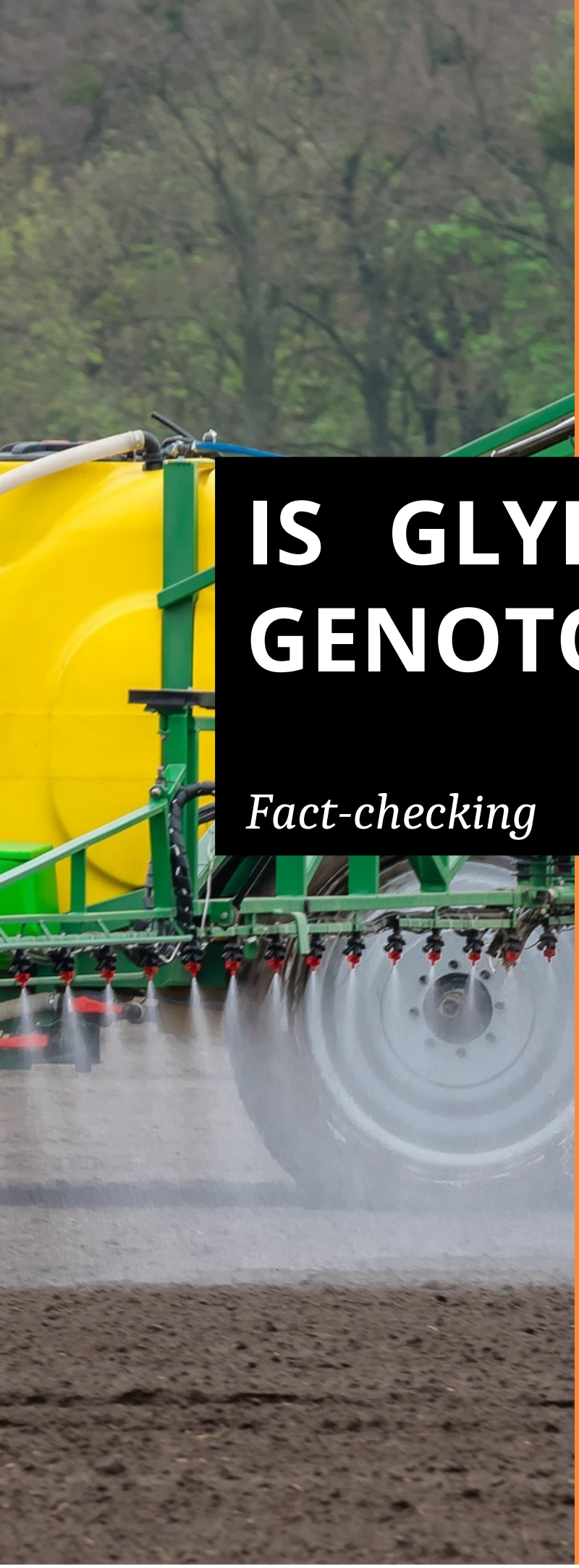

3 MAY 2022

IS GLYPHOSATE GENOTOXIC ?

Fact-checking

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générations FUTURES

INTRODUCTION

Safe!

"Glyphosate does not present a carcinogenic risk"; *"It is not genotoxic"; "Glyphosate is inert"*; *"Glyphosate is biodegradable"*; *"It is the safest herbicide on the market"*. etc.

These are all sentences that are regularly used by the defenders of this herbicide, the idea being to **make this substance seem relatively harmless to** the environment and even to health. And for this, they have a **strong argument: the opinions of the** regulatory **agencies** which have classified it as only irritating to the eyes and which have given their opinion in favour of renewing its marketing authorisation.

... really?

But if this substance really was harmless, **why was Monsanto condemned by the courts** for having sold its product as biodegradable?

Why did the International Agency for Research on Cancer (**IARC**) classify it in 2015 as a probable human carcinogen? **Why did Inserm**, the French medical research institute, state during the public consultation on the renewal dossier that *"glyphosate may have endocrine disrupting properties that have an impact on reproductive function"*?

Answers

What is it really about? Why the controversy and the differing opinions? This is what **we will try to understand in this** fact-checking **paper,** focusing on the issue of the suspected genotoxicity of glyphosate.

EVALUATION

How is the genotoxic potential of a substance evaluated?

The term "**mutation**" refers to permanent and transmissible changes in the structure or the amount of genetic material in an organism. A mutagenic substance is a substance capable of causing or increasing the frequency of mutations. These mutations can be caused by :

- **gene mutations** (changes in the DNA sequence of one or more genes), or
- **clastogenic effects** (structural changes to chromosomes caused by DNA breaks or chromosomal rearrangements), or
- **aneugenic effects** (changes in the number of chromosomes in the cell).

The broader term "**genotoxicity**" includes mutations that are permanent changes but also damages to the DNA that may be reversible through DNA repair processes or that may result in cell death and thus do not result in a permanent change in the genetic content (1).

