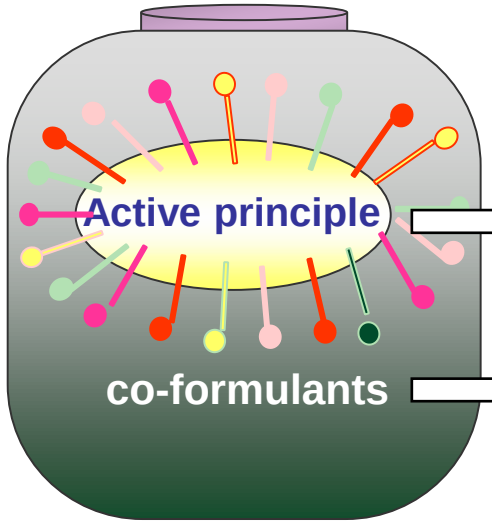
Multiple Organ Toxicity from Glyphosate and Roundup at Regulatory Relevant Doses

Prof Michael Antoniou

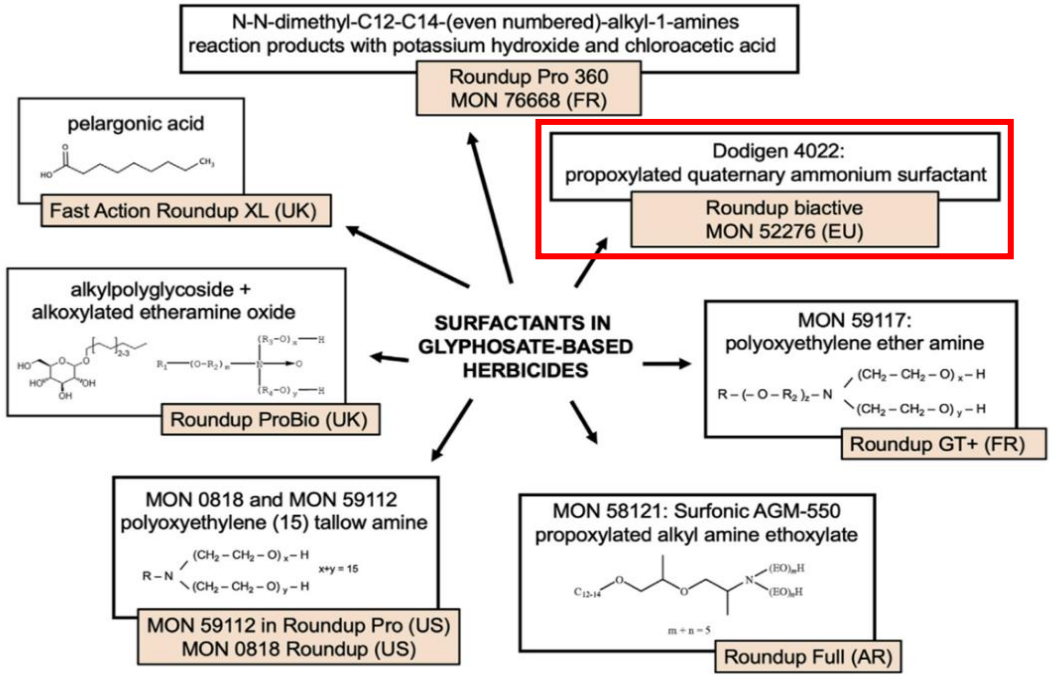
King’s College London

Pesticides: farmers never use active principles alone



Toxic to a target species
e.g. glyphosate on plants

Known to stabilize and enhance cell/plant cell wall penetration of the active principles



Pesticide co-formulants/adjuvants:

- **Up to 1000x more toxic than active ingredient**
- **Usually not disclosed**
- **Largely ignored by regulators during pesticide risk assessment; no long-term toxicity testing required**

Mesnage R *et al. (2019) Food Chem Toxicol.* **128**: 137-1445

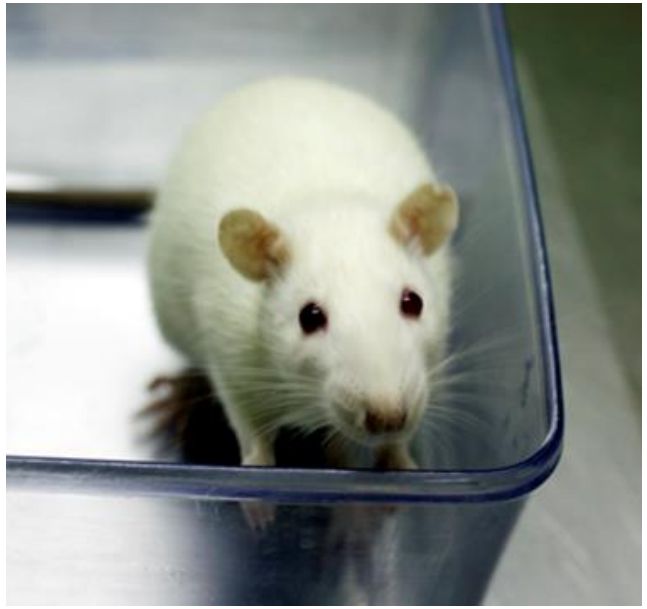
Sub-chronic toxicity testing of glyphosate and Roundup MON 52276

