exception was chronic cough). Twelve percent of the sample reported a chronic respiratory disease and of those, 84 reported a medically confirmed asthma diagnosis (2.6 percent of the sample).

Sunyer J, M. Torrent, R. Garcia-Esteban, N. Ribas-Fitó, D. Carrizo, I. Romieu et al. "Early exposure to Dichlorodiphenyldichloroethylene, breastfeeding and asthma at age six." *Clin Exp Allergy* 2006 36(10): 1236–1241.

Researchers from Spain and the United Kingdom conducted a longitudinal study from a sample of 468 Minorcan children (Balearic Island in the northwest Mediterranean sea with no local pollution sources) to examine the association between prenatal exposure to DDE and other organochlorine compounds and asthma. Asthma was defined as the presence of a wheeze, persistent wheezing, or parental report of doctor-diagnosed asthma at age four. All children were born with quantifiable levels of DDE and PCB compounds. Wheezing at age four was reported for 11.6 percent of all children. Wheezing at four years of age increased with DDE concentration, particularly at the highest quartile, which was also found for persistent wheezing. This association was maintained even after adjusting for potential confounding variables. These results corroborated the association established between DDE and asthma in German school children conducted by Karmaus et al. in 2001.

I-Fra5

Appendix B: Top Pesticides Used in Agriculture & at Home

Key
? – Insufficient data
ND – No data available
I – Insecticide
H – Herbicide
F – Fungicide
PGR – Plant growth regulator
FUM – Fumigant

Table B-1: Most Commonly Used Pesticide Active Ingredients - Agriculture Listed by volume of use[1]

Pesticide & use level range (millions of lbs active ingredient)	PAN HHP[2]	Type	High[3] acute toxicity	Carcin-ogen	Acute neuro-toxicant (ChE inhibitor)	Devel. or reprod. toxicant	Endocrine disruptor	Primary crops	Food residues[4]
Glyphosate (180-185)		H				?	?	Hay/pasture, soybeans, corn	ND
Atrazine (73-78)	Y	H		Y		?	suspected	Corn, sugarcane	Spinach, wheat, onions, lettuce, water
Metam-sodium (50-55)	Y	FUM	Y	Y		Y	suspected	Potatoes, carrots, tomatoes, onions, peanuts	ND
Metolachlor, (S) (30-35)	Y	H		possible		?	suspected	Tomatoes, beans, corn, cotton	Oats, celery, water, corn
Acetochlor (28-33)	Y	H		Y		?	suspected	Corn, popcorn	Water
Dichlorpropene (27-32)		FUM	Y	Y		?	?	Strawberries, sweet potatoes, tree nuts	
2,4-D (25-29)	Y	H		possible		?	suspected	Grasses, wheat, citrus fruits, tree nuts	Potatoes, water
Methyl bromide (11-15)	Y	FUM	Y			Y	suspected	Tomatoes, strawberries, almonds, peppers, watermelon, cucumbers	ND
Chloropicrin (9-11)	Y	FUM	Y	?		?	?	Tobacco, tomatoes, strawberries, bell peppers	ND
Pendimethalin (7-9)	Y	H		possible		?	suspected	Soybeans, corn, cotton, peanuts	Carrots, collard greens, kale
Ethephon (7-9)		PGR			Y	?	?	Cotton, walnuts, grapes, tomatoes	ND
Chlorothalonil (7-9)	Y	F	Y	Y		?	?	Tomatoes, watermelons, onions	Cranberries, celery, green beans
Metam Potassium (7-9)		FUM	Y	Y		Y	?	Lettuce, potatoes	ND
Chlorpyrifos (7-9)	Y	I			Y	?	suspected	Tree nuts, apples, alfalfa, broccoli, citrus, grapes, sweet corn	Apples, bell peppers, cranberries, kale, grapes, peaches
Copper Hydroxide (6-8)		F				?	?	Tree nuts, grapes, peaches	ND
Simazine (5-7)	Y	H				Y	suspected	Corn, citrus, grapes, tree nuts	Blueberries, kale, water, oranges
Trifluralin (5-7)	Y	H		possible		?	suspected	Soybeans, cotton, green beans, broccoli, tomatoes	Carrots, spinach, wheat, soybeans, broccoli
Propanil (4-6)	Y	H		possible		?	suspected	Rice, oats, barley, wheat	Wheat
Mancozeb (4-6)	Y	F		Y		Y	suspected	Apples, tomatoes, onions, watermelon	ND
Acephate (2-4)	Y	I		possible	Y	?	suspected	Cotton, tobacco, cranberries, mint	Green beans, bell peppers
Diuron[5] (2-4)	Y	H		Y		Y	suspected	Oranges	Asparagus, oranges, water, potatoes
MCPA (2-4)	Y	H	Y	possible		?	?	Flax, barley, wheat, rice	water
Paraquat (2-4)	Y	H	Y			?	suspected	Corn, soybeans, cotton, apples	ND
Dimethenamid (2-4)	Y	H		possible		?	?	Corn, soybeans, sugarbeets	Soybeans, water

Table B-2: Most Commonly Used Pesticide Active Ingredients – Home & Garden

Listed by volume of use

Pesticide & use level range (millions of lbs active ingredient)	PAN HHP	Type	High acute toxicity	Carcinogen	Acute neurotoxicant (ChE inhibitor)	Devel. or reprod. toxicant	Endocrine disruptor
2,4-D (8-11)	Y	H		possible		?	suspected
Glyphosate (5-8)		H				?	?
Carbaryl (4-6)	Y	I		Y	Y	Y	suspected
Mecoprop-P (MCPP) (4-6)	Y	H		possible		?	?
Pendimethalin (3-5)	Y	H		possible		?	suspected
Pyrethroids[6] (2-4)	Y	I	Y	Y		Y	suspected
Malathion (2-4)	Y	I	Y	possible	Y	Y	suspected
Dicamba (1-3)		H				Y	?
Malathion (2-4)	Y	I	Y	possible	Y	Y	suspected
Trifluralin (1-3)	Y	H		possible		?	suspected
Pelargonic Acid (< 1)		H/F		?		?	?

Notes

1 See Table 3.6 and 3.7 in *Pesticide Industry Sales & Usage, 2006 and 2007 Market Estimates*, U.S. EPA, Washington, DC Feb 2011. See www.epa.gov/opp00001/pestsales/07pestsales/market_estimates2007.pdf. Aldicarb was removed from the list as registration was withdrawn in 2010.

2 PAN International has compiled and published a list of Highly Hazardous Pesticides (HHPs) that are harmful to human health and the environment, and targeted for global reduction and elimination. See www.panna.org/issues/publication/pan-international-list-highly-hazardous-pesticides.

3 PAN's online pesticide database provides an explanation of these categories and additional toxicity, use and regulatory information for these and other pesticides. See www.pesticideinfo.org.

4 Based on USDA's Pesticide Data Program, as listed on www.whatsonmyfood.org.

5 Noted health effects not applicable for products with < 7% diuron, and applied to foliage.

6 Health hazards of specific pyrethroids vary, the effects indicated here represent those with most hazardous potential effects.

I-Fra5

Appendix C

Online Resources & Tools

This compilation highlights a number of key online resources available through government agencies and public interest groups. It is not intended to be comprehensive.

Pesticide use data

California pesticide use reporting: *calpip.cdpr.ca.gov*

EPA Pesticide Industry Sales & Usage: *www.epa.gov/opp00001/pestsales*

USDA National Agricultural Statistics Service: *www.nass.usda.gov*

Pesticide health harms

Agency for Toxic Substances & Disease Registry, ToxFAQs: *www.atsdr.cdc.gov/az/c.html*

Collaborative on Health & the Environment, Toxicant & Disease Database: *www.healthandenvironment.org/tddb*

EPA Pesticides & Human Health Issues: *www.epa.gov/opp00001/health/human.htm*

EPA Recognition & Management of Pesticide Poisonings: *npic.orst.edu/rmpp.htm*

Ontario College of Family Physicians, Systematic Review of Pesticide Human Health Effects: *www.ocfp.on.ca/docs/pesticides-paper/pesticides-paper.pdf*

PAN International Highly Hazardous Pesticides: *www.panna.org/issues/publication/pan-international-list-highly-hazardous-pesticides*

PAN's pesticide database: *www.pesticideinfo.org*

Physicians for Social Responsibility, Pesticides & Human Health: A Resource For Health Care Professionals: *www.psr-la.org/resources/reports-training-materials/#Pesticides*

The Endocrine Disruption Exchange (TEDX): *www.endocrinedisruption.com/pesticides.introduction.php*

Pesticides & children's health

Beyond Pesticides, Learning/Developmental Disorders resource page: *www.beyondpesticides.org/health/learningdevelopmental.htm*

Center for Environmental Research & Children's Health: *cerch.org/research-programs/chamacos*

EPA Pesticides & Children: *www.epa.gov/opp00001/health/children.htm*

National Academy of Sciences: *www.nap.edu/catalog.php?record_id=2126*

PAN's Children's health page: *www.panna.org/children*

Pesticide food residues

FDA Total Diet Study: *www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/TotalDietStudy/default.htm*

Whats On My Food? database (also includes health effect data): *www.whatsonmyfood.org*

USDA Pesticide Data Program: *www.ams.usda.gov/AMSv1.0/pdp*

Childhood disease & disorders

American Academy of Pediatrics: *www.aap.org*

CDC Child Health Statistics: *www.cdc.gov/nchs/fastats/children.htm*

Children's environmental health

Children's Environmental Health Network: *www.cehn.org*—A national multidisciplinary organization whose mission is to protect the developing child from environmental health hazards and promote a healthier environment.

Children's Environmental Health Project: *www.cape.ca/children*—A project of the Canadian Association of Physicians for the Environment, CEHP is intended to introduce clinicians (and their patients) to children's environmental health issues. Information on the health effects from environmental exposures is presented in a systems approach.

Healthy Child, Healthy World: *healthychild.org*—Protecting children's health and wellbeing from harmful environmental exposures through education and prevention strategies.

Healthy Kids: *www.healthy-kids.info—Provides resources and programs to help educators, health p*rofessionals, community officials, organizations, policy makers and parents work together to ensure schools are safe for children's healthy development.

Learning & Developmental Disabilities Initiative: *www.healthandenvironment.org/initiatives/learning*—An international partnership fostering collaboration among LDD organizations, researchers, health professionals and environmental health groups to address concerns about the impact environmental pollutants may have on children's neurological health.

Making our Milk Safe (MOMS): *www.safemilk.org*—A national grassroots movement of mothers working to create a healthier, safer environment for children, MOMS engages in education, advocacy and corporate campaigns.

Pediatric Environmental Health Specialty Units: *www.aoec.org/PEHSU.htm*—ATSDR and EPA support this network to provide education for health professionals, public health officials and others about the topic of children's environmental health.

Physicians for Social Responsibility: *www.psr.org/resources/pediatric-toolkit.html*—PSR has developed a pediatric environmental health toolkit that combines easy-to-use reference guides for health providers and user-friendly health education materials on preventing exposures to toxic chemicals and other substances that affect infant and child health. The toolkit is endorsed by the American Academy of Pediatrics.

Safer Chemicals, Healthy Families: *www.saferchemicals.org*—A coalition pressing for reform of national chemicals policy. SCHF represents more than 11 million individuals including parents, health professionals, advocates for people with learning and developmental disabilities, reproductive health advocates, environmentalists and businesses.

The Children's Environmental Health Institute: *cehi.org*—Works to identify, validate and develop solutions to address adverse health effects to children occurring as a consequence of exposure to hazardous environmental substances.

I-Fra5


Pesticide Action Network
NORTH AMERICA
Pesticide Action Network North America
1611 Telegraph Ave, Suite 1200
Oakland CA 94612-2130
510.788.9020

Comment Letter I-Fra5 Fraser, Mary

Response 1

The Pesticide Action Network (PAN) Report covers a long list of pesticides used in agriculture and at home with the thesis that these chemicals are undermining the health of children. The great majority of the products mentioned are not in use or proposed for use by the District, and there is no way to compare the broad generalizations of harm to human health, including endocrine disruption, with the very specific use of pesticides and herbicides for vector control in outdoor environments.

Throughout the brochure, the PAN provides narratives and graphics illustrating numerous diseases in adults and children. The approach by PAN strongly suggests that chemical exposures are the cause of nearly every disease and condition. The comparisons are all based on nonspecific correlations that are not scientifically defensible. The brochure's use of national annual chemical use data and the comparison it provides with national data on diseases and adverse health effects, provides no defensible causality. When the arguments provided in the brochure suggest that national or even regional chemical use can be used to indicate causality to so many diverse diseases, they lose scientific credibility. The comparisons might also relate to hundreds of other genetic and environmental factors. This approach to developing correlation is fatally flawed.

Furthermore, the fatal flaws in the illustrations and correlations provided in this brochure include several correlations that are not supported by the accompanying data. Correlation is not causation. Causation can only be claimed if credible and reproducible data have been developed showing a direct link to documented exposures at the individual level. The claims in the brochure do not show direct correlation and, therefore, cannot be verified.

The materials proposed for use in the District's program are subjected to rigorous initial and ongoing testing and scrutiny for safety by both the US Environmental Protection Agency and the California Department of Pesticide Regulation.

I-Fra6

Marin/Sonoma Mosquito and Vector Control District's
Integrated Vector Management Program Draft PEIR

WRITTEN COMMENT

Name/Affiliation: Mary Fraser Date: 9/17/15

Address: [redacted]

City: Mill Valley Zip Code: 94941 Telephone: [redacted]

Please provide comments and concerns on the content of the Draft PEIR and the potential environmental effects of the Proposed Program.

I have attached copies of 4 emails that I sent to the email address given to me. I did not receive a reply stating that they had been received, so I am sending them again to you with this written comment.

M Fraser

1

peir@msmosquito.com

Please use backside of page for additional comments, if needed. This comment form may be handed in at the public hearing to District staff or mailed to the attention of Philip Smith, to arrive on or prior to **October 2, 2015** at the Marin/Sonoma Mosquito and Vector Control District, 595 Helman Lane, Cotati CA 94931.

Print

Page 1 of 1

I-Fra6

Subject: Comment on Vector Management Program Draft PEIR

From: Mary Mac (mizmerrymac@yahoo.com)

To: peir@msmosquito.com;

Bcc: fegger@pacbell.net; barbogard@gmail.com; kerry.stoebner@gmail.com; w1rothman@gmail.com;

Date: Thursday, September 17, 2015 10:53 AM

Dear Marin Sonoma Mosquito and Vector Control,
I am writing to inform you of my concerns about your Draft PEIR. My concerns are many and I will be sending you multiple emails.
1. You have given the public inadequate time and notice of these hearings and their content. You published a 'legal notice' only on August 28th and expect the public to be able to read and prepare comments no later than Oct. 2, 2015 on a document that is over 500 pages. You made the document available in libraries in CD form. Most libraries have reservation systems in order to use a computer, time is limited to one hour per reservation and to print out any pages is a ten cent fee per page. All of this combined makes for great difficulty in reading and analyzing your Draft PEIR. I am requesting that you extend the official notification period to at least 120 days, that you take out 1/4 page 'display' ads in newspapers and that you send notifications of the Draft PEIR to multiple NGO's and non-profits. I also want you to supply copies of the Draft PEIR to interested individuals at no charge. I have not had adequate time to analyze your entire PEIR.

2. I attended your Public Hearing on Sept. 12, 2015 and watched a power point presentation that was misleading. The presentation stated that you use chemicals approved for organic food production and that you consulted beekeepers. During a very brief review of the Draft PEIR in my one hour computer time allotment at the public library, I found that you propose to use glyphosate. That is a chemical pesticide that is not allowed in organic food production. The World Health Organization thru its research arm, The International Agency for Research on Cancer has declared glyphosate to be carcinogenic to animals, giving glyphosate a 1 rating for animals and a 2A rating for humans.

In reviewing the Draft PEIR I noted that the information on glyphosate is inaccurate in the following ways: a. "Section 4.6.2.3 Human Toxicity. The shikimic acid pathway is specific to plants and some microorganisms; therefore, glyphosate is thought to have very low toxicity to mammals (USEPA 1993)" This is old information. Dr. Stephanie Seneff, MIT and Anthony Samsel have found that the shikimic pathway is present in bacteria in the human gut. Here is their peer reviewed, published paper.
http://people.csail.mit.edu/seneff/Entropy/entropy-15-01416.pdf
Dr. Seneff and Anthony Samsel have published numerous other articles about glyphosate. Here are other articles:
http://people.csail.mit.edu/seneff/ITX_2013_06_04_Seneff.pdf
http://surgicalneurologyint.com/surgicalint_articles/glyphosate-pathways-to-modern-diseases-iii-manganese-neurological-diseases-and-associated-pathologies/
http://people.csail.mit.edu/seneff/Hoy_wildlife_2015.pdf
http://people.csail.mit.edu/seneff/glyphosate/Chen_I_wan_Reference_info_glyphosate_June18_2014.pdf

Glyphosate is rarely used alone. It is almost always used in combination with inerts and adjuvants. A team of independent scientists did studies on the complete formulation of Roundup, which is the most widely used pesticide that has glyphosate as its main ingredient. The testing found that the complete formulations had toxicity levels that were up to 1000x more toxic. Here are two of the studies:

Major Pesticides Are More Toxic to Human Cells Than Their Declared Active Principles
RobinMesnage,[1] Nicolas Defarge,[1] Joël Spiroux de Vendômois,[2] and Gilles-Eric Séralini[1] http://dx.doi.org/10.1155/2014/179691

https://d3n8a8pro7vhmx.cloudfront.net/yesmaam/pages/680/attachments/original/1407922431/2012._Mesnage_et_al._Ethoxylated_adjuvants_of_glyph based_herbicides_are_active_principles_of_human_cell_toxicity.pdf?1407922431
Ethoxylated adjuvants of glyphosate based herbicides are active principles of human cell toxicity.

