GLYPHOSATE - SCIENCE AND HARM

This is a supplement to the: TEMPLATE for use by MA towns to adopt a "NEW Organic Land Management Regulation for Turf and Landscapes on Town-Owned/Operated Property"; "Alternatives To Glyphosate-Based Herbicides"; "Massachusetts Towns with Glyphosate Controls"; and "1ppt Concentration POSTER". While those documents cite a considerable amount of important scientific literature, there was a need for something to enable a quick review of some older and of some newer research reports. Here, we begin with a 2015 review of scientific literature. Importantly, that article attracted the attention of U.S. Congressman Ted Lieu who recognized the egregious nature of this herbicide; he arranged for a 2016 GEO-led team of scientists to meet/educate Congressional Staff and EPA Scientific Staff in DC; he later issued a press release recommending the public to stop using RoundUp® because of the risk of cancer; and requested investigation of U.S. government agencies that had allowed registration and marketing of glyphosate-based herbicides. This underscores the value of educating ourselves and government decision-makers; in this case, it also paved the way for GEO's legislative and other activities to restrict glyphosate use in CA and elsewhere. These efforts continued after GEO relocated to Massachusetts in 2018 and joined forces in mid-2019 with POCCA Cape Cod (already active against glyphosate in MA). A major collaborative effort of POCCA and GEO (that includes this document) has become the ways and means for engaging town governments to replace applications of glyphosate (and other toxic pesticides) with organically-based practices on town-owned/operated properties. For this current effort, scientific literature is key — as is a broader understanding of the impact of glyphosate on people, animals, microbes, the general environment and the climate crisis. Hopefully, this collection of selected publications will help to fill-in lingering knowledge gaps; becoming better informed is the prelude to making change happen.

INTRODUCTION ——————————————————————————————

1) Frantz S. 2015. **Misery in a bottle.** *LA Progressive:* October 20, 2015.
https://www.laprogressive.com/glyphosate/

A thoroughly documented 2015 review of: glyphosate science; associated health, environmental and political issues; organic importance; and advocacy for glyphosate to be added to California's Prop 65 list of carcinogens (it was added to the list about a year later).

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2) Rushton S, A Spake & L Chariton. 2016. **The unintended consequences of using glyphosate (the main ingredient in the herbicide Roundup).** Sierra Club, January 2016.
https://content.sierraclub.org/grassrootsnetwork/sites/content.sierraclub.org.activistnetwork/files/teams/documents/The_Unintended_Consequences_of_Using_Glyphosate_Jan-2016.pdf

An excellent, well-documented review paper, covering: history; mobility in soil and water; environmental persistence, fate, and deleterious effects on numerous species (including people, especially children); and the advent of "super weeds".

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3) Montgomery, D. 2017. **Sustaining life - from soil microbiota to gut microbiome.** *Pesticides and You:* Summer 2017, 17pp.
https://beyondpesticides.org/assets/media/documents/journal/bp-37.2-su17%20SustainingLife.pdf

This piece contains excerpts from a talk at Beyond Pesticides' 35th National Pesticide Forum, "Healthy Hives Healthy Lives, Healthy Land: Ecological and Organic Strategies for Regeneration" by David Montgomery, PhD, a McArthur Fellow, professor of geomorphology at the University of Washington, and author of several books, including Growing a Revolution, The Hidden Half of Nature, and Dirt. Rachel Carson, in her book Silent Spring wrote, "By their very

nature, chemical controls are self-defeating, for they have been devised and applied without taking into account the complex biological systems against which they have been blindly hurled. The chemicals may have been pretested against a few individual species, but not against living communities." In this vein, Dr. Montgomery, in this talk, brings modern scientific understanding to one of the most critical public health and environmental issues of the modern era—how complex microbial or biological systems that Ms. Carson identified are essential to the health of the soil microbiota and the gut microbiome in humans. Dr. Montgomery's complete talk can be viewed on Beyond Pesticides' YouTube channel at bp-dc.org/Forum17.

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4) Anon. 2019. **Monsanto papers/Secret documents**.
https://www.baumhedlundlaw.com/toxic-tort-law/monsanto-roundup-lawsuit/monsanto-secret-documents/

The collection of documents known as The Monsanto Papers or The Monsanto Secret Documents are available and explain Monsanto and EPA ill deeds in detail. Baum Hedlund Aristei & Goldman is one of the leading law firms representing people across the nation in lawsuits against Monsanto. These personal injury and wrongful death lawsuits claim that exposure to the herbicide weed killer, Roundup, causes non-Hodgkin's lymphoma (NHL). These documents, obtained via Discovery (pre-trial civil procedure allowing the parties to obtain evidence from each other) allow people to see what is happening "behind the curtain" of secrecy that normally shrouds ongoing litigation. This collection provides links to internal Monsanto emails, text messages, company reports, studies and other memoranda.

