GLYPHOSATE:
A SELECTIVE REVIEW
OF RECENT RESEARCH

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INTRODUCTION

Discussions between citizens and Vermont Pesticide Advisory Council in the last year about railroad vegetation management have occasionally focused on glyphosate, the principle (or active) ingredient in Roundup, Accord, Rodeo and similar herbicides used on railroad, highway and utility rights-of-way (ROWs), as well as in corn and soybean production. Water quality staff consider glyposate to be less dangerous than other herbicides in the environment or to humans, and have asked the question: "what is the nexus between glyphosate, water quality and railroads?" It's a fair question, and one I hope to address in this paper, which provides summaries of recent research findings on glyphosate-based herbicides, and attempts to "make the connections."

HUMAN HEALTH

Endocrine Disruption in Humans

Glyphosate-based herbicides are toxic to human cell lines at concentrations considerably below concentrations used in agriculture. They also caused human cell endocrine disruption from 0.5ppm on the androgen receptor in some cells, and from 2ppm, certain endocrine activities on both estrogen receptors were inhibited. Part of a complex interplay of hormones and enzymes in mammals, aromatase transcription was disrupted from 10ppm. Toxic effects to the cells were seen at 10ppm, and DNA damages were seen at 5ppm. These findings urge reconsideration of levels of glyphosate residues allowed in food for humans, and in the environment.

*[*Gasnier C et al (2009). Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. Toxicology 262; 184-191. *http://www.ncbi.nlm.nih.gov/pubmed/19539684]*

"Glyphosate is toxic to human placental cells within 18 hours with concentrations lower than those found in agricultural use, and this effect increases with concentration and time or in the presence of Roundup adjuvants." Glyphosate inhibited aromatase activity with a median inhibiting concentration of 0.04% with Roundup. The added ingredients in Roundup greatly facilitated glyphosate penetration of the cell membrane. Exposure of cells to Roundup also resulted in a decrease of the cytochrome P450 enzymes at concentrations 100 times lower than the recommended use in agriculture. These enzymes are present in most tissues of the body and play important roles in hormone synthesis.

*[*Richard, Sophie et al (2005). Environmental Health Perspectives 113: 6; 716-720. *http://www.ncbi.nlm.nih.gov/pubmed/15929894]*

Damage to Human Gut Microbiome & Relation to Human Health

Cytochrome P450 or CYP enzymes have a critical role in helping the human body to detoxify foreign organisms and toxic substances. Glyphosate inhibits CYP enzymes and thus enhances the damaging effects of other environmental toxins in our bodies, making us more susceptible to disease. Its mode of action is disruption of the shikimate pathway used by plants, but can also affect our gut bacteria, which are biologically similar to plants. These gut bacteria aid in digestion, synthesize vitamins, detoxify xenobiotics, and assist the immune system in maintaining health. Clear evidence is offered that glyphosate

disrupts gut biota and suppresses the CYP enzyme class, contribute to serious chronic diseases such as obesity, autism, Alzheimer's, depression, Parkinson's, liver disease, cancer. Periodically EPA raises the level of accepted residues in animal feed and food for humans in order to accommodate increased industry use, without monitoring actual food levels, and without concern for human health.
*[*Samsel, Anthony and Stephanie Seneff (2013). Glyphosate's Suppression of Cytochrome P450 Enzymes and Amino Acid Biosynthesis by the Gut Microbiome: Pathways to Modern Diseases. Entropy 15; 1416-1463. *http://www.mdpi.com/1099-4300/15/4/1416]*

Chronic Kidney Disease

Glyphosate can combine with metals in hard drinking water supplies to cause chronic kidney disease (CKD), destroying kidney tissue in farmers living where certain metals in water are toxic to the liver. This syndrome has been observed in Sri Lanka and in Central America. The contaminants in the water contributing to CKD are arsenic, cadmium and pesticides. Dehydration due to high temperatures, drinking hard water and lack of adequate hydration are associated factors. (Some areas of Vermont have naturally occurring arsenic in the water.)
[Jayasumana C et al (2014). Glyphosate, Hard Water and Nephrotoxic Metals: Are They the Culprits... International Journal of Environmental Research and Public Health 11; 2125-2147 *http://www.mdpi.com/1660-4601/11/2/2125]*