In Section 4.6.2.3 it is stated " There is currently no published scientific. evidence indicating that glyphosate is carcinogenic or mutagenic". I am attaching a copy of the WHO report that disputes this and that labels glyphosate as carcinogenic to animals and a probable human carcinogen. I don't understand why this report has not been included in the Draft PEIR. The initial release was in March 2015 and the entire monograph was released in July 2015. There have been numerous media reports about the WHO finding!
In Section 4.6.2.1 there is a table of the degradation of glyphosate. Monsanto, the manufacturer of glyphosate has been sued by both the State of New York and the county of France over falsely advertising that their product, Roundup, (which has glyphosate as its active principle) is biodegradable. The county of France won their case, even though it was appealed to the highest court in France and the State of New York settled the case with Monsanto. The degradation of glyphosate is dependent on the chemical nature of the environment it is in. In some cases it can take up to 20 years or more to biodegrade.

All of my referenced attachments should be included in my comments.

Please do not use glyphosate. I want the Directors of the District to approve the No Chemical option that is proposed in your Draft PEIR.
Mary Fraser
110 Seminary Drive, Apt. 2A
Mill Valley, CA 94941
415 686-8072

https://us-mg5.mail.yahoo.com/neo/launch?.rand=b0l89v31rgnh5 9/17/2015

I-Fra6

Subject: Comments on Draft PEIR
From: Mary Mac (mizmerrymac@yahoo.com)
To: peir@msmosquito.com;
Date: Thursday, September 17, 2015 11:02 AM

I want the Directors of the District to approve the No Chemicals option in the Draft PEIR. Here is a copy of a speech I did on why pesticides should be banned along with its references. Please use this as one of my comments.

WHY PESTICIDES SHOULD BE BANNED.

9/16/15

Thank you Mr. President, Fellow Toastmasters and Welcome Guests,

I want to share this graphic with you. For those of you who can't read it, it says: It's Raining Roundup. With a picture of a little girl playing in the rain. Underneath it says: Monsanto's Roundup found in 75% of air and rain samples. (1) I find this very disturbing. I would think you would find it disturbing too. Pesticides raining on our heads.

I am the mother of three children and have had the unfortunate experience of having a child in the intensive care unit for 35 days. Since I never, ever want to have that experience again, I've educated myself on health hazards. Today I want to share my findings about pesticides with you. I come from a large family of attorneys, so all of my facts in this speech have been verified. I have a copy of this speech available with references to all the facts that I quote.

This graphic was created from data found in official gov't studies. The United States Geographical Survey has done testing in several states and this is what they have consistently found. 75% of the air and rain samples had Roundup in it. (2)

Let me give you a little background on pesticides.

The word Pesticide means death to pests. It is an umbrella term. Under the umbrella, we have herbicides or death to plants, insecticides or death to animals and fungicides or death to fungi. Many people think that a more apt term for pesticide is ecocide. Death to the environment.

Now imagine that this jar is a can of pesticide. Inside this jar, there is the main ingredient or what is technically called the 'active principle'. This is the chemical that the EPA requires manufacturer's to do testing on. The manufacturer's do the testing. The EPA only reviews the tests. Along with the active principle, there are inerts and/or adjuvants added to the formula. Under EPA rules, the inerts and adjuvants do not need to be tested. They do not need to be disclosed to the public as they can be considered to be trade secrets or proprietary business information. So what you have, is a can of chemicals of completely unknown toxicity, untested and undisclosed. (3) Now many of you have gone to your doctor and when he wants to write you a prescription, he always asks about other drugs you are taking. He doesn't want any unforeseen chemical reactions. I guess the EPA didn't get that memo. This is the dirty little secret of pesticides and one of the dirty little secrets about the EPA. Now, after the manufacturer has created this pesticide of unknown toxicity, they are often patented. Once patented, no one can do research on the formula without the patent holders permission. Fat chance.

Let me go back to the graphic. " It's Raining Roundup." The active principle or main ingredient in Roundup is glyphosate. The World Health Organization has just declared that glyphosate causes cancer in animals. (4) They declared it to be a 'probable human carcinogen' only because they could not find enough enough scientific studies on humans and glyphosate. It is unethical to try to induce cancer in humans and the few studies they had just didn't have enough data.

The patent has run out on Roundup and a group of independent scientists did studies on it. What they found was that Roundup was 1000x more toxic than its main ingredient glyphosate. 1000x time more toxic. (5) Roundup is the most widely used pesticide in this country. We use millions of pounds of it per year. (6) It's what genetically modified crops like corn, soy , canola, sugar beets, alfalfa and cotton are designed to withstand heavy doses of. (7) 80% of all processed foods contain GMO ingredients. (8) It's allowed on 160 different food crops in this country. (9) Farmers spray it on many crops right before harvest to eliminate weeds and dry out the crops. Right before harvest. So many of us are now gluten intolerant. Well, Roundup is oftentimes sprayed on wheat right before harvest. You're not gluten intolerant. You're being poisoned. (9) And no one tests for residues of Roundup on our food. That's another dirty little secret about the EPA. (10) We test for other pesticides but not Roundup.

So what are the public health consequences of all this toxicity? According to the American Cancer Society, the official rate of cancer in 2015 is now 1 in 2. That's for men. 1 in 2. For women its 1 in 3. (11) And that is just cancer. Roundup has been linked to 32 other chronic diseases (12) and has been found in breast milk, urine (13) and now it's raining Roundup.

So what can you do? If you have Roundup at home, carefully take it to the hazardous waste facility. Eat only organic food and you will avoid about 80% of pesticides. This is particularly important for children as they eat more per lb. of body weight than adults do and their bodies do not have the same capacity to detox pesticides as adults do. (14)

And exercise your democratic rights. The Marin Co Board of Supervisors has the issue of banning pesticides on its agenda on Oct. 6th. Let them know what you think. The CA EPA has opened comments about adding Roundup to its list of Prop. 65 chemicals and our Mosquito abatement program is proposing to use Roundup. I have notices on all of these actions and I have information on eating organic food .

Let me close with another graphic. This is a quote from Dr. Don Huber, a professor emeritus of Plant Pathology, Purdue University.

"Future generations will judge us- not on how many millions of tons of pesticides we sprayed, but on how willing we were- how perfectly willing we were, to sacrifice our children". (16)

Thank you. You have my permission to share this speech with anyone or to contact me if you wish a copy of this speech in electronic form.

I-Fra6

Mary Fraser

Email: mizmerrymac@yahoo.com

References:

1. https://www.facebook.com/pages/Monsanto-Class-Action/992106394134272?sk=photos_stream
Monsanto class action lawsuit Facebook photos
2. http://www.usgs.gov/newsroom/article.asp?ID=2909#.VfnIlNVViko
http://www.greenmedinfo.com/blog/roundup-weedkiller-found-75-air-and-rain-samples-gov-study-finds
http://www.ncbi.nlm.nih.gov/pubmed/24549493
http://www.ncbi.nlm.nih.gov/pubmed/18453431
http://www.ncbi.nlm.nih.gov/pubmed/21128261

3. **Poison Spring: The Secret History of Pollution and the EPA Hardcover – April 8, 2014**
by E.G. Vallianatos (Author), McKay Jenkins (Author)

1. International Agency for Research on Cancer, **IARC Monographs Volume 112: evaluation of five organophosphate insecticides and herbicides.**

2. Hindawi Publishing Corporation

BioMed Research International
Volume 2014, Article ID 179691, 8 pages
Major Pesticides Are More Toxic to Human Cells Than Their Declared Active Principles
RobinMesnage,1 Nicolas Defarge,1 Joël Spiroux de Vendômois,2 and Gilles-Eric Séralini1 http://dx.doi.org/10.1155/2014/179691

https://d3n8a8pro7vhmx.cloudfront.net/yesmaam/pages/680/attachments/original/1407922431/2012._Mesnage_et_al._Ethoxylated_adjuvants_of_glyph based_herbicides_are_active_principles_of_human_cell_toxicity.pdf?1407922431
Ethoxylated adjuvants of glyphosate based herbicides are active principles of human cell toxicity.

1. **http://www.sciencedirect.com/science/article/pii/S027869151530034X**

Potential toxic effects of glyphosate and its commercial formulations below regulatory limits

7. **https://d3n8a8pro7vhmx.cloudfront.net/ncap/pages/26/attachments/original/1428423381/glyphosate.pdf?1428423381** **JOURNAL OF PESTICIDE REFORM/ WINTER 2004 • VOL. 24, NO. 4 HERBICIDE FACTSHEET**

1. Ibid

2. http://d3n8a8pro7vhmx.cloudfront.net/yesmaam/pages/680/attachments/original/1393210109/Glyphosate_II_Samsel-Seneff_Toxicology_FNL.pdf?1393210109
Glyphosate, pathways to modern diseases II: Celiac sprue and gluten intolerance
Anthony SAMSEL 1 and Stephanie SENEFF 2 1 Independent Scientist and Consultant, Deerfield, NH 03037, USA 2 Computer Science and Artificial Intelligence Laboratory, MIT, Cambridge, MA, USA ITX060413R01 • Received: 24 September 2013 • Revised: 10 November 2013 • Accepted: 12 November 2013

3. http://www.ecfr.gov/cgi-bin/text-idx?SID=195f8b224b4d7d5ab8cfff3bf0f92f68&node=se40.24.180_1364&rgn=div8
Electronic Code of Federal Regulations 180.364 Glyphosate, tolerances for residues.

4. www.consumerreports.org/cro/news/2015/03/glyphosate-in-your-diet/index.htm
Consumer Reports: Is there glyphosate in your diet? No one knows how much of this pesticide is in the produce we eat.

5. **American Cancer Society publication: Cancer Facts & Figures 2015, page 14.**
http://www.cancer.org/acs/groups/content/@editorial/documents/document/acspc-044552.pdf

6. **http://people.csail.mit.edu/seneff/Swanson_et_al_2014.pdf**
Journal of Organic Systems, 9(2), 2014 ORIGINAL PAPER
Genetically engineered crops, glyphosate and the deterioration of health in the United States of America
Nancy L. Swanson1 , Andre Leu2 *, Jon Abrahamson3 and Bradley Wallet4

http://www.examiner.com/article/data-trends-show-correlation-between-increase-organ-disease-and-gmos

7. **Glyphosate Testing Report: Findings in American Mothers' Breast Milk, Urine and Water.**
Conducted by Moms Across America and Sustainable Pulse
April 7, 2014.
Zen Honeycutt, Moms Across America, Henry Rowlands, Sustainable Pulse

8. Pesticide Action Network North America, **"A Generation in Jeopardy, How pesticides are undermining our children's health & intelligence".**
http://www.panna.org/publication/generation-in-jeopardy

Sincerely,

Mary Fraser

110 Seminary Drive, Apt. 2A

Mill Valley, CA 94941

415 686-8072

I-Fra6

Subject: Comment on Draft PEIR

From: Mary Mac (mizmerrymac@yahoo.com)

To: Peir@msmosquito.com;

Date: Thursday, September 17, 2015 11:13 AM

Dear District Board of Directors and Staff,
Please choose the No Chemical option from your draft PEIR. Pesticides drift, so you cannot guarantee anyone that you will not affect them. Pesticides get into our air and our water. I am attaching a speech that I recently wrote about pesticides, drift and their threat to the $45 million dollar agricultural market in Marin.
My name is Mary Fraser and I am a resident of Marin County. I am here today to ask you to ban the use of pesticides on Marin County Open Spaces and Parks. I am asking Parks and Open Space to become organic.

I want to share this graphic with you. For those of you who can't read it, it says: It's Raining Roundup. With a picture of a little girl playing in the rain. Below it, it says Monsanto's Roundup in 75% of Air and Rain Samples. This graphic has been created from a study done by the US Geological Survey. The following quotes are from their study.

"Pesticides can become airborne through volatilization and wind erosion of particles both during and after their application. Volatilization from treated areas is a continuous process and can be a major dissipative route for many pesticides. Airborne pesticides can be carried by wind and deposited in unintended areas by dry (gas and particle) and wet (rain and snow) deposition." This is what is commonly known as drift.

Marin County has organic agriculture that is worth $46 million dollars per yr. $46 million dollars per yr. There are 66 registered organic operations. The drift from the use of pesticides on Open Space and Parks may jeopardize that $46 million dollars or it may drift into more populated areas and contribute to health problems. No one knows where it will go. We just know that pesticides drift.

Coincidentally, I also just happened to sign up for the Marin Organic newsletter. I want to read you the reply I received.

Hi Mary,

I just wanted to send a quick note to thank you for signing up.

When the founders of Marin Organic first met in the mid-1990's they envisioned a county free of herbicides and pesticides and hoped that organic would become the new norm. Well, today we're

I-Fra6

proud to say that the majority of productive farmland in Marin County is certified organic. We're still here carrying on that mission, and part of that is educating folks through our newsletters.

Jeffrey Westman-Executive Director Marin Organic

Please join Marin Organics vision of Marin County as a place free of herbicides and pesticides.

Attached are documents regarding the USGS studies that I referenced above.

Mary Fraser

110 Seminary Drive, Apt 2A

Mill Valley, CA 94941

http://www.usgs.gov/newsroom/article.asp?ID=2909#.VfnIlNVViko

1. http://www.greenmedinfo.com/blog/roundup-weedkiller-found-75-air-and-rain-samples-gov-study-finds
   http://www.ncbi.nlm.nih.gov/pubmed/24549493
   http://www.ncbi.nlm.nih.gov/pubmed/18453431
   http://www.ncbi.nlm.nih.gov/pubmed/21128261

I-Fra6

Subject: Comment on Draft PEIR

From: Mary Mac (mizmerrymac@yahoo.com)

To: Peir@msmosquito.com;

Bcc: fegger@pacbell.net;

Date: Thursday, September 17, 2015 11:27 AM

Dear District Board of Directors and Staff,
I urge you to choose the No Chemical option from the Draft PEIR. Many other places are understanding how detrimental to the environment and human health the use of pesticides are. Attached is a list of many other places that have either banned or restricted pesticides. Here is the text of a speech that I gave about the other places that have banned pesticides and their reasons for doing so.

Board of Supervisors and Ladies and Gentlemen,

My name is Mary Fraser. I am a resident of Marin Co.

I am here today to urge you to ban pesticides in Marin County. Today I want to talk about other places that have banned pesticides and why they chose to do so. Each place is a little different but they all chose public health over the use of pesticides.

As you may know, the Marin Municipal Water District recently chose to exclude the use of pesticides from the 26,000 acres they manage. This is a continuation of a 9 yr. hiatus in the use of pesticides. No one wants pesticides in our water supply.

Richmond CA has banned pesticides. This effort was led by 2 doctors, one of whom is the retired chief of cardiology for Kaiser Permanente. Dr. Jeff Ritterman wrote and published an extensive article outlining the public health reasons for banning pesticides. I had the privilege to hear Dr. Ritterman speak recently. He started his talk by saying that the compelling evidence against pesticide use was the birth defects that are showing up in South America. We are talking about babies born with no skull. Babies born with no arms AND no legs. Babies born with one big cyclops eye. Defects nearly identical to the ones that happened in VietNam after we defoliated the country with Agent Orange. And we did not ban the entire formula of Agent Orange. Only 2, 4 T. We still use 2, 4 D on our agriculture.

So in Argentina, because of the health consequences, 30,000 doctors have called for a ban on pesticides. 30,000 doctors.

In El Salvador, 20,000 men have died in the last 5 years because they started using pesticides in the sugar cane fields. In El Salvador they've banned 32 different pesticides.

Sri Lanka has the same issue. Thousands are dying from kidney disease. One of the reasons that they have such terrible kidney diseases is because they have serpentine soil. We have serpentine soil in Marin. Sri Lanka has banned pesticides.

I-Fra6

France has banned them. The Netherlands has banned non commercial use. German ministers are calling for a ban in the entire European Union. All of these countries operate on the precautionary principle, where chemicals have to be proven safe before they can be used. In America, we have the opposite policy. Chemicals undergo minimal testing by the manufacturer and then the public has to prove that a chemical is unsafe before it can be forced off of the market. This can take decades and have untold consequences.

So I'm asking the Board of Supervisors to be our heroes and ban pesticides on all of the property that the County of Marin owns, leases and manages, including Open Space.

Places where the use of glyphosate and/or pesticides are restricted or banned.

Marin County:

Marin Municipal Water District, Marin County, CA. 22,000 acres of watershed- Pesticides banned. Vote taken 7/7/15 http://www.marinij.com/environment-and-nature/20150708/marin-water-district-herbicides-wont-be-used-on-mount-tam

City of Fairfax, CA- Pesticides banned on commons.

City of Belvedere, CA- Pesticides banned on commons.

City of Sausalito, CA-Pesticides under review

Reed School District, Tiburon, CA- Pesticides not used on school district grounds.

Larkspur-Corte Madera School District, Corte Madera, CA-Pesticides not used on school grounds.

Mill Valley School District, Mill Valley, CA- Pesticides not used on school grounds.

States:

Connecticut: Pesticides banned on municipal playgrounds:

http://www.beyondpesticides.org/dailynewsblog/2015/07/connecticut-bans-toxic-lawn-pesticides-in-municipal-playgrounds-statewide

New York: Pesticides banned on school grounds

Countries:

Country of the Netherlands- Glyphosate banned for non-commercial use.
http://sustainablepulse.com/2014/04/04/dutch-parliament-bans-glyphosate-herbicides-non-commercial-use/#.VZ2Wca5Vikp

I-Fra6

Country of Sri Lanka-Glyphosate banned
http://www.publicintegrity.org/2014/03/13/14418/sri-lanka-bans-monsanto-herbicide-citing-potential-link-deadly-kidney-disease.

County of El Salvador- Glyphosate banned by legislature.