USAGE

5) Benbrook, CM. **Trends in glyphosate herbicide use in the United States and globally.** *Environ Sci Eur:* 28, 3 (2016). doi:10.1186/s12302-016-0070-0
https://enveurope.springeropen.com/articles/10.1186/s12302-016-0070-0

Since 1974 in the U.S., over 1.6 billion kilograms of glyphosate active ingredient have been applied, or 19 % of estimated global use of glyphosate (8.6 billion kilograms). Globally, glyphosate use has risen almost 15-fold since so-called "Roundup Ready," genetically engineered glyphosate-tolerant crops were introduced in 1996. Two-thirds of the total volume of glyphosate applied in the U.S. from 1974 to 2014 has been sprayed in just the last 10 years. The corresponding share globally is 72 %. In 2014, farmers sprayed enough glyphosate to apply ~1.0 kg/ha (0.8 pound/acre) on every hectare of U.S.-cultivated cropland and nearly 0.53 kg/ha (0.47 pounds/acre) on all cropland worldwide. Genetically engineered herbicide-tolerant crops now account for about 56 % of global glyphosate use. In the U.S., no pesticide has come remotely close to such intensive and widespread use. This is likely the case globally, but published global pesticide use data are sparse. Glyphosate will likely remain the most widely applied pesticide worldwide for years to come, and interest will grow in quantifying ecological and human health impacts. Accurate, accessible time-series data on glyphosate use will accelerate research progress.

HEALTH EFFECTS

6) IARC. 2015. **Some organophosphate insecticides and herbicides/ IARC Working Group on the Evaluation of Carcinogenic Risks to Humans.** *IARC monographs on the evaluation of carcinogenic risks to humans*: V.112. Lyon, France - 2017.
https://monographs.iarc.fr/wp-content/uploads/2018/07/mono112.pdf

Lyon, France, 20 March 2015 – The International Agency for Research on Cancer (IARC), the

specialized cancer agency of the World Health Organization, has assessed the carcinogenicity of five organophosphate pesticides. The herbicide glyphosate and the insecticides malathion and diazinon were classified as probably carcinogenic to humans (Group 2A).

For the herbicide glyphosate, there was limited evidence of carcinogenicity in humans for non-Hodgkin lymphoma. The evidence in humans is from studies of exposures, mostly agricultural, in the USA, Canada, and Sweden published since 2001. In addition, there is convincing evidence that glyphosate also can cause cancer in laboratory animals. On the basis of tumours in mice, the United States Environmental Protection Agency (U.S. EPA) originally classified glyphosate as possibly carcinogenic to humans (Group C) in 1985. After a re-evaluation of that mouse study, the U.S. EPA changed its classification to evidence of non-carcinogenicity in humans (Group E) in 1991. The US EPA Scientific Advisory Panel noted that the re-evaluated glyphosate results were still significant using two statistical tests recommended in the IARC Preamble. The IARC Working Group that conducted the evaluation considered the significant findings from the U.S. EPA report and several more recent positive results in concluding that there is sufficient evidence of carcinogenicity in experimental animals. Glyphosate also caused DNA and chromosomal damage in human cells, although it gave negative results in tests using bacteria. One study in community residents reported increases in blood markers of chromosomal damage.

Glyphosate currently has the highest global production volume of all herbicides. The largest use worldwide is in agriculture. The agricultural use of glyphosate has increased sharply since the development of crops that have been genetically modified to make them resistant to glyphosate. Glyphosate is also used in forestry, urban, and home applications.

What does Group 2A mean?
Group 2A means that the agent is probably carcinogenic to humans. This category is used when there is limited evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in experimental animals. Limited evidence means that a positive association has been observed between exposure to the agent and cancer but that other explanations for the observations (called chance, bias, or confounding) could not be ruled out. This category is also used when there is limited evidence of carcinogenicity in humans and strong data on how the agent causes cancer.(micronuclei) after glyphosate formulations were sprayed nearby.

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7) Portier CJ, BK Armstrong, BC Baguley, et al. 2016. **Differences in the carcinogenic evaluation of glyphosate between the International Agency for Research on Cancer (IARC) and the European Food Safety Authority (EFSA)**. *J Epidem Commun Health:* 70 (8). https://jech.bmj.com/content/70/8/741.full

The International Agency for Research on Cancer (IARC) Monographs Programme identifies chemicals, drugs, mixtures, occupational exposures, lifestyles and personal habits, and physical and biological agents that cause cancer in humans and has evaluated about 1000 agents since 1971. For Monograph 112, 17 expert scientists evaluated the carcinogenic hazard for four insecticides and the herbicide glyphosate. The WG concluded that the data for glyphosate meet the criteria for classification as a probable human carcinogen. The European Food Safety Authority (EFSA) is the primary agency of the European Union for risk assessments regarding food safety. In October 2015, EFSA reported on their evaluation of the Renewal Assessment Report (RAR) for glyphosate that was prepared by the Rapporteur Member State, the German Federal Institute for Risk Assessment (BfR). EFSA concluded that 'glyphosate is unlikely to pose a carcinogenic hazard to humans and the evidence does not support classification with regard to its carcinogenic potential'. Serious flaws in the scientific evaluation in the RAR incorrectly characterize the potential for a carcinogenic hazard from exposure to glyphosate. Since the RAR is the basis for the European Food Safety Agency (EFSA) conclusion, it is critical that these shortcomings are corrected.

