



## FAQs on the King's College Study on the Impact of Glyphosate and GBHs on the Gut Microbiome and Serum Metabolome

### ***Why did the King's College team carry out this study?***

Two reasons. First, many of the health problems linked to glyphosate and glyphosate-based herbicides (GBHs) seem to involve the human GI system. This led the King's College team to focus on the gut microbiome and its influence on blood composition.

### ***What sort of health problems?***

Strong evidence has confirmed that people applying GBHs are sometimes heavily exposed, and as a result are at heightened risk of damage to their DNA and non-Hodgkin lymphoma (NHL).

GBH use near residential communities appears to increase the risk of autism among children. It also raises the odds of a woman giving birth to low-birth weight, preterm babies.

Last, even low-level exposures to glyphosate in food might be contributing to a host of GI tract, liver, and kidney problems.

### ***What's the second reason?***

GBHs are by far the most heavily used pesticide in history, both in the U.S. and globally. Most people on the planet are exposed on many days every year to low-levels of glyphosate via food, drinking water, and/or the air.

Even low exposures may be contributing in subtle ways to chronic disease and long-term health problems.

When such impacts could be impacting a significant share of humanity, it is important to use the best science possible to assure that pesticide regulators have not missed something that might end up contributing to health problems for millions of people.

***What is the key finding in this study?***

Dietary exposure to low levels of glyphosate and GBHs can alter the composition of GI tract microbiomes in ways that may be linked to adverse health outcomes.

The effects of glyphosate on the gut microbiome were found to be caused by the same mechanism of action by which glyphosate acts as a weed killer.

***Did glyphosate act as an antibiotic?***

Glyphosate is often claimed to work as an antibiotic and kill bacteria. This is because it was patented as an antiparasitic agent to kill a range of microbial parasites, and because some studies have found that bacteria were killed by glyphosate.

However, what happens in a petri dish does not necessarily reflect what happens in the gut.

For glyphosate to kill gut bacteria, it would need to cause a significant shortage in the production of compounds called aromatic amino acids. These compounds are synthesized within the shikimate pathway in plants and some other organisms, including bacteria but not mammals.

But humans do not produce aromatic amino acids themselves. Instead, we source these compounds from our diet, and especially fresh fruits and vegetables, nuts, and other nutrient-dense foods. The same is true for most of our gut bacteria.

We have evolved together. Because humans have lost their ability to produce aromatic amino acids during the course of evolution, most gut bacteria have lost this ability too. This was recently confirmed by another study by the King's College team (<https://www.sciencedirect.com/science/article/pii/S2666027X20300049>).

Examining 734 human gut microbiome samples, they found that most bacteria in the human gut have lost their ability to make aromatic amino acids. This led the team to realize that glyphosate's impact on the composition of bacteria in the human microbiome is happening for some other reason, or more likely, complex and changing combinations of reasons.

***So why did the composition of the gut microbiome change in the rat study?***

Glyphosate was indeed found to inhibit the shikimate pathway in the new rat study. But this likely did not kill bacteria, because they do not need the shikimate pathway to get the aromatic amino acids they need. Like us, they get them from the food we eat.

However, glyphosate's impact had other consequences. A multitude of small molecules accumulated, or were depleted in the rat gut. It is not clear whether these changes caused a pathological effect. However, the changes in certain marker molecules can serve as the first signature to evaluate if glyphosate is having an effect on the gut microbiome in humans.

The composition of the gut microbiome may have changed for another reason. When the King's College team examined the 734 human gut microbiome samples in their last study, they predicted that glyphosate might fuel the growth of some Proteobacteria. This is because the glyphosate molecule contains one atom of phosphorus, and this phosphorus can be used as a source of energy for this type of bacteria. This is one plausible reason explaining why the composition of the rat gut microbiome changed after the exposure to glyphosate in the new King's College study.

In addition, some of the bacteria which grew in presence of glyphosate in the rat gut microbiome were previously found to use glyphosate as a source of energy in studies on soil bacteria. This capacity of glyphosate to be, in effect, a food source for certain bacteria is also thought to possibly be connected to toxic algae blooms in lakes in many regions of the world, including the Great Lakes and many reservoirs in the American Heartland.

### ***What is shotgun metagenomics?***

Shotgun metagenomics entails the study of the genetic material sampled from, or recovered from environmental samples.

In the case of this study, the environmental sample explored was the contents of the large intestine from glyphosate-treated rats, referred to as "ceca" in the paper.

The "genetic material" picked up by this method included rat DNA, the DNA from microorganisms inhabiting the rat gut, as well as DNA in the food the rats consumed. One of the tricky challenges the team had to tackle was separating out the rat-DNA and the DNA in the food fed to the rats from microbial DNA. Why separate these out?

Because the team's focus was changes in the microbial composition of the rat microbiome. To study **THAT**, the team had to isolate changes in microbial DNA from the other DNA in the contents of the large intestine.

### ***How do scientists carry out a shotgun metagenomics analysis?***

Step 1 -- Scientists use rapid genomic sequencing tools to identify all the different types of DNA, including gene sequences, in a sample.

Step 2 -- The likely sources of the identified genetic material (i.e. which bacterial species it comes from) are identified.

Step 3 -- The relative diversity and abundance of different types and sources of genetic material is determined.

In this glyphosate and GBH microbiome study, only the sequences of the genes made by microbial DNA were used to identify and quantify the different species of microorganisms composing the gut microbiome.

