Before the Panel established pursuant to Chapter 31 (Dispute Settlement) of the Agreement between the United States of America, the United Mexican States and Canada (USMCA)

EXPERT REPORT ON THE TOXICITY OF AGENTS CONTAINED IN GENETICALLY ENGINEERED CORN AND THE HEALTH RISKS ASSOCIATED TO ITS CONSUMPTION

Mexico – Measures Concerning Genetically Engineered Corn (MEX-USA-2023-31-01)

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Credentials of the author

1. I am Professor of Molecular Genetics and Toxicology at King's College London, a world-leading university with a speciality in the life sciences. I graduated with honours from the University of Oxford in Biochemistry and obtained a PhD in molecular biology from the University of Reading. I have been a member of the faculty of King's College London and have led my own research group for the last 30 years.

2. I am a career-long molecular biologist who has made seminal contributions to the field of gene structure and regulation. My discoveries have led to various biotechnological applications, including in the fields of gene therapy and the industrial manufacture of therapeutic proteins. I hold inventor status on numerous patents covering gene expression platforms (now expired, but with others pending). I therefore have first-hand experience with all manner of genetic modification technologies, especially those of a transgenic nature.

3. My expertise in molecular biology and genetic modification technologies has allowed me to critically evaluate the claims of this technology within an agricultural context. In this regard I have acted in an unpaid advisory capacity to many non-government organisations, political parties, and governments on all five continents.

4. For the last 10 years, I have established a molecular and cellular toxicology programme in my group, with a focus on plasticisers (including bisphenols) and pesticides, particularly glyphosate-based herbicides. This has led to my group becoming a world leader on glyphosate herbicide toxicology, highlighting hitherto unforeseen mechanisms of glyphosate herbicide toxicity and negative health outcomes. Overall, my group's work has shown that the safety exposure limits set by regulators for pesticide active ingredients are too high for the protection of health and the environment.

5. I have made limited but significant contributions to the study of herbicide-tolerant GM corn.

6. I have over 160 publications in the peer-reviewed literature, with around 50 papers on pesticide toxicology.

7. In summary, given my expertise in molecular genetics, genetic engineering technologies and pesticide toxicology, I feel eminently qualified to provide this expert report on the toxicity of agents contained in genetically engineered corn and the health risks associated with its consumption.

Declaration of interests

8. For my work on plasticisers, I have received research funding from Breast Cancer UK. My work on pesticides has largely been funded by the Sustainable Food

Alliance (USA). However, I conduct the work and publish it independently and without influence of the funders. I have no financial conflict of interest related to the topics covered in this report. This report is based on my own independent work, and the views I express are my genuinely held independent views. Prior to the preparation of this report, I had not collaborated in legal proceedings with the Government of Mexico, nor with lawyers representing it in the present dispute. Mexico has informed me that the hearing for the case is scheduled to take place from 26 to 28 June, dates on which I am unavailable. Nevertheless, I am available on any other date that may be convenient.

Summary

9. This report provides an overview of key studies and other evidence on the toxicity of agents contained in genetically modified (GM) corn and other GM crops, including Bt/VIP insecticidal toxins and herbicide residues, in particular glyphosate. There is no intention to claim that all studies that have investigated the safety of GM crops show toxic or other adverse effects. Nevertheless, this report compiles a large body of evidence from well controlled laboratory animal toxicity studies that show evidence of harm to multiple physiological systems. This shows that it is not possible to generalise about the safety of GM crops and that each individual variety needs to be tested.

10. Studies are presented that show major compositional differences between GM and non-GM crop varieties, including corn. These studies demonstrate that the GM transformation process can result in unintended compositional changes at both a protein and metabolite level, stemming from the DNA damaging effects of this procedure. Thus claims that GM foods are substantially equivalent to their non-GM counterparts and are therefore safe are not supported by the scientific evidence. These changes may have unknown health consequences for the consumer.

11. The report summarises findings from well controlled animal feeding studies, including some commissioned by industry to support regulatory authorisations, that show signs of toxicity from the consumption of GM corn and another GM crop (soy). These studies repeatedly show that the main organs and systems affected by the consumption of the GM food are the liver, kidneys, digestive tract, and immune system.

12. The report progresses to consider the health implications of glyphosate-based herbicide residues that will inevitably be present in GM glyphosate-tolerant corn and other crops. It is beyond the scope of this report to review the vast number of studies undertaken to investigate glyphosate herbicide toxicity. Instead the report summarises work by my own research group and others of particular relevance.

13. In studies on animals exposed to regulatory-relevant doses of glyphosate and commercial glyphosate-based herbicide formulations, adverse effects were observed in multiple organs and systems, including gut microbiome structure and functional

dysbiosis, oxidative stress in the gut and internal organs, non-alcoholic fatty liver disease, increased markers of genotoxicity (DNA damage), and carcinogenicity. It is particularly noteworthy that these toxic effects were more pronounced in animals exposed to the commercial glyphosate-based herbicide formulations than those exposed to glyphosate alone. This demonstrates the high degree of toxicity of the co-formulants present in these formulations, the composition of which is generally kept secret by manufacturers, and the residues of which are never monitored in foodstuffs by regulatory agencies.

14. In vivo toxicity studies of glyphosate and commercial glyphosate-based herbicide formulations presented in the report consistently demonstrate that the currently held regulatory “no observed adverse effect level” (NOAEL) for glyphosate, and by default, the acceptable daily intake level (ADI), is incorrect and set too high. Indeed, based on the current available evidence, the safe dose of glyphosate, when consumed over the long term, is unknown.

15. The report also summarises key epidemiological studies. One such study has shown statistically significant associations between glyphosate-based herbicide use and non-Hodgkin lymphoma. In addition, scientists have found links between occupational use of glyphosate-based herbicides and a marker of DNA damage (genotoxicity) called mosaic loss of chromosome Y (mLOY). mLOY has been associated with blood cancers such as lymphoma, myeloma, and leukemia, as well as with Alzheimer's disease. Furthermore, in a study that analysed the urine of applicators of glyphosate-based herbicides, it was found that markers of oxidative stress were significantly elevated. This included the marker of oxidative stress known as 8-OHdG, which is also an indicator of direct DNA damage.

16. Furthermore, the report summarises studies that have investigated the toxicity of glyphosate in combination with other pesticides used on corn, all of which were administered to laboratory animals at the acceptable daily intake (ADI) dose. These mixtures of pesticides, including the glyphosate, resulted in numerous negative health outcomes. This shows that the regulatory practice of setting ADI values based on toxicity studies of individual pesticide active ingredients is invalid and puts public health at risk.

17. It is important to bear in mind that all the GM corn toxicity studies summarised in this report have tested single-Bt trait or single-herbicide-tolerance trait varieties. However, the current reality is that the vast majority of GM corn that will be imported into Mexico from the US will be stacked-trait varieties, engineered to contain multiple Bt/VIP toxins and herbicide tolerances. Without scientific foundation, regulators have assumed that the toxicity of the stacked-trait varieties is no greater than the toxicity of any individual Bt/VIP or the residues arising from an individual herbicide tolerance trait.

18. In conclusion, this report provides evidence that Mexican citizens will be exposed to multiple types of toxicity from the consumption of imported US GM corn. This combinatorial source of toxicity has not been properly addressed by regulators.

The health implications of this regulatory oversight for Mexican citizens are unknown. However, based on the evidence presented in this report, the consumption of imported US GM corn at the high levels typical for Mexican citizens has the potential to result in serious negative health outcomes.

Definitions

19. European Union law defines a genetically modified organism (GMO) as an organism in which “the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination” and requires the risks of each GMO to be assessed on a case-by-case basis.[1]

20. In the US there is no legal definition of "genetically modified organism".[2] However, "biotechnology" is defined in the US Code of Federal Regulations as a product of recombinant DNA technology. The US Environmental Protection Agency (EPA) defines a GMO as “a plant, animal, or microorganism that has had its genetic material (DNA) changed using technology that generally involves the specific modification of DNA, including the transfer of specific DNA from one organism to another”.[3]

21. In Mexico, “modern biotechnology” is defined as “the application of in vitro techniques of nucleic acids, including recombinant deoxyribonucleic acid (DNA and RNA) and the direct injection of nucleic acids into cells and organelles, or the fusion of cells beyond the taxonomic family, exceeding the natural physiological barriers of reproduction or recombination; these are not techniques commonly used in traditional reproduction and selection, and are used to originate genetically modified organisms.”[4]

22. The Cartagena Protocol on Biosafety established the basis to regulate the release and international trade of living GMOs. According to this protocol, of which Mexico is a signatory, a genetically modified organism is defined as “any living organism that possesses a novel combination of genetic material obtained using modern biotechnology”. The protocol defines “modern biotechnology” as “In vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or… Fusion of cells beyond the

[1] European Parliament and Council. Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Off J Eur Communities. April 2001:1–38. http://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32001L0018. **MA-01**.
[2] USDA (2019). Ask USDA. https://ask.usda.gov/s/article/What-is-the-legal-definition-of-Genetically-Modified-Organism. **MA-02**.
[3] US EPA (2023). Genetically modified organisms. https://www.epa.gov/regulation-biotechnology-under-tsca-and-fifra/genetically-modified-organisms. **MA-03**.
[4] Conahcyt (2005). Law on biosafety of genetically modified organisms. https://conahcyt.mx/cibiogem/images/cibiogem/eng/Docs/Ing_LBOGM_P.pdf.**MA-04**.

taxonomic family, that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection."[5]

23. The aim of genetic modification is to confer a new (novel) trait on the organism or to modify an existing trait.

US-grown GM corn

24. Most US-grown corn is genetically engineered with one or both of two traits:

- insecticidal Bt toxin-producing, including vegetative insecticidal proteins (VIP)
- herbicide tolerance.

25. The first Bt corn varieties were engineered to produce one or two different Bt toxins, but as pests became increasingly resistant to these Bt toxins, Bt corn varieties were engineered with "stacked" (multiple) GM traits to contain up to seven Bt/VIP insecticidal proteins. In addition, US corn typically contains genes for tolerance to up to 4 different herbicides.[6]

26. Historically, the main herbicide that GM corn has been engineered to tolerate has been glyphosate, but as weeds have become resistant to glyphosate, genes for tolerance to other herbicides, such as glufosinate and 2,4-D, have been added.[7]

27. As a result of such trait stacking, the levels of Bt/VIP toxins and herbicide residues in corn grain have increased exponentially since the introduction of GM corn in the US. Stacked trait varieties of GM corn are more commonly planted in the US now than the varieties expressing one or two different Bt toxins, so a consumer of US corn is likely to be eating multiple Bt/VIP toxin proteins as well as the residues of multiple herbicides.

28. SmartStax GM corn produces six Bt toxin proteins targeting the European corn borer and the corn rootworm. Total Bt toxin protein production is estimated at 4.2 kg/ha, 19 times the average chemical insecticide rate of application on non-GM corn in 2010.[8] These high levels of Bt toxins expressed in SmartStax have never been tested in animals or humans for long-term effects on health.

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[5] CBD (2000). Cartagena Protocol on Biosafety to the Convention on Biological Diversity: text and annexes. Montreal: Secretariat of the Convention on Biological Diversity. https://www.cbd.int/doc/legal/cartagena-protocol-en.pdf. **MA-05**.