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8) Benbrook CM. 2019. **How did the US EPA and IARC reach diametrically opposed conclusions on the genotoxicity of glyphosate-based herbicides?** *Environ Sci Eur* (2019) 31: 2.
https://doi.org/10.1186/s12302-018-0184-7
EPA and IARC reached diametrically opposed conclusions on glyphosate genotoxicity for three primary reasons: (1) in the core tables compiled by EPA and IARC, the EPA relied mostly on registrant-commissioned, unpublished regulatory studies, 99% of which were negative, while IARC relied mostly on peer-reviewed studies of which 70% were positive (83 of 118); (2) EPA's evaluation was largely based on data from studies on technical glyphosate, whereas IARC's review placed heavy weight on the results of formulated glyphosate-based herbicide (GBH) and AMPA assays; (3) EPA's evaluation was focused on typical, general population dietary exposures assuming legal, food-crop uses, and did not take into account, nor address generally higher occupational exposures and risks. IARC's assessment encompassed data from typical dietary, occupational, and elevated exposure scenarios. More research is needed on real-world exposures to the chemicals within formulated GBHs and the biological fate and consequences of such exposures.

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9) Samsel A & S Seneff. 2014. **Glyphosate, pathways to modern diseases IV: cancer and related pathologies.** *J Biol Phys and Chem:* 15 (2015) 121–159.
doi: 10.4024/11SA15R.jbpc.15.03

https://www.drperlmutter.com/wp-content/uploads/2016/03/Glyphosate_pathways_to_modern_diseases_IV_cancer_and_related_pathologies.pdf

Glyphosate is the active ingredient in the pervasive herbicide, Roundup, and its usage, particularly in the United States, has increased dramatically in the last two decades, in step with the widespread adoption of Roundup®-Ready core crops. The World Health Organization recently labelled glyphosate as "probably carcinogenic." In this paper, we review the research literature, with the goal of evaluating the carcinogenic potential of glyphosate. Glyphosate has a large number of tumorigenic effects on biological systems, including direct damage to DNA in sensitive cells, disruption of glycine homeostasis, succinate dehydrogenase inhibition, chelation of manganese, modification to more carcinogenic molecules such as N-nitrosoglyphosate and glyoxylate, disruption of fructose metabolism, etc. Epidemiological evidence supports strong temporal correlations between glyphosate usage on crops and a multitude of cancers that are reaching epidemic proportions, including breast cancer, pancreatic cancer, kidney cancer, thyroid cancer, liver cancer, bladder cancer and myeloid leukaemia. Here, we support these correlations through an examination of Monsanto's early studies on glyphosate, and explain how the biological effects of glyphosate could induce each of these cancers. We believe that the available evidence warrants a reconsideration of the risk/benefit trade-off with respect to glyphosate usage to control weeds, and we advocate much stricter regulation of glyphosate.

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10) Samsel A & S Seneff. 2017. **Glyphosate, pathways to modern diseases VI: Prions, amyloidoses and autoimmune neurological diseases.** *J Biol Phys and Chem:* 17 (2017) 8–32. DOI: 10.4024/25SA16A.jbpc.17.01
https://www.researchgate.net/publication/316601847_Glyphosate_pathways_to_modern_diseases_VI_Prions_amyloidoses_and_autoimmune_neurological_diseases

Usage of the herbicide glyphosate on core crops in the USA has increased exponentially over the past two decades, in step with the exponential increase in autoimmune diseases including autism, multiple sclerosis, inflammatory bowel disease, type 1 diabetes, coeliac disease, neuromyelitis optica and many others. In this paper we explain how glyphosate, acting as a

neuromyelitis optica and many others. In this paper we explain how glyphosate, acting as a non-coding amino acid analogue of glycine, could erroneously be integrated with or incorporated into protein synthesis in place of glycine, producing a defective product that resists proteolysis. Whether produced by a microbe or present in a food source, such a peptide could lead to autoimmune disease through molecular mimicry. We discuss similarities in other naturally produced disease-causing amino acid analogues, such as the herbicide glufosinate and the insecticide L-canavanine, and provide multiple examples of glycine-containing short peptides linked to autoimmune disease, particularly with respect to multiple sclerosis. Most disturbing is the presence of glyphosate in many popular vaccines including the measles, mumps and rubella (MMR) vaccine, which we have verified here for the first time. Contamination may come through bovine protein, bovine calf serum, bovine casein, egg protein and/or gelatin. Gelatin sourced from the skin and bones of pigs and cattle given glyphosate-contaminated feed contains the herbicide. Collagen, the principal component of gelatin, contains very high levels of glycine, as do the digestive enzymes: pepsin, trypsin and lipase. The live measles virus could produce glyphosate-containing haemagglutinin, which might induce an autoimmune attack on myelin basic protein, commonly observed in autism. Regulatory agencies urgently need to reconsider the risks associated with the indiscriminate use of glyphosate to control weeds.