Country of Bermuda- Glyphosate imports suspended.
http://www.todayinbermuda.com/news/health/item/1471-health-minister-importation-of-roundup-weed-spray-suspended

Country of Brazil. Chief prosecutor wants glyphosate banned.
http://www.globalresearch.ca/brazils-public-prosecutor-wants-to-ban-monsantos-chemicals-following-recent-glyphosate-cancer-link/5449440

Country of Germany and the European Union (EU). EU ban proposed by ministers.
http://www.globalresearch.ca/german-ministers-call-for-eu-wide-ban-on-monsantos-deadly-glyphosate-herbicide-roundup/5451831

1. Country of Argentina. 30,000 medical doctors call for ban on glyphosate.
http://www.globalresearch.ca/30000-doctors-in-argentina-demand-that-glyphosate-be-banned/5445542

Country of Columbia. Glyphosate banned for use on illicit crops. http://www.bbc.com/news/world-latin-america-32677411

Country of France. Sale of glyphosate banned.
http://www.naturalnews.com/050248_french_legislation_glyphosate_ban_Monsanto_GMOs.html

States:

Connecticut: Pesticides banned on municipal playgrounds:

http://www.beyondpesticides.org/dailynewsblog/2015/07/connecticut-bans-toxic-lawn-pesticides-in-municipal-playgrounds-statewide

New York: Pesticides banned on school grounds

Cities:

City of Richmond, CA- Moratorium on organophosphate pesticides

Chicago, IL,

Takoma Park, MD

Laguna Hills, CA

I-Fra6

Durham, CT

Paris, France

University of Vermont Law School and Emory University campuses have banned all pesticides

Santa Barbara, CA

Carrboro, NC

Plainville, CT

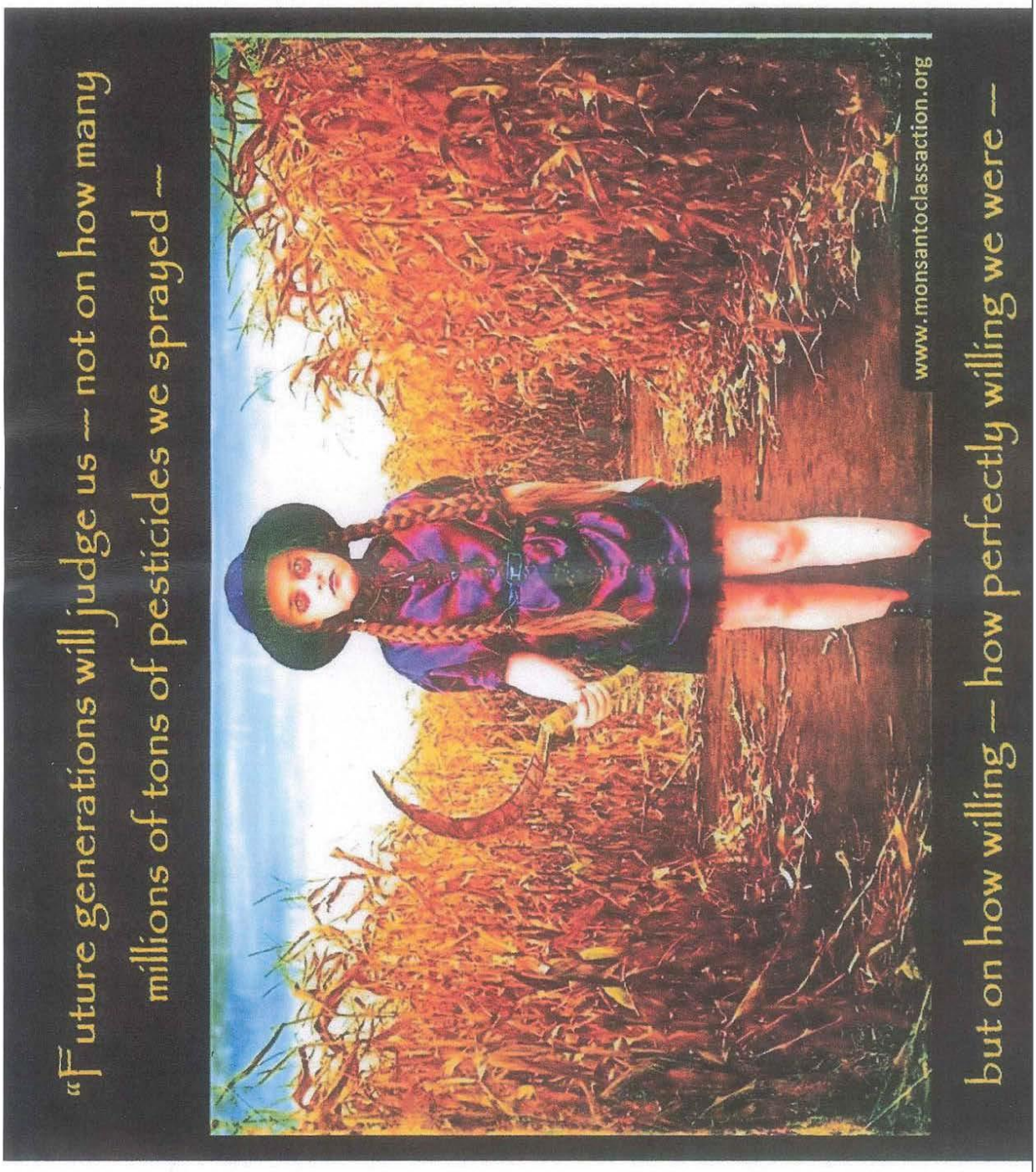
Santa Fe, NM

The township of Marblehead, Massachusetts instituted one of the earliest ordinances calling for the use of Organic Land Care on township lands and has successfully implemented fully organic techniques for turf management.

Between 1996 and 2005 San Francisco municipal agencies achieved an 85% reduction in gallons of liquid pesticide, a 55% decrease in pounds of solid pesticides, and a 90% decrease in the use of Roundup®, one of the herbicides most widely used by municipalities. San Francisco has been so successful that in 2005 New York City passed a similar local law based largely on San Francisco's ordinance.

Mary Fraser
110 Seminary Drive Apt 2A
Mill Valley, CA 94941
415 686-8072

I-Fra6


"Future generations will judge us – not on how many
millions of tons of pesticides we sprayed –
but on how willing – how perfectly willing we were –
www.monsantoclassaction.org

Comment Letter I-Fra6 Fraser, Mary

Response 1

These emails were received and have been addressed as responses to comments I-Fra1 through I-Fra4.

I-Fra7

Marin/Sonoma Mosquito and Vector Control District's Integrated Vector Management Program Draft PEIR

WRITTEN COMMENT

Name/Affiliation: Mary Fraser Date: 9/17/15

Address: [redacted]

City: Mill Valley Zip Code: 94941 Telephone: [redacted]

Please provide comments and concerns on the content of the Draft PEIR and the potential environmental effects of the Proposed Program.

Attached scientific study on pesticides and erectile dysfunction.

Attached graphic - "It's raining Roundup"

1

Please use backside of page for additional comments, if needed. This comment form may be handed in at the public hearing to District staff or mailed to the attention of Philip Smith, to arrive on or prior to **October 2, 2015** at the Marin/Sonoma Mosquito and Vector Control District, 595 Helman Lane, Cotati CA 94931.

Asian Pacific Journal of Reproduction 2015; ■(■): 1-9

I-Fra7 1

HOSTED BY

ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Reproduction

journal homepage: www.apjr.net

Review http://dx.doi.org/10.1016/j.apjr.2015.07.012

Potential pathways of pesticide action on erectile function – A contributory factor in male infertility

R.P. Kaur, V. Gupta, A.F. Christopher, P. Bansal*

University Centre of Excellence in Research, Baba Farid University of Health Sciences, Faridkot, India

ARTICLE INFO

Article history:
Received 12 Dec 2014
Received in revised form 10 Jun 2015
Accepted 20 Jul 2015
Available online xxx

Keywords:
Pesticides
Erectile function
Infertility

ABSTRACT

One of the important objectives of this manuscript is to focus on the place of erectile dysfunction as an important factor for infertility. The review is about correlating the indiscriminate use of pesticides and to find out and highlight the evidences for mechanism of action of these pesticides for erectile dysfunction and find out the most used and most dangerous pesticide from erectile dysfunction point of view. The review suggests that erectile dysfunction is having a significant place as a causal factor for infertility. Study infers that pesticides are having multiple mechanisms of action through which these cause erectile dysfunction. It also reflects that acetamiprid is having most devastating effect causing erectile dysfunction as it acts through multiple inhibitory pathways. The review successfully highlights the indiscriminate regional use of pesticides.

1. Introduction

Male fertility is reported to be declining day by day. According to recent estimates every year about 60–80 million couples all over world suffer from infertility of which probably between 15 and 20 million are in India alone [1]. Researchers have shown that male factors account for 40%–50% of infertility in human [2,3] and one of the major problems contributing towards male infertility is erectile dysfunction afflicting as much as 10% of the male population [4]. The data are more alarming above the age of 40 as nearly 52% of men are afflicted. There is enumerable number of factors like psychological factors, physiological, pathological, social, environmental, nutritional etc that play a major role in pathogenesis of erectile dysfunction. Today environment is laced with heavy metals, radioactivity, poisonous fumes of organic chemicals, pesticides that may attribute for erectile dysfunction.

Incidentally it has been reported that a number of birth defects and infertility problems are being faced in the pesticide afflicted areas in India and abroad [5]. Reports also depict that pesticides may cause erectile dysfunction [6]. A number of mechanisms have evolved for erectile dysfunction by various pesticide residues in the body. The purpose of this compilation is to put all the mechanisms of action of different pesticides at one platform so as to enable the researchers, physicians and regulatory authorities to design check points for dreaded chemicals being pumped in the environment. It will also give an idea about a pesticide that is having potential multiple toxic effects through different mechanisms.

2. Major causes of male infertility

Before going into details of different mechanism of actions, it is pertinent to enumerate some common factors that affect male fertility. Male infertility is commonly due to deficiencies in the semen and semen quality is used as a surrogate measure of male fecundity [7]. Some of the pre-testicular factors impede adequate support of the testes and include situations of poor hormonal support and poor general health including hypogonadism; drugs such as cimetidine that decrease follicle stimulating hormone (FSH) levels, and nitrofurantoin that decreases sperm motility; adopted life style (marijuana, cigarette smoking); and strenuous activities such as strenuous bicycle riding [8]. Testicular factors affect quality and quantity of semen produced by the testes and include age, genetic defects of the Y-chromosome (Klinefelter syndrome), neoplasm e.g. seminoma, cryptorchidism, varicocele which account for 14% [9], mumps viral infection [10] and may be idiopathic which accounts for 30% of male infertility [11]. USP 26 a peptidase enzyme expressed by USP 26 an X-linked gene in testis has been found to be defective in some cases of birth defects [12]. Besides this, there are some Post-testicular factors that decrease male fertility due to conditions that affect the male genital system after testicular sperm production and include defects of the genital tract as well as problems in

*Corresponding author: Dr. Parveen Bansal, Joint Director, University Centre of Excellence in Research, Baba Farid University of Health Sciences, Faridkot 151203, India.
Tel: +91 0 8872016290, +91 0 8427775823
E-mails: bansal66@yahoo.com, ucer_bfuhs@rediffmail.com
Peer review under responsibility of Hainan Medical College.

Please cite this article in press as: Kaur RP, et al., Potential pathways of pesticide action on erectile function – A contributory factor in male infertility, Asian Pacific Journal of Reproduction (2015), http://

ejaculation: e.g. impotence, Vas deferens obstruction, lack of Vas deferens, infection e.g. prostitis, ejaculatory duct obstruction and hypospadias [13].

Other important factor are conditions that affect the hypothalamus and pituitary gland will eventually affect the gonadotropin releasing hormone (GnRh) and hence the levels of follicle stimulating hormone, luteinizing hormone and prolactin hormone. These conditions include Kallmann syndrome (isolated gonadotropin deficiency), hyperprolactenemia and hypopituitarism. Hyperprolactinemia may be due to diseases affecting the hypothalamus and pituitary gland or secondary to disease of other organs such as the liver, kidneys and thyroid [14]. Hyperprolactinemia may cause hypogonadism, erectile dysfunction, decreased libido and infertility [15].

The etiological importance of environmental factors in infertility has also been stressed [16]. The implication of toxins such as glues, volatile organic solvents, silicones, physical agents, chemical dusts and pesticides in infertility has already been established [17]. Radiations and excessive heat to the genitalia have damaging effect on the testicles. Hence individuals having direct contact with or exposure to such chemicals have high chances of having primary or secondary infertility as the case may be. Estrogen-like hormone-disrupting chemicals such as phthalates are of particular concern for infertility in men and for effects on offspring of women. Exposure to phthalates can occur via dietary consumption, dermal absorption or inhalation and has been linked with impaired spermatogenesis and increased sperm DNA damage [18,19]. The mechanism for this is probably due to increase in the generation of reactive oxygen species (ROS) within the testis and a concomitant decrease in antioxidant levels, culminating in impaired spermatogenesis as observed in rats [20]. The contribution of tobacco smoking and alcohol intake to infertility has also been demonstrated. Tobacco smoking was observed to damage sperm DNA [21]. Though some of the damage is irreversible, but stopping smoking can prevent further damage [22]. It has been reported that smokers are 60% more likely to be infertile than non-smokers. Smoking reduces the chances of IVF producing a live birth by 34% and increases the risk of an IVF pregnancy miscarriage by 30% [23]. Smokers have decreased levels of antioxidants such as Vitamin E and Vitamin C, placing their spermatozoa at additional risk of oxidative damage.

Sexually transmitted diseases (STD) have also proved to be a leading cause of infertility. They are often asymptomatic but may display few symptoms, with the risk of failing to seek proper treatment in time to prevent decreased fertility [22]. Some of the identified STDs (such as syphilis, trichomoniasis, chancroid, chlamydia, gonorrhea, herpes simplex virus, human papilloma virus, lymphogranuloma venerum) are treatable while many are not and may eventually lead to death. Similarly the urinary tract has a relative anatomical association with the reproductive tract. Escherichia coli and Staphylococcus aureus are reported to be the most prevalent Gram negative and Gram positive organisms implicated in UTI respectively [24].

Obstructive azoospermia may result from previous vasectomy; epididymal, vassal, or ejaculatory duct pathology relating to genitourinary infection; iatrogenic injury during inguinal or scrotal surgery and congenital anomalies [25]. Azoospermia (low sperm counts), abnormal spermatozoa morphology (shape) and low sperm motility are usually asymptomatic conditions to most males but of great etiological importance. It is well recognized that sperm DNA can be damaged oxidatively by oxidative stress [26] and nonoxidatively by mechanisms such as aberrant apoptosis and incomplete sperm protamination [27].

3. Microorganisms and infertility

Microbial infections have been reported to reduce sperm viability. Staphylococcus aureus is the most prevalent Gram positive organism, while Escherichia coli is the most prevalent Gram negative organism isolated in the semen of males with primary infertility [28]. Chronic epididymitis secondary to Chlamydia trachomatis infection had been shown to blockage of the epididymis and thus obstructive azoospermia [29]. However, Ureaplasma urealyticum infections induce leukocytospermia and consequently lead to sperm damage, decrease sperm counts and invariably impaired sperm motility [30]. Herpes simplex virus (HSV) was reported to have been found in the semen of some infertile men and was related to low sperm count and poor motility [31]. Mumps viral infections in adolescent and adult males carry about 30% risk of developing orchitis or epididymitis, which can result in testicular atrophy and sterility [32].

4. Chemotherapy and infertility

Studies have shown that the antral follicle count decreases after the third series of chemotherapy, whereas follicle stimulating hormone (FSH) reaches menopausal levels after the fourth series; inhibin B and anti Mullerian hormone levels have been reported to decrease following chemotherapy [33]. Drugs with high risk of infertility include procarbazine, cyclophosphamide, ifosfamide, busulfan, melphalan, chlorambucil and chlormethine; drugs like doxorubicin, cisplatin and carboplatin have medium risk while therapies with plant derivatives (such as vincristine and vinblastine), antibiotics (such as bleomycin and dactinomycin) and antimetabolites (such as methotrexate, mercaptopurine and 5-fluoruracil) have low risk of gonadotoxicity [19].

5. Psychological/physical/behavioral problems

Several sexual problems exist that can affect male fertility. These problems are most often both psychological and physical in nature. It is difficult to separate the physiological and physical components. Stress can be an important reason for infertility. Ejaculatory incompetence is a rare psychological condition that prevents men from ejaculating during sexual intercourse even though they can ejaculate normally through masturbation. This condition sometimes responds well to behavioral therapy.

Above all one of the main upcoming reasons for male infertility is pesticides. Experimental evidence in the laboratory has linked the chemicals present in pesticides to reduced sperm quality, testicular cancer and reproductive abnormalities. The chemicals work by "blocking" the activity of hormones, known as androgens, which influence the development of the male reproductive system. Several studies have suggested that human semen quality and fecundity is declining [34–46]. A pesticide is "any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or

Please cite this article in press as: Kaur RP, et al., Potential pathways of pesticide action on erectile function – A contributory factor in male infertility, Asian Pacific Journal of Reproduction (2015), http://

animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies". They fall into three major classes: insecticides, fungicides, and herbicides, Based upon the target organism classification these are also rodenticides, nematicides, molluscicides and acaricides [47]. Another classification categorizes pesticides according their chemical structure. Insecticides include organochlorines, organophosphates, and carbamates. Organophosphate and carbamates are less toxic and largely replaced organochlorines.