[6] DiFonzo C (2023). The handy Bt trait table for US corn production. 2 Feb.https://www.texasinsects.org/uploads/4/9/3/0/49304017/bttraittable_feb_2023.pdf **MA-06**

[7] DiFonzo C (2023). The handy Bt trait table for US corn production. 2 Feb. https://www.texasinsects.org/uploads/4/9/3/0/49304017/bttraittable_feb_2023.pdf **MA-06.**

[8] Benbrook C (2012). Impacts of genetically engineered crops on pesticide use in the US – The first sixteen years. Environmental Sciences Europe 24. http://www.enveurope.com/content/24/1/24 **MA-07**.

29. According to the submission of Friends of the Earth to the USMCA Secretariat:[9]

> "Residue levels of Bt/VIP toxins in corn grain – 2 ppm to 100 ppm – exceed maximum food tolerances for widely used corn insecticides by 40- to 2,000-fold (most tolerances governing insecticide residues in corn grain are 0.05 ppm or less). Likewise, several contemporary GE corn varieties express 50- to 100-fold more Bt/VIP toxin per hectare compared to typical corn insecticide application rates.
>
> "In addition to multiple Bt/VIP toxins, GE [genetically engineered] corn also often contains GLY [glyphosate] residues. In 2022 testing by the USDA,[10] residues of GLY were present in 73 of 309 samples of corn grain (24%) at an average level of 0.06 ppm (max value of 0.12 ppm). Monitoring by the FDA in recent years has identified GLY in corn in ~60% of samples, and GLY/AMPA were by far the most common residues detected in animal feed. In testing carried out in 2013-2015 in MX, Gonzalez-Ortega et al. report[11] that 27% of corn-based products tested positive for GLY or AMPA."

Corn consumption in Mexico

30. The levels of Bt/VIP toxin residues and glyphosate/other pesticide residues from US-grown corn consumed in Mexico will far exceed the levels consumed by Americans because corn makes up a larger proportion of the diet in Mexico and in contrast with the US, it is consumed in minimally processed forms.

31. US regulators have not established the toxicological implications of consuming these levels of residues because they have not required relevant animal feeding studies with corn grains to be conducted.

32. It is challenging to investigate toxicity in humans because it is not considered ethically acceptable to deliberately expose humans to toxic or potentially toxic agents, e.g. pesticides or Bt toxins. However, the potential toxicity of stacked GM corn varieties warrants caution, especially in regions where minimally processed corn grain is directly consumed in large quantities as a daily food staple, and the risk

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[9] Friends of the Earth (2024). Comments Submitted to the USMCA Secretariat on Behalf of Friends of the Earth. 13 Mar. https://www.iatp.org/sites/default/files/2024-04/Written%20Views%20FOE.pdf **MA-08**.

[10] Corn data are from Appendix C in USDA (2024), "Pesticide Data Program Annual Summary: Calendar Year 2022", https://www.ams.usda.gov/sites/default/files/media/2022PDPAnnualSummary.pdf ; mean level calculated by C. Benbrook in the Dietary Risk Index system https://hh-ra.org/projects/the-dietary-risk-index-dri/ **MA-09**.

[11] González-Ortega et al (2017). Pervasive presence of transgenes and glyphosate in maize-derived food in Mexico. Agroecology and Sustainable Food Systems 41(9-10):1146-1161. https://www.tandfonline.com/doi/full/10.1080/21683565.2017.1372841 ; full paper can be downloaded at https://www.researchgate.net/publication/319413251_Pervasive_presence_of_transgenes_and_glyphosate_in_maize-derived_food_in_Mexico **MA-10**.

of exposure to transgenic proteins and pesticide residues is therefore high.

Sources of potential toxicity from the consumption of GM food crops

33. There are three main sources of potential toxicity that arise from the consumption of GM food crops, including GM corn:

i. The product of the foreign transgene in the GM crop: for example, the Bt/VIP proteins, which can be immunogenic and/or allergenic.

ii. Inadvertent production of novel toxins or allergens stemming from the widescale mutagenic effects (DNA damage) arising from the GM transformation process. The GM transformation process is known to produce hundreds or thousands of sites of DNA damage in the resultant GM crop.[12] Some of this DNA damage will inevitably alter patterns of gene function, resulting in altered biochemistry and composition.[13] These alterations could include the production of novel toxins and/or allergens.[14]

iii. The residues of the pesticides, particularly glyphosate-based herbicides, that the GM crops are designed to be grown with, and which will inevitably be present in the final marketed product.

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[12] Latham JR et al (2006). The mutational consequences of plant transformation. J Biomed Biotechnol: 1–7. http://www.ncbi.nlm.nih.gov/pubmed/16883050 **MA-11**; Wilson AK et al (2006). Transformation-induced mutations in transgenic plants: Analysis and biosafety implications. Biotechnol Genet Eng Rev 23: 209–238. http://www.ncbi.nlm.nih.gov/pubmed/22530509, **MA-12**.

[13] Zolla L et al (2008), Proteomics as a complementary tool for identifying unintended side effects occurring in transgenic maize seeds as a result of genetic modifications. J Proteome Res 7: 1850-61. http://www.ncbi.nlm.nih.gov/pubmed/18393457 **MA-13**; Mesnage R et al (2016). An integrated multi-omics analysis of the NK603 Roundup-tolerant GM maize reveals metabolism disturbances caused by the transformation process. Scientific Reports 6. http://www.nature.com/srep/2016/161219/srep37855/full/srep37855.html **MA-14**; Agapito-Tenfen SZ et al (2014). Effect of stacking insecticidal cry and herbicide tolerance epsps transgenes on transgenic maize proteome. BMC Plant Biology 14:346 doi:10.1186/s12870-014-0346-8. http://www.biomedcentral.com/1471-2229/14/346/abstract **MA-15**; Lehesranta SJ et al (2005). Comparison of tuber proteomes of potato varieties, landraces, and genetically modified lines. Plant Physiol 138, 1690–1699. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1176438/pdf/pp1381690.pdf **MA-16**; Wang L et al (2015). Comparative proteomics of Bt-transgenic and non-transgenic cotton leaves. Proteome Science 13:15, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4422549/pdf/12953_2015_Article_71.pdf **MA-17**; Gong C et al (2012). Proteomics insight into the biological safety of transgenic modification of rice as compared with conventional genetic breeding and spontaneous genotypic variation. J Proteome Res 11, 3019–3029. https://pubs.acs.org/doi/10.1021/pr300148w **MA-18**.

[14] Latham JR et al (2006). The mutational consequences of plant transformation. J Biomed Biotechnol: 1–7. http://www.ncbi.nlm.nih.gov/pubmed/16883050 **MA-11**; Wilson AK et al (2006). Transformation-induced mutations in transgenic plants: Analysis and biosafety implications. Biotechnol Genet Eng Rev 23: 209–238. http://www.ncbi.nlm.nih.gov/pubmed/22530509 **MA-12**; Zolla L et al (2008), Proteomics as a complementary tool for identifying unintended side effects occurring in transgenic maize seeds as a result of genetic modifications. J Proteome Res 7: 1850-61. http://www.ncbi.nlm.nih.gov/pubmed/18393457 **MA-13.**

34. Below we provide evidence of signs of toxicity or actual toxicity arising from the above three sources, either alone or in combination.

Animal feeding studies with single-trait GM Bt and HT corn varieties

35. The vast majority of animal feeding studies on GM Bt and herbicide-tolerant (HT) corn are on single-trait Bt corn varieties and not on stacked varieties with high expression levels of Bt/VIP toxins and/or multiple pesticide residues.

36. It is reasonable to expect that stacked trait GM crops will multiply risks over and above any risks that apply to single-trait GM crops.

37. Some animal feeding studies on single-trait GM Bt and HT corn varieties have shown adverse effects and indications of toxicity and/or immunogenicity. The findings are summarised below.

Altered blood biochemistry, multiple organ damage, and potential effects on male fertility

38. Rats fed the GM Bt corn MON810: Ajeeb YG (developed by Monsanto for the Egyptian market) for 45 and 91 days showed differences in organ and body weights and in blood biochemistry, compared with rats fed the non-GM parent variety grown side-by-side in the same conditions. The authors noted that the changes could indicate "potential adverse health/toxic effects", which need further investigation.[15]

39. Histopathological investigations by the same researchers found toxic effects in multiple organs in rats fed the GM Bt corn for 91 days. Effects included abnormalities in, and fatty degeneration of, liver cells, congestion of blood vessels in kidneys, and excessive growth and necrosis (death) of intestinal structures called villi. Examination of the testes revealed necrosis and desquamation (shedding) of the spermatogonial cells that are the precursors of sperm cells and thus the foundation of male fertility.[16]

Immune disturbances

40. Young and old mice fed GM Bt corn for periods of 30 and 90 days showed a marked disturbance in immune system cells and in biochemical activity. Bt corn

[15] Gab-Alla AA et al (2012). Morphological and biochemical changes in male rats fed on genetically modified corn (Ajeeb YG). J Am Sci. 8(9):1117–1123. https://www.academia.edu/3138607/Morphological_and_Biochemical_Changes_in_Male_Rats_Fed_on_Genetically_Modified_Corn_Ajeeb_YG_ **MA-19**.

[16] El-Shamei ZS et al (2012). Histopathological changes in some organs of male rats fed on genetically modified corn (Ajeeb YG). J Am Sci. 8(10):684–696. https://www.academia.edu/3405345/Histopathological_Changes_in_Some_Organs_of_Male_Rats_Fed_on_Genetically_Modified_Corn_Ajeeb_YG_ **MA-20**.

consumption was also linked to an increase in serum cytokines (protein molecules that can influence the immune response), an effect associated with allergic and inflammatory responses.[17]

41. A separate study in rats fed GM Bt rice for 28 or 90 days found a Bt-specific immune response in the non-GM-fed control group as well as the GM-fed groups. The researchers concluded that the immune response in the control animals was due to their inhaling particles of the powdered Bt toxin-containing feed consumed by the GM-fed group. They recommended that for future tests involving Bt crops, GM-fed and control groups should be kept separate.[18] This indicates that animals can be sensitive to small amounts of GM proteins, so even low levels of contamination of conventional crops with GMOs could be harmful to health.