5-6 week old Sprague Dawley rats

Tap water

Glyphosate or MON 52276
(0.5, 50 and 175 mg/kg bw/day)

Mixture (ADI)
(azoxystrobin, boscalid chlorpyrifos, **glyphosate** imidacloprid, thiabendazole)



90 days

X12 per group

OECD and GLP compliant

Gut microbiome
Caecum metabolomics
Caecum metagenomics

General toxicity
Liver and kidney histopathology
Biochemistry
Serum metabolomics

Liver molecular profiles
Transcriptomics
DNA Methylome
miRNAome
DNA damage

Mesnage R et al (2021) *Environ Health Perspect*. **129**: 17005
Mesnage R et al. (2022) *Toxicol Sci*. **186**: 83-101

Glyphosate and MON 52276 disturb gut microbiome at doses regulators say are safe

- Glyphosate and Roundup MON 52276 disrupt the gut microbiome by the same mechanism by which the chemical acts as a weedkiller – inhibition of the shikimate biochemical pathway.
- Roundup MON 52276 and glyphosate altered the microbiome at all doses tested, causing shifts in bacterial populations.
- **These effects happen even at low doses (NOAEL, ADI) which regulators assert have no adverse effects and are safe.**
- Study is first to describe one key mechanism by which glyphosate and Roundup affect function of the gut microbiome.
- Humans and other animals don't have the shikimate pathway, enabling industry and regulators to state that glyphosate is nontoxic to humans. But fungi and some strains of gut bacteria do have this pathway.

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). DOI: 10.1289/EHP6990

Global Glyphosate Study

Glyphosate and Roundup formulations decrease bacterial diversity and increase fungal diversity

- **Sprague-Dawley rats exposed prenatally until adulthood (90-days)**
- **Three doses of glyphosate (0.5, 5, 50 mg/kg body weight/day)**
- **Three glyphosate equivalent doses of Roundup Bioflow and RangerPro**

Bacterial composition diversity reduced.

Fungal composition diversity increased.

Exposure to glyphosate and Roundup formulations starting at foetal stage results in more dramatic changes in the gut microbiome compared to exposure starting in young adulthood.

Glyphosate and RangerPro and Roundup Bioflow (MON 52276) reduced colonisation resistance and allowed opportunistic (potentially pathogenic) fungi to grow in the gut.

Mesnage R et al. (2022) *Front. Microbiol*. **13**: 888853

Roundup MON 52276 more disruptive to gut and blood biochemistry than glyphosate alone

- Molecular biochemical composition profiles (metabolomics) showed an oxidative stress response in both the gut and blood, with Roundup MON 52276 more disruptive than glyphosate alone.
- Oxidative stress damages not only cells and organs, but also DNA, which can lead to serious disease such as cancer.
- Epidemiological studies show oxidative stress and DNA damage response following occupational exposure to glyphosate-based herbicides (Chang VC et al., 2023)

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). DOI: 10.1289/EHP6990

Glyphosate and Roundup change gene function, damage DNA

- Blood metabolomics suggested glyphosate and Roundup MON 52276 damaged the rats' livers.
- Follow-up study: analysis of liver from the same rats to see if harm had occurred.
- **Standard tests** demanded by regulators for market authorisation of pesticides:
  - Blood biochemistry
  - Kidney and liver histopathology (microscopic examination of tissue)
- **Non-standard tests** not demanded by regulators:
  - Liver and kidney molecular profiling and analysis of gene expression (transcriptomics)
  - Epigenetics (DNA methylation) analysis
  - Using the ToxTracker cell line system to look for changes linked with cancer formation
  - Tests to detect direct damage to DNA.

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. *Toxicol Sci*. **186**: 83-101.

Findings of the standard tests

- Histopathology and blood biochemistry analysis: dose-dependent adverse effects from Roundup MON 52276 - and statistically significant increase in fatty liver disease and liver cell death (steatosis, necrosis).
- Fatty liver disease induced by Roundup MON 52276 confirmed previous observation that an ultra-low dose of Roundup Grand Travaux Plus (~40,000x below the glyphosate EU ADI), fed to rats over 2 years, caused non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) (Mesnage et al., 2017).
- Strong association between glyphosate/Roundup exposure and NAFLD/NASH found in epidemiological studies (e.g. Eskenazi B et al., 2023)
- Increase in liver and kidney lesions was seen in animals fed glyphosate, although this did not reach statistical significance. A longer experiment using more animals may have resulted in statistical significance.

