

# Glyphosate Herbicides Change Gene Function and Cause DNA Damage – New Study

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by [Sustainable Pulse](#)

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Glyphosate-based herbicides such as Roundup activate mechanisms involved in cancer development, including DNA damage – and these effects occur at doses assumed by regulators to have no adverse effects, a [new study](#) shows. The DNA damage was caused by oxidative stress, a destructive imbalance in the body that can cause a long list of diseases, [GMWatch reported Tuesday](#).

The study also found that the isolated active ingredient of Roundup – glyphosate alone – damaged DNA. This finding, according to the EU's pesticide law, should result in a ban on glyphosate and all its formulations. In addition, the results obtained in the study could strengthen the legal cases of the cancer sufferers in the US who are suing Bayer/Monsanto because they believe that exposure to Roundup caused their disease. Three such cases have already been decided in favour of the plaintiffs.

The new study is currently published on the pre-print website [bioRxiv](#) and has not yet been peer reviewed.

### **How the study was done**

The new study, led by Dr Michael Antoniou and Dr Robin Mesnage at King's College London, builds 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, resulted in the animals suffering gut microbiome disturbances and oxidative stress, with indications that the liver was affected and possibly [damaged](#).

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

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).

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

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

### **Roundup causes fatty liver disease – confirmed**

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.

The finding of fatty liver disease from exposure to the MON 52276 formulation of Roundup confirmed the same researchers' previous [observation](#) 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.

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.

### **Non-standard tests most revealing**

Worryingly for public health, it was the non-standard molecular profiling tests that are not required by pesticide regulators that were most revealing.

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 or "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 was due to glyphosate and not the additional substances (adjuvants) present in the Roundup formulation.

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

These findings potentially constitute a bombshell that could end the authorization of glyphosate in the EU. That's because the EU pesticide regulation (1107/2009) has what's known as hazard-based cut-off criteria. This means that if a pesticide active ingredient is shown to cause a certain type of harm to

health at whatever dose, it must be banned. One of the named types of harm is damage to DNA. The discovery that glyphosate alone damages DNA in a living animal should, if regulators follow the law, result in a ban on glyphosate.

Third, both glyphosate and Roundup were found to cause epigenetic changes known as DNA methylation. Epigenetics describes consists of 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 a concern because such alterations are typically found at high frequency in cancer tissues.

## **Cancer**

The researchers performed further laboratory tests in mouse cell lines, which are designed to highlight effects that can lead to cancer formation. Glyphosate and three Roundup formulations were assessed in these tester cell lines. It was found that two formulations of Roundup herbicide, but not glyphosate, activated oxidative stress and misfolded protein responses, both clear markers of carcinogenicity.

## **Ending animal testing not yet feasible**

Interestingly, glyphosate was shown to damage DNA in living animals but not in the cell culture 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. This is because animals (including humans) are whole organisms whose complexity cannot be replicated in a flask, petri dish, or test tube. While many people (GMWatch included) would like to see an end to animal testing, as long as pesticides and other chemicals are allowed to be released into the environment, such a move would put public health at risk.

## **Roundup more toxic than glyphosate**

In summary, in general Roundup was found to be more toxic than glyphosate, confirming and building on previous observations. However, taken together, the results from the various assays conducted 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.

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.

The study further highlights the power of in-depth molecular profiling “omics” methods to detect changes that are missed by relying solely on conventional biochemical and histopathological measurements conducted in standardised industry tests on pesticide active ingredients. The study paves the way for future investigations by identifying gene expression changes and altered DNA methylation sites, which can serve as biomarkers and potential predictors of negative health outcomes resulting from exposure to glyphosate-based herbicides.

## **Investigating Roundup health effects in people**

Commenting on the implications of the results, study lead Dr Michael Antoniou said, “The biomarkers we identified can be tested for in people, but we don’t know if this particular pattern of biomarkers is unique to glyphosate-based herbicide exposure. Thus the biomarkers would need to be correlated with a history of exposure to glyphosate-based herbicides and measurements of glyphosate in urine.

“If high levels of glyphosate were found in the urine, and this correlated with the biomarkers identified in the new

study and the person's history of glyphosate herbicide exposure, this would indicate that exposure to glyphosate-based herbicides might be responsible for any health effects that are both indicated by our findings and found in the person. These findings should be tested first by investigations of herbicide applicators, as their exposure can be high and details of the particular herbicides used are often recorded, which would enable clearer results to be obtained."