42. GM Bt toxin produced in bacteria has been found to induce a potent immune response in the intestine of mice[19] and to amplify the immune response of mice to other substances.[20] A 2021 study noted that some Cry proteins can stimulate the immune system and the response can be as potent as that elicited by cholera toxin. As a result, Cry proteins are being developed as adjuvants in human and animal vaccines.[21]

43. The potent immunogenic potential of Bt toxin could account for some of the physiological disturbances seen in GM crop animal feeding studies. Even natural Bt may not be completely benign. 1999 study found IgE-antibodies against a Bt endotoxin in farmworkers exposed to natural Bt sprays.[22]

Liver and kidney toxicity

44. A review of 19 studies (including the GMO industry's own tests submitted in support of regulatory authorisation of GM crops) on mammals fed with commercialised GM soy and corn found consistent signs of toxicity in the liver and

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[17] Finamore A et al (2008). Intestinal and peripheral immune response to MON810 maize ingestion in weaning and old mice. J Agric Food Chem. 56:11533–39. doi:10.1021/jf802059w **MA-21**.
[18] Kroghsbo S et al (2008). Immunotoxicological studies of genetically modified rice expressing PHA-E lectin or Bt toxin in Wistar rats. Toxicology 245:24-34. doi:10.1016/j.tox.2007.12.005, **MA-22**.
[19] Vázquez-Padrón RI et al (1999). Intragastric and intraperitoneal administration of Cry1Ac protoxin from Bacillus thuringiensis induces systemic and mucosal antibody responses in mice. Life Sci. 64(21):1897-1912. http://www.ncbi.nlm.nih.gov/pubmed/10353588 **MA-23**; Vázquez-Padrón RI et al (2000). Characterization of the mucosal and systemic immune response induced by Cry1Ac protein from Bacillus thuringiensis HD 73 in mice. Braz J Med Biol Res. 33:147-155. https://www.scielo.br/j/bjmbr/a/f8zxDxPtQcJK7ywHYNM8dtj/?lang=en **MA-24**; Vázquez-Padrón RI et al (2000). Cry1Ac protoxin from Bacillus thuringiensis sp. kurstaki HD73 binds to surface proteins in the mouse small intestine. Biochem Biophys Res Commun. 271:54-58. doi:10.1006/bbrc.2000.2584. **MA-25**.
[20] Vázquez-Padron RI et al (1999). Bacillus thuringiensis Cry1Ac protoxin is a potent systemic and mucosal adjuvant. Scand J Immunol. 49:578-584. http://www.ncbi.nlm.nih.gov/pubmed/10354369 **MA-26.**
[21] Gonzalez-Vazquez MC et al (2021). Importance of Cry proteins in biotechnology: Initially a bioinsecticide, now a vaccine adjuvant. Life 11(10):999. https://doi.org/10.3390/life11100999 **MA-27**.
[22] Bernstein IL et al (1999). Immune responses in farm workers after exposure to Bacillus thuringiensis pesticides. Environmental Health Perspective 107(7):575–82. https://ehp.niehs.nih.gov/doi/epdf/10.1289/ehp.99107575 **MA-28**.

kidneys. Such effects may mark the onset of chronic disease, though longer-term studies are required to assess this potential more thoroughly. Such long-term feeding trials on GMOs are not required by regulators in North America or the EU.[23]

45. In a separate study, the same research group re-analysed Monsanto's own rat feeding trial data, submitted to obtain approval in Europe for three commercialised GM Bt corn varieties. The re-analysis concluded that the corn varieties caused signs of toxicity in the liver and kidneys.[24] The data suggest that approval of these GM corn varieties – and stacked varieties based on them – should be withdrawn from the market because they are not substantially equivalent to non-GM corn and may be toxic.

Toxic effects on liver and kidneys and altered blood biochemistry

46. Rats fed GM Bt corn over three generations showed damage to liver and kidneys and alterations in blood biochemistry.[25]

Disturbances in digestive system and changes to liver and pancreas

47. Female sheep fed Bt GM corn over three generations showed disturbances in the functioning of the digestive system, while their lambs showed cellular changes in the liver and pancreas.[26]

EU-funded animal feeding studies with GM Bt and HT corn

48. The EU has funded three research projects on GM Bt and HT corn varieties: G-TwYST, GRACE, and GMO90+. They included the following rat feeding studies:

- G-TwYST – 90-day subchronic and two-year combined chronic toxicity and carcinogenicity studies on Roundup-tolerant GM corn NK603
- GRACE – 90-day[27] and one-year[28] studies on Bt corn MON810

[23] Séralini GE et al (2011). Genetically modified crops safety assessments: Present limits and possible improvements. Environ Sci Eur. 2011;23. doi:10.1186/2190-4715-23-10. https://enveurope.springeropen.com/articles/10.1186/2190-4715-23-10 **MA-29**.
[24] de Vendomois JS et al (2009). A comparison of the effects of three GM corn varieties on mammalian health. Int J Biol Sci. 5:706–26. http://www.ncbi.nlm.nih.gov/pubmed/20011136 **MA-30**.
[25] Kilic A, Akay MT (2008). A three generation study with genetically modified Bt corn in rats: Biochemical and histopathological investigation. Food Chem Toxicol. 46:1164–70. doi:10.1016/j.fct.2007.11.016. https://www.sciencedirect.com/science/article/abs/pii/S0278691507005443?via%3Dihub **MA-31**.
[26] Trabalza-Marinucci M et al (2008). A three-year longitudinal study on the effects of a diet containing genetically modified Bt176 maize on the health status and performance of sheep. Livest Sci. 113:178–190. doi:10.1016/j.livsci.2007.03.009 **MA-32**.
[27] Zeljenková D et al (2014). Ninety-day oral toxicity studies on two genetically modified maize MON810 varieties in Wistar Han RCC rats (EU 7th Framework Programme project GRACE). Arch Toxicol. 1-26. doi:10.1007/s00204-014-1374-8 **MA-33**.
[28] Zeljenková D et al (2016). One-year oral toxicity study on a genetically modified maize MON810 variety in Wistar Han RCC rats (EU 7th Framework Programme project GRACE). Arch Toxicol. 90(10):2531-2562. doi:10.1007/s00204-016-1798-4 **MA-34**.

- GMO90+ – 6-month (180 days) studies on MON810 and NK603 corn, looking at biomarkers of health effects.

49. All the studies were claimed by the authors and GMO advocates to show no adverse effects from the GM diets tested. However, this is not the case. Close reading of the study publications shows that signs of toxicity were seen in GM-fed animals in all three studies, as described below.

GRACE studies

50. The authors of the GRACE studies stated that no adverse effects were seen in the GM-fed animals. But the rats fed MON810 corn over 90 days showed various statistically significant differences. These include signs of liver toxicity, as evidenced by changes in levels of liver enzymes. The authors dismissed these findings on the grounds that they were not dose dependent, or were within the range of historical control data, or were not supported by the finding of lesions in the gross necroscopy or histopathological analysis.[29]

51. However, these are not valid reasons to dismiss statistically significant differences. Nonlinear dose responses are common in toxicological studies and lesions may not become visible within the short study duration of 90 days.

52. As noted by the German research NGO Testbiotech, GM-fed rats also showed a dose-dependent decrease in total serum protein and relative pancreas weight, with the latter accompanied by an increase in blood glucose levels. The authors failed to discuss the statistically significant and dose-dependent decrease of the relative pancreas weight and dismissed the significant increase in blood glucose because it was seen only in males of Trial A, conducted with one line of GM MON810 Bt toxin corn. However, the pancreas weight was not only decreased in males of Trial A, but also in males of Trial B, conducted with a second line of GM MON810 corn, although this change was not statistically significant.

53. Testbiotech also noted that surprisingly, the authors did not discuss these changes in conjunction in their paper, in spite of the vital role of the pancreas in the regulation of blood glucose levels. Instead the discussion remained silent on the statistically significant and dose-dependent decrease of the relative pancreas weight and the authors dismissed the significant increase in blood glucose because it was seen only in males of Trial A. However, the pancreas weight was not only decreased in males of Trial A, but also in males of Trial B, conducted with a second line of GM corn, although this change was not statistically significant. In the experiment as a whole, no “safe” level for this GM corn was found. Nonetheless the researchers

[29] Zeljenková D et al (2014). Ninety-day oral toxicity studies on two genetically modified maize MON810 varieties in Wistar Han RCC rats (EU 7th Framework Programme project GRACE). Arch Toxicol. 1-26. doi:10.1007/s00204-014-1374-8 **MA-33**.

dismissed all these findings as not toxicologically relevant, without a valid scientific justification.[30]

54. Testbiotech's interpretation of these findings was disputed by one of the 90-day GRACE study authors, Pablo Steinberg,[31] as well as by the team of authors in their publication of the results of the one-year feeding trial carried out under the same project. The authors' reasoning included that some of the findings of the 90-day study (notably the pancreas and glucose abnormalities) were not borne out by the findings of the one-year study, when those parameters normalised, and that no histopathological alterations were observed in rats fed the GMO diets.[32]

55. However, it is not acceptable to dismiss abnormalities that later normalise; if it were, we could dismiss as unimportant any human disease that the person eventually recovers from.

56. Moreover, there were concerning findings in the one-year study. The male rats fed the 33% GMO diets had a lower body weight than the male controls, while there were no differences in the body weight between male rats fed the 11% GMO diet and those fed the control diet. The mean body weight of female rats fed the 11% GMO and 33% GMO diets was lower than that of the animals fed the control diet throughout the study. These differences correlated with a lower feed intake in these GM-fed groups, but they were not statistically significant and the authors concluded they were of no toxicological relevance.

57. It is possible, though, that the differences could have reached statistical significance in a longer duration study with more animals.

58. In addition, statistically significant differences were observed in the GM-fed animals over the one-year period, including:

59. Haematology

- At 3 months the percentage of eosinophils was significantly higher in female rats fed the 33 % GMO diet than in the animals fed the control diet
- At 6 months the percentage of white blood cells was significantly higher in male rats fed the 11% GMO and the 33% GMO diets, and the percentage of

[30] Bauer-Panskus A, Then C (2014). Comments Regarding the GRACE Publication "Ninety-Day Oral Toxicity Studies on Two Genetically Modified Maize MON810 Varieties in Wistar Han RCC Rats (EU 7th Framework Programme Project GRACE)". Testbiotech. https://web.archive.org/web/20220617054534/https://www.testbiotech.org/sites/default/files/Testbiotech_Doubts_%20EU_Research_Project_GRACE_2.pdf **MA-35**.

[31] Steinberg P (2015). Response to a report and press release by Bauer-Panskus and Then (2014) criticizing the presentation and interpretation of the results of recently published 90-day feeding studies with diets containing genetically modified MON810-maize varieties and their comparators (Zeljenková et al. 2014). Arch Toxicol. 89(1): 137–139. doi: 10.1007/s00204-014-1429-x. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4282875/ **MA-36**.

[32] Zeljenková D et al (2016). One-year oral toxicity study on a genetically modified maize MON810 variety in Wistar Han RCC rats (EU 7th Framework Programme project GRACE). Arch Toxicol. 90(10):2531-2562. doi:10.1007/s00204-016-1798-4 **MA-34**.

eosinophils, which was significantly lower in female rats fed the 33% GMO diet when compared to the corresponding animals fed the control diet
- At 12 months the percentage of eosinophils was increased in male as well as in female rats fed the conventional 2 and the 11% GMO diets when compared to the corresponding animals fed the control diet.

60. Clinical biochemistry

- At 3 months, Cl (chloride) levels in male rats fed the 33% GMO diet were significantly decreased when compared to male rats fed the control diet
- At 6 months, the AST (a liver enzyme, increased levels of which can indicate liver damage) activity was significantly increased in male rats fed the 11% GMO diet, the P level was significantly increased in male rats fed the 33% GMO diet; GLU level was significantly lower in female animals fed the 33% GMO diet than in those fed the control diet
- At 12 months, the GLU level was significantly reduced and the P level significantly increased in male rats fed the 11% GMO diet as compared to those fed the control diet; CREA (creatinine) level was significantly higher in animals fed the 11% GMO diet compared with controls
- With the classical statistical methods, an increase in the AST activity was observed in female rats fed the 33% GMO diet for 12 months when compared to the corresponding control group.

61. Urinalysis

- A tendency to a higher pH was observed in urine samples from rats fed the 11% GMO and 33% GMO diets compared to those of animals fed the control and the conventional 2 diets.

62. Relative organ weights

- With the classical statistical methods, the relative left kidney weight of female rats fed the 33% GMO diet was increased compared to controls.

63. It is possible that extending the study length to 2 years or the full lifetime of the animals and increasing the numbers of animals used would have clarified the seriousness or otherwise of these findings.

64. It is also the case that the control diets were not tested for content of GMOs besides GM MON810 Bt toxin corn, so more obvious effects of the GMO diet could be masked. In addition, the diets used were found to contain residues of many different pesticides and these differed between the diets. The authors assumed that the levels found did not affect the animals' health in any way, but this assumption is unproven. This factor could also introduce "noise" into the experimental findings.