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11) L Zhang, I Rana, E Taioli, RM Shaffer & L Sheppard. 2019. **Exposure to glyphosate-based herbicides and risk for Non-Hodgkin Lymphoma: A meta-analysis and supporting evidence.** *Mutation Research/Rev in Mut Res:* (Available online 10 February 2019). https://www.sciencedirect.com/science/article/pii/S1383574218300887?mc_cid=23c18e62e7&mc_eid=ff8c3a64ef

Glyphosate is the most widely used broad-spectrum systemic herbicide in the world. Recent evaluations of the carcinogenic potential of glyphosate-based herbicides (GBHs) by various regional, national, and international agencies have engendered controversy. We investigated whether there was an association between high cumulative exposures to GBHs and increased risk of non-Hodgkin's lymphoma (NHL) in humans. We conducted a new meta-analysis that includes the most recent update of the Agricultural Health Study (AHS) cohort published in 2018 along with five case-control studies. Using the highest exposure groups when available in each study, we report the overall meta-relative risk (meta-RR) of NHL in GBH-exposed individuals was increased by 41% (meta-RR=1.41, 95% confidence interval, CI: 1.13–1.75). For comparison, we also performed a secondary meta-analysis using high-exposure groups with the earlier AHS (2005), and we calculated a meta-RR for NHL of 1.45 (95% CI: 1.11–1.91), which was higher than the meta-RRs reported previously. Multiple sensitivity tests conducted to assess the validity of our findings did not reveal meaningful differences from our primary estimated meta-RR. To contextualize our findings of an increased NHL risk in individuals with high GBH exposure, we reviewed publicly available animal and mechanistic studies related to lymphoma. We documented further support from studies of malignant lymphoma incidence in mice treated with pure glyphosate, as well as potential links between glyphosate / GBH exposure and immunosuppression, endocrine disruption, and genetic alterations that are commonly associated with NHL or lymphomagenesis. Overall, in accordance with findings from experimental animal and mechanistic studies, our current meta-analysis of human epidemiological studies suggests a compelling link between exposures to GBHs and increased risk for NHL.

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12) Parvez S, RR Gerona, C Proctor, M Friesen, JL Ashby, JL Reiter, Z Lui & PD Winchester. 2018. **Environmental Health Glyphosate exposure in pregnancy and shortened gestational length: a prospective Indiana birth cohort study.** *Environmental Health*: 17, Article number: 23 (2018). https://ehjournal.biomedcentral.com/articles/10.1186/s12940-018-0367-0

The mean age of participants was 29 years, and the majority were Caucasian. Ninety three percent of the pregnant women had GLY levels above the limit of detection (0.1 ng/mL). Mean urinary GLY was 3.40 ng/mL (range 0.5–7.20 ng/mL). Higher GLY levels were found in women who lived in rural areas (p =0.02), and in those who consumed >24 oz. of caffeinated beverages per day (p =0.004). None of the drinking water samples had detectable GLY levels. We observed no correlations with fetal growth indicators such as birth weight percentile and head circumference. However, higher GLY urine levels were significantly correlated with shortened gestational lengths (r=−0.28, p=0.02). This is the first study of GLY exposure in US pregnant women using urine specimens as a direct measure of exposure. We found that >90% of pregnant women had detectable GLY levels and that these levels correlated significantly with shortened pregnancy lengths. Although our study cohort was small and regional and had limited racial/ethnic diversity, it provides direct evidence of maternal GLY exposure and a significant correlation with shortened pregnancy. Further investigations in a more geographically and racially diverse cohort would be necessary before these findings could be generalized.

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13) Duforeste M, A Nadaradjane, G Bougras-Cartron, J Briand, C Olivier, Jean-Sébastien Frenel, F. M. Vallette, SA Lelièvre & Pierre-François Cartron. 2019. **Glyphosate Primes Mammary Cells for Tumorigenesis by Reprogramming the Epigenome in a TET3-Dependent Manner.** *Front Genet:* 27 September 2019.
https://doi.org/10.3389/fgene.2019.00885
https://www.frontiersin.org/articles/10.3389/fgene.2019.00885/full

The acknowledgment that pollutants might influence the epigenome raises serious concerns regarding their long-term impact on the development of chronic diseases. The herbicide glyphosate has been scrutinized for an impact on cancer incidence, but reports demonstrate the difficulty of linking estimates of exposure and response analysis. An approach to better apprehend a potential risk impact for cancer is to follow a synergistic approach, as cancer rarely occurs in response to one risk factor. The known influence of glyphosate on estrogen-regulated pathway makes it a logical target of investigation in breast cancer research. We have used nonneoplastic MCF10A cells in a repeated glyphosate exposure pattern over 21 days. Glyphosate triggered a significant reduction in DNA methylation, as shown by the level of 5-methylcytosine DNA; however, in contrast to strong demethylating agent and cancer promoter UP peptide, glyphosate-treated cells did not lead to tumor development. Whereas UP acts through a DNMT1/PCNA/UHRF1 pathway, glyphosate triggered increased activity of ten-eleven translocation (TET)3. Combining glyphosate with enhanced expression of microRNA (miR) 182-5p associated with breast cancer induced tumor development in 50% of mice. Culture of primary cells from resected tumors revealed a luminal B (ER+/PR-/HER2-) phenotype in response to glyphosate-miR182-5p exposure with sensitivity to tamoxifen and invasive and migratory potentials. Tumor development could be prevented either by specifically inhibiting miR 182-5p or by treating glyphosate-miR 182-5p-cells with dimethyloxallyl glycine, an inhibitor of TET pathway. Looking for potential epigenetic marks of TET-mediated gene regulation under glyphosate exposure, we identified MTRNR2L2 and DUX4 genes, the hypomethylation of which was sustained even after stopping glyphosate exposure for 6 weeks. Our findings reveal that low pressure but sustained DNA hypomethylation occurring via the TET pathway primes cells for oncogenic response in the presence of another potential risk factor. These results warrant further investigation of glyphosate-mediated breast cancer risk.