### ***What do scientists learn from the results that shotgun metagenomics produces?***

The mix of DNA and genetic material in our GI tract is a marker of what we have been eating, the chemicals we have been exposed to via food, air, and our drinking water, and how healthy our lifestyles are (nutrition, sleep, stress, medical conditions, drug use).

The diversity and balance of microorganisms in the human GI tract, AKA our microbiome, are linked in complex ways to whether:

- We are well nourished,
- Our bodies are able to effectively combat infections, pandemics, or cancerous cell growths, and
- We respond effectively to the tasks and stresses we face every day.

### ***What does the mix of genetic material in a person's microbiome tell scientists?***

Rapid progress is being made by scientists all over the world in recognizing abnormal, unhealthy patterns of DNA and genetic material in the human microbiome. Some patterns in microbiome genetic material have been linked to heightened risk of specific health problems.

An important example has recently been highlighted in high-quality scientific papers -- COVID patients with healthy microbiomes are more likely to recover quickly, and tend to suffer fewer and less serious lingering symptoms, compared to COVID patients with abnormal microbiomes unable, among other things, to mount a full and effective immune response.

But one point deserves emphasis -- Scientists are identifying linkages between abnormal patterns in the human GI tract microbiome, but do not fully understand, yet, when the abnormal mix of DNA in a person's microbiome is the ***cause*** of a health problem, or one of the ***symptoms caused by the health problem***.

### ***What is metabolomics?***

It is the study of small molecules called metabolites. Metabolites are studied in cells, biofluids like blood and urine, organs, and tissues. They are products of chemical reactions occurring during the life cycle of a living organism. However, the metabolomics method is very powerful and does not discriminate between small molecules from different sources. It measures biological molecules from an animal's body, food components, and even foreign compounds (AKA xenobiotics) like drugs, pollutants, or pesticides.

The presence and mix of metabolites in a sample gives scientists important insights into the health of the metabolic processes that keep the human body functioning, nourished, and bestowed with sufficient energy to get through the day.

### ***Why did the King's College team deploy both shotgun metagenomics and metabolomics in their glyphosate study?***

Because they understood they needed at least two prisms through which to explore the connections between a rat's microbiome, its exposure to glyphosate, and the impact on an animal's health.

Each tool helped sharpen the team's understanding of what the sequencing data was telling them.

### ***How is this study unique?***

The depth and sophistication of the sequencing in this study is unprecedented. Earlier studies focused on the impact of glyphosate on the composition of the gut microbiome have measured and tracked changes in approximately 50,000 DNA sequences. The King's College team looked at approximately 20,000,000 DNA sequences covering every gene in the microorganisms in the rats. The DNA code contains 4 letters. The quantity of DNA analyzed for each rat gut microbiome sample represented approximately 5 billion letters.

And that's not all. Earlier studies have focused on the composition of microbiomes. The King's College team also evaluated for the first time the alterations in function of the gut microbiome caused by glyphosate. They accomplished this by measuring via metabolomics the levels of ~700 small molecules. Plus, this was done in both ceca and blood, giving the team a more comprehensive picture of the full-body consequences of the gut microbiome alterations caused by exposure to glyphosate.

### ***Why is the impact of glyphosate and GBHs on shikimate important?***

Shikimate is a key compound in the shikimate pathway. It serves as a precursor for the synthesis of many compounds including polyphenols and key aromatic amino acids that

play vital roles in health promotion.

***What are the consequences of the big decrease in levels of solanidine and carotenoids?***

This is a major finding, but it's hard to understand its implications. Clearly, GBHs in the microbiome appear to reduce the presence of these compounds. One hypothesis is that GBHs like Roundup promote the growth of bacteria species that metabolize (i.e. break down) solanidine and carotenoids.

One can extrapolate the clinical significance of decreased levels of these compounds. **Carotenoids** are a group of pigments present in a wide variety of bacteria, algae, fungi, and plants. They play an important role in human health by decreasing oxidative stress, a critical factor of the pathogenic processes of various chronic disorders such as cancer and cardiovascular disease. (<https://pubmed.ncbi.nlm.nih.gov/24473231/>)

Whether the observed decrease in carotenoids is sufficient to influence health outcomes cannot be determined at this time. Interestingly, another study has found a similar phenomenon recently in soil organisms exposed to GBHs. (<https://www.sciencedirect.com/science/article/abs/pii/S0045653520314478>)

***Was the toxicity of glyphosate and Roundup different?***

Both glyphosate alone and a formulated herbicide containing glyphosate were tested. The herbicide formulation was chosen to be representative of the EU market. The commercial formulation of glyphosate exerted more effects than glyphosate on both the gut microbiome and the composition of blood.

The blood of animals exposed to the commercial formulation of glyphosate showed evidence of “oxidative stress” that were not observed with glyphosate alone. Oxidative stress can not only lead to organ damage, but also DNA damage, a known major contributor to cancer.

***What determines the health of a person's microbiome health, and does it change?***

There is a short window in which a child's microbiome is established. The process is largely complete by or about age 3. After that time, the microbial species in a person's GI tract do not change very much, but the relative abundance of species can and does shift.

When a person gets an infection and is prescribed an antibiotic, the mix and balance of microbiota in the person's gut will change. This is because every antibiotic works especially well on some bacteria, but less well on others. Those bacteria that are relatively unaffected when a person takes an antibiotic tend to do better in the absence of competition from those bacteria that are more heavily impacted.

***What are the next steps?***

This study is the first to demonstrate a mechanism for glyphosate effects in the gut microbiome. The biomarkers of such effects could help scientists track glyphosate impacts in the human population, and ultimately understand if the ingestion of low glyphosate levels via contaminated food or water is triggering adverse human health effects.