65. A separate study found that laboratory rodent feeds are highly contaminated with pesticides, toxic metals, PCBs, and GMOs. The researchers found that all the

feeds contained significant concentrations of several of these products at levels likely to cause diseases by disrupting the endocrine and nervous system of the animals.[33]

66. In spite of these issues with the GRACE study, differences were still found in the GMO-fed animals that may indicate adverse health effects. It is possible that if the diets were carefully controlled for contaminants, the effects found would have been more obvious.

67. An additional concern with the GRACE and G-TwYST projects were alleged conflicts of interest and bias among some of the scientists involved and even the journal that published the studies.[34]

GMO90+ studies

68. In the GMO90+ studies, two GM corn varieties, NK603 (glyphosate-tolerant) and MON810 (Bt toxin), were tested in rats over a 6-month period.

69. Statistically significant differences found in the GM corn-fed animals when compared with animals fed non-GM corn include alterations in the blood and urine biochemistry as well as changes in urine metabolites and hormone levels.[35] Such alterations could lead to ill health.

70. The concentration of intra-testicular testosterone displayed a difference at 180 days with a lower level in rats fed with the 11% MON810 diet than the controls.[36]

71. In addition, differentially expressed genes (DEGs) were found in animals fed a GM diet. Some alterations in gene expression were evidently caused by changes in the GM corn caused by spraying with glyphosate herbicide, since the sprayed NK603 corn had different effects on the rats than the unsprayed NK603 corn.

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[33] Mesnage R et al (2015). Laboratory rodent diets contain toxic levels of environmental contaminants: implications for regulatory tests. PLoS ONE 10.1371/journal.pone. 0128429. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0128429 **MA-37**.

[34] Testbiotech has carried out work on this. See, for example: https://www.testbiotech.org/en/news/testbiotech-files-complaint-about-grace-project/ **MA-38**; https://www.testbiotech.org/wp-content/uploads/2016/10/TBT-Background-GRACE_final_0.pdf **MA-39** ; **MA-40** https://www.testbiotech.org/en/news/grace-tries-end-feeding-study-debate/ ; **MA-41** https://www.testbiotech.org/en/news/grace-consortium-cannot-invalidate-criticism-feeding-trials/ See also the Ombudsman's response to Testbiotech's complaint alleging that the EU Commission, which financed the GRACE project, awarded the project to scientists with conflicts of interest: https://www.testbiotech.org/wp-content/uploads/2016/10/Ombudsman-decision-regarding-GRACE-July-2016.pdf The Ombudsman found no maladministration by the Commission but recommended that it take measures to explain why it thought there were no conflicts of interest and that it take measures to improve transparency and prevent conflicts of interest **MA-42**.

[35] Coumoul X et al (2018). The GMO90+ Project: Absence of evidence for biologically meaningful effects of genetically modified maize-based diets on Wistar rats after 6-months feeding comparative trial. Toxicological Sciences 168(2): 315–338. https://doi.org/10.1093/toxsci/kfy298 **MA-43**.

[36] Coumoul X et al (2018). The GMO90+ Project: Absence of evidence for biologically meaningful effects of genetically modified maize-based diets on Wistar rats after 6-months feeding comparative trial. Toxicological Sciences 168(2): 315–338. https://doi.org/10.1093/toxsci/kfy298 **MA-43.**

72. In some cases, the authors dismissed the significance of the DEGs because the number was low, but this is not valid because a change in the expression of only one gene can lead to far-reaching effects – even the difference between health and disease. For example, a change in the expression of a single gene was found to be associated with coronary artery disease in a group of people who have the disease, as compared with a group of people who do not have it.[37] In addition, it is well established that mutations in single or a few genes, which either increase or decrease their expression, can contribute to serious illnesses, including cancer.

73. The authors also dismissed findings of DEGs due to an assumed lack of biological relevance, “owing to the lack of information related to these changes or to the lack of difference in biologically linked variables for all comparisons”.[38] This is not scientifically valid as it assumes that we know all there is to know about the effects of gene expression.

G-TwYST study

74. A long-term 2-year rat feeding study (G-TwYST) found that males fed the GM Roundup-tolerant corn NK603 sprayed with the herbicide were significantly more likely to die before the end of the two-year experiment than males fed non-GM corn. The paper reporting the results of the G-TwYST study stated, "The mortality rate of the male rats fed the 33% NK603 + Roundup diet was significantly higher than that of the corresponding control group" fed non-GM corn."[39]

75. However, this finding was buried in the detail of the study and was not mentioned in the abstract. The abstract states – falsely – that "no adverse effects related to the feeding of the NK603 corn cultivated with or without Roundup for up to 2 years were observed".

76. The statistical report performed for the study offers a clearer statement on the size of the difference between males fed GM corn + Roundup (the "NK33+" diet): "There was an indication... that the mortality rate for NK33+ in males (54%) is larger than for the control non-GM feed (36%)."

77. The statistical report also says that there was no sign that the GM-fed groups without Roundup applications had an overall higher mortality rate than non-GM-fed groups. But, the statistical report authors add, "There was an indication for a Roundup effect in males... Keeping in mind that the mortality in the male control

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[37] Guo J et al (2018). Association of expression of ZNF606 gene from monocytes with the risk of coronary artery disease. Clin Biochem 60:44-51. doi: 10.1016/j.clinbiochem.2018.08.005. https://pubmed.ncbi.nlm.nih.gov/30130524/ **MA-44.**

[38] Coumoul X et al (2018). The GMO90+ Project: Absence of evidence for biologically meaningful effects of genetically modified maize-based diets on Wistar rats after 6-months feeding comparative trial. Toxicological Sciences 168(2): 315–338. https://doi.org/10.1093/toxsci/kfy298 **MA-43.**

[39] Steinberg P et al (2019). Lack of adverse effects in subchronic and chronic toxicity/carcinogenicity studies on the glyphosate-resistant genetically modified maize NK603 in Wistar Han RCC rats. Biologics 93: 1095–1139. https://link.springer.com/article/10.1007/s00204-019-02400-1 **MA-45.**

group was 36%, the mean percentages dead were 31% for NK603 without Roundup and 45% for NK603 with Roundup."

78. There was also a significantly higher average mortality in the two GM feed + Roundup groups compared to the two GM feed groups without Roundup (45% vs 31%).

79. The authors of the statistical report state, "There was an indication that Roundup could have increased the hazard and the mortality rate at 24 months for the males."[40]

80. In contrast with the males, the mortality rate for females fed the GM NK603 diet + Roundup was lower than the control group, though not significantly so.

81. The increased mortality in the males fed NK603 + Roundup was related to pituitary tumours, according to the Discussion section of the paper. The NK603 + Roundup diet did not increase the incidence of pituitary tumours, but did increase the number of deaths related to those tumours.

82. The G-TwYST authors claimed that the males fed NK603 + Roundup showed increased deaths because they ate more, leading to a "strong increase" in body weight between the 12th and 24th month of the feeding trial, compared with the non-GM-fed control group.

83. Such over-eating, they stated, typically leads to an earlier onset and higher incidence of pituitary tumours, as well as reduced survival.[41]

84. However, they did not investigate why the male rats fed NK603 corn + Roundup over-ate and got too fat. The rats in the other groups were also allowed unrestricted access to food, but did not over-eat or get fat. No explanation for this effect of the NK603 corn + Roundup diet is offered.

85. In all three of the EU-funded research projects described above, adverse and potentially adverse findings in animals fed the 33% (high dose) GM corn diet are especially concerning for populations, such as Mexicans, who eat a high proportion of corn in their diet.

[40] Goedhart PW, Voet H van der (2018). G-TwYST study A combined chronic toxicity and carcinogenicity study in rats fed GM maize NK603: main statistical report. Wageningen University & Research. https://library.wur.nl/WebQuery/wurpubs/547099 **MA-46.**

[41] Steinberg P et al (2019). Lack of adverse effects in subchronic and chronic toxicity/carcinogenicity studies on the glyphosate-resistant genetically modified maize NK603 in Wistar Han RCC rats. Biologics 93: 1095–1139. https://link.springer.com/article/10.1007/s00204-019-02400-1 **MA-45.**

GM HT corn NK603 is not substantially equivalent to non-GM corn

86. Substantial equivalence is the principle whereby a GM crop variety is assumed to be compositionally equivalent to a non-GM parent, apart from the intended genetic change. This principle underlies GMO authorisations in many countries and regions, including the US and (even if non-explicitly) in the EU. Thus, any study that demonstrates non-substantial equivalence questions the basis of the assumption of safety – and consequent market authorisation – of a GMO product.

87. In order to establish substantial equivalence, regulators only require industry to undertake a gross compositional analysis (e.g. total protein, fat, carbohydrates, etc.) akin to the nutritional information on the packet of a processed food product. However, it is not the total protein/fat/carbohydrate, etc. that accurately informs on the substantial equivalence and possible toxicity of a GM food, but the actual types of proteins/fats/carbohydrates, etc. that are present. In order to accurately establish whether a GM food is substantially equivalent to its non-GM counterpart, an in-depth molecular compositional analysis is required, employing non-targeted, comprehensive protein profiling (proteomics) and small biochemical profiling (metabolomics) methods. Below are summarised some of the studies that have used such "omics" methods in comparing the composition of GM and non-GM equivalent foods. The results consistently show that the use of cutting-edge omics analytical methods reveal that GMOs and their non-GM comparators can be far from compositionally substantially equivalent, undermining the claims of safety for these GMOs.

88. An in-depth analysis (Mesnage et al 2016) of the types of proteins (proteomics) and small biochemical molecules (metabolomics) in NK603 glyphosate-tolerant corn (sprayed or unsprayed with Roundup) revealed major differences from the non-GMO parent variety, showing that they were not substantially equivalent.[42]

89. Although the authors of the EU-funded animal feeding studies mentioned above did not do their own "omics" compositional analysis on the NK603 corn and the non-GM control variety, differences were found in the animals fed this GM corn and the non-GMO counterpart. It is possible that the compositional changes found in the in-depth analysis could account for some of these differences.

90. In the analysis (Mesnage et al 2016), a total of 117 proteins and 91 small molecule biochemicals (metabolites) were found to be significantly altered in NK603 corn by the GM transformation process. In addition, one protein and 31 metabolites had their expression significantly altered by the spraying of the Roundup herbicide. The GMO and non-GMO corn varieties were grown in the same location and under

[42] Mesnage R et al (2016). An integrated multi-omics analysis of the NK603 Roundup-tolerant GM maize reveals metabolism disturbances caused by the transformation process. Scientific Reports 6:37855. DOI: 10.1038/srep37855 **MA-14.**

the same conditions, meaning that environmental factors such as different growing soils did not cause these differences.[43]

91. The results of the analysis showed disturbances in energy utilisation and oxidative stress (damage to cells and tissues by reactive oxygen; oxidative stress can cause many diseases, including cancer) in the GMO corn. There were also large increases in certain polyamines, notably putrescine and cadaverine. Although some polyamines may have beneficial effects in certain contexts, others, such as putrescine and cadaverine, can produce various toxic effects. For example, they enhance the effects of histamine, thus heightening allergic reactions, and both have been implicated in the formation of carcinogenic substances called nitrosamines with nitrite in meat products.[44]

92. If this GM corn were to cross with native Mexican varieties, some of the genetic disturbances induced by the GM transformation process would be incorporated into native varieties, with unknown consequences. Given that a single event GM corn such as NK603 shows this degree of disturbance in composition, a stacked variety with multiple GM traits might be expected to show a greater degree of disturbance. Thus, the consequences of a GM stacked variety crossing with native Mexican varieties could have a greater impact on the genetic integrity of the latter.