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14) Kurenbach B, AM Hill, W Godsoe, S van Hamelsveld JA Heinemann. 2018. **Agrichemicals and antibiotics in combination increase antibiotic resistance evolution.** *PeerJ* 6:e5801
https://doi.org/10.7717/peerj.5801

Antibiotic resistance in our pathogens is medicine's climate change: caused by human activity,

and resulting in more extreme outcomes. Resistance emerges in microbial populations when antibiotics act on phenotypic variance within the population. This can arise from either genotypic diversity (resulting from a mutation or horizontal gene transfer), or from differences in gene expression due to environmental variation, referred to as adaptive resistance. Adaptive changes can increase fitness allowing bacteria to survive at higher concentrations of antibiotics. They can also decrease fitness, potentially leading to selection for antibiotic resistance at lower concentrations. There are opportunities for other environmental stressors to promote antibiotic resistance in ways that are hard to predict using conventional assays. Exploiting our previous observation that commonly used herbicides can increase or decrease the minimum inhibitory concentration (MIC) of different antibiotics, we provide the first comprehensive test of the hypothesis that the rate of antibiotic resistance evolution under specified conditions can increase, regardless of whether a herbicide increases or decreases the antibiotic MIC. Short term evolution experiments were used for various herbicide and antibiotic combinations. We found conditions where acquired resistance arises more frequently regardless of whether the exogenous non-antibiotic agent increased or decreased antibiotic effectiveness. This is attributed to the effect of the herbicide on either MIC or the minimum selective concentration (MSC) of a paired antibiotic. The MSC is the lowest concentration of antibiotic at which the fitness of individuals varies because of the antibiotic, and is lower than MIC. Our results suggest that additional environmental factors influencing competition between bacteria could enhance the ability of antibiotics to select antibiotic resistance. Our work demonstrates that bacteria may acquire antibiotic resistance in the environment at rates substantially faster than predicted from laboratory conditions.

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15) Mesnage R, M Teixeira, D Mandrioli, L Falcioni, QR Ducarmon, RD Zwittink, C Amiel, Jean-Michel Panoff, F Belpoggi, MN Antoniou. 2019 Preprint. **Shotgun metagenomics and metabolomics reveal glyphosate alters the gut microbiome of Sprague-Dawley rats by inhibiting the shikimate pathway.**
doi: https://doi.org/10.1101/870105
https://www.biorxiv.org/content/10.1101/870105v1
https://www.gmwatch.org/en/news/latest-news/19261?fbclid=IwAR27DyCI04eLANzrJF-Zx75YmQRJKNdRXHfSndvkqec_UceivXqgLCyTuV4

There is intense debate as to whether glyphosate can interfere with aromatic amino acid biosynthesis in microorganisms inhabiting the gastrointestinal tract, which could potentially lead to negative health outcomes. We have addressed this major gap in glyphosate toxicology by using a multi-omics strategy combining shotgun metagenomics and metabolomics. We tested whether glyphosate (0.5, 50, 175 mg/kg bw/day), or its representative EU commercial herbicide formulation MON 52276 at the same glyphosate equivalent doses, has an effect on the rat gut microbiome in a 90-day subchronic toxicity test. Clinical biochemistry measurements in blood and histopathological evaluations showed that MON 52276 but not glyphosate was associated with statistically significant increase in hepatic steatosis and necrosis. Similar lesions were also present in the liver of glyphosate-treated groups but not in the control group. Caecum metabolomics revealed that glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase in the shikimate pathway as evidenced by an accumulation of shikimic acid and 3-dehydroshikimic acid. Levels of caecal microbiome dipeptides involved in the regulation of redox balance (γ-glutamylglutamine, cysteinylglycine, valylglycine) had their levels significantly increased. Shotgun metagenomics showed that glyphosate affected caecum microbial community structure and increased levels of *Eggerthella spp*. and *Homeothermacea spp.*. MON 52276, but not glyphosate, increased the relative abundance of *Shinella zoogleoides*. Since *Shinella spp.* are known to degrade alkaloids, its increased abundance may explain the decrease in solanidine levels measured with MON 52776 but not glyphosate. Other glyphosate formulations may have different effects since Roundup® GT Plus inhibited bacterial growth *in vitro* at concentrations at which MON 52276 did not GT Plus inhibited bacterial growth *in vitro* at concentrations at which MON 52276 did not present

any visible effect. Our study highlights the power of a multiomics approach to investigate effects of pesticides on the gut microbiome. This revealed the first biomarker of glyphosate effects on rat gut microbiome. Although more studies will be needed to ascertain if there are health implications arising from glyphosate inhibition of the shikimate pathway in the gut microbiome, our findings can be used in environmental epidemiological studies to understand if glyphosate can have biological effects in human populations.