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. *Toxicol Sci*. **186**: 83-101.

Association of Lifetime Exposure to Glyphosate and Aminomethylphosphonic Acid (AMPA) with Liver Inflammation and Metabolic Syndrome at Young Adulthood: Findings from the CHAMACOS Study

Eskenazi B et al. *Environ Health Perspect*. **131**: 37001, 2023

“Childhood exposure to glyphosate and AMPA may increase risk of liver and cardiometabolic disorders in early adulthood, which could lead to more serious diseases later in life.”

Non-standard tests were most revealing

- Roundup MON 52276 altered the expression of 96 genes in the liver linked to oxidative stress and DNA damage, as well as disruption of circadian rhythms ("body clocks”).
- Most affected genes reflective of DNA damage in liver also had their expression similarly altered in kidneys.
- Core gene expression changes reflective of oxidative stress and DNA damage were the same in Roundup and glyphosate-treated animals; **thus due to glyphosate and not the co-formulants in the Roundup MON 52276**.
- Direct DNA damage (AP-sites) in the liver increased with glyphosate exposure.
- **Discovery that glyphosate alone damages DNA should, under EU pesticide law, result in a ban on the chemical.**

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. *Toxicol Sci*. **186**: 83-101.

Epigenetic changes suggest cancer-causing ability

- Glyphosate and Roundup MON 52276 caused epigenetic (DNA methylation); alterations in gene function.
- DNA methylation changes were found at over 5,000 genomic sites for glyphosate and over 4,000 for Roundup MON 52276.
- DNA methylation alterations typically found at high frequency in cancer.
- Small RNA profiling in liver showed changes in the microRNA profile reflective of carcinogenesis.

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. *Toxicol Sci*. **186**: 83-101.

Cell line tests show cancer-causing ability of Roundup formulations

- **ToxTracker** cell line test system (Toxys, The Netherlands); highlights gene expression changes that can lead to cancer formation.
- Two formulations of Roundup herbicide including MON 52276, but not glyphosate, activated oxidative stress and misfolded protein responses, both clear markers of carcinogenicity.
- Glyphosate damaged DNA in living animals but not in the cell system. This shows that in vitro tests (lab tests not performed in living organisms) cannot fully substitute for tests in a living animal because certain effects will be missed.

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. *Toxicol Sci*. **186**: 83-101.

Regulatory “safe” doses of glyphosate and Roundup shown to be unreliable

- All doses of glyphosate and Roundup MON 52276 tested caused some adverse effects; **current EU glyphosate / Roundup NOAEL and ADI are incorrect**.
- Glyphosate/Roundup caused (i) gut microbiome composition and biochemical function changes, (ii) oxidative stress in gut and body, (iii) gene expression changes reflective of DNA damage, (iv) direct DNA damage, (v) epigenetic (DNA methylation, miRNA) alterations
- **Collectively all measures point to glyphosate, and more so to Roundup, as potent risk factors for fatty liver disease and carcinogenicity**
- Stronger adverse effects were seen from Roundup MON 52276 than glyphosate; **why do regulators fail to require meaningful toxicological data, including long-term testing, on co-formulants?**
- **Adverse effects from glyphosate/Roundup exposure are slow to become evident and may give a false sense of safety.**

Cutting-edge molecular profiling methods show toxic effects that standard regulatory tests miss

- Adverse effects were revealed even over the short period of 90 days because we used cutting-edge molecular analytical techniques (“omics”) to measure the composition of blood and contents of the gut.
- These techniques are more sensitive than the standard toxicity tests performed by industry to support regulatory approvals of pesticides.
- Tests required by regulators will miss important toxic effects.
- **When will regulators stop living in the dark ages and come into the 21st century and demand molecular profiling methods to be used in risk assessment?**

- **Why were our studies presented here not included in the ECHA opinion on glyphosate toxicity?**

Acknowledgements

Gene expression And Therapy Group

Robin Mesnage
Scarlett Ferguson
Mariam Ibragim

Istituto Ramazzini
COOPERATIVA SOCIALE ONLUS

Fiorella Belpoggi
Daniele Mandrioli
Laura Falcioni
Simona Panzacchi

Barts and The London
School of Medicine and Dentistry

Genome Centre

Charles Mein

Leids Universitair Medisch Centrum

Quinten R Ducarmon
Romy Daniëlle Zwittink

Sustainable Food Alliance