Birth Defects

In areas of large uses of glyphosate-based herbicides in Argentina, increased birth defects have been noted. Scientists find that glyphosate in highly diluted solutions (430 micromolars in 1/5000 agricultural concentration) caused brain malformations and nervous system abnormalities in frogs in the laboratory. Early frog embryos injected with pure glyphosate showed the same defects, indicating that glyphosate rather than surfactant was responsible. Chick embryos suffered similar defects, with loss of eyes and smaller heads. Glyphosate increased retinoic acid (RA), an oxidized form of Vitamin A, and also inhibited several genes involved in cranial development, causing failure of forebrain development into two lobes. RA activity is regulated by CYP26 enzymes, members of the cytochrome P450 family, which are inhibited by glyphosate.
[Paganelli A et al (2010). Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling. Chemical Research in Toxicology 23: 1586-1595. *http://pubs.acs.org/doi/abs/10.1021/tx1001749*] See also: Mae-Wan H (2010). Lab Study establishes glyphosate link to birth defects. http://nfuontario.ca/upload/files/userfiles/12542-lab-study-establishes-glyphosate-link-to-birth-defects.pdf

Non-Hodgkins Lymphoma

The association between glyphosate, Roundup and different types of cancer has been the subject of much research internationally. The International Agency for Research on Cancer has come to a consensus that glyphosate is a Class 2A, or probable human carcinogen, based on what they have learned from a survey of many studies. Researchers in Sweden found a significant association between exposure to glyphosate and small lymphocytic lymphoma, chronic lymphocytic lukaemia, as well as with B-cell lymphoma. T-cell lymphomas were associated with exposure to all herbicides.
[Eriksson M et al (2008). Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis. International Journal of Cancer 123; 1657-1663. *http://onlinelibrary.wiley.com/doi/10.1002/ijc.23589/pdf]*

ENVIRONMENTAL FATE & MOVEMENT

Atmospheric transport

The atmosphere is an integral part of the hydrologic cycle, moving pesticides across wide areas through either volatilization or through pesticide-contaminated dust particles. Rain can remove such particles to the earth some distance from their original place of application. US Geological Services scientists monitored glyphosate and its degradate aminomethylphosphonate acid (AMPA) in air and rain samples during the growing season in the Mississippi delta in 1995 and again in 2007. Samples in air and rain were derivatized and analyzed by online Solid Phase Extraction (SPE) coupled to liquid chromatography/ mass spectometry. Glyphosate was detected in 86% of air samples and in 77% of rain samples in 2007 but were not included in 1995 tests. Highest concentrations of glyphosate and AMPA occurred in April and May; but there were detectable amounts in the air during the rest of the season. Total herbicide contamination was greater in 2007 than in 1995, and was dominated by glyphosate, due to increase in genetically modified crops.
[Majewski MS et al, 2014. Pesticides in Mississippi air and rain: comparison between 1995 and 2007. Environmental Toxicology and Chemistry 33: 6; 1283-1293.
http://www.ncbi.nlm.nih.gov/pubmed/24549493]

Transport to waters

Glyphosate and its breakdown product aminomethyl-phosphonate acid (AMPA) were found in effluent downstream from wastewater treatment plants (WWTPs) at higher rates than in surface waters upstream of such facilities. The study may have been the first to document that urban use of glyphosate contributes to glyphosate and AMPA in surface waters, and also suggests that glyphosate and AMPA are more mobile and persistent in aquatic environ-ments than previously believed.
[Kolpin DW et al (2006). Urban contributions of glyphosate and its degradate AMPA to streams in the U.S.A. Science of the Total Environment 354; 191-197.
http://www.ncbi.nlm.nih.gov/pubmed/16398995]

Glyphosate and AMPA were the most common contaminants in the Orge River watershed in France during a 2007-2008 study. Samples taken from surface water, wastewater, storm sewer and wastewater treatment plants showed glyphosate and AMPA at levels above the *European standard of 0.1ug/L for drinking water.* Railroad and highway applications were established as the source of the contaminants, which were highest during rainfall events. During dry periods detergent seemed to be the main source of AMPA in surface waters.
[Botta F et al (2009). Transfer of glyphosate and its degradate AMPA to surface waters through urban sewerage systems. Chemosphere 77: 1; 133-139.
http://www.ncbi.nlm.nih.gov/pubmed/19482331]