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16) Kubsad D, EE Nilsson, SE King, I Sadler-Riggleman, D Beck & MK Skinner. 2019. **Assessment of Glyphosate Induced epigenetic transgenerational Inheritance of pathologies and sperm epimutations: Generational Toxicology.** *Sci Rep* 9, 6372 (2019) doi:10.1038/s41598-019-42860-0
https://www.ncbi.nlm.nih.gov/pubmed/31011160

Ancestral environmental exposures to a variety of factors and toxicants have been shown to promote the epigenetic transgenerational inheritance of adult onset disease.
The current study using a transient exposure of gestating F0 generation female rats found negligible impacts of glyphosate on the directly exposed F0 generation, or F1 generation offspring pathology. In contrast, dramatic increases in pathologies in the F2 generation grand-offspring, and F3 transgenerational great-grand-offspring were observed. The transgenerational pathologies observed include prostate disease, obesity, kidney disease, ovarian disease, and parturition (birth) abnormalities. Epigenetic analysis of the F1, F2 and F3 generation sperm identified differential DNA methylation regions (DMRs). A number of DMR associated genes were identified and previously shown to be involved in pathologies. Therefore, we propose glyphosate can induce the transgenerational inheritance of disease and germline (e.g. sperm) epimutations. Observations suggest the generational toxicology of glyphosate needs to be considered in the disease etiology of future generations.

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17) Mesnage R, G Renney, Gilles-Eric Séralini, M Ward & MN Antoniou. 2017/2018. **Multiomics reveal non-alcoholic fatty liver disease in rats following chronic exposure to an ultra-low dose of Roundup herbicide.** *Sci Rep*: 2017 Jan 9;7:39328. doi:10.1038/srep39328.
https://www.ncbi.nlm.nih.gov/pubmed/28067231

The impairment of liver function by low environmentally relevant doses of glyphosate-based herbicides (GBH) is still a debatable and unresolved matter. Previously we have shown that rats administered for 2 years with 0.1 ppb (50ng/L glyphosate equivalent dilution; 4ng/kg body weight/day daily intake) of a Roundup GBH formulation showed signs of enhanced liver injury as indicated by anatomorphological, blood/urine biochemical changes and transcriptome profiling. Here we present a multiomic study combining metabolome and proteome liver analyses to obtain further insight into the Roundup-induced pathology. Proteins significantly disturbed (214 out of 1906 detected, $q<0.05$) were involved in organonitrogen metabolism and fatty acid β-oxidation. Proteome disturbances reflected peroxisomal proliferation, steatosis and necrosis. The metabolome analysis (55 metabolites altered out of 673 detected, $p<0.05$) confirmed lipotoxic conditions and oxidative stress by showing an activation of glutathione and ascorbate free radical scavenger systems. Additionally, we found metabolite alterations associated with hallmarks of hepatotoxicity such as γ-glutamyl dipeptides, acylcarnitines, and proline derivatives. Overall, metabolome and proteome disturbances showed a substantial overlap with biomarkers of non-alcoholic fatty liver disease and its progression to steatohepatosis and thus confirm liver functional dysfunction resulting from chronic ultra-low dose GBH exposure.

IMPACT ON ANIMALS

18) Gaupp-Berghausen M, M Hofer, B Rewald, & J G Zaller. 2015. **Glyphosate-based herbicides reduce the activity and reproduction of earthworms and lead to increased soil nutrient concentrations.** *Sci Rep:* 2015; 5: 12886.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4542661/

Herbicide use is increasing worldwide both in agriculture and private gardens. However, our knowledge of potential side-effects on non-target soil organisms, even on such eminent ones as earthworms, is still very scarce. In a greenhouse experiment, we assessed the impact of the most widely used glyphosate-based herbicide Roundup on two earthworm species with different feeding strategies. We demonstrate, that the surface casting activity of vertically burrowing earthworms (*Lumbricus terrestris*) almost ceased three weeks after herbicide application, while the activity of soil dwelling earthworms (*Aporrectodea caliginosa*) was not affected. Reproduction of the soil dwellers was reduced by 56% within three months after herbicide application. Herbicide application led to increased soil concentrations of nitrate by 1592% and phosphate by 127%, pointing to potential risks for nutrient leaching into streams, lakes, or groundwater aquifers. These sizeable herbicide-induced impacts on agroecosystems are particularly worrisome because these herbicides have been globally used for decades.

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19) Motta EVS, K Raymann, and NA Moran. 2018. **Glyphosate perturbs the gut microbiota of honey bees.** *Proc. Nat. Acad. Sci. (PNAS):* October 9, 2018 115 (41) 10305-10310.
https://doi.org/10.1073/pnas.1803880115
https://www.pnas.org/content/115/41/10305

