# Frequently Asked Questions

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# 1. The Controversy over the Genotoxicity of Glyphosate

## What is genotoxicity?

Genotoxicity is the scientific term for the ability of a chemical to damage the genetic material (DNA) of an organism. DNA damage in somatic (body) cells is considered to be the central molecular mechanism for the development of cancer and also accelerates ageing.
DNA-damage in germ cells (sperm and eggs) leads to infertility and heritable diseases in the offspring. Therefore, the question of whether a substance is genotoxic and thus can damage DNA plays a central role in the safety testing of chemicals.

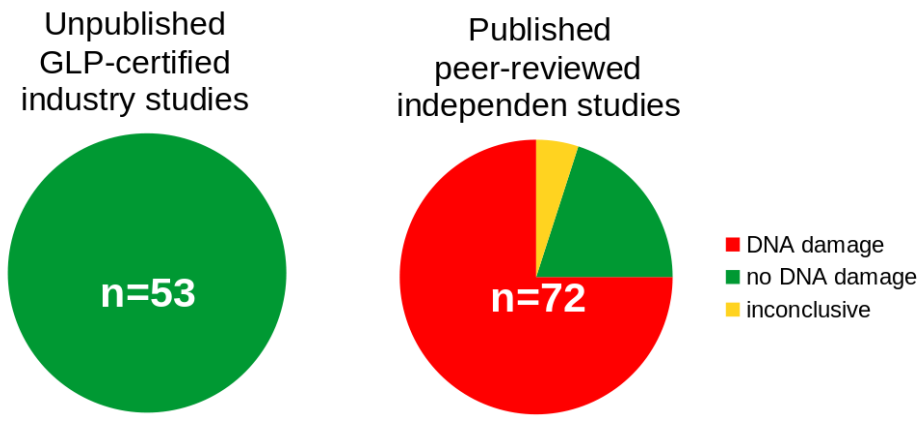
## How can you find out if a chemical is genotoxic?

Different genotoxicity tests for chemicals were developed in the last decades. They comprise in vitro experiments, with bacterial indicator cells (bacterial DNA has a similar structure as that of higher organisms) and experimental models with cultured cells from vertebrates (including humans) as well as in vivo experiments with laboratory rodents. Human studies are not conducted to classify new chemicals, but can provide valuable information about potential occupational and lifestyle related effects. In general, in vivo experiments with laboratory rodents (mice and rats) are regarded as more reliable than in vitro experiments with cultivated cells and bacteria. However, some in vivo experiments are not very reliable (e.g. the micronucleus assay with bone marrow which was frequently conducted with glyphosate), and detect only 5-6 out of 10 carcinogens.

## Is glyphosate genotoxic?

The WHO's International Agency for Research on Cancer (IARC) in its Monograph on Glyphosate (p. 398) says yes: "There is strong evidence that exposure to glyphosate or glyphosate-based formulations is genotoxic based on studies in humans and studies in experimental animals." IARC has based this assessment on all available published studies, most of which report DNA-damaging effects of glyphosate. IARC stated that genotoxicity is a major molecular mechanism for the carcinogenicity of glyphosate.
However, the EU regulators questioned the results of the studies on which the IARC had relied and based their judgement on studies that were not available to the IARC: the (until recently) secret studies of the glyphosate manufacturers. These had been conducted or commissioned

by the manufacturers themselves and kept under wraps for decades under the cover of intellectual property rights and trade secrets.
The following graph illustrates the discrepancy between these unpublished manufacturer's studies (98% of which report the absence of genotoxicity) and the published studies from the scientific literature (75% of which report evidence of genotoxicity and 20% report the absence of genotoxicity, while 5% are inconclusive). The underlying data are taken from EFSA's final RAR.