Mechanism of toxicity of GM Bt crops uncertain

93. The GM Bt crop feeding studies cited above have not definitively proven that the engineered Bt toxin is the specific cause of harm that has been observed. This is because the animals have been fed the whole GM Bt crop, which contains thousands of components, not just the GM Bt toxin in isolation. These studies lacked a group of animals that were fed a standard diet spiked with the GM Bt toxin extracted from the GM crop, which would be very difficult to undertake. Nevertheless, these studies do show that GM Bt crops contain substances that cause adverse effects, regardless of the molecular cause of the problem, which could be the GM Bt toxin, unknown novel toxins arising from the GM transformation process, or a combination of the two.

Bt toxins in GM plants not the same as natural Bt

94. An analysis published in 2017 challenged claims that the Bt toxins in GM Bt crops are the same as natural Bt toxins sprayed by organic and conventional farmers – and that they are specific to a few insect pests. The authors systematically

[43] Mesnage R et al (2016). An integrated multi-omics analysis of the NK603 Roundup-tolerant GM maize reveals metabolism disturbances caused by the transformation process. Scientific Reports 6:37855. DOI: 10.1038/srep37855 **MA-14.**

[44] Mesnage R et al (2016). An integrated multi-omics analysis of the NK603 Roundup-tolerant GM maize reveals metabolism disturbances caused by the transformation process. Scientific Reports 6:37855. DOI: 10.1038/srep37855 **MA-14.**

compared GMO and natural Bt proteins and showed that many of the elements contributing to the narrow toxicity of natural Bt proteins have been removed by GMO developers in the process of inserting Bt toxins into crops. Thus, developers have made GMO insecticides that, in the words of a Monsanto patent, are “super toxins”. The authors of the analysis concluded that references to any GMO Bt toxins being “natural” are incorrect and scientifically unsupportable.[45]

Bt toxin found circulating in pregnant women’s blood

95. The US EPA assumes in the context of the regulation of GMOs that in mammals, including humans, Bt toxins are harmlessly broken down in the digestive tract.[46] However, this is false. A laboratory study simulating human digestion found that the Bt toxin protein was highly resistant to being broken down in realistic stomach acidity conditions and still produced an immune response.[47]

96. A study conducted in Canada found Bt toxin protein circulating in the blood of pregnant and non-pregnant women and the blood supply to foetuses.[48] Whether the Bt toxin originated from GM crops is not known. But wherever it came from, it did not break down fully in the digestive tract – and entered the body of the consumer.

A GM Bt corn, identified as a possible allergen, was found in food supply 10 years after being withdrawn

97. A GM Bt corn called StarLink was found to have contaminated the US food supply in 2000, shutting export markets, forcing the recall of hundreds of food products, with the cost to the food industry being estimated at a potential $1 billion.[49]

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[45] Latham JR et al (2017). The distinct properties of natural and GM cry insecticidal proteins. Biotechnol Genet Eng Rev. 33(1):62-96. doi:10.1080/02648725.2017.1357295 **MA-47.**
[46] US Environmental Protection Agency (EPA). Bt Plant-Incorporated Protectants: October 15, 2001 Biopesticides Registration Action Document. Washington, DC: US Environmental Protection Agency (EPA); 2001. https://www3.epa.gov/pesticides/chem_search/reg_actions/pip/bt_brad.htm **MA-48.**
[47] Guimaraes V et al (2010). In vitro digestion of Cry1Ab proteins and analysis of the impact on their immunoreactivity. J Agric Food Chem. 2010;58:3222-3231. doi:10.1021/jf903189j **MA-49.**
[48] 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):528–533. https://pubmed.ncbi.nlm.nih.gov/21338670/ **MA-50**; Goldstein DA et al (2012). Letter to the Editor. Comment: Aris and Leblanc “Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada”. Reproductive Toxicology 33: 120–121. https://pubmed.ncbi.nlm.nih.gov/22074695/ **MA-51**; Aris A (2012). Response to comments from Monsanto scientists on our study showing detection of glyphosate and Cry1Ab in blood of women with and without pregnancy. Reprod Toxicol. 33:122-123. https://www.sciencedirect.com/science/article/abs/pii/S0890623811003911?via%3Dihub **MA-52.**
[49] Laidlaw S (2001). StarLink fallout could cost billions: Future of modified crops thrown in doubt, report says. The Toronto Star, 9 Jan. Version of 15 Mar 2008 archived in Wayback Machine. https://web.archive.org/web/20080315114549/http://www.mindfully.org/GE/StarLink-Fallout-Cost-Billions.htm **MA-53.**

98. This was not an example of a field trial contamination case, but of a partial release that had taken place under the false assumption that GM genes could be controlled and contained. Regulators had allowed StarLink to be grown for animal feed and industrial use but had not approved it for human food because of suspicions that the Bt insecticidal protein it contained might cause allergic reactions. The US Centers for Disease Control (CDC) carried out tests on blood serum taken from a small number of those people who had reported reactions and concluded that there was no evidence that StarLink was the cause.[50] However, a Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) expert panel convened by the US Environmental Protection Agency (EPA) challenged the methodology and sensitivity of the CDC's tests and concluded that there was a "medium likelihood" that the Bt protein was an allergen.[51]

99. The company that developed StarLink, Aventis, withdrew the registration for the variety in 2000.[52] But in an example of the difficulties of recalling a GMO once it has been released, it was still detected in samples gathered from Saudi Arabian markets in 2009 and 2010.[53]

Health and environmental risks of stacked trait GM Bt crops

100. A review of the environmental effects and risks of stacked trait GM Bt crops containing multiple Bt toxins concluded that given the "unprecedented concentrations of potent bioactive bacterial toxins" in these plants, "we see a high probability that this increase of active ingredients will adversely affect the communities of organisms associated with these agroecosystems, alone and in conjunction with the likewise significant loads of herbicide and neonicotinoid residues. While such stacked varieties offer benefits to farmers for agronomic problems, these benefits may come with serious health and environmental risks that we find prudent to be experimentally

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[50] Centers for Disease Control and Prevention (CDC) (2001). Investigation of human health effects associated with potential exposure to genetically modified corn: A report to the US Food and Drug Administration. Version of 9 May 2024 archived in Wayback Machine. https://web.archive.org/web/20240509011209/https://www.cdc.gov/nceh/ehhe/cry9creport/pdfs/cry9creport.pdf **MA-54.**

[51] FIFRA Scientific Advisory Panel (2001). A set of scientific issues being considered by the Environmental Protection Agency regarding assessment of additional scientific information concerning StarLinkTM corn. SAP Report No. 2001-09. Arlington, Virginia: US Environmental Protection Agency (EPA). https://archive.epa.gov/scipoly/sap/meetings/web/pdf/one.pdf **MA-55.**

[52] Carpenter JE, Gianessi LP (2001). Agricultural biotechnology: Updated benefit estimates. National Center for Food and Agricultural Policy. Version of 21 Feb 2014 archived in Wayback Machine. https://web.archive.org/web/20140221194641/http://ucbiotech.org/biotech_info/PDFs/Carpenter_2001_Updated_Benefits.pdf **MA-56.**

[53] Elsanhoty RM et al (2013). Prevalence of genetically modified rice, maize, and soy in Saudi food products. Appl Biochem Biotechnol. doi:10.1007/s12010-013-0405-x. https://pubmed.ncbi.nlm.nih.gov/23904260/ . Full paper can be downloaded at: https://www.researchgate.net/publication/254261696_Prevalence_of_Genetically_Modified_Rice_Maize_and_Soy_in_Saudi_Food_Products **MA-57.**

studied prior to field release and market approval."[54]

Animal feeding studies with GM glyphosate-tolerant crops other than corn

101. Some animal feeding studies have been carried out on GM glyphosate-tolerant crops other than corn. Some concerning findings are summarised below.

GM HT soy: Disturbed liver, pancreas and testes function

102. Mice fed GM glyphosate-tolerant soy showed disturbed liver, pancreas and testes function. The researchers found abnormally formed nuclei and nucleoli (structures within the nuclei) in liver cells, which indicates increased metabolism and potentially altered patterns of gene expression. The authors stated that the mechanisms for the liver and pancreas findings remain unknown, though they suggested that, based on previous research on the effects of Roundup, residues of the herbicide may be responsible for the findings in the testes.[55]

GM HT soy: Liver ageing

103. Mice fed GM glyphosate-tolerant soy over a long-term (24-month) period showed changes in the expression of proteins relating to hepatocyte (liver cell) metabolism, stress response, and calcium signalling, indicating more acute signs of ageing in the liver, compared with the control group fed non-GM soy. While the authors stated that the mechanism remains unknown,[56] it is possible that Roundup residues in the soy were responsible.

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[54] Hilbeck A, Otto M (2015). Specificity and combinatorial effects of Bacillus thuringiensis Cry toxins in the context of GMO environmental risk assessment. Environ Health 71. doi:10.3389/fenvs.2015.00071 **MA-58.**

[55] Malatesta M et al (2003). Fine structural analyses of pancreatic acinar cell nuclei from mice fed on genetically modified soybean. Eur J Histochem. 47:385–388. http://www.ejh.it/index.php/ejh/article/viewFile/851/971 **MA-59**; Malatesta M et al (2002). Ultrastructural morphometrical and immunocytochemical analyses of hepatocyte nuclei from mice fed on genetically modified soybean. Cell Struct Funct. 27:173–80. http://www.ncbi.nlm.nih.gov/pubmed/12441651 **MA-60**; Vecchio L et al (2004). Ultrastructural analysis of testes from mice fed on genetically modified soybean. Eur J Histochem. 48:448-454. http://www.ncbi.nlm.nih.gov/pubmed/15718213 **MA-61.**

[56] Malatesta M et al (2008). A long-term study on female mice fed on a genetically modified soybean: effects on liver ageing. Histochem Cell Biol. 130:967–977. https://link.springer.com/article/10.1007/s00418-008-0476-x **MA-62.**

Roundup and other glyphosate-based herbicide formulations are more toxic than glyphosate alone

104. The industry tests carried out to support regulatory authorisations of glyphosate-based herbicides are carried out with glyphosate alone, the presumed "active ingredient". But commercial glyphosate herbicide formulations as sold and used do not consist of glyphosate alone. They also contain many other ingredients, known as co-formulants or adjuvants.

105. Glyphosate on its own cannot penetrate plant cell walls in order to exert its herbicidal activity. The co-formulants in commercial glyphosate-based herbicide formulations act as surfactants, weakening plant cell walls and thus allowing glyphosate to enter plants.