Glyphosate, the primary herbicide used globally for weed control, targets the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme in the shikimate pathway found in plants and some microorganisms. Thus, glyphosate may affect bacterial symbionts of animals living near agricultural sites, including pollinators such as bees. The honey bee gut microbiota is dominated by eight bacterial species that promote weight gain and reduce pathogen susceptibility. The gene encoding EPSPS is present in almost all sequenced genomes of bee gut bacteria, indicating that they are potentially susceptible to glyphosate. We demonstrated that the relative and absolute abundances of dominant gut microbiota species are decreased in bees exposed to glyphosate at concentrations documented in the environment. Glyphosate exposure of young workers increased mortality of bees subsequently exposed to the opportunistic pathogen *Serratia marcescens*. Members of the bee gut microbiota varied in susceptibility to glyphosate, largely corresponding to whether they possessed an EPSPS of class I (sensitive to glyphosate) or class II (insensitive to glyphosate). This basis for differences in sensitivity was confirmed using in vitro experiments in which the EPSPS gene from bee gut bacteria was cloned into *Escherichia coli*. All strains of the core bee gut species, *Snodgrassella alvi*, encode a sensitive class I EPSPS, and reduction in *S. alvi* levels was a consistent experimental result. However, some *S. alvi* strains appear to possess an alternative mechanism of glyphosate resistance. Thus, exposure of bees to glyphosate can perturb their beneficial gut microbiota, potentially affecting bee health and their effectiveness as pollinators.

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20) Farina WM, MS Balbuena, LT Herbert, CM Goñalons & DE Vázquez. **2019. Effects of the herbicide glyphosate on honey bee sensory and cognitive abilities: Individual impairments with implications for the hive.** *Insects:* 2019, *10*(10), 354.
https://doi.org/10.3390/insects10100354
https://www.mdpi.com/2075-4450/10/10/354

The honeybee Apis mellifera is an important pollinator in both undisturbed and agricultural

ecosystems. Its great versatility as an experimental model makes it an excellent proxy to evaluate the environmental impact of agrochemicals using current methodologies and procedures in environmental toxicology. The increase in agrochemical use, including those that do not target insects directly, can have deleterious effects if carried out indiscriminately. This seems to be the case of the herbicide glyphosate (GLY), the most widely used agrochemical worldwide. Its presence in honey has been reported in samples obtained from different environments. Hence, to understand its current and potential risks for this pollinator it has become essential to not only study the effects on honeybee colonies located in agricultural settings, but also its effects under laboratory conditions. Subtle deleterious effects can be detected using experimental approaches. GLY negatively affects associative learning processes of foragers, cognitive and sensory abilities of young hive bees and promotes delays in brood development. An integrated approach that considers behavior, physiology, and development allows not only to determine the effects of this agrochemical on this eusocial insect from an experimental perspective, but also to infer putative effects in disturbed environments where it is omnipresent.

WATER, SOIL, SEDIMENTS & RAINFALL ——————————————————————

21) Scribner, EA, WA Battaglin, JE Dietze, & EM Thurman. 2003. **Reconnaissance data for glyphosate, other selected herbicides, their degradation products, and antibiotics in 51 streams in nine Midwestern States, 2002**. U.S. Geological Survey Open-File Report 03-217, 101 pp. https://toxics.usgs.gov/highlights/glyphosate02.html

Glyphosate Herbicide Found in Many Midwestern Streams, Antibiotics Not Common.
U.S. Geological Survey (USGS) investigated 51 streams (154 water samples) in nine Midwestern States during runoff events. Glyphosate was detected in 36 percent of the samples, while its degradation product, aminomethylphosphonic acid (AMPA) was detected in 69 percent of the samples. The antibiotics, measured to determine if they also would attain peak concentrations during spring runoff events following pesticide application, were detected in only 1 percent of water samples.

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22) Kolpin, DW, EM Thurman, EA Lee, MT Meyer, ET Furlong & ST Glassmeyer. 2006. **Urban contributions of glyphosate and its degradate AMPA to streams in the United States**:
Science of the Total Environment: v. 354, no. 2-3, p. 191-197
doi: 10.1016/j.scitotenv.2005.01.028
https://toxics.usgs.gov/highlights/glyphosate_wastewater.html

USGS scientists sampled the wastewater discharged into streams from 10 wastewater treatment plants. Samples were collected from the treated wastewater and from the stream water—both upstream and downstream of the wastewater discharge.
Found in Wastewater Discharged to Streams: first to demonstrate that the discharge from wastewater treatment plants serving urban areas is a source of glyphosate to streams. Glyphosate was more frequently detected in the *wastewater* (27 percent) and in the *downstream* samples (20 percent) than it was in the *upstream* samples (12 percent). The discharge of the streams and the wastewater outfalls in this study were generally lower when compared to the discharge of the streams in a study of the occurrence of glyphosate in streams draining agricultural areas. In this study of glyphosate, AMPA (aminomethyl phosphonic acid), a toxic degradate of glyphosate, was found in higher concentrations and more frequently (68 percent) in wastewater than was glyphosate (18 percent).
No detections were observed in two reference streams located in areas with little human influence.