### **"Safe" and "no effect" doses shown to be harmful**

In the 90-day rat feeding study, different groups of animals were fed three different doses of glyphosate and the glyphosate-equivalent dose of Roundup MON 52276. The lowest dose was the concentration that regulators assume to be safe to ingest on a daily basis over a lifetime (the EU acceptable daily intake or ADI: 0.5 mg per kg of bodyweight per day). The middle dose was the dose that EU regulators concluded had no observable adverse effect (the "no observable adverse effect" level or NOAEL) in industry-sponsored rat feeding studies (50 mg per kg of bodyweight per day). The highest dose was 175 mg, the dose that US regulators concluded had no observable adverse effect.

Adverse effects were found from Roundup exposure at all dose levels in a dose-dependent fashion. These findings show that the glyphosate ADI for the EU is not safe to ingest if it comes as part of a formulated herbicide – as is the case with public exposures to herbicides. Likewise, it shows that the EU and US regulators were only able to conclude that glyphosate had "no observable adverse effect" at the levels mentioned above because the tests that they require industry to carry out are inadequate and insufficiently sensitive.

### **Implications for Roundup/cancer litigation**

Summarising the implications of the new study, Dr Antoniou commented, "Our results are the first to simultaneously show

glyphosate and Roundup toxicity in a whole mammalian model system and provide a mechanism – oxidative stress – by which DNA damage has been observed in other systems, such as mammalian tissue culture cells.

“These findings have implications for the Roundup/cancer litigation in the US. They show that glyphosate and Roundup score positive in various tests of carcinogenicity (transcriptome/epigenome changes, oxidative stress, protein misfolding, and DNA damage) in a living animal (rat) that is accepted as a surrogate for human health effects. In my view, this strengthens the case that exposure to Roundup herbicides can lead to the type of cancer suffered by many plaintiffs, non-Hodgkin lymphoma.”

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### **The new study:**

In-depth comparative toxicogenomics of glyphosate and Roundup herbicides: histopathology, transcriptome and epigenome signatures, and DNA damage.

Robin Mesnage, Mariam Ibragim, Daniele Mandrioli, Laura Falcioni, Fiorella Belpoggi, Inger Brandsma, Emma Bourne, Emanuel Savage, Charles A Mein, Michael N Antoniou.

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[www.biorxiv.org/content/10.1101/2021.04.12.439463v1](https://www.biorxiv.org/content/10.1101/2021.04.12.439463v1)

### **Abstract**

Background Health effects from exposure to glyphosate-based herbicides is an intense matter of debate. Toxicity including genotoxicity of glyphosate alone has been repeatedly tested over the last 40 years. Contrastingly, few studies have conducted comparative investigations between glyphosate and its commercial herbicide formulations, such as Roundup. We thus performed the first in-depth comparative toxicogenomic evaluation of glyphosate and a typical European Union Roundup formulation by determining alterations in transcriptome and

epigenome profiles.

## **Methods**

Glyphosate and the European Union reference commercial formulation Roundup MON 52276 (both at 0.5, 50, 175 mg/kg bw/day glyphosate equivalent concentration) were administered to rats in a subchronic 90-day toxicity study. Standard clinical biochemistry and kidney and liver histopathology was performed. In addition, transcriptomics and DNA methylation profiling of liver and selective gene expression analysis of kidneys was conducted. Furthermore, a panel of six mouse embryonic reporter stem cell lines validated to identify carcinogenic outcomes (DNA damage, oxidative stress, and protein misfolding) were used to provide insight into the mechanisms underlying the toxicity of glyphosate and 3 Roundup formulations.

## **Results**

Histopathology and serum biochemistry analysis showed that MON 52276 but not glyphosate treatment was associated with a statistically significant increase in hepatic steatosis and necrosis. Similar lesions were also present in the liver of glyphosate-treated groups but not in the control group. MON 52276 altered the expression of 96 genes in liver, with the most affected biological functions being TP53 activation by DNA damage and oxidative stress as well as the regulation of circadian rhythms. The most affected genes in liver also had their expression similarly altered in kidneys. DNA methylation profiling of liver revealed 5,727 and 4,496 differentially methylated CpG sites between the control group and the group of rats exposed to glyphosate and MON 52276, respectively. Direct DNA damage measurement by apurinic/apyrimidinic lesion formation in liver was increased with glyphosate exposure. Mechanistic evaluations showed that two Roundup herbicides but not glyphosate activated oxidative stress and misfolded protein responses.



## **Conclusions**

Taken together, the results of our study show that Roundup herbicides are more toxic than glyphosate, activating mechanisms involved in cellular carcinogenesis and causing gene expression changes reflecting DNA damage. This further highlights the power of high-throughput 'omics' methods to detect metabolic changes, which would be missed by relying solely on conventional biochemical and histopathological measurements. Our study paves the way for future investigations by reporting a panel of gene expression changes and DNA methylation sites, which can serve as biomarkers and potential predictors of negative health outcomes resulting from exposure to glyphosate-based herbicides.

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