106. The co-formulants present in glyphosate-based herbicides are usually designated by manufacturers as "inert" on pesticide packaging and their identity is most frequently withheld, based on claims that this is confidential, proprietary industry information. This is a major concern, as a large and increasing body of evidence unequivocally shows that the co-formulants in themselves can be far more toxic than glyphosate.[57] Also, the formulations as sold and used are more toxic than glyphosate alone. In addition, the co-formulants increase the toxicity of glyphosate by enabling enhanced dermal (skin) penetration.[58] There will be minimally an additive and potentially a synergistic toxic effect. Despite clear evidence of co-formulants' toxicity and their presence in the environment in areas of GM glyphosate-tolerant crop cultivation,[59] their presence in foodstuffs is not monitored, which constitutes a major regulatory oversight. Indeed, co-formulants could be said to constitute the "dark matter" of pesticide toxicity.[60]

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[57] Mesnage R et al (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 ; Mesnage R et al (2019). Insight into the confusion over surfactant co-formulants in glyphosate-based herbicides. Food and Chemical Toxicology 128: 137-145. https://www.sciencedirect.com/science/article/pii/S0278691519301814?via%3Dihub **MA-63.**

[58] Mesnage R, Antoniou MN (2017). Ignoring adjuvant toxicity falsifies the safety profile of commercial pesticides. Front Public Health 5: 361. doi: 10.3389/fpubh.2017.00361 **MA-64**; Mesnage R et al (2019). Insight into the confusion over surfactant co-formulants in glyphosate-based herbicides. Food and Chemical Toxicology 128: 137-145. https://www.sciencedirect.com/science/article/pii/S0278691519301814?via%3Dihub **MA-65.**

[59] Tush D, Meyer MT (2016). Polyoxyethylene tallow amine, a glyphosate formulation adjuvant: soil adsorption characteristics, degradation profile, and occurrence on selected soils from agricultural fields in Iowa, Illinois, Indiana, Kansas, Mississippi, and Missouri. Env Sci Technol 50:5781–5789. https://pubmed.ncbi.nlm.nih.gov/27163278/ **MA-66**; Tush D, Maksimowicz MM, Meyer MT (2018). Dissipation of polyoxyethylene tallow amine (POEA) and glyphosate in an agricultural field and their co-occurrence on streambed sediments. Sci Tot Environ 636:212–219. https://www.sciencedirect.com/science/article/abs/pii/S0048969718314232 **MA-67.**

[60] Mesnage R, Antoniou MN (2017). Ignoring adjuvant toxicity falsifies the safety profile of commercial pesticides. Front Public Health 5: 361. doi: 10.3389/fpubh.2017.00361 **MA-64**; Mesnage R et al (2019). Insight into the confusion over surfactant co-formulants in glyphosate-based herbicides. Food and Chemical Toxicology 128: 137-145. https://www.sciencedirect.com/science/article/pii/S0278691519301814?via%3Dihub **MA-65.**

107. An in vitro study showed that all of 9 glyphosate-based herbicide formulations tested were more toxic than glyphosate alone.[61]

108. In another in vitro study, eight out of nine major pesticides tested in their complete formulations, including Roundup, were up to 1000 times more toxic to human cells than their isolated active ingredients. This increased toxicity of the complete formulation compared with the active ingredient alone was found to be a general principle of pesticide toxicology.[62]

109. An in vivo study on Roundup and glyphosate in rats showed that Roundup activates mechanisms involved in cancer development, including DNA damage – and these effects occur at doses assumed by regulators to have no adverse effects. The DNA damage was caused by oxidative stress. The study also found that the isolated active ingredient of Roundup – glyphosate alone – damaged DNA.

110. Roundup was found to be more toxic than glyphosate, confirming and building on previous observations. However, taken together, the results from the various assays show that both glyphosate and Roundup herbicides activate mechanisms involved in cancer development, causing gene expression changes reflecting oxidative stress and DNA damage. Also, glyphosate alone induced DNA damage.[63]

111. A more in-depth analysis of this study is below ("Animal feeding studies with Roundup and glyphosate: *DNA damage and oxidative stress caused by both Roundup and glyphosate").*

Animal feeding studies with Roundup and glyphosate

112. Numerous animal feeding studies have been carried out with Roundup and glyphosate, many of which show adverse effects. Summarised below are a few key studies.

Very low dose of Roundup causes liver and kidney damage

113. Rats fed an extremely low dose of Roundup glyphosate herbicide over a long-term 2-year period showed a marked increased incidence of structural and blood/urine biochemical changes, indicating damage to liver and kidney structure and functional pathology. This was confirmed by a total gene expression (transcriptome) profile analysis of liver and kidney tissues from these rats, which indicated damage to liver and kidney structure and function compared with controls.

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[61] Mesnage R et al (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 **MA-63.**
[62] Mesnage R et al (2014). Major pesticides are more toxic to human cells than their declared active principles. BioMed Res Int. doi:10.1155/2014/179691 **MA-68.**
[63] Mesnage R et al (2022). Comparative toxicogenomics of glyphosate and Roundup herbicides by mammalian stem cell-based genotoxicity assays and molecular profiling in Sprague-Dawley rats. Toxicological Sciences 186(1): 83-101. https://doi.org/10.1093/toxsci/kfab143 **MA-69.**

114. The dose of Roundup administered was 0.1 ppb Roundup (50 ng/L glyphosate equivalent) via drinking water (giving a daily intake of 4 ng/kg bw/day of glyphosate). This dose of glyphosate was 125,000 times lower than the current acceptable daily intake in Mexico and the European Union (500 μg/kg bw/day) and 250,000 times lower than the acceptable daily intake (chronic reference dose) in the USA (1000 μg/kg bw/day).

115. The authors concluded that long-term exposure to Roundup at an ultra-low, environmentally relevant dose "can result in liver and kidney damage with potential significant health implications for animal and human populations".[64]

Very low dose of Roundup causes non-alcoholic fatty liver disease

116. In a follow-up investigation of the same female rat liver tissues, the prediction of structural and functional damage from the transcriptomics analysis was confirmed using protein profiling (proteomics) and biochemical (metabolomics) analyses. Non-targeted proteomics and metabolomics analyses revealed that exposure to the glyphosate-based herbicide Roundup over a 2-year period, representing a dose of glyphosate that was thousands of times below what is permitted by regulators worldwide, resulted in non-alcoholic fatty liver disease (NAFLD).

117. In summary, these results demonstrated that long-term consumption of an ultra-low dose of Roundup at a glyphosate daily intake level of only 4 nanograms per kilogram of bodyweight per day, which is 125,000 times below EU/Mexican and 250,000 below US permitted levels, results in NAFLD.[65]

118. NAFLD currently affects 25% of the US population[66] and 14% to 27% of the general population in the industrialised world.[67] Prevalence in Mexico appears to be higher – over 50% of the population may be affected.[68] Risk factors include being overweight or obese, having diabetes, or having high cholesterol or high triglycerides (a constituent of body fat) in the blood. However, some people develop NAFLD even if they do not have any of these known risk factors. These studies on Roundup

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[64] Mesnage R et al (2015). Transcriptome profile analysis reflects rat liver and kidney damage following chronic ultra-low dose Roundup exposure. Environmental Health 14:70. http://www.ehjournal.net/content/pdf/s12940-015-0056-1.pdf **MA-70.**

[65] Mesnage R et al (2015). Transcriptome profile analysis reflects rat liver and kidney damage following chronic ultra-low dose Roundup exposure. Environmental Health 14:70. http://www.ehjournal.net/content/pdf/s12940-015-0056-1.pdf **MA-70.**; Mesnage R et al (2017). Multiomics reveal non-alcoholic fatty liver disease in rats following chronic exposure to an ultra-low dose of Roundup herbicide. Scientific Reports 7, Article number: 39328. https://www.nature.com/articles/srep39328 **MA-71.**

[66] American Liver Foundation (undated). Non-alcoholic fatty liver disease. https://liverfoundation.org/wp-content/uploads/2022/06/NAFLD-At-A-Glance-2015.pdf **MA-72.**

[67] Weiß J et al (2014). Non-alcoholic fatty liver disease. Dtsch Arztebl Int. 111(26): 447–452. doi: 10.3238/arztebl.2014.0447. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4101528/ **MA-73.**

[68] Bernal-Reyes R et al (2019). The Mexican consensus on nonalcoholic fatty liver disease. Revista de Gastroenterologia de Mexico 84(1): 69-99. DOI: 10.1016/j.rgmxen.2019.02.003. http://www.revistagastroenterologiamexico.org/en-the-mexican-consensus-on-nonalcoholic-articulo-S2255534X19300118#:~:text=Nonalcoholic%20fatty%20liver%20disease%20(NAFLD,its%20prevalence%20could%20surpass%2050%25 **MA-74**.

suggest that exposure to this herbicide may be a hitherto unrecognised risk factor for NAFLD.[69]

DNA damage and oxidative stress caused by both Roundup and glyphosate

119. A study on Roundup and glyphosate showed that Roundup activates mechanisms involved in cancer development, including DNA damage – and these effects occur at doses assumed by regulators to have no adverse effects. The DNA damage was caused by oxidative stress. The study also found that the isolated active ingredient of Roundup – glyphosate alone – damaged DNA.[70]

120. The study built on the findings of a previous study by the same authors. In the previous study, the researchers compared the effects in rats of a Roundup formulation, MON 52276, with those of its "active ingredient", glyphosate, tested alone. The findings showed that glyphosate and Roundup herbicide, given at doses that regulators say are safe to ingest, resulted in the animals suffering gut microbiome disturbances and oxidative stress, with indications that the liver was affected and possibly damaged.[71]

121. In the followup study, the researchers analysed the liver tissue from the same rats to see if indeed damage had occurred. [72]

122. The researchers carried out some of the standard tests that regulators require the pesticide industry to conduct to gain market authorisation for their products – namely blood biochemistry and kidney and liver histopathology (microscopic examination of tissue).

123. They also carried out in-depth tests (molecular profiling) that are not demanded by regulators or typically carried out by the industry. One type of test looked for adverse effects at a profound molecular level of biological functioning through analysis of gene expression (transcriptomics) and epigenetics (DNA methylation) in the liver and kidneys. Another type of test, using specialised

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[69] Mesnage R et al (2015). Transcriptome profile analysis reflects rat liver and kidney damage following chronic ultra-low dose Roundup exposure. Environmental Health 14:70. http://www.ehjournal.net/content/pdf/s12940-015-0056-1.pdf **MA-70**; Mesnage R et al (2017). Multiomics reveal non-alcoholic fatty liver disease in rats following chronic exposure to an ultra-low dose of Roundup herbicide. Scientific Reports 7, Article number: 39328. https://www.nature.com/articles/srep39328 **MA-71.**

[70] Mesnage R et al (2022). Comparative toxicogenomics of glyphosate and Roundup herbicides by mammalian stem cell-based genotoxicity assays and molecular profiling in Sprague-Dawley rats. Toxicological Sciences 186(1): 83-101. https://doi.org/10.1093/toxsci/kfab143 **MA-69.**

[71] Mesnage R et al (2021). Use of shotgun metagenomics and metabolomics to evaluate the impact of glyphosate or Roundup MON 52276 on the gut microbiota and serum metabolome of Sprague-Dawley rats. Environmental Health Perspectives 129(1). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7839352/ **MA-75.**

[72] Mesnage R et al (2022). Comparative toxicogenomics of glyphosate and Roundup herbicides by mammalian stem cell-based genotoxicity assays and molecular profiling in Sprague-Dawley rats. Toxicological Sciences 186(1): 83-101. https://doi.org/10.1093/toxsci/kfab143 **MA-69.**

genetically engineered cell lines, was intended to highlight changes in function linked with cancer formation.

124. In addition, the researchers carried out tests that can detect direct damage to DNA.

125. The standard tests, histopathology and blood biochemistry analysis, found adverse effects from the Roundup treatment, namely a dose-dependent and statistically significant increase in fatty liver disease and liver cell death.

126. The finding of fatty liver disease from exposure to the MON 52276 formulation of Roundup confirmed the same researchers' previous finding that an ultra-low dose of another Roundup formulation, Roundup Grand Travaux Plus, administered to the same strain of Sprague-Dawley rats over a 2-year period, caused non-alcoholic fatty liver disease.[73]

127. An increase in liver and kidney lesions was also detected in animals treated with glyphosate, although this did not reach statistical significance. However, the authors commented that an experiment of longer duration using more animals may have resulted in statistical significance.

128. However, it was the non-standard molecular profiling tests that are not required by pesticide regulators that were most revealing.