Pesticides may differ according to their chemical structure, their mechanism of action and the toxicity they exhibit, but typically each pesticide consists of one (or more) active ingredient, which exerts the pesticidal activity, and an inert ingredient, which is inactive and helps in handling the active ingredient. Several studies have shown that the inert ingredient is not as inactive as it was previously believed to be [47,48]. Over 700 active ingredients are in use worldwide as pesticides, each with distinct chemical and toxicological properties [49].

In addition to the desired effects of crop protection and pest management, pesticides have some recognized adverse impacts on human health and the environment. Humans have a great risk of exposure through several pathways in occupational, agricultural and household use. Inhalation, oral, dermal and ocular is four possible routes for pesticide exposure. Ingestion of food and water is thought to be the main routes of pesticide exposure in the general population, while dermal absorption is suspected to be the main source of occupational exposure [49]. Over 25% of fruits, vegetables, and cereals are known to contain detectable residues of at least two pesticides and more than 300 different pesticides are known to contaminate food products sold in the EU. [50]. In the majority of cases, however, human exposure is unintentional and unintended [51,52]. Pesticides disrupt different mechanisms in the body.

6. Different mechanisms of action of pesticides causing erectile dysfunction

6.1. Pesticides can be the reason of erectile dysfunction

Penile erection is a complex neurovascular phenomenon. It involves the coordination of three hemodynamic events: increased arterial inflow, sinusoidal smooth muscle relaxation and decreased venous outflow. It also implies the interaction of the brain, nerves, neurotransmitters, smooth muscles and striated muscles. An alteration in any of these components may affect the response of the erectile tissue and cause erectile dysfunction [53–57]. The effect of the normal aging process on erectile function is unknown and the cause of age-related dysfunction is likely to be multifactorial in origin [58]. Pesticides act via different mechanism like oxidative stress, lowering testosterone levels, etc. [59–61]. Pesticides are responsible for decreasing testosterone concentration either by inhibiting release of FSH or LH [62]. Pesticides are also responsible for apoptosis of leydig cells and hence decreasing overall concentration testosterone. Pesticides also cause increase secretion of hypothalamic corticotrophin releasing hormone which stimulates release of adrenocorticotropic hormone (ACTH) and so cortisol [63] which inhibits GnRH and so LH and testosterone decreases. So, all the above written pathways are responsible for decreasing testosterone concentration. Alterations in blood vessels, hormonal changes, neurologic dysfunction, medication and associated systemic diseases are the main causes of erectile dysfunction [64].

6.2. Pesticides inhibit acetylcholine esterase

An acetylcholine esterase inhibitor (AChEI) or anti-cholinesterase is a chemical that inhibits the acetylcholine esterase enzyme from breaking down acetylcholine (Figure 1), thereby increasing both the level and duration of action of the neurotransmitter acetylcholine. Existence of reversible, quasi-irreversible and irreversible inhibitors of ACh like chloropyrifos, Malathion has been reported [65]. Increase in acetylcholine inhibits the release of gonadotrophin releasing hormone (GnRH) and that inhibits release of luteinizing hormone (LH) and follicle stimulating hormone (FSH). It results into inhibition of gametogenesis and steroidogenesis [66,67]. So, it is quiet likely that synthesis of testosterone being a steroid hormone may get hampered that could further result in erectile dysfunction. Pesticides like chlorpyrifos and carbofuran inhibit acetylcholine esterase [68,69]. Hence increased acetylcholine suppresses the reproductive functions [53–57].

6.3. Oxidative stress

Oxidative stress occurs when cells are exposed to excessive levels of reactive oxygen species (ROS) as a result of an imbalance between pro-oxidants and the protective mechanisms conferred by antioxidants [70]. ROS are formed during regular metabolism due to the univalent reduction of oxygen molecule. Superoxide (O^{2-}) is the most important among the ROS. Organophosphate induces production of ROS and hence causes reproductive tissue damage [71]. Atrazine and imidachloroprid is also responsible for oxidative stress by reducing levels of glutathione [59,60]. Hydrogen peroxide (H_2O_2), hypochlorous acid (HOCL), and peroxynitrite ($OONO^-$) are other important free radicals implicated in the pathophysiological mechanism of vascular disease. The vascularendothelium is the major source for these free radicals. Besides this, platelets and leukocytes are the other important sources of ROS [72]. Superoxide radicals are generated because of incomplete oxygen reduction in the electron transport system. Membrane bound enzymes, such as nicotinamide adenine dinucleotide hydrogenase–nicotinamide adenine dinucleotide phosphate hydrogenase oxidase, are the major source of superoxide radicals in activated phagocytic cells [73]. It has been reported that up regulation of these enzymes is associated with an increased risk of vascular disease [74,75]. Superoxide dismutase (SOD) is an important enzyme that removes the superoxide radicals from the human

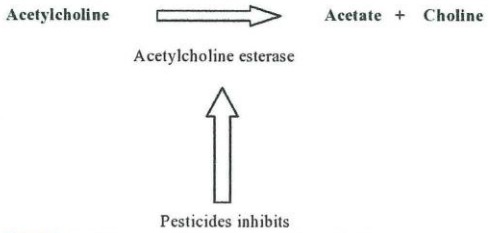


Figure 1. Inhibitory action of pesticides on Acetylcholine esterase.

Please cite this article in press as: Kaur RP, et al., Potential pathways of pesticide action on erectile function – A contributory factor in male infertility, Asian Pacific Journal of Reproduction (2015), http://

body. There are 3 types of SOD isoenzymes: cytosolic Cu Zn-SOD, mitochondrial Mn SOD, and extracellular SOD. Extracellular SOD reportedly plays a critical role in maintaining the redox state of vascular interstitium and thereby prevents the pathophysiological effects of superoxide in the vasculature. Extracellular SOD converts superoxide to H_2O_2.

The interaction between NO and ROS is one of the important mechanisms implicated in the pathophysiological process of erectile dysfunction [76]. NO interacts with superoxide to form peroxynitrite, which has been reported to play a central role in atherogenesis [72]. The stability of peroxynitrite allows a greater opportunity for it to diffuse through a cell to find a target. The unusual stability of peroxynitrite is due to its being folded into the &-conformation (Figure 2), which cannot directly isomerize to the much more stable form, nitrate [77]. Peroxynitrite reacts with the tyrosyl residue of proteins, which inactivates superoxide dismutase and leads to increased amount of superoxide [78]. This further increases the formation of peroxynitrite and reduces the available NO concentration. Peroxynitrite causes smooth-muscle relaxation and is less potent than NO. The effect of NO and peroxynitrite have been studied on stripped cavernosal tissue from rabbits [79]. They reported that relaxation induced by NO is short lived and immediate in onset compared with that due to peroxynitrite, which is prolonged and slow in onset. Moreover, the tissues returned to original tension immediately with NO, whereas with peroxynitrite, the tissues were unable to recover their original tension. These mechanisms ultimately produce an ineffective relaxation in cavernosal tissue, which produces erectile dysfunction shown in Figure 3.

6.4. Apoptosis and necrosis

The new findings are consistent with the well-known involvement Ca^{2+} in cell death from oxidative stress. Oxidative stress causes Ca^{2+} influx into the cytoplasm from the extracellular environment and from the endoplasmic reticulum [80]. Rising Ca^{2+} concentration in the cytoplasm in turn causes Ca^{2+} influx into the mitochondria and nuclei. In the mitochondria, Ca^{2+} accelerates the disruption of normal oxidative metabolism leading to necrotic cell death. In nuclei, Ca^{2+} modulates gene transcription and nucleases that control apoptosis (programmed cell death that involves fragmentation of DNA). Insecticides and pesticides has been shown to act as reproductive toxicants in male rats and histologically induce severe focal necrosis of the germinal cells in seminiferous tubules associated with tubular atrophy (shown in Figure 3). [81–83] NO interact with peroxide to form peroxynitrile. Peroxynitrite and superoxide have been reported to increase the incidence of apoptosis in the endothelium. This leads to denudation of endothelium and reduction of available NO [77,79]. Currently, the following are considered biomarkers of vascular endothelial dysfunction: insulin resistance, homocysteinemia, lipoprotein (a), endogenous nitric oxide (NO) synthesis inhibitors, vasodilators (nitrites, nitrates, and 6-keto prostaglandin F1a), adhesion molecules (vascular adhesion molecule-1 [VCAM-1], intercellular adhesion molecule-1 [ICAM-1], and P- and E-selectins), and thrombotic hemostatic factors [84]. Since, endothelium is made up of endothelial cells and these cells are responsible for NO production via eNOS. Superoxide is reported to have a direct vasoconstriction effect through mobilization of calcium ions [85]. This can potentially produce ED. According to the literature, the decreased availability of NO is the key pathophysiological process that leads to erectile dysfunction [86].

6.5. Endocrine disrupter

An endocrine disruptor was defined by the U.S. Environmental Protection Agency (EPA) as "an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction and developmental process" [87]. The pesticide, Glyphosate is a known or likely potential cause of endocrine effects [88]. There is already evidence that Glyphosate may act as an endocrine disruptor for both males and females by altering aromatase activity, estrogen regulated genes, and testosterone levels in rats [89] however Roundup has been reported to act via different mechanisms. Roundup exposure during pregnancy and lactation at a level that did not induce maternal toxicity in Wistar rats nevertheless induced adverse reproductive effects in male offspring, including decreased daily sperm production during adulthood, increase in abnormal sperms, and low testosterone serum level at puberty. In exposed female offspring, only a delay in vaginal canal opening was observed [90]. Pesticides disrupt level of different neurotransmitters and later these increased neurotransmitters effect the follicle stimulating hormone and luteinizing hormone by feedback inhibition [53–57,66,67].

6.6. Effect on leydig cells

Leydig cells are responsible for production of testosterone. These cells are under influence of luteinizing hormone (LH). Luteinizing hormone acts on enzyme cholesterol demolase

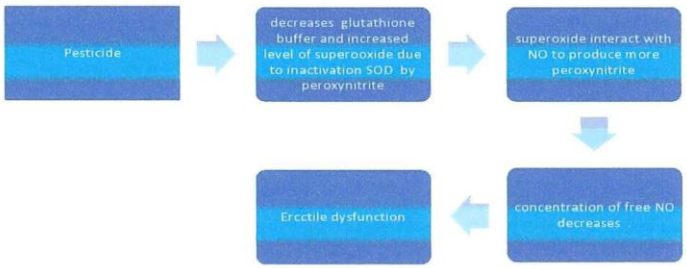


Figure 2. Pesticides causing oxidative stress.

Please cite this article in press as: Kaur RP, et al., Potential pathways of pesticide action on erectile function – A contributory factor in male infertility, Asian Pacific Journal of Reproduction (2015), http://

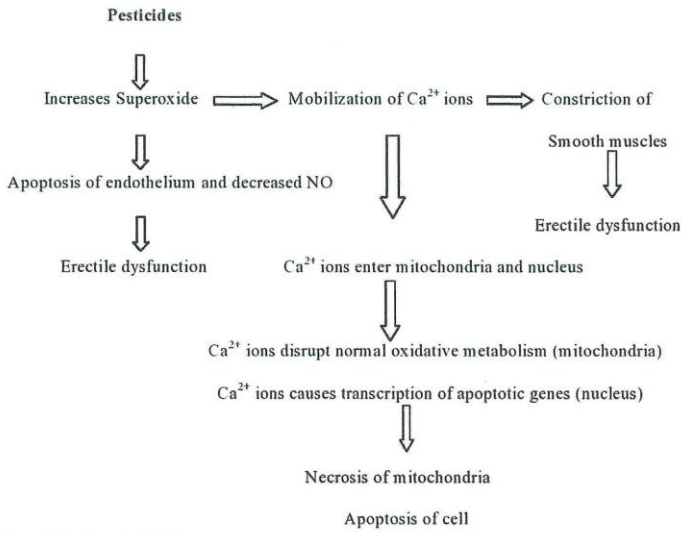


Figure 3. Apoptotic and necrotic action of pesticides.

activity which results in testosterone synthesis from cholesterol. Round exposure damages testosterone producing Leydig cells from mature rat testis at concentrations a tenth of agricultural use and beginning 1 hour after exposure [61]. Insecticide like atrazine, carbaryl and methoxychlor effects leydig cells. Metabolic products of methoxychlor downregulates the Cytochrome P450 enzymes, the enzymes involved in synthesis of testosterone [91].

6.7. Effect on testosterone and other hormones

Testosterone, the male hormone, is the major driver of male reproductive development and function. Suppression of its levels within the adult testis shuts down spermatogenesis the cause is unknown - and low sperm counts often show evidence of abnormal Leydig cells, which produce testosterone in the testis [92]. Dimethoate causes testicular damage, damage to sperm production & reduction in testosterone levels [93]. Glyphosate reduces production of testosterone [61]. At the very low, non-toxic concentration of 1 ppm, both Roundup and Glyphosate decreased testosterone level by 35%. It also inhibits production of other hormone [94]. Previous studies indicated that most insecticides inhibit the non-specific esterase activity in leydig cells that, in turn, result in reduced testosterone production [91,95]. Organophosphate is responsible for decreasing total serum testosterone and estradiol levels [96]. In fact, IMI acts as a nicotine acetylcholine receptor agonist and somehow likely to interfere with the release of gonadotropin-release hormone from hypothalamus and/or with release of LH/FSH from the pituitary (Figure 4), resulting in the reduction of sperm production in the testes [97]. Pesticides like, carbamates, pyrethroids, organophosphatases, Thio-and dithiocarbamates, chlorphenoxy acids and chlormethylphosporic acids reduces testosterone concentrations in male after acute exposure during exposure season [98]. Pesticides may also be involved in erectile dysfunction by altering levels of testosterone which functions as activator of enzyme nitric oxide synthase, enzyme responsible

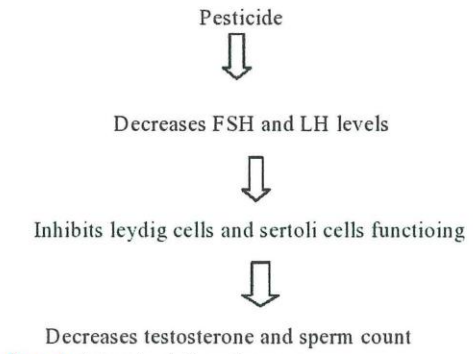


Figure 4. Action of pesticides on hormones.

for production of nitric oxide [99]. NOS are of two types iNOS and cNOS [100,101]. cNOS is calcium dependent and it has two isoforms i.e. eNOS (endothelial) and nNOS (neuronal) [102]. Nitric oxide synthases are responsible for the synthesis of nitric oxide from L-arginine [103]. Experimental evidence suggests that the constitutive isoforms of NOS may be responsible for NO production in penile erection [104,105]. Recently, several studies have revealed that nitric oxide (NO) is an important neural messenger which mediates penile erection [106–110]. Erection is mediated by the release of NO from non-adrenergic non-cholinergic nerve terminals, the endothelium of penile blood vessels, and corporal smooth muscle, producing smooth muscle relaxation and vasodilation [111]. NO stimulates the formation of guanylate cyclase in smooth muscle cells, converting GTP to 3′5′-cyclic GMP (cGMP) [111]. A cascade of cGMP-dependent intracellular events then leads to a decrease in intracellular calcium, ultimately causing smooth muscle relaxation, in part through changes in potassium conductance [111,112]. A recent study also hypothesized that androgens maintained and facilitated male

Please cite this article in press as: Kaur RP, et al., Potential pathways of pesticide action on erectile function – A contributory factor in male infertility, Asian Pacific Journal of Reproduction (2015), http://

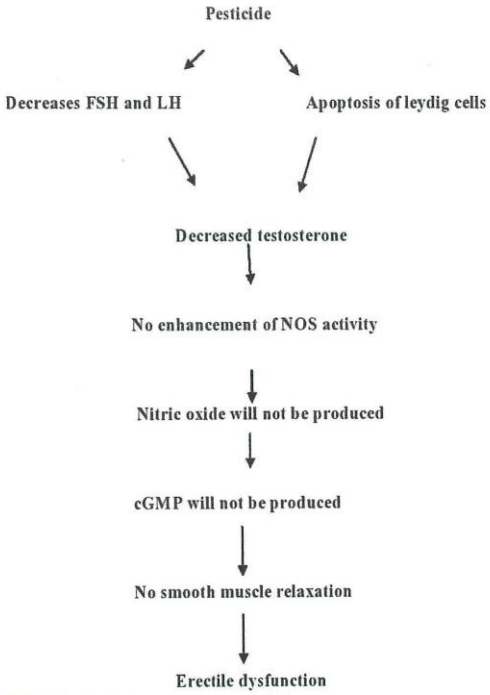


Figure 5. Mechanism of action of pesticides.

sexual potency through enhancement or maintenance of NOS activity in the corpus cavernous tissue in the penis [86]. Testosterone ensures penile erection through maintenance of nitric oxide synthase (NOS) activities in the peripheral nervous system and that deprivation of the androgen may reduce the number of corporal smooth muscle cells through apoptosis [113]. In addition, several studies have shown that androgen replacement facilitated neural activities in some areas of the brain which mediate sexual function [114,115]. Neurogenic NO is an important mediator of penile erection [116]. Hence indirect effect of pesticides occurs as shown in Figure 5.