## How did the EU-authorities justify their distrust of published studies?

In principle, the EU-authorities are legally obliged to consider all relevant scientific evidence from industry studies and published studies to perform their assessment. The reasoning with which the authorities largely withdrew confidence from the 72 published studies from the scientific literature was essentially based on a (highly disputed) rating system proposed by three BASF employees in 1997, the so-called Klimisch Criteria. This rating system excludes a priori all non-guideline studies and/or non-GLP studies from being rated "reliable" (GLP stands for "Good Laboratory Practise" and is a certified standard designed to prevent fraud in industry contract laboratories). Since academic research is neither conducted under GLP conditions nor bound by narrow OECD Test Guidelines, the Klimisch rating "reliable" is reserved exclusively for industry studies.
Against this background, the Glyphosate Task Force (GTF) had submitted an assessment to the German authority BfR in which all 72 published genotoxicity studies were devalued as unreliable using the Klimisch criteria. The German authority had subsequently copied this assessment of the GTF word for word and presented in its "Renewal Assessment Report" as an independent assessment of the authority.
This approach became known as the "copy-paste/plagiarism scandal". It was the subject of an expertise on plagiarism commissioned by Members of the European Parliament (The BfR up to now denies any wrongdoing).

## How did the authorities justify their unilateral reliance on the secret industry studies?

EU authorities chose an assessment system which effectively “weeds out” any studies which are not performed in the way industry’s studies are required by law to be performed. Their assessment is based on the (highly disputed) Klimisch Criteria, proposed by three BASF employees in 1997, that systematically discriminates against studies from academic science. Because, in order to be classified as “reliable” under Klimisch, the studies must be carried out according to OECD Test Guidelines and in a certified GLP environment.

# 2. About the study

## Who are the authors of the study?

Prof. Siegfried Knasmueller and Dr. Armen Nersesyan are the authors of the study. Prof. Siegfried Knasmueller (Siegfried Knasmueller) studied biology and chemistry at the University of Vienna; his thesis concerned bacterial mutagenicity tests. Siegfried Knasmueller was visiting scientist at the Institute of Environmental Health (School of Medicine) of the University of Cleveland (Illinois) and at the US EPA (North Carolina). Since 1983 Siegfried Knasmueller works at the Institute of Cancer Research, Medical University of Vienna. He is a head of the Environmental Toxicology Group. Siegfried Knasmueller published 255 articles in peer-reviewed journals (Scopus) as well as four text books on genetic toxicology. He has a Hirsch-index of 54, and was cited > 9,000 times. Currently Siegfried Knasmueller is editor of the journal “Mutation Research – Genetic Toxicology” and co-editor of the journal of “Food and Chemical Toxicology”.

Dr. Armen Nersesyan (Armen Nersesyan ) studied biophysics at Yerevan State University and at the Institute of Normal and Pathological Physiology, Moscow (USSR). He also studied animal science at the University of Utrecht (the Netherlands) and molecular epidemiology at the NCI, Bethesda (US) and the IARC (France). His thesis concerned modification of genotoxic and carcinogenic effects of chemicals and radiation with different effect modifiers of biological origin. Armen Nersesyan was visiting scientist at the Rowett Research Institute, Aberdeen (UK), the Institute of Pharmacology and Toxicology, University of Wuerzburg (Germany) and the National Cancer Research Institute, (Genoa, Italy). In 1974 – 2003 Armen Nersesyan worked at the National Center of Oncology, Yerevan, Armenia and since 2004 at the Institute of Cancer Research of Medical University of Vienna till his retirement in 2017. Armen Nersesyan published 144 articles in peer-reviewed journals and has a Hirsch-index of 32, he was cited > 2,500 times.

## Who commissioned the study?

SumOfUs crowd-funded the study. Founded in 2011, SumOfUs is an advocacy non-profit organization and online community with millions of members worldwide. Our campaigns combine and amplify ordinary peoples' voices to make sure regulators and corporations hear them.
SumOfUs campaigner David Norton requested the industry studies from EFSA in 2019, and then transferred them to Prof. Knasmueller and Dr. Nersesyan, to evaluate them in 2020. Knasmueller was recommended by Helmut Burtscher, at Global 2000. SumOfUs paid Dr. Nersesyan approximately 3,500 EUR by wire transfer.
Previously, SumOfUs also campaigned to prevent Bayer's merger with Monsanto and crowd-funded a paper by competition law experts about that merger.

## What role did NGOs play?

After SumOfUs had received the study manuscript from Siegfried Knasmüller and Armen Neresyan, SumOfUs contacted experts from CEO, GLOBAL 2000, HEAL and PAN Europe - all of them had been active in the European Citizens Initiative "Ban Glyphosate"- to discuss the significance of the findings and its implications for the glyphosate authorization and for EU risk assessment in general. Subsequently, these FAQs as well as an explanatory background paper were drafted by experts from these NGOs.