129. First, Roundup was found to alter the expression of 96 genes in the liver specifically linked to DNA damage and oxidative stress, as well as disruption of circadian rhythms ("body clocks"). The most affected genes in liver also had their expression similarly altered in kidneys. Crucially, a core set of genes whose expression was altered by Roundup was similarly changed in the glyphosate-treated animals. This strongly suggests that the key changes in gene function reflective of oxidative stress and DNA damage were due to glyphosate and not the additional substances (co-formulants) present in the Roundup formulation.

130. Second, direct DNA damage to the liver was found to increase with glyphosate exposure.

131. Third, both glyphosate and Roundup were found to cause epigenetic changes known as DNA methylation. Epigenetics describes layers of molecular structures associated with DNA that control the underlying function of genes. The defining feature of epigenetic changes is that they can alter how genes work but do not involve changes to the actual DNA sequence. These types of changes were found at over 5,000 genomic sites for glyphosate and over 4,000 for Roundup. This is of concern because such alterations are typically found at high frequency in cancer tissues.

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[73] Mesnage R et al (2017). Multiomics reveal non-alcoholic fatty liver disease in rats following chronic exposure to an ultra-low dose of Roundup herbicide. Scientific Reports 7, Article number: 39328. https://www.nature.com/articles/srep39328 **MA-71.**

132. In summary, Roundup was found to be more toxic than glyphosate, confirming and building on previous observations. However, taken together, the results from the various assays show that both glyphosate and Roundup herbicides activate mechanisms involved in cancer development, causing gene expression changes reflecting oxidative stress and DNA damage. Also, glyphosate alone was clearly able to induce DNA damage.[74]

These findings directly challenge the global regulatory practice of only assessing the isolated declared active ingredient (glyphosate) and not the complete commercial formulations (Roundup) as sold and used.

Toxic effects of glyphosate and commercial glyphosate herbicide formulations are more pronounced when exposure starts at a pre-natal stage

133. In an effort to better reflect a real-world exposure scenario, the scientists who initiated the Global Glyphosate Study [ref website] designed experiments whereby exposure of rats to glyphosate alone or to two different commercial glyphosate-based herbicide formulations representative of the EU and the USA began at an early stage of pregnancy, and with some animals analysed at different stages of life post-natally. Rats were exposed to three doses of glyphosate (0.5, 5, and 50 mg/kg body weight/day) and the commercial formulations at the same glyphosate-equivalent dose. Below are summarised the outcomes from this study that have been published to date.

Glyphosate and commercial glyphosate-based herbicide formulations result in marked gut microbiota compositional dysbiosis

134. Exposure of rats, starting in early adulthood, to glyphosate and the EU representative glyphosate herbicide formulation (Roundup Bioflow) resulted in modest compositional changes in the microbiome.[75]

135. In contrast, exposure to glyphosate and the EU (Roundup Bioflow) and US (RangerPro) representative commercial formulations, starting pre-natally, resulted in marked microbiota compositional changes at both a bacterial and fungal level. The fitness of major commensals of the microbiome in the large intestine was highly compromised, which in turn reduced competition and allowed opportunistic fungi to grow in the gut, in particular in animals exposed to the herbicide formulations. This indicated that changes in gut microbiome composition might influence the long-term

[74] Mesnage R et al (2022). Comparative toxicogenomics of glyphosate and Roundup herbicides by mammalian stem cell-based genotoxicity assays and molecular profiling in Sprague-Dawley rats. Toxicological Sciences 186(1): 83-101. https://doi.org/10.1093/toxsci/kfab143 **MA-69.**
[75] Mesnage R et al (2021). Use of shotgun metagenomics and metabolomics to evaluate the impact of glyphosate or Roundup MON 52276 on the gut microbiota and serum metabolome of Sprague-Dawley rats. Environmental Health Perspectives 129(1). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7839352/ **MA-75.**

toxicity, carcinogenicity, and multigenerational effects of glyphosate-based herbicides.[76]

136. Rats exposed to glyphosate and the two commercial formulations were analysed for leukemia incidence and mortality at 104 weeks of age. In the animals exposed to glyphosate, a significantly increased trend in incidence of lymphoblastic leukemia was observed in males. In the Roundup Bioflow-treated animals, significantly increased trends were observed in incidence of lymphoblastic leukemia (males and females), monocytic leukemia (males), total myeloid leukemia (males), and all leukemias combined (males and females). In the RangerPro-treated animals, significantly increased trends were observed in incidence of lymphoblastic leukemia (males and females), monocytic leukemia (males) and all leukemias combined (males). 43% of leukemia deaths in the glyphosate and glyphosate-based herbicide-treated groups occurred before the first year of age (52 weeks).[77] (Note: this study has not yet been peer-reviewed.)

Regulatory implications

137. The in vivo toxicity studies of glyphosate and commercial glyphosate-based herbicide formulations summarised above consistently show that adverse health outcomes result from administration of the "no observed adverse effect level" (NOAEL) dose (in the EU and Mexico, this is 50 mg/kg bodyweight/day). These results challenge regulators' position that this level of glyphosate has produced no adverse effects in the animals on the basis of the studies that industry submits to support market authorisation. This suggests that the industry studies lack sufficient sensitivity for an accurate determination of safety.

138. Since the in vivo toxicity studies summarised above demonstrate that the current EU and Mexican NOAEL is incorrect, the ADI, which is calculated on the basis of the NOAEL, is also incorrect and set too high.

139. It should be noted that the glyphosate NOAEL held by the US authorities is 100 mg/kg bodyweight/day. Thus the studies summarised here also invalidate the US glyphosate NOAEL and its corresponding ADI.

140. For these reasons, assurances of the safety of levels of residues of glyphosate-based herbicides present in US-grown corn imported into Mexico are questionable, especially when long-term exposure is considered.

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[76] Mesnage R et al (2022). Glyphosate and its formulations Roundup Bioflow and RangerPro alter bacterial and fungal community composition in the rat caecum microbiome. Front. Microbiol. 13:888853. doi: 10.3389/fmicb.2022.888853 **MA-76.**

[77] Panzacchi S et al (2023). Leukemia in Sprague-Dawley rats exposed long-term from prenatal life to glyphosate and glyphosate-based herbicides. bioRxiv, 16 Nov. https://www.biorxiv.org/content/10.1101/2023.11.14.566013v1.full **MA-77.**

Epidemiological studies in humans on Roundup exposure

141. The body of epidemiological literature on the effects of Roundup exposure on humans is vast and beyond the scope of this report to summarise. However, below are summaries of a few key recent studies.

Markers of DNA damage from glyphosate-based herbicide exposure

142. A study funded by the US National Institutes of Health reported indications of DNA damage among men applying Roundup and other glyphosate-based herbicides. DNA damage is concerning because it can lead to cancer.[78] A commentary on the paper published in the same issue of the journal that published the study called it "a critical step forward in filling knowledge gaps of glyphosate carcinogenicity in humans".[79]

143. The study's authors were inspired to conduct their study because although mechanistic studies in human cells and animals support the genotoxic effects of glyphosate, "evidence in human populations is scarce". They analysed the blood, urine, and mouth cells of pesticide applicators in Iowa and North Carolina. They found links between lifetime occupational use and a marker of DNA damage (genotoxicity) called mosaic loss of chromosome Y (mLOY). mLOY is a chromosomal alteration that is commonly detected in the blood cells of ageing men. It has been associated with blood cancers such as lymphoma, myeloma, and leukemia, as well as with Alzheimer's disease.

144. The findings showed that greater lifetime glyphosate-based herbicide use was associated with higher prevalence of mLOY affecting at least 10% of cells. Associations were strongest among applicators aged 70 years or over, those who were never smokers and those who were not obese. The authors observed a dose-response relationship, with higher odds of mLOY as total lifetime days of glyphosate use increased.

The authors state that their findings on mLOY provide new insights into the biological mechanisms through which glyphosate may contribute to genomic instability, which is another key characteristic of carcinogens, beyond the mechanisms of direct DNA damage (genotoxicity) and oxidative stress.[80]

Oxidative stress biomarkers increase with exposure to glyphosate-based herbicide

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[78] Chang VC et al (2023). Glyphosate use and mosaic loss of chromosome Y among male farmers in the Agricultural Health Study. Environmental Health Perspectives 131(12). CID: 127006 https://doi.org/10.1289/EHP12834 **MA-78.**

[79] Schinasi LH, De Roos AJ (2023). Invited Perspective: Important new evidence for glyphosate hazard assessment. Environmental Health Perspectives 131(12). CID: 121305. https://doi.org/10.1289/EHP1425 **MA-79.**

[80] Chang VC et al (2023). Glyphosate use and mosaic loss of chromosome Y among male farmers in the Agricultural Health Study. Environmental Health Perspectives 131(12). CID: 127006 https://doi.org/10.1289/EHP12834 **MA-78.**

145. Research by US National Institutes of Health scientists found that people exposed to glyphosate-based herbicide have biomarkers in their urine of oxidative stress.

146. The study measured glyphosate levels in the urine of applicators of glyphosate-based herbicides and other study participants and determined that high levels of the pesticide were associated with signs of a reaction in the body called oxidative stress, a condition that causes damage to DNA. Urinary concentrations of each oxidative stress biomarker increased with increasing levels of urinary glyphosate.

147. Among recently exposed applicators, glyphosate use within 1 day (vs 5-7 days) of urine collection was associated with elevated concentrations of the oxidative stress biomarkers 8-OHdG and malondialdehyde. Also, recent glyphosate use (regardless of further classification by days since last use) was associated with increased levels of the oxidative stress biomarker 8-isoprostane.[81] The increased levels of 8-OHdG are particularly noteworthy and of concern since it is not only a marker of oxidative stress but also of DNA damage.

High exposure to glyphosate-based herbicide increases risk of non-Hodgkin lymphoma by 41%

148. A meta-analysis of studies on the cancer-causing potential of glyphosate herbicides, including the 2018 update of the Agricultural Health Study (AHS) cohort, found that individuals with high exposures to the herbicides have a 41% increased risk of developing non-Hodgkin lymphoma (NHL).[82]

149. The paper is published in the journal Mutation Research/Reviews in Mutation Research, whose editor-in-chief is EPA toxicologist David DeMarini. Three of the study authors were members of the EPA's scientific advisory panel on glyphosate. The EPA concluded that glyphosate does not cause cancer, but a group of those advisors told the EPA it had failed to follow its own rules and proper scientific practices in its assessment.[83]

150. The authors of the meta-analysis state in their paper, "Together, all of the meta-analyses conducted to date, including our own, consistently report the same

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[81] Chang VC et al (2023). Glyphosate exposure and urinary oxidative stress biomarkers in the Agricultural Health Study. J Natl Cancer Inst. 115(4): 394–404. doi: 10.1093/jnci/djac242. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10086635/ **MA-80.**

[82] Zhang L et al (2019). Exposure to glyphosate-based herbicides and risk for Non-Hodgkin Lymphoma: A meta-analysis and supporting evidence. Mutation Research/Reviews in Mutation Research 781:186-206. https://www.sciencedirect.com/science/article/abs/pii/S1383574218300887?mc_cid=23c18e62e7&mc_eid=ff8c3a64ef **MA-81.**

[83] Gillam C (2019). Weedkiller 'raises risk of non-Hodgkin lymphoma by 41%'. The Guardian, 14 Feb. https://www.theguardian.com/business/2019/feb/14/weed-killing-products-increase-cancer-risk-of-cancer **MA-82.**

key finding: exposure to glyphosate, more precisely to GBHs [glyphosate-based herbicides], is associated with a statistically significant increased risk of NHL."