6.8. Effects on tunica albuginea

The tunica albuginea is the tough fibrous envelope of connective tissue that surrounds the corpora cavernosa of ths penis. It consists of approximately 5% elastin, an extensible tissue that is primarily made up of the amino acids glycine, valine, alanine, and proline. The majority of the remaining tissue is collagen, which is made up of lysine, proline, glycine, alanine, and other amino acids [117]. The tunica albuginea is directly involved in maintaining an erection. On administration of imidachloroprid to the rats, the investigations revealed increased thickness of tunica albuginea [118]. Alterations in the microarchitecture of the tunica albuginea, including a decrease in the elastic fibers, may contribute to impotence in men [119]. Microstructural disorders of tunica albuginea have been reported in patients affected by impotence. In impotent patients, a reduction in the elastic fibers in the TA appears to produce disorders in the arrangement of the collagenic fibers. These alterations in the architecture of the TA in impotent patients can give rise top erection disorders [120]. During erection intracorporal pressure of patients with venogenic erectile dysfunction was significantly lower. Tunica albuginea collagen fibers exhibited degenerative and atrophic changes which presumably lead to tunica albuginea subluxation and floppiness. These tunica albuginea changes seem to explain cause of lowered intracorporal pressure which apparently results from loss of tunica albuginea veno-occlusive mechanism [121]. Atrazine also effects tunica albuginea. Atrazine increases thickness of tunica albuginea [70]. Tunica albuginea of patients showed degenerative and atrophic changes of collagen fibers; elastic fibers were scarce or absent [121].

6.9. Pesticides and neurotransmitters

Neurotransmitters are endogenous chemicals that transmit signals across a synapse from one neuron (brain cell) to another 'target' neuron. Decrease or increase in level of neurotransmitter effect the normal functioning of body. Different neurotransmitters like GABA when increased in body regulate FSH and LH in negative manner by suppressing release of Gonadotrophin releasing hormone. Hence suppress normal reproductive functions [53–57]. Acephate and metamidophos stimulate the secretion of hypothalamic corticotropin-releasing hormone, which in turn stimulate adrenocorticotropic hormone (ACTH) and so cortisol [49]. Receptor of cortisol are located on GnRH neurons, RFRP-3(mammalian ortholog of GnIH) and on gonadotrops. High cortisol level inhibits the secretion of GnRH, LH and testosterone while positively regulates RFRP-3 secretion, thus suppressing reproductive system [122]. Decrease in testosterone may be the reason of erectile dysfunction as testosterone is activator of nitric oxide synthase enzyme involved in production of nitric oxide [99].

The manuscript is suggestive of a significant place of erectile dysfunction as a causal factor for infertility. In this communication it is inferred that pesticides are having multiple mechanisms of action through which these chemicals cause erectile dysfunction. It is also reflected that out of mentioned widely used pesticides, acetamiprid is having most devastating effect causing erectile dysfunction as it acts through multiple inhibitory pathways.

References

[1] Sharath KC, Najafi M, Malini SS. Association of obesity with male infertility among infertile couples is not significant in Mysore, South India. *Adv Stud Biol* 2013; **5**: 319-325.
[2] Brugh VM, Lipshultz LI. Male factor infertility. *Med Clin North Am* 2004; **88**: 2367-2385.
[3] Hirsh A. Male subfertility. *BMJ* 2003; **327**: 669-672.
[4] Read J. Sexual problems associated with infertility, pregnancy and ageing. *BMJ* 1999; **318**: 587-589.
[5] Beyond pesticides. Pesticide-induced diseases: birth/fetal effects. [Online] Avaliable from: http://www.beyondpesticides.org/health/birthdefects.php [Accessed on 10th March].
[6] Soliman SS, Awad Allah AS, Ebied ZM. Erectile dysfunction in workers chronically - exposed to pesticides and organic solvents in Damietta Governorate. *Mansoura J Forensic Med Clin Toxicol* 2008; **16**: 63-76.
[7] Cooper TG, Noonan E, Von Eckardstein S, Auger J, Baker HW, Behre HM, et al. World Health Organization reference values for

Please cite this article in press as: Kaur RP, et al., Potential pathways of pesticide action on erectile function – A contributory factor in male infertility, Asian Pacific Journal of Reproduction (2015), http://

human semen characteristics. *Hum Reprod Update* 2010; **16**: 231-245.
[8] Leibovitch I, Mor Y. The vicious cycling: bicycling related urogenital disorders. *Eur Urol* 2005; **47**: 277-287.
[9] Costabile RA, Spevak M. Characterization of patients presenting with male factor infertility in an equal access, no cost medical system. *Urology* 2001; **58**: 1021-1024.
[10] Masarani M, Wazait H, Dinneen M. Mumps orchitis. *J R Soc Med* 2006; **99**: 573-575.
[11] Cavallini G. Male idiopathic oligoasthenoteratozoospermia. *Asian J Androl* 2006; **8**: 143-147.
[12] Zhang J, Qiu SD, Li SB, Zhou DX, Tian H, Huo YW, et al. Novel mutations in ubiquitin-specific protease 26 gene might cause spermatogenesis impairment and male infertility. *Asian J Androl* 2007; **9**: 809-814.
[13] Andrology Australia. Male infertility. [Online] Available from: https://www.andrologyaustralia.org/reproductive-problems/male-infertility/[Accessed on 8th May, 2015].
[14] Olooto WE, Amballi AA, Banjo TA. A review of female infertility; important etiological factors and management. *J Microbiol Biotech Res* 2012; **2**: 379-385.
[15] Scott IZ, Jacob R. Hyperprolactinemia and erectile dysfunction. *Rev Urol* 2000; **2**: 39-42.
[16] Hruska KS, Furth PA, Seifer DB, Sharara FI, Flaws JA. Environmental factors in infertility. *Clin Obstet Gynecol* 2000; **43**: 821-829.
[17] Mendiola J, Torres-Cantero AM, Moreno-Grau JM, Ten J, Roca M, Moreno-Grau S, et al. Exposure to environmental toxins in males seeking infertility treatment: a case-controlled study. *Reprod Biomed Online* 2008; **16**: 842-850.
[18] Hauser R, Meeker JD, Singh NP, Silva MJ, Ryan L, Duty S, et al. DNA damage in human sperm is related to urinary levels of phthalate monoester and oxidative metabolites. *Hum Reprod* 2007; **22**: 688-695.
[19] Brydoy M, Fossa SD, Dahl O, Bjoro T. Gonadal dysfunction and fertility problems in cancer survivors. *Acta Oncol* 2007; **46**: 480-489.
[20] Lee E, Ahn MY, Kim HJ, Kim IY, Han SY, Kang TS, et al. Effect of di (*n*-butyl) phthalate on testicular oxidative damage and antioxidant enzymes in hyperthyroid rats. *Environ Toxicol* 2007; **22**: 245-255.
[21] Gaur DS, Talekar M, Pathak VP. Effect of cigarette smoking on semen quality of infertile men. *Singap Med J* 2007; **48**: 119-123.
[22] Akhter N, Jebunnaher S. Evaluation of female infertility. *J Med* 2012; **13**: 200-209.
[23] Expert group on commissioning NHS infertility provision. *Regulated fertility services: a commissioning aid*. United Kingdom: Department of Health; 2009.
[24] Momoh ARM, Odike MAC, Samuel SO, Momoh AA, Okolo PO. Resistant pattern of urinary tract infection bacterial isolates to selected quinolones. *Benin J Postgrad Med* 2007; **9**: 22-27.
[25] American Society for Reproductive Medicine. The management of infertility due to obstructive azoospermia. *Fertil Steril* 2008; **90**: 121-124.
[26] Oger I, Cruz CD, Panteix G, Menezo Y. Evaluating sperm DNA integrity: relationship between 8 hydroxyguanosine quantification and sperm chromatin structure assay. *Zygote* 2003; **11**: 367-371.
[27] Ozmen B, Koutlaki N, Youssry M, Diedrich K, Al-Hasani S. DNA damage of human spermatozoa in assisted reproduction: origins, diagnosis, impacts and safety. *Reprod Biomed Online* 2007; **14**: 384-395.
[28] Momoh ARM, Idonije BO, Nwoke EO, Osifo UC, Okhai O, Omoroguiwa A, et al. Pathogenic bacteria-a probable cause of primary infertility among couples in Ekpoma. *J Microbiol Biotech Res* 2011; **1**: 66-71.
[29] Ochsendorf FR, Ozdemir K, Rabenau H, Fenner T, Doer HW. Chlamydia trachomatis and male infertility: chlamydia-IgA antibodies in seminal plasma are *C. trachomatis* specific and associated with an inflammatory response. *J Eur Acad Dermatol Venerol* 1999; **12**: 143-152.
[30] Wolf HG. The biologic significance of white blood cells in semen. *Fertil Steril* 1995; **63**: 1143-1157.
[31] Nikiforos JK, Eftichia P, Cathrin A, Dimosthenis K. Detection of herpes simplex virus, cytomegalovirus, and Epstein-Barr virus in the semen of men attending an infertility clinic. *Fertil Steril* 2003; **79**: 1566-1570.
[32] Senanayake SN. Mumps: a resurgent disease with protean manifestation. *Med J Aust* 2008; **189**: 456-459.
[33] Rosendahl M, Andersen C, La Cour Freiesleben N, Juul A, Lossl K, Andersen A. Dynamics and mechanisms of chemotherapy-induced ovarian follicular depletion in women of fertile age. *Fertil Steril* 2010; **94**: 156-166.
[34] Carlsen E, Giwercman A, Keiding N, Skakkebeak NE. Evidence for decreasing quality of semen during the past 50 years. *BMJ* 1992; **305**: 609-613.
[35] Auger J, Kunstmann JM, Czyglik F, Jouannet P. Decline in semen quality among fertile men in Paris during the past 20 years. *Engl N. J Med* 1995; **332**: 281-285.
[36] Adamopoulus DA, Pappa A, Nicopoulou S, Andreou E, Karamertzanis M, Michopoulos J, et al. Seminal volume and total sperm number trends in men attending sub fertility clinics in the Greater Athens area during the period 1977-1993. *Hum Reprod* 1996; **9**: 1936-1941.
[37] Irvine S, Cawood E, Richardson D, MacDonald E, Aitken J. Evidence of deteriorating semen quality in the United Kingdom: birth cohort study in 577 men in Scotland over 11 years. *BMJ* 1996; **312**: 467-471.
[38] Becker S, Berhane K. A meta-analysis of 61 sperm count studies revised. *Fertil Steril* 1997; **67**: 1103-1108.
[39] Swan SH, Elkin EP, Fenster L. Have sperm densities declined? A reanalysis of global trend data. *Environ Health Perspect* 1997; **105**: 1228-1232.
[40] Swan SH, Elkin EP, Fenster L. The question of declining sperm density revisited: an analysis of 101 studies published 1934-1996. *Environ Health Perspect* 2000; **108**: 961-966.
[41] Aitken RJ, Koopman P, Lewis SE. Seeds of concern. *Nature* 2004; **432**: 48-52.
[42] Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum Reprod* 2001; **16**: 972-978.
[43] Skakkebaek NE, Jorgensen N, Main KM, Rajpert-De Meyts E, Leffers H, Andersson A-M, et al. Is human fecundity declining? *Int J Androl* 2006; **29**: 2-11.
[44] Jorgensen N, Asklund C, Carlsen E, Skakkebaek NE. Coordinated European investigations of semen quality: results from studies of Scandinavian young men are a matter of concern. *Int J Androl* 2006; **29**: 54-61.
[45] Hauser R. The environment and male fertility: recent research on emerging chemicals and semen quality. *Semin Reprod Med* 2006; **24**: 156-167.
[46] Swan SH. Does our environment affect our fertility? Some examples to help reframe the question. *Semin Reprod Med* 2006; **24**: 142-146.
[47] Cox C, Surgan M. Unidentified inert ingredients in pesticides: implications for human and environmental health. *Environ Health Perspect* 2006; **114**: 1803-1806.
[48] Surgan MH. Toxicity tests: "inert" and active ingredients. *Environ Health Perspect* 2005; **113**: 657-658.
[49] Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillette LJ Jr, et al. Male reproductive health and environmental xenoestrogens. *Environ Health Perspect* 1996; **104**: 741-803.
[50] Ramazzini C. Collegium ramazzini statement on the control of pesticides in the European Union, a call for action to protect human health. *Am J Ind Med* 2009; **52**: 176-177.
[51] Ribas-Fito N. Silent Invaders: pesticides, livelihoods and women's health, London: ZED Books, 2002. *Int J Epidemiol* 2006; **35**: 504-505.
[52] Department of Agriculture and Cooperation. Summary of monitoring of pesticide residues at national level. India: Ministry of Agriculture, Govt. of India; 2010. [Online] Avaliable at: http://

Please cite this article in press as: Kaur RP, et al., Potential pathways of pesticide action on erectile function – A contributory factor in male infertility, Asian Pacific Journal of Reproduction (2015), http://

 I-Fra7

www.indiaenvironmentportal.org.in/files/Summary%20of%20Monitoring%20of%20pesticide%20residues%20at%20NAtional%20Level25november2010.pdf.

[53] Kuhar MJ, Ritz MC, Boja JW. The dopamine hypothesis of the reinforcing properties of cocaine. *Trends Neurosci* 1991; **14**: 299-302.

[54] Corrigall WA, Franklin KBI, Coen KM, Clarke PBS. The mesolimbic dopamine system is implicated in the reinforcing effects of nicotine. *Psychopharmacology* 1992; **107**: 285-289.

[55] Murphy LL, Munoz RM, Adrian BA, Villanu MA. Function of cannabinoid receptors in the neuroendocrine regulation of hormone secretion. *Neurobiol Dis* 1998; **5**: 432-446.

[56] Di Chiara G. Role of dopamine in the behavioural actions of nicotine related to addiction. *Euro J Pharmacol* 2000; **393**: 295-314.

[57] Watkins SS, Koob GF, Markou A. Neural mechanisms underlying nicotine addiction: acute positive reinforcement and withdrawal. *Nicotine Tob Res* 2000; **2**: 19-37.

[58] Melman A, Gingell JC. The epidemiology and pathophysiology of erectile dysfunction. *J Urol* 1999; **161**: 5-11.

[59] Dehkhargani SF, Malekinejad H, Shahrooz R, Sarkhanloo RA. Detrimental effect of atrazine on testicular tissue and sperm quality: implication for oxidative stress and hormonal alterations. *Iran J Toxicol* 2011; **5**: 426-435.

[60] Sasidhar BN, Anand KA, Gopala RA, Amaravathi P, Hemanth I. Chronic experimental feeding of imidachloroprid induced oxidative stress and amelioration with vitamin C and *Withania somnifera* in layer birds. *Int J Sci Enviorn Technol* 2014; **3**: 1679-1684.

[61] Clair E, Mesnage R, Travert C, Seralini GE. A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells *in vitro*, and testosterone decrease at lower levels. *Toxicol Vitro* 2012; **26**: 269-279.

[62] Slimani S, Boulakoud MS, Abdennour C. Pesticide exposure and reproductive biomarkers among male farmers from north-east Algeria. *Ann Biol Res* 2011; **2**: 290-297.

[63] Spassova D, White T, Singh AK. Acute effects of acephate and metamidophos on acetylcholinesterase activity, endocrine system and amino acid concentration in rats. *Comp Biochem Physiol C Toxicol Pharmacol* 2000; **126**: 79-89.

[64] Lue TF. Physiology of penile erection and pathophysiology of erectile dysfunction and priapism. In: Walsh PC, editor. *Campbell's urology*. 7th ed. Beijing: Science Press; 2001, p. 1167-1168.

[65] Pohanka M. Acetylcholinesterase inhibitors; a patent review (2008–present). *Expert Opin Ther Pat* 2012; **22**: 871-886.

[66] Mitsushima D, Hei DL, Terasawa E. GABA is an inhibitory neurotransmitter restricting the release of luteinizing hormone-releasing hormone before the onset of puberty. *Proc Nat Aca Sci U. S. A* 1994; **91**: 395-399.

[67] Terasawa E, Fernandez DL. Neurobiological mechanisms of the onset of puberty in primates. *Endocr Rev* 2001; **22**: 111-151.

[68] Elayan OEA, Karyono S, Sujuti H. The Effect of carbofuran on testosterone serum concentration and histological change of Leydig cell in mice. *IOSR J Pharm Biol Sci (IOSR-JPBS)* 2013; **7**: 01-04.

[69] Mandal TK, Das NS. Correlation of testicular toxicity and oxidative stress induced by chlorpyrifos in rats. *Hum Exp Toxicol* 2011; **30**: 1529-1539.

[70] Zalba G, Beaumont J, San Jose G, Fortuno A, Fortuno MA, Diez J. Vascular oxidant stress: molecular mechanisms and pathophysiological implications. *J Physiol Biochem* 2000; **56**: 57-64.

[71] Bal R, Naziroglu M, Turk G, Yilmaz O, Kuloglu T, Etem E, et al. Insecticide imidacloprid induces morphological and DNA damage through oxidative toxicity on the reproductive organs of developing male rats. *Cell Biochem Funct* 2012; **30**: 492-499.

[72] Beckman JS, Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am J Physiol* 1996; **271**: 1424-1437.

[73] Kojda G, Harrison D. Interactions between NO and reactive oxygen species: pathophysiological importance in atherosclerosis, hypertension, diabetes and heart failure. *Cardiovasc Res* 1999; **43**: 562-571.

[74] Warnholtz A, Nickenig G, Schulz E, Macharzina R, Brasen JH, Skatchkov M, et al. Increased NADH oxidasemediated superoxide production in the early stages of atherosclerosis: evidence for involvement of the renin-angiotensin system. *Circulation* 1999; **99**: 2027-2033.

[75] Hink U, Li H, Mollnau H, Oelze M, Matheis E, Hartmann M, et al. Mechanisms underlying endothelial dysfunction in diabetes mellitus. *Circ Res* 2001; **88**: 14-22.

[76] Jones RW, Rees RW, Minhas S, Ralph D, Persad RA, Jeremy JY. Oxygen free radicals and the penis. *Expert Opin Pharm* 2002; **3**: 889-897.

[77] Tsai JHM, Harrison JG, Martin JC, Hamilton TP, Woerd MV. Role of conformation of peroxynitrite anion (ONOO-) with its stability and toxicity. *J Am Chem Soc* 1994; **116**(9): 4115-4116.

[78] Zou M, Martin C, Ullrich V. Tyrosine nitration as a mechanism of selective inactivation of prostacyclin synthase by peroxynitrite. *Biol Chem* 1997; **378**: 707-713.