## Why was this study not commissioned much earlier?

For decades, the glyphosate manufacturers had been able to keep their studies under wraps, making it impossible for independent scientists to scrutinize them. When Corporate Europe Observatory in early 2016 demanded the release of all cancer studies on glyphosate, this was refused by EFSA with reference to "trade secrets" and "intellectual property rights" of the glyphosate manufacturers. This only changed in March 2019 by a landmark ruling of the European Court of Justice brought by activist Tony Tweedale and members of the Green Group in the European Parliament. Since then, EFSA is obliged to disclose all manufacturer studies upon request. As a result, the NGO SumOfUs was able to obtain from EFSA all industry studies on the genotoxicity of glyphosate, and subject them to the scrutiny of renowned and independent experts on genotoxicity.

# 3. Regulatory Studies – Top secret and highly conflicted

## Who can guarantee that the industry does not simply fabricate the desired results in its "own" studies?

Large-scale data manipulation in US laboratories in the 1970s led to the introduction of a laboratory standard called “Good Laboratory Practise” (GLP) for industry contract laboratories to discourage falsification and manipulation of industry studies. GLP provides a legal framework for planning, conducting and monitoring regulatory studies. The manipulation and falsification of data should be prevented with the mandatory daily documentation of all activities and observations and with the archiving of protocols, findings and tissues from animal experiments. There is no doubt that GLP has not only improved the traceability and scientific accuracy of regulatory studies, but also made the falsification of data more difficult and, above all, more risky for a contract laboratory.
However, the interpretation of these - hopefully unmanipulated - data by the staff of the contract laboratories is not controlled by GLP. And the glyphosate case in particular had revealed that contract laboratory staff had judged the same rodent studies as evidence of the absence of carcinogenicity which, according to the IARC experts, actually showed the opposite, namely “convincing evidence that glyphosate also can cause cancer in laboratory animals".
In the case of genotoxicity tests, many studies which did not allow drawing firm conclusions were declared by the study directors as negative. As long as studies are not conducted independent of industry, it should be the task of regulators to make an independent assessment of the raw data to ensure their correct interpretation, which, however, is hampered by insufficient resources.

## Does GLP assure the quality of science?

No. GLP only aims at a correct acquisition and documentation of measurements and data, to prevent the intended or unintended falsification of measurement results. But science comprises much more - first and foremost, a methodology that appropriately addresses the scientific question that is concerned. In other words: reliable and unfalsified data acquisition does not help, if the scientific question is wrong or the method is inappropriate.

## Is GLP really a quality assurance system that is fraud-proof and cannot be falsified?

The argument that the certified laboratory standard GLP is a guarantee against manipulation and falsification of manufacturer studies was shaken at the end of 2019 when the laboratory fraud scandal at the LPT-Hamburg became public. Several former employees of the contract laboratory had testified (partly anonymously and partly on camera), that they had falsified

experimental data on order and that dead animals had been replaced by healthy animals during ongoing experiments (without documenting this). Subsequently, a check by GLOBAL 2000, CEO and PAN Europe revealed that 21 manufacturer studies from the last glyphosate approval had originated from the LPT Hamburg; among those three studies on glyphosate genotoxicity. The LPT scandal has shown that the certified quality assurance standard GLP is far from being fraud-proof. Nevertheless, GLP has been a substantial step to make falsification more difficult and risky for contract labs and to improve the integrity of industry studies.

## Why do industry studies need to adhere to OECD-Test Guidelines?

The legally prescribed data requirements for the approval of pesticide active substances stipulate binding Test Guidelines according to which the corresponding studies with the active substances or products are to be carried out. This is to ensure that studies are correctly performed and interpreted, guidelines were developed by authorities (OECD, EFSA, UKEMS, EPA, etc) which defined quality criteria and criteria for the classification of clear positive and clear negative results. The most relevant guidelines for Europe are those of OECD. The guidelines describe standardized and validated test procedures which were accepted for routine testing of chemicals. It is highly relevant that meaningful industrial studies are conducted in agreement with these guidelines. OECD guidelines are updated and improved as necessary according to the state of the art. The EU pesticide regulation requires that the risk assessment is carried out in the light of current scientific and technical knowledge. Therefore, the regulatory studies that form the basis for this assessment must be in accordance with the applicable Test Guidelines and reflect the state of the art in science.