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23) Battaglin WA, MT Meyer, KM Kuivila & JE Dietze. 2014. **Glyphosate and Its Degradation Product AMPA Occur Frequently and Widely in U.S. Soils, Surface Water, Groundwater, and Precipitation.** *Paper No. JAWRA-13-0028-P of the Journal of the American Water Resources Association (JAWRA)*: 275 - 290.
https://pubs.er.usgs.gov/publication/70046159
https://onlinelibrary.wiley.com/doi/full/10.1111/jawr.12159

The largest and most comprehensive assessment of the environmental occurrence of glyphosate and aminomethylphosphonic acid (AMPA) in the United States conducted to date, summarizing the results of 3,732 water and sediment and 1,018 quality assurance samples collected between 2001 and 2010 from 38 states. Glyphosate and AMPA were detected frequently in soils and sediment, ditches and drains, precipitation, rivers, and streams; and less frequently in lakes, ponds, and wetlands; soil water; and groundwater. Results indicate that glyphosate and AMPA are usually detected together, mobile, and occur widely in the environment. Concentrations of glyphosate were below the levels of concern for humans or wildlife; however, pesticides are often detected in mixtures [*Note: EPA'S "Safe Level" is not scientifically valid*].

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24) Bonansea RI, I Filippi, DA Wunderlin, DJG Marino & MV Amé. 2018. **The Fate of Glyphosate and AMPA in a Freshwater Endorheic Basin: An Ecotoxicological Risk Assessment.** *Toxics:* 2018 Mar; 6(1): 3. doi: 10.3390/toxics6010003
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5874776/

Glyphosate is the most widely used herbicide worldwide. However, there are some uncertain aspects with respect to its environmental fate. To evaluate the existence and distribution of this pesticide and its metabolite, aminomethylphosphonic acid (AMPA), their presence in fresh water, sediment, and suspended particulate matter (SPM) was measured in samples collected in a river running across a large city and through areas with intensive and extensive agriculture. The aquatic risk associated to the occurrence of these compounds was estimated using the hazard quotient (HQ) calculation for water and sediment. From the analyzed samples, overall 35% contained glyphosate, AMPA, or both compounds. Concentrations of the analytes were spread in different percentages depending on the environmental matrices considered, with levels ranging from 12 to 20 times higher for glyphosate and AMPA in sediment and SPM, as compared with the levels found in water. The most polluted area was situated within a green belt zone of the city; while in second place were sites located in areas of extensive agriculture. Aquatic organisms inhabiting areas both inside and outside agricultural areas are threatened by water glyphosate concentrations. Benthic organisms inside the greenbelt zone and inside the lower basin are threatened by the concentrations of glyphosate in sediment. Even when the concentrations measured in water were below the levels of concern for wildlife, results showed the risk of agricultural practices to aquatic biota. An update of the limits established for freshwater biota protection is needed.

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25) Alonso LL, PM Demetrio, MA Etchegoyen & DJ Marino. 2018. **Glyphosate and atrazine in rainfall and soils in agroproductive areas of the pampas region in Argentina**. *Sci Total Environ:* 2018 Dec 15;645:89-96. doi: 10.1016/j.scitotenv.2018.07.134. Epub 2018 Jul 14.
https://www.ncbi.nlm.nih.gov/pubmed/30015122

The presence in the atmosphere of glyphosate (GLP) and atrazine (ATZ) was investigated-those pesticides dominating the market in Argentina-through rain, as the main climatic phenomenon associated with wet deposition, both through analyzing source-receptor relationships with soil along with the climatic influences that may condition that transport and through estimating the annual deposition on the surface of the Argentine pampas. Rainwater samples (n = 112) were

collected throughout each rainfall in urban areas of the pampas having different degrees of land use and with extensive crop production plus subsurface-soil samples (n = 58) from the relevant periurban sites. The herbicides-analyzed by liquid-chromatography-mass-spectrometry-were detected in >80% of the rain samples at median-to-maximum concentrations of 1.24-67.3 µg·L-1 (GLP) and 0.22-26.9 µg·L-1(ATZ), while aminomethylphosphonic acid (AMPA) was detected at 34% (0.75-7.91 µg·L-1). In soils, GLP was more frequently registered (41%; 102-323 µg·kg-1) followed by ATZ (32%; 7-66 µg·kg-1) and then AMPA (22%; 223-732 µg·kg-1). The maximum GLP concentrations quantified in rainwater exceeded the previously reported levels for the USA and Canada. No associations were observed between soil and rainwater concentrations in the same monitoring areas-despite the soil's action as a source, as evidenced through the AMPA present in rainwater. Median GLP concentrations were significantly associated with isohyets, in an increasing gradient from the east to the west-as such in an inverse pattern to that of the annual rainfall volumes; whereas ATZ-rainwater levels exhibited no characteristic spatial configuration. The estimated annual deposition of GLP by rainfall indicated that more than one source of an herbicide can lead to its presence in the atmosphere and points out the relevance of rainfall's contribution to the surface levels of a pollutant.

MEDIA ——————————————————————————————————————

26) Wilcox, M. 2019. **The uphill battle for communities that ban pesticides.** *Environmental Health News:* June 17, 2019*. https://www.ehn.org/the-uphill-battle-for-communities-that-ban-harmful-pesticides-2638803514.html*

As cities and counties across the U.S. restrict or ban pesticides, many are realizing passing a law is but a battlefield victory in a prolonged war. Local advocacy organizations such as POCCA are particularly important since many federal and state authorities are-out of-touch with, or are ignoring, current independent science regarding the universal threat of glyphosate to all life forms. As people become aware of the risks of glyphosate exposure, increasing numbers of municipalities are imposing restrictions or outright bans. Media coverage is both informative and encouraging for other communities to take action to protect themselves from glyphosate's insidious harm.

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