[79] Khan MA, Thompson CS, Mumtaz FH, Mikhailidis DP, Morgan RJ, Bruckdorfer RK, et al. The effect of nitric oxide and peroxynitrite on rabbit cavernosal smooth muscle relaxation. *World J Urol* 2001; **19**: 220-224.

[80] Ermak G, Davies KJA. Calcium and oxidative stress: from cell signalling to cell death. *Mol Immunol* 2002; **38**: 713-721.

[81] Bustos OE, Gonzalez HP. Effect of a single dose of malathion on spermatogenesis in mice. *Asian J Androl* 2003; **5**: 105-107.

[82] Narayana K, Prashanthi N, Bairy LD, Souza U. An organophosphate insecticide methyl parathion (0-0- dimethyl 0-4-nitrophenylphosphorothioate) induces cytotoxic damage and tubular atrophy in the testis despite elevated testosterone level in rats. *J Toxicol Sci* 2006; **31**: 177-189.

[83] Swart Y, Kruger TF, Menkveld R, Schabort I, Lombard CJ. Effect of Lead and organophosphates on sperm morphology. *Syst Biol Reprod Med* 1991; **26**: 67-70.

[84] Costa C, Virag R. The endothelial-erectile dysfunction connection: an essential update. *J Sex Med* 2009; **6**: 2390-2404.

[85] Katusic ZS, Vanhoutte PM. Superoxide anion is an endothelium derived contracting factor. *Am J Physiol* 1989; **257**: 33-37.

[86] Mills TM, Reilly CM, Lewis RW. Androgen and penile erection: a review. *J Androl* 1996; **17**: 633-638.

[87] Kavlock RJ, Daston GP, DeRosa C, Fenner-Crisp P, Gray LE, Kaattar S, et al. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the U.S. EPA-sponsored workshop. *Environ Health Perspect* 1996; **104**: 715-740.

[88] Environmental protection agency. Draft list of initial pesticide active ingredients and pesticide inerts to be considered for screening under the federal food, drug, and cosmetic act; extension of comment period. *Fed Regist* 2007; **72**(116): 33486-33503.

[89] Romano RM, Romano MA, Bernadi MM, Furtado PV, Oliveira CA. Prepubertal exposure to commercial formulation of the herbicide and decreased testosterone serum level at puberty alters testosterone levels and testicular morphology. *Arch Toxicol* 2010; **84**: 309-317.

[90] Dallegrave E, Mantese FD, Oliveira RT, Andrade AJ, Dalsenter PR, Langeloh A. Pre- and postnatal toxicity of the commercial glyphosate formulation in Wistar rats. *Arch Toxicol* 2007; **81**: 665-673.

[91] Akingbemi BT, Ge RS, Klinefelter GR, Gunsalus GL, Hardy MP. A metabolite of methoxychlor, 2,2-bis(p-hydroxyphenyl)-1,1, 1-trichloroethane, reduces testosterone biosynthesis in rat leydig cells through suppression of steady-state messenger ribonucleic acid levels of the cholesterol side-chain cleavage enzyme. *Biol Reprod* 2000; **62**: 571-578.

[92] Institute of Science in Society. Glyphosate/Roundup & human male infertility. ISIS Report 19/03/14 [Online] Available at: http://www.i-sis.org.uk/Glyphosate_Roundup_and_Human_Male_Infertility.php. [Accessed on 10th November, 2014].

Please cite this article in press as: Kaur RP, et al., Potential pathways of pesticide action on erectile function – A contributory factor in male infertility, Asian Pacific Journal of Reproduction (2015), http://

[93] Afifi NA, Ramadan A, Abd- El Aziz MI, Saki EE. Influence of on testicular and epididymal organs, testosterone, plasma level and their tissue residues in rats. *Dtsch Tierarztl Wochenschr* 1991; **98**: 419-420.

[94] Walsh LP, McCormick C, Martin C, Stocco DM. Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression. *Environ Health Perspect* 2000; **108**: 769-776.

[95] Chapin RE, Phelps JL, Somkuti SG, Heindel JJ, Burka LT. The interaction of Sertoli and Leydig cells in the testicular toxicity of tri-o-cresyl phosphate. *Toxicol Appl Pharmacol* 1990; **104**: 483-495.

[96] Padungtod C, Lasley BL, Christiani DC, Ryan LM, Xu X. Reproductive hormone profile among pesticide factory workers. *J Occup Environ Med* 1998; **40**: 1038-1047.

[97] Ngoula F, Watcho P, Dongmo MC, Kenfack A, Kamtchouing P, Tchoumboue J. Effects of pirimiphos-methyl (an organophosphate insecticide) on the fertility of adult male rats. *Afr Health Sci* 2007; **7**: 3-9.

[98] Evamarie S, Wolfgang S, Egon K, Matthias B, Margitta JM, Hans JR. Disruption of male sex hormones with regard to pesticides: pathophysiological and regulatory aspects. *Toxicol Lett* 1999; **107**: 225-231.

[99] Zvara P, Sioufi R, Schipper HM, Begin LR, Brock GB. Nitric oxide mediated erectile activity is a testosterone dependent event: a rat erection model. *Int J Impot Res* 1995; **7**: 209-219.

[100] Stuehr DJ. Mammalian nitric oxide synthases. *BBA-Bioenergetics* 1999; **1411**: 217-230.

[101] Forstermann U, Gath I, Schwarz P, Closs EL, Kleinert H. Isoforms of nitric oxide synthase. *Biochem Pharmacol* 1995; **50**: 1321-1332.

[102] Bansal P, Gupta V, Acharaya MV, Kaur H, Bansal R, Sharma S. Garlic- potential substitute to synthetic aphrodisiacs for erectile dysfunction. *J Pharm Res* 2010; **3**: 3072-3074.

[103] Masters BS, McMillan K, Sheta EA, Nishimura JS, Roman LJ, Martasek P. Neuronal nitric oxide synthase, a modular enzyme formed by convergent evolution: structure studies of a cysteine thiolate-liganded heme protein that hydroxylates L-arginine to produce NO as a cellular signal. *FASEB J* 1996; **10**: 552-558.

[104] Burnett AL, Tillman SL, Chang TSK, Epstein JI, Lowenstein Cj, Bredt DS, et al. Immuno histochemical localization of nitric oxide synthase in the autonomic innervation of the human penis. *J Urol* 1993; **150**(1): 73-76.

[105] Bush P, Aronson WJ, Buga GM, Rajfer J, Ignarro LJ. Nitric oxide is a potent relaxant of human and rabbit corpus cavernosum. *J Urol* 1992; **147**: 1650-1655.

[106] Ignarro LJ, Bush PA, Buga GM, Wood KS, Fukuto JM, Rajfer J. Nitric oxide and cyclic GMP formation upon electrical field stimulation cause relaxation of corpus cavernosum smooth muscle. *Biochem Biophys Res Commun* 1990; **170**: 843-850.

[107] Holmquist F, Stief CG, Jonas U, Andersson KE. Effects of the nitric oxide synthase inhibitor NGnitro-L-arginine on the erectile response to cavernous nerve stimulation in the rabbit. *Acta Physiol Scand* 1991; **143**: 299-304.

[108] Kim N, Azadzoi KM, Goldstein I, Saenz DTI. A nitric oxide-like factor mediates nonadrenergicnoncholinergic neurogenic relaxation of penile corpus cavernosum smooth muscle. *J Clin Invest* 1991; **88**: 112-118.

[109] Burnett AL, Lowenstein CJ, Bredt DS, Chang TS, Snyder SH. Nitric oxide: a physiologic mediator of penile erection. *Science* 1992; **257**: 401-403.

[110] Rajifer J, Aronson WJ, Bush P, Dorey FJ, Ignorro LJ. Nitric oxide as a mediator of relaxation of the corpus cavernosum in response to nonadrenergic, noncholinergic neurotransmission. *N Engl J Med* 1992; **326**: 90-94.

[111] Burnett AL. Nitric oxide in the penis: physiology and pathology. *J Urol* 1997; **157**: 320-324.

[112] Seftel AD, Viola KA, Kasner SE, Ganz MB. Nitric oxide relaxes rabbit corpus cavernosum smooth muscle via a potassium-conductive pathway. *Biochem Biophys Res Commun* 1996; **219**: 382-387.

[113] Traish AM, Park K, Dhir V, Kim NN, Moreland RB, Goldstein I. Effects of castration and androgen replacement on erectile function in a rabbit model. *Endocrinology* 1999; **140**: 1861-1868.

[114] Okamura H, Yokosuka M, McEwen BS, Hayashi S. Colocalization of NADPH-diaphorase and estrogen receptor immunoreactivity in the rat ventromedial hypothalamic nucleus: stimulatory effects of estrogen on NADPH-diaphorase activity. *Endocrinol* 1994; **135**: 1705-1708.

[115] Pu S, Xu B, Kalra SP, Kalra PS. Evidence that gonadal steroids modulate nitric oxide efflux in the medial preoptic area: effects of N-methyl-Daspartate and correlation with Luteinizing hormone secretion. *Endocrinol* 1996; **137**(5): 1949-1955.

[116] Sullivan ME, Thompson CS, Dashwood MR, Khan MA, Jeremy JY, Morgan RJ, et al. Nitric oxide and penile erection: Is erectile dysfunction another manifestation of vascular disease? *Cardivasc Res* 1999; **43**: 658-665.

[117] Boston University School of Medicine. Male genital anatomy. [Online] Available from: http://www.bumc.bu.edu/sexualmedicine/physicianinformation/male-genital-anatomy/ [Accessed on 10th January, 2004].

[118] Najafi G, Razi M, Hoshyar A, Shah mohamadloo S, Feyzi S. The effect of chronic exposure with imidacloprid insecticide on fertility in mature male rats. *Int J Fertil Steril* 2010; **4**: 9.

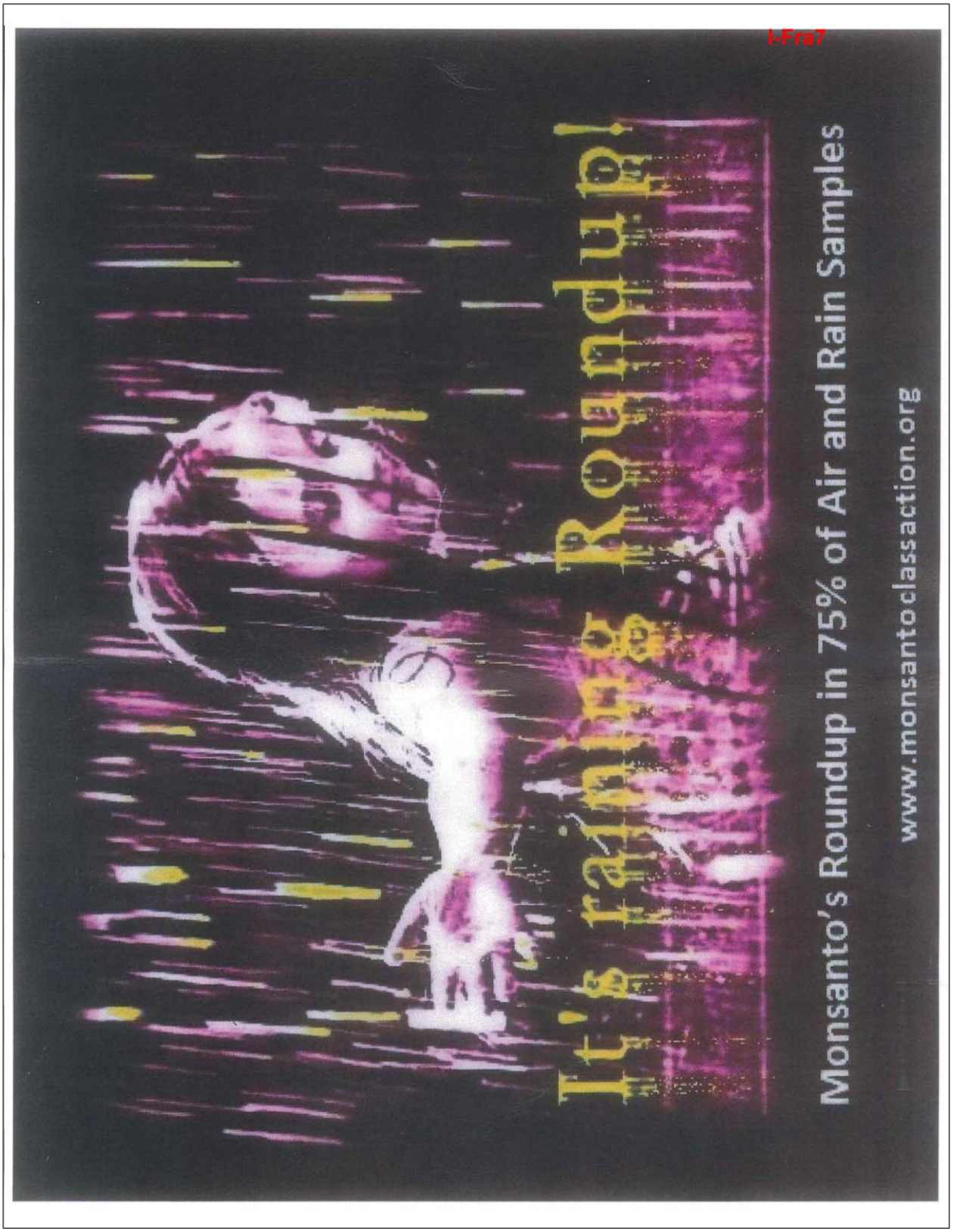
[119] Lacono F, Barra S, Dirosa G, Boscaino A, Lotti T. Microstructural disorders of tunica albuginea in patients affected by impotence. *Eur Urol* 1994; **26**: 233-239.

[120] Lacono F, Barra S, Cafiero G, Lotti T. Scanning electron microscopy of the tunica albuginea of the corpora cavernosa in normal and impotent subjects. *Urol Res* 1995; **23**: 221-226.

[121] Shafik A, Shafik I, El Siba O, Shafik AA. On the pathogenesis of penile venous leakage: role of the tunica albuginea. *BMC Urol* 2007; **7**: 14.

[122] Zhang JJ, Wang Y, Xiang HY, LI MX, LI WH, MA KG, et al. Oxidative stress: role in acetamiprid-induced impairment of the male mice reproductive system. *Agricul Sci China* 2011; **10**: 786-796.

Please cite this article in press as: Kaur RP, et al., Potential pathways of pesticide action on erectile function – A contributory factor in male infertility, Asian Pacific Journal of Reproduction (2015), http://


I-Fra7
It's raining Roundup!
Monsanto's Roundup in 75% of Air and Rain Samples
www.monsantoclassaction.org

Comment Letter I-Fra7 — Fraser, Mary

Response 1

The article is “Potential pathways of pesticide action on erectile function – A contributory factor in male infertility.” 2015. RP Kaur, V Gupta, AF Christopher, P Bansal*, Baba Farid Univ of Health Sciences Faridkot*, India.

The authors submit an extensive summary of their suggestion that erectile dysfunction is an important contributor to infertility. They proceed to correlate what they describe as the “indiscriminant use of pesticides” with infertility and then narrow their hypothesis to a few chemicals associated with pesticides.

The paper provides an exhaustive discussion of the possible causes of infertility with numerous examples of the dozens of ways that fertility may be impacted. The list of factors cited that “may” contribute to infertility is focused on erectile dysfunction (and not fertility). The authors inadvertently make a strong case for the impact of dozens of direct and indirect “confounding factors” on fertility.

Although the paper outlines more than a dozen physiologic and metabolic processes that may be involved in fertility, they inappropriately attempt to link “possible” causality to dozens of the processes discussed in their article. They fail to provide any substantive support to the secondary and tertiary links to pesticides. In fact, the authors fail to provide defensible arguments to primary exposures to pesticide or the links to the dozen mechanisms and processes they discuss. Throughout the paper they use words such as “infer”, “suggests”, “possible”, “quite likely” and numerous other unsubstantiated terms that fail to link their hypothesis to direct or confirmed pesticide exposure. After five pages of discussion, the authors state as their summary that “the review successfully highlights the indiscriminate regional use of pesticides” which does not link causality to their hypothesis. As a result, it is doubtful that this paper would be accepted for publication in the USA. Moreover, the active ingredients cited in the paper are not proposed for use in the District’s Integrated Vector Management Program (IVMP).

Furthermore, it should be noted that the District’s IVMP represents a tiered, carefully considered and focused approach to the surveillance and control of vector species, not the “indiscriminate regional use of pesticides.”