Maine Board of Pestcide Control (BPC) monitored glyphosate runoff into surface waters from railways to determine whether their 10-foot buffer adequately protects waters and whether glyphosate was leaching into groundwater. Due to several problems the results of the study were inconclusive. The BPC decided *not* to allow variances from the *10-foot buffer* until railroad applicators submitted a new quality monitoring plan for approval.
[Maine Board of Pesticide Control (2003). A Pilot Study: Railroad Right of Way Herbicides and Maine's Surface and Ground Water.
www.maine.gov/dacf/php/pesticides/documents2/water_quality/rightofway.pdf]

Glyphosate movement into surface waters from railbeds was studied in England. Fifteen micrograms per liter (ug /L or ppb) glyphosate were detected in water 6 days after treatment, and after 81 days, 0.8 ug/L was found.

[Ramwell CT et al (2004). Herbicide loss following application to a railway. Pest Management Science 60: 6; 556-564. *http://onlinelibrary.wiley.com/doi/10.1002/ps.850/abstract]*

Groundwater contamination may be less frequently found than surface water. Research in Europe has developed testing protocols for detecting glyphosate at parts per trillion. Efforts in groundwater monitoring in Catalonia (northeast Spain) in real groundwater samples have found glyphosate in 41% of samples with concentrations as high as 2.5ug/L (above EU drinking water standard) and a mean concentration of 200 ng/L.
[Sanchis J et al (2012). Determination of glyphosate in groundwater samples... Analytical and Bioanalytical Chemistry 402: 7; 2334-2345. *http://www.ncbi.nlm.nih.gov/pubmed/22101424]*

Scientists with Ohio EPA found that spring applications of glyphosate used on Ohio cornfields adjacent to Lake Erie were associated with algal blooms in the Lake. The phosphonate component averages about 15% of glyphosate, which can be available as a nutrient source for algae living in the lake.
[Ohio Lake Erie Phosphorus Task Force Report 2010. p.41.
*http://epa.ohio.gov/portals/35/lakeerie/ptaskforce/Task_Force_Final_Report_April_2010.pd*f]

AQUATIC WILDLIFE

Glyphosate has differing toxicities to three species of sturgeon. Longer exposure times caused greater toxic effects and lower glyphosate amounts were needed to reach lethality. The POEA surfactant in Roundup formulation is the more toxic ingredient to aquatic organisms. Technical grade glyphosate is less toxic as pH increased, but Roundup became more toxic as pH increased from 6.5 to 9.5. With increased exposure times and higher glyphosate concentrations, abnormal behaviors increased. Glyphosate may reduce populations by decreasing fry mass and size of yolk sac, and by initiation of unsafe behaviors.
[Filizadeh Y and Rajabi Islami H. (2010). Toxicity determination of three sturgeon species exposed to glyphosate. Iranian Journal of Fisheries Sciences 10: 3; 383-392. *http://www.jifro.ir/browse.php?a_id=211&sid=1&slc_lang=en]*

Roundup exposure at 10 mg/L for 6, 24 and 96 hours caused genotoxic damage in erythrocytes (red blood cells) and in gill cells of the tropical fish *Prochilodus lineatus.* Effects on branchial cells were considerably higher than controls after 6 and 24 hours with the comet assay. The comet assay with gill cells proved to be an important tool to reveal DNA damage in fish tissues in periods of exposure not revealed by tests with erythrocytes (red blood cells).
[Cavalcante D.G.S.M. et al (2008). Genotoxic effects of Roundup on the fish *Prochilodus lineatus.* Mutation Research 655: 41-46. *http://www.uel.br/laboratorios/lefa/Cavalcante%20et%20al%202008%20in%20press.pdf]*

Glyphosate caused several toxic effects in juvenile Nile tilapia fish: tissue changes in gills, liver, kidney and brain. The lethal concentration for the 96 hour EC50 value was 1.05 mg/L. At 2mg/L effects were seen in gills, liver and kidney. Respiratory distress, erratic swimming, excessive secretion of mucus, weakness, and instant death were observed at concentrations of 9, 30, 97 and 310 mg/L. In gill tissues, glyphosate caused impairment in gaseous exchange efficiency and other anomalies.
[Ayoola S.O (2008). Toxicity of glyphosate herbicide on Nile tilapia *(Oreochromis niloticus)* juvenile. African Journal of Agricultural Research 3: 12; 825-834.
http://www.academicjournals.org/article/article1380968357_Ayoola.pdf]