## What ended the secrecy around the industry studies?

Criticism of the secrecy surrounding the studies used by manufacturers to prove the safety of their products has been around since the early 1980s, when systematic scientific fraud surrounding such "safety studies" in the USA - known as the "IBT scandal" - led to the closure of contract laboratories and prison sentences for their management.
But it was to take another 4 decades for the secrecy of manufacturers' studies in the EU to finally end, and it obviously took the glyphosate controversy to do so. Because many EU citizens lost confidence in their regulatory authorities when they found out that the WHO's cancer research agency IARC declared glyphosate a probable carcinogen referring to publicly available studies and the EU authorities claimed the opposite, referring to industry studies that were kept secret.
In March 2019, an ECJ ruling clarified that EFSA must disclose manufacturers' studies upon request, and in May 2019, the EU amended the General Food Law to require proactive publication of all manufacturers' studies in all food-related authorization processes (this amendment was the EU Commission's response to the Ban Glyphosate ECI).

# 4. The evaluation of genotoxicity studies by Armen Nersesyan and Siegfried Knasmueller

## What was the total volume (number of pages) of the study reports that have been reviewed?

In total, 54 studies were analysed, 53 of which had been subject to the former EU authorization process of glyphosate and are therefore listed in EFSA's Renewal Assessment Report (RAR). They comprise the results of 24 bacterial tests (Ames test), 2 studies with the bacterial Rec-A test (one of them was not evaluated by EFSA), 3 studies with so-called Hprt assay in mammalian cells, one DNA repair test in rat primary hepatocytes, 5 investigations with chromosomal aberration analyses in mammalian cells, 2 concerned dominant lethal mutations (DLM) tests with mice, 17 in vivo experiments with rodents (15 were micronucleus assays and 2 chromosomal aberrations analyses in bone marrow cells of mice). Taken together, the 53 industry studies cover 1,796 pages.

## What criteria were used for the evaluation?

The 53 industry genotoxicity studies have been classified by Siegfried Knasmueler and Armen Nersesyan in regard to their scientific quality and compliance with the current OECD and other guidelines and with the recommendations of international expert groups as follows:

- The results of a study were classified as "reliable" if the study in question was performed in compliance with the applicable OECD guideline .
- The results of a study were classified as "partly reliable" if the study in question showed moderate deviations from the applicable OECD Test Guideline which was valid when the RAR came out (a deviation that is not expected to strongly affect the correctness of a result obtained in a specific test-system is described as “moderate”).
- The results of a study were classified as "not reliable" if the study in question showed substantial deviations from the OECD Test Guideline, which was valid when the RAR came out (a deviation that could be expected to impair the sensitivity and/or accuracy of the test system is described as “substantial”).

## Why is compliance with the OECD Test Guidelines so important for regulatory studies?

The mandatory adherence to Test Guidelines makes sense, especially for the manufacturers' studies, since the manufacturers are subject to considerable conflicts of interest when investigating potential harmful effects of their own pesticide active ingredient. This is particularly true when it comes to effects that - as in the case of glyphosate - can result in the loss of market

authorization. Strict and controllable adherence to the Test Guidelines therefore limits the possibility of influencing results in a desired direction through the choice of study design.

## What is the big fuss about the non-reliable studies? Glyphosate has been tested so many times, and, according to Armen Nersesyan & Siegfried Knasmueller, there are 2 reliable and 17 partly reliable studies, isn't that enough?

No, it is not. If one takes a closer look at the total of 19 genotoxicity tests to which Siegfried Knasmueller and Armen Nersesyan ascribed (at least partial) reliability, one finds that 17 of these tests were carried out in vitro with bacteria (12 tests) or mammalian cell lines (5 tests). Apart from the fact that, according to IARC, bacterial tests also show negative results with glyphosate in independent studies, in vitro tests with bacteria and mammalian cell cultures are in general of secondary importance compared to in vivo studies with rodents. However, only 2 of the 19 genotoxicity tests identified as at least "partially reliable" are in vivo studies (micronucleus assays with rodent polychromatic erythrocytes). These two studies show negative results, but the sensitivity of this test system to identify genotoxic carcinogens is known to be rather low. Other in vivo methods (e.g. experiments with transgenic animals and single cell gel electrophoresis assays) which are accepted in guidelines are more reliable but not performed with glyphosate by the industry.