151. They conclude, "The overall evidence from human, animal, and mechanistic studies presented here supports glyphosate's carcinogenic potential in mediating NHL. Given that humans are exposed to adjuvant-containing mixtures known to provoke synergistic toxic effects in vivo and in vitro, future studies of GBHs in experimental animals should be conducted."[84]

152. A review (Williams et al, 2016) by the consultancy Intertek, hired by Bayer/Monsanto, relied heavily on the Agricultural Health Study to claim there is no link between NHL and glyphosate herbicide exposure. However, the meta-analysis based on updated data shows that this claim was incorrect.

153. International Agency for Research on Cancer's (IARC's) classification of glyphosate as a probable human carcinogen was incorrect, and that glyphosate was "unlikely to pose a carcinogenic risk to humans".[85]

154. This claim is in line with the conclusion of "no effect" from glyphosate on NHL that was reached by the authors of the previously published AHS study reports.[86]

155. However, the authors of the new meta-analysis point out that the results of the previous analyses were biased by the inclusion of people with very low exposure, which can dilute the risk estimates. When people with high exposures are considered independently, a link between glyphosate herbicides and NHL is found.[87]

Court invalidates US EPA health assessment of glyphosate

156. In June 2022 a US federal appellate court invalidated the US EPA's favourable human health safety assessment for glyphosate, ruling that the EPA did

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[84] Zhang L et al (2019). Exposure to glyphosate-based herbicides and risk for Non-Hodgkin Lymphoma: A meta-analysis and supporting evidence. Mutation Research/Reviews in Mutation Research 781:186-206. https://www.sciencedirect.com/science/article/abs/pii/S1383574218300887?mc_cid=23c18e62e7&mc_eid=ff8c3a64ef **MA-81.**

[85] Williams GM et al (2016). A review of the carcinogenic potential of glyphosate by four independent expert panels and comparison to the IARC assessment. Critical Reviews in Toxicology 46 - Issue sup1: An Independent Review of the Carcinogenic Potential of Glyphosate. https://www.tandfonline.com/doi/full/10.1080/10408444.2016.1214677?role=tab&tab=permissions&aria-labelledby=reprints-perm&scroll=top **MA-83.**

[86] Andreotti G et al (2018). Glyphosate use and cancer incidence in the Agricultural Health Study. J Natl Cancer Inst 110(5):509-516. doi: 10.1093/jnci/djx233 **MA-84**; De Roos AJ et al (2004). Cancer incidence among glyphosate-exposed pesticide applicators in the Agricultural Health Study. Environmental Health Perspectives 113(1): 49–54. doi: 10.1289/ehp.7340 **MA-85.**

[87] Zhang L et al (2019). Exposure to glyphosate-based herbicides and risk for Non-Hodgkin Lymphoma: A meta-analysis and supporting evidence. Mutation Research/Reviews in Mutation Research 781:186-206. https://www.sciencedirect.com/science/article/abs/pii/S1383574218300887?mc_cid=23c18e62e7&mc_eid=ff8c3a64ef **MA-81.**

not properly follow scientific guidelines when it determined glyphosate was not carcinogenic. The court found that EPA officials discounted several important studies and said, “most studies EPA examined indicated that human exposure to glyphosate is associated with an at least somewhat increased risk of developing NHL”. The court said that the EPA ignored expert advice from scientific advisers and used “inconsistent reasoning” in concluding that the chemical poses “no risks to human health”. Overall, the EPA determination that glyphosate was “not likely to be carcinogenic” was “flawed” in multiple ways, the court said. The court ruled that the EPA should produce a revised assessment by 1 October 2022.[88]

157. The EPA withdrew the relevant part of its glyphosate assessment but said it was unable to meet the October 2022 court-imposed deadline.[89]

Mixtures of pesticides can harm health even when each pesticide is present at levels deemed safe

158. For regulatory purposes, pesticide active ingredients are tested individually for safety. However, in real life, people are exposed to a mixture of different pesticides. Studies show that such mixtures can be toxic even when each individual pesticide is present at a level that regulators deem safe to ingest. The following studies illustrate this point.

Omics analyses of gut-liver axis show metabolic disturbance from a low-dose pesticide mixture

159. A study found that mixtures of pesticide residues commonly found in foods can have adverse effects on health even when each individual pesticide is present at a level considered safe by regulators.

160. The study also found that the use of "omics" molecular analytical techniques can reveal adverse effects on health that are missed by the standard toxicological tests used to support regulatory authorisations of pesticides.

161. The researchers set out to see if omics and standard toxicological tests would reveal signs of ill health in rats fed a mixture of pesticides over the relatively short-term period of 90 days. The mixture consisted of six pesticide active ingredients – azoxystrobin, boscalid, chlorpyrifos, glyphosate, imidacloprid and thiabendazole. All these pesticides are used on corn, with glyphosate able to be used on GM glyphosate-tolerant corn at higher levels than would be used on non-glyphosate-tolerant corn.

[88] NRDC and PANNA vs US EPA (2022). No. 20-70801. Opinion by Judge Friedland. https://www.thenewlede.org/wp-content/uploads/2022/06/9th-circuit-on-glyphosate.pdf **MA-86.**
[89] US EPA (2023). EPA withdraws glyphosate interim decision released on September 23, 2022. https://www.epa.gov/pesticides/epa-withdraws-glyphosate-interim-decision **MA-87.**

162. A mixture was tested because this reflects real-world conditions, in which people are exposed to cocktails of pesticides. Each individual pesticide was present at the EU acceptable daily intake (ADI) level, the level that is deemed by regulators to be safe to consume on a daily basis.

163. The study found that the standard toxicological measures – analysis of water and feed consumption, body weight, histology, and blood biochemistry – showed little or no evidence of harm. In contrast, the omics analyses showed biochemical changes in the gut and blood and gene function changes in the liver that indicated the possible onset of harm.

164. Metabolomics analysis of the blood of the pesticide-exposed rats showed that many metabolites had their levels altered by exposure to the pesticide mixture. In particular, a decrease in blood pyridoxal (a form of vitamin B6) was seen. This may indicate that exposure to the pesticide mixture could in the long term result in vitamin B6 deficiency, which has been linked with autism spectrum disorder.

165. Omics analysis of the biochemical signalling between the gastrointestinal tract and the animal's body as a whole suggested a cell danger response. This response included adaptation to oxidative stress, which manifested as alterations in tryptophan-nicotinamide biochemical pathways. Oxidative stress results from excessive production of reactive oxygen, an imbalance in the body that can damage vital molecules and cell structures, which in turn can lead to serious disease such as cancer.

166. The results showed a link between the gut biochemical disturbance and overall health status – the gut findings correlated with the blood biochemistry and liver profiling.

167. Transcriptomics analysis of the liver showed that 257 genes had their expression changed. Gene functions affected included those involved in the regulation of response to hormones.

168. Analysis of the DNA methylation (a process that can change the activity of DNA without changing the gene sequence) of the liver showed a metabolic adaptation that could exceed the cells' capacity for restoring balance, ultimately leading to disease such as liver damage or cancer.[90]

Alterations in small RNA profiles in liver following exposure to a low-dose pesticide mixture

169. Small RNAs are biomarkers to monitor health and track the development of diseases. A study found changes in small RNAs (miRNAs) in the liver of rats exposed to a pesticide mixture, as revealed by "omics" analyses. The mixture was

[90] Mesnage R et al (2021). Multi-omics phenotyping of the gut-liver axis reveals metabolic perturbations from a low-dose pesticide mixture in rats. Communications Biology 4, Article number: 471. https://www.nature.com/articles/s42003-021-01990-w **MA-88.**

composed of six pesticides frequently detected in foodstuffs (azoxystrobin, boscalid, chlorpyrifos, glyphosate, imidacloprid and thiabendazole, all of which are used on corn). Nine miRNAs with known health roles were altered by the pesticide mixture treatment, including 7 that were downregulated and 2 that were upregulated. These miRNAs were predicted to regulate genes, which were found to have their expression altered by the pesticide mixture and which have known health implications in the regulation of hepatic metabolism.[91]

170. This work supported and extended previously published conclusions that high- throughput "omics" analyses can reveal molecular perturbations, which can potentially act as sensitive and accurate markers of health risks arising from exposure to environmental pollutants such as pesticides.[92]

Perinatal exposure to mixture of 3 herbicides at the ADI level caused oxidative stress in liver

171. People who eat US-grown corn will likely be ingesting residues of glyphosate and 2,4-D, as Dow DuPont's/Corteva's GM Enlist corn is engineered to tolerate both pesticides.[93]

172. A study investigated hepatotoxic (toxic to liver) effects following perinatal exposure to glyphosate alone or in combination with 2,4-D and dicamba from gestational day-6 until adulthood in rats. This perinatal exposure (the period of time from becoming pregnant to after giving birth) – reflects the real-life conditions in which people are exposed to pesticides. Animals were administered with glyphosate at the EU (and Mexican) acceptable daily intake (ADI; 0.5 mg/kg bw/day) and no-observed-adverse-effect level (NOAEL; 50 mg/kg bw/day). A mixture of glyphosate with 2,4-D (0.3 mg/kg bw/day) and dicamba (0.02 mg/kg bw/day) with each at their EU ADI was evaluated.

173. The researchers analysed redox reactions in the rats, a type of oxidative stress that can cause diseases, including cancer. Gene expression analysis of genes associated with oxidative damage to DNA was also performed.

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[91] Mesnage R et al (2021). Alterations in small RNA profiles in liver following a subchronic exposure to a low-dose pesticide mixture in Sprague-Dawley rats. Toxicol Lett. 353:20-26. doi: 10.1016/j.toxlet.2021.10.001. https://pubmed.ncbi.nlm.nih.gov/34626815/ **MA-89**

[92] Mesnage R et al (2021). Multi-omics phenotyping of the gut-liver axis reveals metabolic perturbations from a low-dose pesticide mixture in rats. Communications Biology 4, Article number: 471. https://www.nature.com/articles/s42003-021-01990-w **MA-88.**

[93] Corteva (2024). 2024 Product use guide. https://www.enlist.com/content/dam/dpagco/enlist/na/us/en/files/fact-sheets/DOC-Enlist-PUG-NA-US.pdf **MA-90.**

174. Analysis of liver samples showed that exposure to the mixture of the three herbicides induced a disturbance in redox balance. Toxic effects were also evident in the groups of animals exposed to the glyphosate NOAEL dose.[94]

175. Results from an investigation of the same animals as in the above-cited study, relating to gut structure, function, and integrity, will become available later this year.

2,4-D induces carcinogenic cellular changes

176. The importance of taking into account effects of toxicity from a mixture of glyphosate and 2,4-D (as above) is further highlighted by the findings in a battery of cell line tests designed to assess the carcinogenic potential of chemicals. This study found that 2,4-D, either alone or in combination with glyphosate and dicamba, elicited a potent oxidative stress and misfolded protein response, which are both recognised predictors of carcinogenicity.[95]

SIGNED DATE

May 28, 2024

Professor Michael Antoniou

[94] Nechalioti PM et al (2023). Evaluation of perinatal exposure of glyphosate and its mixture with 2,4-D and dicamba on liver redox status in Wistar rats. Environmental Research 228, 115906. https://www.sciencedirect.com/science/article/abs/pii/S0013935123006989?via%3Dihub **MA-91**

[95] Mensage R et al (2021). Genotoxicity evaluation of 2,4-D, dicamba and glyphosate alone or in combination with cell reporter assays for DNA damage, oxidative stress and unfolded protein response. Food and Chemical Toxicology 157: 112601 **MA-92**