I-Fra8

EFFECTS OF GLYPHOSATE BIBLIOGRAPHY

1. Antoniou, M.et.al. Teratogenic Effects of Glyphosate-Based Herbicides Environmental & Analytical Toxicology, J. Environ Anal Toxicol. 2012.Jun 23, 2012 - Environmental & Analytical. Toxicology. Antoniou et al., J Environ Anal Toxicol 2012, S:4 http://dx.doi.org/10.4172/2161-0525.S4-006. (on birth defects)
2. Aris, A., & Leblanc, S. (2011). Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. Reproductive Toxicology, 31(4), 528-533. (on presence of glyphosate and Bt insecticidal proteins in pregnant women and cord blood)
3. Barberis, C. L., Carranza, C. S., Chiacchiera, S. M., & Magnoli, C. E. (2013). Influence of herbicide glyphosate on growth and aflatoxin B1 production by Aspergillus section Flavi strains isolated from soil on in vitro assay. Journal of Environmental Science and Health, Part B, 48(12), 1070-1079. (on toxic fungi appearing in soil sprayed with glyphosate)
4. Barrett, Mike Monsanto's Infertility-Linked Roundup Found in All Urine Samples Tested. Mike Barrett, Natural Society. 1/26/2012. (a news article following the Ithaka journal study of glyphosate in German urine)
5. Battaglin, W. A., Kolpin, D. W., Scribner, E. A., Kuivila, K. M., & Sandstrom, M. W. (2005). Glyphosate, other herbicides, and transformation products in midwestern streams, 20021. (glyphosate found in streams, aquifers)
6. Battaglin, W. A., Rice, K. C., Focazio, M. J., Salmons, S., & Barry, R. X. (2009). The occurrence of glyphosate, atrazine, and other pesticides in vernal pools and adjacent streams in Washington, DC, Maryland, Iowa, and Wyoming, 2005–2006. Environmental monitoring and assessment, 155(1-4), 281-307. (Presence of glyphosate in streams, aquifers)
7. Brändli, D., & Reinacher, S. (2012). Herbicides found in human urine. Ithaka Journal, 1(2012), 270-272. (Glyphosate in human urine)
8. de Liz Oliveira Cavalli, V. L., Cattani, D., Heinz Rieg, C. E., Pierozan, P., Zanatta, L., Benedetti Parisotto, E., & Zamoner, A. (2013). Roundup disrupts male reproductive functions by triggering calcium-mediated cell death in rat testis and Sertoli cells. Free Radical Biology and Medicine, 65, 335-346. (damage done by glyphosate to testicular tissue)
9. Carrasco, A. (2013). Teratogenesis by glyphosate based herbicides and other pesticides. Relationship with the retinoic acid pathway. GMLS 2012, 24. (birth defects in Argentina)
10. Chang, F. C., Simcik, M. F., & Capel, P. D. (2011). Occurrence and fate of the herbicide glyphosate and its degradate aminomethylphosphonic acid in the

I-Fra8

atmosphere. Environmental Toxicology and Chemistry, 30(3), 548-555. (presence of glyphosate in the air)

11. Chaufan, G., Coalova, I., & de Molina, M. D. C. R. (2014). Glyphosate Commercial Formulation Causes Cytotoxicity, Oxidative Effects, and Apoptosis on Human Cells Differences With its Active Ingredient. International journal of toxicology, 33(1), 29-38. (strong toxic effect of glyphosate together with additives)
12. Clair, É., Mesnage, R., Travert, C., & Séralini, G. É. (2012). A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells< i> in vitro and testosterone decrease at lower levels. Toxicology in vitro, 26(2), 269-279.(glyphosate damage in testicular cells)
13. Clements C, Ralph S, Pertas M, 1997. Genotoxicity of select herbicides in Rana catesbeiana tadpoles using the alkaline single-cell gel DNA electrophoresis (comet) assay. Environ Mol Mutagen 1997; 29(3):277-288.) (cytotoxicity of glyphosate on animal cells)
14. Copping, L. G. (2014). Sri Lanka Bans the Sale and Use of Glyphosate. Outlooks on Pest Management, 25(2), 187-191. (recent ban on Glyphosate in certain Sri Lankan counties-high serpentine soils)
15. Cox, C. (1995). Glyphosate, part 1: toxicology. Journal of Pesticide Reform, 15(3), 14-20. (Ecological effects of glyphosate on birds, bees, fish, insects, tadpoles, plants)
16. Criswell, J. T., & Campbell, J. (2013). Pesticide Applicator Certification Series, Oklahoma Cooperative Extension Service, EPP-7457. (toxic levels of glyphosate on skin , eyes etc.)
17. Dekker, E. J., Vaessen, M. J., van den Berg, C., Timmermans, A., Godsave, S., Holling, T., ... & Durston, A. (1994). Overexpression of a cellular retinoic acid binding protein (xCRABP) causes anteroposterior defects in developing Xenopus embryos. Development, 120(4), 973-985. (birth defects in amphibians)
18. Foulk, K. E., & Reeves, C. (2009). Identifying the role of glyphosate-containing herbicides on honeybee mortality rates and colony collapse disorder. In Proceedings of Junior Science, Engineering, and Humanities Symposium, Camdenton, MO, USA (pp. 2-23).
19. Garry, V. F., Harkins, M. E., Erickson, L. L., Long-Simpson, L. K., Holland, S. E., & Burroughs, B. L. (2002). Birth defects, season of conception, and sex of children born to pesticide applicators living in the Red River Valley of Minnesota, USA. Environmental health perspectives, 110(Suppl 3), 441. (birth defects in children of farm workers)
20. Garry, V. F. (2004). Pesticides and children. Toxicology and applied pharmacology, 198(2),152-163. (birth defects- children without limbs)

I-Fra8

21. Girona, Jordi, Article Affiliation: Institute of Environmental Assessment and Water Research (IDAEA-CSIC), C/ 18-26, 08034, Barcelona, Spain. (pervasiveness of glyphosate in ground water)

22. Hardell, L.&Eriksson,M (1999), A case-control study of non-Hodgkin lymphoma and exposure to pesticides. Cancer 85(6), 1353-1360 (glyphosate and non-Hodgkin lymphoma)

23. Hedges, C., Lindorff, D., Horn, S., Baker, D., Harrop, F., & Morris, P. (2012). 62 comments on" Explosive: Monsanto 'Knowingly Poisoned Workers' Causing Devastating Birth Defects. Way, 3, 50am. (Birth defects in Argentina, Does not inform workers on correct usage)

24. Heitanen, et al., 1983. Effects of phenoxyherbicides and glyphosate on the hepatic and intestinal biotransformation activities in the rat. Acta Pharmacol Toxicol (Copenh) 1983 Aug; 53(2):103-12.) (effects of glyphosate on P-450 detoxification pathways in animals and humans)

25. Hedges, C., Lindorff, D., Horn, S., Baker, D., Harrop, F., & Morris, P. (2012). 62 comments on" Explosive: Monsanto 'Knowingly Poisoned Workers' Causing Devastating Birth Defects. Way, 3, 50am.

26. Ho, M. W. (2010). Lab study establishes glyphosate link to birth defects. ISIS. (birth defects in Argentina)

27. Hwang, Dr.Hyun Min and Thomas Young, Biodegradability of Roundup , of UC Davis. 4/19/2011 (study commissioned by MMWD on Biodegradability of Roundup)

28. Khan, S. U. (1981). N-nitrosamine formation in soil from the herbicide glyphosate and its uptake by plants. In ACS symposium series-American Chemical Society (USA). (cancer causing nitrosamine in soils with glyphosate exposure)

29. Jayasumana, C., Gunatilake, S., & Senanayake, P. (2014). Glyphosate, hard water and nephrotoxic metals: are they the culprits behind the epidemic of chronic kidney disease of unknown etiology in sri lanka?. International journal of environmental research and public health, 11(2), 2125-2147. (Sri Lankan kidney deaths in counties with serpentine soils)

30. Johal, G. S., & Huber, D. M. (2009). Glyphosate effects on diseases of plants. European Journal of Agronomy, 31(3), 144-152. (glyphosate greatly increases phytophthora ramorum on oak trees. Sudden Oak Death)

31. Kassaby, F. Y. (1985). Interaction of four herbicides with Phytophthora cinnamomi. Australasian Plant Pathology, 14(2), 21-22. (glyphosate greatly increases phytophthora which causes Sudden Oak Death)

32. Kimmel, G. L., Kimmel, C. A., Williams, A. L., & DeSesso, J. M. (2012). Evaluation of developmental toxicity studies of glyphosate with attention to cardiovascular

I-Fra8

development. Critical reviews in toxicology, 43(2), 79-95. (Glyphosate and birth defects)

33. Koller, V. J., Fürhacker, M., Nersesyan, A., Mišík, M., Eisenbauer, M., & Knasmueller, S. (2012). Cytotoxic and DNA-damaging properties of glyphosate and Roundup in human-derived buccal epithelial cells. Archives of toxicology, 86(5), 805-813. (precancerous changes in cells in cheek and nose linings with extremely small amounts of glyphosate exposure)

34. Krüger, Monika, et al. "Visceral botulism at dairy farms in Schleswig Holstein, Germany–Prevalence of< i> Clostridium botulinum</i> in feces of cows, in animal feeds, in feces of the farmers, and in house dust." Anaerobe 18.2 (2012): 221-223. (chronic botulism in feces and house dust of farmers using glyphosate)

35. Kyvik KR, Morn BE, 1995. Environmental poisons and the nervous system. Tidsskr Nor Laegeforen 1995. June 10; 115(15):1834-8.) (effects of glyphosate on the nervous system)

36. Larsen, K., Najle, R., Lifschitz, A., & Virkel, G. (2012). Effects of sub-lethal exposure of rats to the herbicide glyphosate in drinking water: glutathione transferase enzyme activities, levels of reduced glutathione and lipid peroxidation in liver, kidneys and small intestine. Environmental toxicology and pharmacology, 34(3), 811-818. (cytotoxic effects of glyphosate on liver, kidney and small intestine cells at extremely small concentrations)

37. Lévesque, C. A., Rahe, J. E., & Eaves, D. M. (1987). Effects of glyphosate on Fusarium spp.: its influence on root colonization of weeds, propagule density in the soil, and crop emergence. Canadian journal of microbiology, 33(5), 354-360. (glyphosate promotes pathogens in soil that can attack all plants)

38. López, S. L., Aiassa, D., Benitez-Leite, S., Lajmanovich, R., Manas, F., Poletta, G., ... & Carrasco, A. E. (2012). Pesticides used in South American GMO-based agriculture: A review of their effects on humans and animal models. Advances in Molecular Toxicology, 6, 41-75. (glyphosate and birth defects Argentina)

39. Moon, Lady Spirit, (8/7/2014) Five Causes of Colony Collapse Disorder, Center for Honey Bee Research, Asheville, North Carolina (glysophate kills critical bacteria in guts of insects including honey bees, enhances death along with neonicotinoids)

40. Mañas, F., Peralta, L., Raviolo, J., Garcia Ovando, H., Weyers, A., Ugnia, L., ... & Gorla, N. (2009). Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetic tests. Ecotoxicology and Environmental Safety, 72(3), 834-837. (birth defects with AMPA, a metabolite of glyphosate)

41. Mariager, T. P., Madsen, P. V., Ebbehøj, N. E., Schmidt, B., & Juhl, A. (2013). Severe adverse effects related to dermal exposure to a glyphosate-surfactant

I-Fra8

herbicide. Clinical toxicology, 51(2), 111-113. (skin lesions from glyphosate-surfactant exposure)

42. McDuffie, H. H., Pahwa, P., McLaughlin, J. R., Spinelli, J. J., Fincham, S., Dosman, J. A., ... & Choi, N. W. (2001). Non-Hodgkin's Lymphoma and Specific Pesticide Exposures in Men Cross-Canada Study of Pesticides and Health. Cancer Epidemiology Biomarkers & Prevention, 10(11), 1155-1163. (Non-Hodgkin's lymphoma and glyphosate use)

43. Mesnage, R., Bernay, B., & Séralini, G. E. (2013). Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. Toxicology, 313(2), 122-128. (Toxicity of glyphosate together with its adjuvants)

44. Mesnage, R., Clair, E., Gress, S., Then, C., Székács, A., & Séralini, G. E. (2013). Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide. Journal of Applied Toxicology, 33(7), 695-699. (cytotoxicity of glyphosate and Bt insecticidal toxins from GMO foods)

45. Monheit, S. (2002). Glyphosate-based Aquatic herbicides. An overview of risk. Noxious Times, 4(4). (damage to salmon from glyphosate)

46. Oliveira, A. G., Telles, L. F., Hess, R. A., Mahecha, G. A., & Oliveira, C. A. (2007). Effects of the herbicide Roundup on the epididymal region of drakes< i> Anas platyrhynchos</i>. Reproductive Toxicology, 23(2), 182-191. (effects of Roundup on formation of testicular tissue)

47. Orsi, L., Troussard, X., Monnereau, A., Berthou, C., Fenaux, P., Marit, G., ... & Clavel, J. (2007). Occupation and lymphoid malignancies: results from a French case-control study. Journal of Occupational and Environmental Medicine, 49(12), 1339-1350. (Roundup and lymphoma)

48. Paganelli, A., Gnazzo, V., Acosta, H., López, S. L., & Carrasco, A. E. (2010). Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling. Chemical Research in Toxicology, 23(10), 1586-1595. (Glyphosate and birth defects in Argentina)

49. Robinson, C. J., & Fagan, J. (2012). Teratogenic effects of glyphosate-based herbicides: divergence of regulatory decisions from scientific evidence. Journal of Environmental & Analytical Toxicology.(glyphosate and birth defects)

50. Rodloff, A. C., & Krüger, M. (2012). Chronic< i> Clostridium botulinum</i> infections in farmers. Anaerobe, 18(2), 226-228. (glyphosate and chronic botulism)

51. Salmon, H. P. H. How Pesticides Threaten Salmon. Biol, 48, 758-775. (glyphosate damage to salmon)

52. Sanchís, J., Kantiani, L., Llorca, M., Rubio, F., Ginebreda, A., Fraile, J., ... & Farré, M. (2012). Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction

I-Fra8

followed by liquid chromatography coupled to tandem mass spectrometry. Analytical and bioanalytical chemistry, 402(7), 2335-2345. (Pervasiveness of glyphosate in groundwater)

53. (prevalence of glyphosate in groundwater)Saver Li, Study Links Roundup 'Weedkiller' To Overgrowth of Deadly Fungal Toxins, Green Med Info. 9/9/2013

54. Safford, H. D., Viers, J. H., & Harrison, S. P. (2005). Serpentine endemism in the California flora: a database of serpentine affinity. Madroño, 52(4), 222-257. (serpentine soils in Marin and certain other parts of California)

55. Samsel, A., & Seneff, S. (2013). Glyphosate's suppression of cytochrome P450 enzymes and amino acid biosynthesis by the gut microbiome: Pathways to modern diseases. Entropy, 15(4), 1416-1463. (effect on P450 detoxification in humans and animals)

56. Schinasi, L., & Leon, M. E. (2014). Non-Hodgkin Lymphoma and Occupational Exposure to Agricultural Pesticide Chemical Groups and Active Ingredients: A Systematic Review and Meta-Analysis. International journal of environmental research and public health, 11(4), 4449-4527.(glyphosate and lymphoma)

57. Séralini, G. E., Clair, E., Mesnage, R., Gress, S., Defarge, N., Malatesta, M., ... & de Vendômois, J. S. (2012). Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. Food and chemical toxicology, 50(11), 4221-4231. (Roundup, a serious carcinogen in rats in very small amounts)

58. Séralini, G. E., Mesnage, R., Defarge, N., Gress, S., Hennequin, D., Clair, E., ... & de Vendômois, J. S. (2013). Answers to critics: Why there is a long term toxicity due to a Roundup-tolerant genetically modified maize and to a Roundup herbicide. Food Chem Toxicol, 53, 476-483

59. Séralini, G. E., Mesnage, R., Clair, E., Gress, S., de Vendômois, J. S., & Cellier, D. (2011). Genetically modified crops safety assessments: present limits and possible improvements. Environmental Sciences Europe, 23(1), 1-10. (glyphosate and genetically altered plants and their effects on human health)

60. Sharpe, R. M., & Irvine, D. S. (2004). How strong is the evidence of a link between environmental chemicals and adverse effects on human reproductive health?. Bmj, 328(7437), 447-451. (glyphosate and birth defects)

61. Smith, J. M. (2011). Monsanto's Roundup Triggers Over 40 Plant Diseases and Endangers Human and Animal Health. URL http://www. foodconsumer. org/newsite/Nonfood/Environment/roundup_0118110818. html. (glyphosate induced diseases in plants, humans and animals)

62. Snijders, C. H., Samson, R. A., Hoekstra, E. S., Quellet, T., Miller, J. D., Baar, A. J. M., ... & Kauffman, H. F. (1996). Analysis of Fusarium causing dermal toxicosis in marram grass planters. Mycopathologia, 135(2), 119-128. (pathological fungi in soil treated with glyphosate)

I-Fra8

63. SF Natural Areas Program, Roundup and Birth Defects. Chemical Research in Toxicology. 5/2010

64. Székács, I., Fejes, Á., Klátyik, S., Takács, E., Patkó, D., Pomóthy, J, & Székács, A. Environmental and Toxicological Impacts of Glyphosate with Its Formulating Adjuvant, World Academy of Science, Engineering and Technology; International Journal of Biological, Veterinary, Agricultural, and Food Engineering, VOl: 8: No 3, 2014 (toxicity of glyphosate to human and animal cells)

65. Székács, A., & Darvas, B. (2012). Forty years with glyphosate. Herbicides–properties, synthesis and control of weeds. Ed. Hasaneen, MNAE-G., InTech, Croatia, 247-284. (glyphosate produces many plant diseases)

66. Tominack RL, Yang GY, Tsai WJ, Chung HM, Deng JF, 1991. Taiwan National Poison Center survey of glyphosate-surfactant herbicide ingestions. J Toxicol Clin Toxicol 1991; 29 (1): 91-109) (effects of glyphosate on human ery throcytes (red blood cells)

67. Thongprakaisang, S., Thiantanawat, A., Rangkadilok, N., Suriyo, T., & Satayavivad, J. (2013). Glyphosate induces human breast cancer cells growth via estrogen receptors. Food and Chemical Toxicology, 59, 129-136. (one part per trillion of glyphosate can be an endocrine disruptor)

68. Vera, M. S., Lagomarsino, L., Sylvester, M., Pérez, G. L., Rodríguez, P., Mugni, H., ... & Pizarro, H. (2010). New evidences of Roundup®(glyphosate formulation) impact on the periphyton community and the water quality of freshwater ecosystems. Ecotoxicology, 19(4), 710-721. (effects of glyphosate on stream water and fish)

69. Vigfusson, N.V. and Vyse, E.R. (1980), "The effect of the pesticides, Dexon, Captan, and Roundup, on sister-chromatid exchanges in human lymphocytes in vitro". MUTATION RESEARCH, v.79 p.53-57.) (effects of Roundup on white blood cells)

70. Vithanage, M., Rajapaksha, A. U., Oze, C., Rajakaruna, N., & Dissanayake, C. B. (2014). Metal release from serpentine soils in Sri Lanka. Environmental monitoring and assessment, 186(6), 3415-3429. (metal release from serpentine soils- Sri Lanka)

71. Walsh, L. P., McCormick, C., Martin, C., & Stocco, D. M. (2000). Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression. Environmental health perspectives, 108(8), 769. (effects of glyphosate on testicular cell development)

72. Whittaker, R. H. (1954). The ecology of serpentine soils. Ecology, 258-288. (serpentine soils, metals and plants that grow in serpentine soils)

News Articles on the Internet and Elsewhere:

73. How To Recognize Marin County Herbicide Use. admin in Hard Truths (Pg. 17)

I-Fra8

74. The Sad Saga of Ignacio Chapela, by John Ross, Anderson Valley Advertiserwww.theava.com/04/0218-chapela.html, Feb 18, 2004

75. Study confirms GMO herbicide glyphosate contaminates groundwater. Jonathan Benson staff writer NaturalNews.com. 12/28/2011

76. Attack of the Superweed: New strains resist Roundup, the world's top-selling herbicide. Jack Kaskey, Businessweek. 9/8/2011.