## Do the OECD Test Guidelines also apply to university research?

Unlike manufacturer studies, studies published in academic journals are not required to be in (strict) compliance with OECD Test Guidelines. While the purpose of OECD Test Guidelines is to provide repeated testing that facilitates comparability of results, university research is typically novel and hypothesis-driven. As such, academic research is carefully peer-reviewed before publication. More over, guidelines for all methods have not been developed. Nevertheless, results generated with these models can be of high relevance. If university research with non-guideline studies repeatedly comes to opposite results, e.g. "genotoxic" vs. a "non-genotoxic" result from OECD-compliant regulatory tests, then this should be taken as an opportunity to critically question the choice of the methods for the regulatory testing.

## What do the 53 industry studies say about the genotoxicity of glyphosate?

Since 34 of the 53 manufacturer studies are "not reliable" and 17 of the remaining tests that are at least "partially reliable" are in vitro tests with bacteria and mammalian cell lines (which are of secondary importance for the assessment of genotoxicity in humans), only two in vivo tests with rodents remain. These are tests in which the genotoxicity of glyphosate was investigated in bone marrow.

This is a very weak basis upon which to contradict the dozens of peer-reviewed studies that identify glyphosate as genotoxic. It becomes even weaker still, when you know that these 2 tests were investigating genotoxicity in bone marrow.
There is some evidence that bone marrow is not a target for the induction of DNA damage by glyphosate, while the herbicide was found to cause DNA damage in liver-derived cells in vitro and in inner organs of laboratory animals, and also in inner organs of many wildlife species (fishes, reptiles, amphibians). The latter test with wildlife species are not included in the OECD guidelines, but positive findings are an alarm signal.

## What consequences do the results of this study have for the approval of glyphosate?

Siegrfried Knasmueller and Armen Nersesyan emphasize that there is growing evidence that glyphosate causes DNA-damage in multiple inner organs, but that bone marrow is not a target for DNA-damage by glyphosate. Importantly, bone marrow is the only organ for which two partly reliable industry studies provide evidence for absence of DNA-damage. The authors conclude that a re-evaluation of the genotoxicity of glyphosate by the EU-authorities is strongly warranted.

**Table 1. Evaluation of the results of industrial studies on genotoxicity of glyphosate by RAR and S. Knasmueller (SK)**

| Study type | BfR/RAR assessment | | | | SK assessment | | | |
|---|---|---|---|---|---|---|---|---|
| | Acceptable/ Valid | Supplementary | Limited value | Not acceptable | Reliable | Partly reliable | Not reliable | Not in OECD |
| **All mutagenicity tests (n=53)** | 45 | 5 (2 also are considered as acceptable) | 2 | 3 | 2 | 17 | 35* | 2 |
| Bacterial mutagenicity tests (n=24) | 22 | 2 (also considered as acceptable; #14 and #21) | 1 | 1 | 2 | 10 | 12 | 0 |
| RecA assay (n=1) | 0 | 1 | 0 | 0 | 0 | 0 | 2* | 1 |
| Gene mutations in mammalian cells *in vitro* (n=3) | 3 | 0 | 0 | 0 | 0 | 2 | 1 | 0 |
| DNA repair assay (n=1) | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| CA *in vitro* (n=5) | 4 | 1 | 0 | 0 | 0 | 3 | 2 | 0 |
| CA *in vivo* (n=2) | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| MN *in vivo* (n=15) | 11 | 1 | 1 | 2 | 0 | 2 | 13 | 0 |
| DLM assay (n=2) | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |

*one of RecA studies was not evaluated by BfR

**This document is maintained in collaboration with Corporate Europe Observatory (CEO), the Health and Environment Alliance (HEAL), GLOBAL 2000, Pesticide Action Network (PAN) Germany and SumOfUs.**

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