Fish exposed to 4mg/L glyphosate (amount used in agriculture) for 45 days experienced severe damage, shrinkage, and degeneration of epithelial cells in the gills. Among other tissue disturbances, damage to cells in the esophagus and severe mucus secretion in the stomach were observed. Glyphosate reduced the enzymes amylase, lipase and protease involved in removing toxins and in digestive processes in the esophagus, stomach and intestine.
[Senapati, T et al (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. *http://www.academia.edu/1058878/Observation_on_the_effects_of_glyphosate_on_fish*]

Frogs exposed to environmentally relevant concentrations of glyphosate-based herbicide formulations showed changes in length of snout, increased time to metamorphosis, tail damage and gonadal abnormalities. These effects were caused by disruption of hormone signaling. Roundup was the most toxic to frogs; the least acutely toxic formulations were Roundup Bioactive, Touchdown and Glyfos BIO, because of different surfactants. Surfactant composition must be considered in evaluation of glyphosate-based herbicides.
[Howe CM et al (2004). Toxicity of glyphosate-based pesticides to four North American Frog Species. Environmental Toxicology & Chemistry 23; 1928-1938
http://www.ncbi.nlm.nih.gov/pubmed/15352482]

Glyphosate, the surfactant POEA and the formulation Roundup were all found to be genotoxic to blood cells of the European eel. Different groups of animals were exposed to 58 and 116 ug/L Roundup, 17.9 and 35.7 ug/L glyphosate, and 9.3 and 18.6 ug/L POEA. Those eels exposed to the lower concentration of the commercial formulation showed significantly higher levels of oxidative damage than those exposed to either the active ingredient or the surfactant. The study demonstrated that most of the genotoxicity comes from the surfactant itself as well as the genotoxic risk of both glyphosate and the POEA surfactant alone.
[Guilherme S et al (2012). Differential genotoxicity of Roundup formulation and its constituents in blood cells of fish... Ecotoxicology 21: 1381-1390. *http://link.springer.com/article/10.1007/s10646-012-0892-5#page-1]*

EFFECTS ON POLLINATORS

Recent research indicates that exposure of bees to field-realistic agricultural concentrations of glyphosate desensitizes bees to sucrose in their foraging areas and causes a significant decrease in memory retention, impairing the bees' learning ability. Foraging bees may be bringing glyphosate-contaminated nectar to the hive, with long-term harm for the hive.
[Herbert LT et al (2014). Effects of field-realistic doses of glyphosate on honeybee appetite and behaviour. Journal of Experimental Biology 217: 3457-3464.
http://jeb.biologists.org/content/jexbio/217/19/3457.full.pdf]

IN SUMMARY

We see, then, from a sampling of independent researchers, that glyphosate has dangers for life now and for future generations: it is a carcinogen, an endocrine disruptor, an air and rain contaminant, a food contaminant, a danger for pollinators, a groundwater contaminant, surface water contaminant and problem for aquatic ecosystems, as well as a possible source of phosphorus not yet accounted for in the TMDL for Lake Champlain. We can no longer assume that glyphosate is less dangerous than other herbicides or that we have no other options.

What is the "nexus" between glyphosate and water quality? Glyphosate gets into water (even if VAAFM does not test for it); it is toxic to human cells at low concentrations (far below EPA's lax drinking water standard of 700ppb), damages digestive biota and enzymes necessary for immune function, can cause cancer, is toxic to fish and pollinators. It may be entering drinking water via Lake Champlain but is not included in tests of drinking water.

The absence of monitoring for glyphosate in waters of the State cannot be construed to mean an absence of glyphosate contamination. VAAFM's difficulty in detecting glyphosate raises questions since it is the most widely used herbicides in the state and US Geological Services are able to detect it in the Midwest. Water quality monitoring between 2001 and 2007 shows that ROW herbicides do indeed enter the waters of the State, contrary to conditions in ROW permits.

The Public Trust Doctrine tells us that waters are to be protected by the State for all people and for future generations. VT Law instructs VPAC to develop benchmarks for reduction of pesticide use and increase in areas managed by other methods.

The nexus is that we all share the water and the air, the water moves, glyphosate moves with soil and water, we are not separate from the water, and we share that water with all life and future generations. Glyphosate has no place in the waters essential for life.