77. 6 Glyphosate-resistant weeds in California. Almond Board of California. 3/20/2013

78. Guest: The failure of the EPA to protest the public from pollution. E.G. Valliantatos in The Seattle Times. 4/12/2014.

79. World renowned scientist lost his job when he warned about GE foods. www.psrast.org/pusztai.htm

80. Dr. Don Huber: GMOs and Glyphosate and Their Threat to Humanity. Carol Grieve. 4/8/2014.

81. War Over Monsanto Gets Ugly: Birth Defects, Superweeds and The Science of Intimidation. Mike Ludwig. 11/9/2010. truth-out.org/archive/.../92751:war-over-monsanto-gets-ugly

82. Tate & Lyle says aflatoxin in U.S. corn complicates grain sourcing uk.reuters.com/.../us-tateandlyle-aflatoxin-idUKBRE8A80192012.

83. Journal Retraction of Seralini GMO-Cancer Study Is Illicit, Unscientific, and Unethical. GMWatch. 11/30/2013.

84. Meet the Soil, Environmental and Atmospheric Sciences Faculty. Robert J Kremer, Ph.D. 2013

85. Report: Pesticide-Birth Defect Link Hidden from Public by European and American Governments. Rodale News. 6/8/2011.

86. Massive Increase In Babies Born Without Brains In Washington State. Sean Brown. 3/5/2014. mrconservative.com/.../35395-pesticides-cause-babies-to-be-born-withou...

87. Increase in rare defects born in babies www.foxnews.com/.../increase-rare-birth-defects-in-washington s... March 14,2014

88. http://www.naturalnews.com/044182_birth_defects_anencephaly_washington_state.html#ixzz39wst4vD1

89. 'Bizarre' Cluster of Severe Birth Defects Haunts Health Experts. Jonel Aleccia, NBC News. 2/17/2014.

I-Fra8

90. EPA to raise Allowable Glyphosate Pesticide Levels in Crops by 3,000%!. Melissa Melton, Truthstream Media. 7/28/2013.

91. Monsanto's Roundup herbicide found to destroy testosterone, male fertility. NaturalNews.com. 3/3/2012.

92. The Demise of Human Sperm: Pesticides lower sperm levels, study finds. Rita Rubin, USA Today. 6/17/2003.

93. Blamed for Bee Collapse, Monsanto Buys Leading Bee Research Firm. Anthony Gucciardi, Natural Society. 4/19/2012.

94. Sri Lanka Bans Monsanto Herbicide Citing Potential Link to Deadly Kidney Disease. Sasha Chavkin, The Center for Public Integrity. 3/19/2014.

95. Dramatic Increase in Kidney Disease in the US and Abroad Linked to Roundup (Glyphosate) 'Weedkiller'. Sayer Ji, Green Med Info. 3/14/2014.

96. Thompson, M. Human Health, Environmental and Animal Impacts of Pesticides in General and Organophosphates in Particular Including Roundup (wordpress.com)

97. Research: Roundup Herbicide Toxicity Vastly Underestimated. Sayer Ji, Green Med Info. 11/15/2012.

98. BREAKING: Glyphosate (Roundup) Carcinogenic in the PARTS PER TRILLION range. Sayer Ji, Green Med Info. 6/13/2013.

99. USGS Technical Announcement: Widely Used Herbicide Commonly Found in Rain and Streams in the Mississippi River Basin. August 29, 2011 http://www.usgs.gov/newsroom/article.asp?ID=2909#.VcZyS6ZViko

I-Fra8

INDEX ON THE EFFECTS OF GLYPHOSATE BIBLIOGRAPHY

Animals and Insects:

Journal Articles- 13, 15, 17, 18, 39, 45, 51, 52, 57, 61

News Articles- 93, 96

Attacks on Scientists:

News Articles- 74, 79, 80, 81, 83

Birth Defects:

Journal Articles- 1, 2, 9, 15, 17, 19, 20, 23, 25, 26, 32, 38, 40, 48, 49, 60, 63

News Articles- 81, 85, 86, 87, 88, 89

Botulism:

Journal Articles- 34, 50

Cancer:

Journal Articles- 28, 33

News Articles- 83, 98

Effects on Body:

Journal Articles- 4, 7, 35, 41, 59, 61, 66, 67, 69

EPA's Lack of Role in Informing the Public:

News Articles-78, 85, 90

Fertility:

Journal Articles- 8, 12, 46, 71

News Articles- 91, 92

Glyphosate In Soil, Streams, and Air:

Journal Articles- 3, 6, 5, 10, 14, 21, 30, 31, 37, 53, 54, 62, 65, 68, 70, 72, 75, 76, 77, 82, 84

I-Fra8

Kidney Disease:

Journal Articles- 29

News Articles- 94, 95

Lymphoma:

Journal Articles- 42, 47, 56

Marin County:

Journal Articles- 27

News Articles- 73

Toxicity:

Journal Articles- 11, 16, 24, 36, 43, 44, 55, 58, 64

News Articles- 97

Comment Materials I-Fra8 — Fraser, Mary

Response 1

The commenter provided a list of documents and an index by email but did not explain how they relate to the PEIR analysis. Therefore, no response can be provided or is required.

I-Joh

Comment from Dale Johnson

i have lived in Bolinas since Jan 3 1982.

This has not been a bad year for mosquitoes.
i want you to maintain the agreement you made with WestMarin citizens to consider our area ORGANIC which it is..spraying would destroy the organic farm certification. 1

DO NOT PUT SPRAY ON US...
Sincerely, Dale Johnson

Comment Letter I-Joh — Johnson, Dale

Response 1

For many years, the District has cooperated closely with organic growers and businesses throughout the Service Area. As a result, no adverse consequences to organic agriculture have been reported. As part of the Proposed Program, the District will continue its consultation and cooperation with organic growers and businesses. Vector control materials certified and labeled for organic use will be used as appropriate in conjunction with organic operations and accompanying organic operation plans.

The District uses several biorational formulations of mosquito larvicides that contain three bacterial active ingredients that are found in nature. Certain formulations containing these active ingredients are labeled for use with organic crops by the Organic Materials Review Institute and the US EPA. Examples of bacteria pathogenic to mosquitoes are *Bacillus sphaericus* (Bs), the several strains of *Bacillus thuringiensis israelensis* (Bti), and *Saccharopolyspora spinosa*. Two bacteria, Bs and Bti, produce proteins that are toxic to most mosquito larvae, while *Saccharopolyspora spinosa* produces compounds known as spinosyns, which effectively control all larval mosquitoes. Bs can reproduce in natural settings for some time following release. The Bti materials the District applies do not contain live organisms but only spores made up of specific protein molecules. All three bacteria are naturally occurring soil organisms that are also commercially produced for use as mosquito larvicides. These are the only three active ingredients approved for use in controlling larval mosquitoes when organic production is in progress.

One adult mosquito control product used by the District (Merus™ 2.0) is approved (by OMRI and the USDA National Organics Program) for use in conjunction with organic operations.

With respect to the issue raised by the commenter about chemical treatments for vector control in West Marin, the District operates under the terms of an agreement with a group known as the West Marin Mosquito Council (WMMC). The most recent version of the Agreement was approved by the District and WMMC in May 2016. The Agreement recognizes that the District may apply all the bacterial products discussed above, plus certain non-organic products, namely Agnique MMF, S-methoprene, and mosquito larvicide oils such as CoCoBear™.

As discussed initially in the PEIR Program Description (Section 2.3.5.1.1), Agnique MMF is a very thinly applied (monomolecular) film product that acts as a larvicide and pupicide. Similarly, mosquito larvicide oils such as CoCoBear™ are applied as larvicides and pupicides where needed and appropriate. The WMMC Agreement also permits the use of s-methoprene, a mosquito growth regulator discussed in the PEIR Section 2.3.5.1.1. In the geographical region referred to by the commenter (West Marin), s-methoprene is applied only when necessary to Onsite Wastewater Treatment Systems (OWTS), also known as septic systems, to control larval mosquitoes. Under the District's policies and practices, Agnique, s-methoprene and larvicide oils are always used judiciously and in such a manner as not to interfere in any way with organic agriculture or production.

I-Leg

Comment from Dan Legacy

Please stop spraying in West Marin. I 1

Dan Legacy
Bolinas

Comment Letter I-Leg — Legacy, Dan

Response 1

With respect to the issue raised by the commenter about chemical treatments for vector control in West Marin, the District operates under the terms of an agreement with a group known as the West Marin Mosquito Council (WMMC). The most recent version of the Agreement was approved by the District and WMMC in May 2016. The Agreement recognizes that the District may apply all the bacterial products discussed above, plus certain non-organic products, namely Agnique MMF, S-methoprene, and mosquito larvicide oils such as CoCoBear.

As discussed initially in the PEIR Program Description (Section 2.3.5.1.1), Agnique MMF is a very thinly applied (monomolecular) film product that acts as a larvicide and pupicide. Similarly, mosquito larvicide oils such as CoCoBear are applied as larvicides and pupicides where needed and appropriate. The WMMC Agreement also permits the use of s-methoprene, a mosquito growth regulator discussed in the PEIR Section 2.3.5.1.1. In the geographical region referred to by the commenter (West Marin), s-methoprene is applied only when necessary to Onsite Wastewater Treatment Systems (OWTS), also known as septic systems, to control larval mosquitoes. Under the District's policies and practices, Agnique, s-methoprene and larvicide oils are always used judiciously and in such a manner as not to interfere in any way with organic agriculture or production.

I-Per

Comment from Michael Peri

We are adverse to any chemical spraying in West Marin. We support organic alternatives, and will do all in our power to bring conscious solutions to the table

1

Comment Letter I-Per — Peri, Michael

Response 1

The District uses several biorational formulations of mosquito larvicides that contain three bacterial active ingredients that are found in nature. Certain formulations containing these active ingredients are labeled for use with organic crops by the Organic Materials Review Institute and the US EPA. Examples of bacteria pathogenic to mosquitoes are *Bacillus sphaericus* (Bs), the several strains of *Bacillus thuringiensis israelensis* (Bti), and *Saccharopolyspora spinosa*. Two bacteria, Bs and Bti, produce proteins that are toxic to most mosquito larvae, while *Saccharopolyspora spinosa* produces compounds known as spinosyns, which effectively control all larval mosquitoes. Bs can reproduce in natural settings for some time following release. The Bti materials the District applies do not contain live organisms but only spores made up of specific protein molecules. All three bacteria are naturally occurring soil organisms that are also commercially produced for use as mosquito larvicides. These are the only three active ingredients approved for use in controlling larval mosquitoes when organic production is in progress.

One adult mosquito control product used by the District (Merus 2.0) is approved (by OMRI and the USDA National Organics Program) for use in conjunction with organic operations.

With respect to the issue raised by the commenter about chemical treatments for vector control in West Marin, the District operates under the terms of an agreement with a group known as the West Marin Mosquito Council (WMMC). The most recent version of the Agreement was approved by the District and WMMC in May 2016. The Agreement recognizes that the District may apply all the bacterial products discussed above, plus certain non-organic products, namely Agnique MMF, S-methoprene, and mosquito larvicide oils such as CoCoBear.

As discussed initially in the PEIR Program Description (Section 2.3.5.1.1), Agnique MMF is a very thinly applied (monomolecular) film product that acts as a larvicide and pupicide. Similarly, mosquito larvicide oils such as CoCoBear are applied as larvicides and pupicides where needed and appropriate. The WMMC Agreement also permits the use of s-methoprene, a mosquito growth regulator discussed in the PEIR Section 2.3.5.1.1. In the geographical region referred to by the commenter (West Marin), s-methoprene is applied only when necessary to Onsite Wastewater Treatment Systems (OWTS), also known as septic systems, to control larval mosquitoes. Under the District's policies and practices, Agnique, s-methoprene and larvicide oils are always used judiciously and in such a manner as not to interfere in any way with organic agriculture or production.

I-Spe

Comment from Suzanne Speh

I live [redacted] in Inverness Park, though I believe technically my address is in Point Reyes Station. Last year I wrote a letter to Cicely Muldoon at Point Reyes National Seashore about my concerns with the huge mosquito problem we were having (I have copied the text of that e-mailbelow). I believe she contacted the mosquito district at that time.
This year the mosquitos have been MUCH less of a problem. I saw that you were asking for public comment prior to October 1st and that is the reason for this e-mail.
I would like you to take whatever steps necessary to keep the mosquito population in this area to a minimum. I feel that your studies will yield solutions that have the lowest environmental impact - you are the experts. I support your recommendations. I do NOT support keeping all chemicals out of West Marin at the expense of not being able to leave my house six months out of the year due to an excessive mosquito population. 1

Thank you

Suzanne Speh
[redacted]

Hi Cicely

When the park was originality planning the conversion of the Giacomini pasture to wetlands, many of us were concerned with the potential for an out of control mosquito population, The first few years were great and we thought we had been worried for no reason. Last year was a little worse, but this year is a nightmare. I live [redacted] in Inverness Park. I frequently walk to Point Reyes in the morning for yoga. We are also in the process of purchasing a second property on Laurel Street. I cannot go outdoors, even in the middle of the day, without being eaten alive by mosquitos. I can no longer walk to Point Reyes because I get so many bites while walking along SFD and the path by White House pool that it drives me right out of my mind - dozens of bites and right through my clothes. I can't garden - just went out for a few minutes to harvest beans for dinner and got at least a half dozen bites. Everyone I talk to who lives or has cause to be on or near properties near the Inverness Park side of the wetland agrees that it has become miserable. I can no longer enjoy my property or the surrounding outdoors. I hope the Park can figure out some way to abate this problem or living here is going to become impossible. It's really not fair as we were here long before the wetland.

Sincerely
Suzanne Speh
[redacted]

Comment Letter I-Spe — Speh, Suzanne

Response 1

The commenter notes that she observed high numbers of mosquitoes and aggressive biting during 2014. She also complains of the partial loss of enjoyment of her property and of the pain caused by multiple bites when outdoors. She suggests that the mosquitoes may be originating from the former pasture land that was converted to wetlands as part of the Giacomini Wetland Restoration Project. The commenter notes that the mosquito populations were lower during the subsequent year (2015).

The commenter's assertions are supported by the observations of District staff during this timeframe and data generated by the District's mosquito surveillance program. It should be noted that the land in question is under the jurisdiction of the National Park Service, specifically the Point Reyes National Seashore. The commenter includes a letter that she sent to the Superintendent of the National Seashore, complaining about the problem. During this 2014 period, District staff also received telephone calls and service requests from other residents of this area.

Although the District periodically conducts surveillance for mosquito species and abundance under a Scientific Collecting Permit, the District is not authorized to conduct mosquito control operations on these federal lands. However, during the design phase of the wetland restoration project, District staff provided technical advice intended to minimize the potential for production of mosquito species (physical control – source reduction measures) referenced by the commenter and collected in the District's mosquito traps.

As the commenter notes, the Point Reyes National Seashore, which employs biologists, is responsible for control of mosquitoes on its property. Although the District enjoys a cooperative working relationship with Seashore staff, it lacks the authority to take direct action to reduce mosquito populations. Annual and seasonal fluctuations in mosquito breeding and populations are normal for the type of wetland, where the potential for mosquito production is high.

During 2014, at the request of the US Fish and Wildlife Service, a District staff member traveled to Bandon, Oregon, to provide technical advice in the case of a newly restored wetland that produced salt marsh mosquitoes (e.g., *Aedes dorsalis*) in extremely high numbers. This type of mosquito can travel up to 20 miles to find a blood meal, and the situation became so dire that the nearby town was featured on the national news for several weeks until the problem was remedied. The Bandon Marsh problem was described in the PEIR in Section 15.4.2 No Chemical Control Program as an example of what can happen when there is no chemical control in a habitat restoration area adjacent to urban development.

The commenter requests that the District not refrain from the use of chemical means to reduce what she cites as excessive mosquito populations. It should be noted that in the locality described by the commenter, due to the jurisdictional issue described above, the District is unable to apply all the components of its full Integrated Vector Management Program, particularly chemical means of mosquito control, on National Park Service lands.

However, the District will provide mosquito surveillance and control in relation to private and public properties in the Inverness area and respond to service requests. The District also attempts to assist residents and visitors to this area and encourage mosquito source reduction through the education and outreach components of the IVMP. This is accomplished via the distribution of brochures, fact sheets, newsletters, participation in local events and fairs, presentations to community organizations, newspaper and radio advertising, public service announcements, social media postings, District website postings, and contact with District staff in response to service requests.