The distinction between mutations and genotoxic effects is therefore linked to whether or not the change in genetic material is **permanent.**
To assess these different genotoxic effects, different tests are available (the main ones are summarised in the table below). These tests can be performed in vitro on cell cultures, or in vivo after administration of the substance to living organisms. Different organisms can be used to assess genotoxic effects in humans (rodents, fish, crustaceans, etc.).

(1) WHO definition (Environmental health criteria 240, Chapter 4: Hazard Identification and Characterization: Toxicological and Human Studies, Section 4.5: genotoxicity; 2020)

The choice of cell model (used in vitro or the type of cell studied after in vivo application) **is key to assessing genotoxicity.**

Genotoxic effects can be studied on germ cells (and thus indicate the potential for transmission of the toxic effect to offspring) or on other cells called somatic cells. Depending on the type of cells studied, the ability to repair DNA damage will be different. Thus, DNA damage may not be detected in cells with high repair capacities.

Therefore, **when a substance is distributed throughout the body, it is important to study the genotoxic effects on several types of cells**, from different organs, in order to compensate for this variability of effects between cells.

Gene mutation tests (mutagenicity)	*Bacterial cell test* Ames test *Somatic cell assay* Mammalian cell gene mutation assay using Hprt and xprt genes Mammalian cell gene mutation assay using the TK gene (mouse lymphoma assay)
Chromosomal damage tests (mutagenicity)	*Somatic cell assay* Mouse lymphoma test Micronucleus tests Chromosome aberration test *Germ cell test* Rodent dominant lethal mutation test Germ cell chromosome aberration test
DNA damage test (genotoxicity)	Comet tests Sister chromatid exchange test Unscheduled DNA synthesis (UDS) assay in mammalian hepatocytes

A single test is not sufficient to assess the genotoxicity of a substance. It is indeed imperative to use tests that allow the genotoxic potential of a substance to be assessed as a whole, because a substance may be negative in gene mutation tests and yet cause chromosomal aberrations.

WHAT DOES THE RESEARCH SAY?

What is the opinion of research bodies on the genotoxicity of glyphosate?

A **large number of studies** on the **genotoxic** potential of glyphosate are available in the independent scientific literature. From all the available data, it seems clear that glyphosate has no potential to cause gene mutations. On the other hand, **its potential to cause clastogenic effects and DNA breaks has been clearly demonstrated in numerous studies**, which have been taken up by two major institutions, the International Agency for Research on Cancer (IARC) — a WHO agency — and Inserm.

IARC'S OPINION (2015)[2]

In its monograph published in 2015 in which glyphosate is considered a probable human carcinogen, IARC **synthesised the existing scientific literature** on the genotoxicity of the herbicide. For the sake of transparency, only publicly available studies in **peer-reviewed journals** were taken into account in this evaluation; confidential **industry studies** were discarded.

In total, data on the effects of glyphosate **in vitro** on human cells(8 publications), mammalian cells (3) and fish cells (1) andin vivo data on mammalian cells (6) and other vertebrate organisms (4) or invertebrates (7) were included.

Concerning in vitro data on human cells, **6 out of 8 publications reported positive effects**. DNA breaks were reported in vitro in 5 comet assays on various human cell types (liver, lymphocytes, cancer line cells) and 1 study showed some clastogenic effects of the glyphosate in the sister chromatid exchange assay. However, the data reported by IARC show no positive effects in the human lymphocyte chromosome aberration and micronucleus assays in vitro.

Also in vitro on mammalian and fish cells, 3 out of 4 publications report positive effects in comet assays.

In vivo in mammals (mice and rats), of the 6 studies cited by IARC, **2 reported positive effects** in comet assays after analysis of liver and kidney cells or in a micronucleus test after 2 injections of the substance. The other 4 showed no effect on bone marrow cells in chromosomal aberration and micronucleus tests after only one administration of the substance to the test animals.

In vivo in other vertebrate organisms such as fish, glyphosate induces DNA breaks as shown by the comet assay in all available studies (4/4).

2. Some organophosphate insecticides and herbicides/ IARC Working Group on the Evaluation of carcinogenic Risks to Humans (2015: Lyon, France)

IARC also notes **many positive effects** (not detailed here) found with **glyphosate-based formulations** and its main metabolite (AMPA).

After analysis of this body of literature, **IARC concludes** that:

"There is strong evidence th glyphosate causes genotoxicity The evidence base includes studies that have shown broadly consiste positive results in human cells in vitro, in mammalian models in vitro and in vivo and studies on other non-mammal organisms."

Furthermore, IARC concludes *that "there is strong evidence that glyphosate can induce oxidative stress, a mechanism of action that explains genotoxicity. Overall, the mechanistic data provide strong evidence for genotoxicity and oxidative stress. There is evidence that these effects can operate in humans.*

These conclusions leave no room for doubt. According to IARC, there is sufficient evidence to conclude that glyphosate is genotoxic.

In 2013, **Inserm** published a first collective report in which glyphosate genotoxicity was discussed. Inserm already noted that glyphosate has a pro-oxidant activity but that this is not necessarily correlated with DNA damage. However, this 2013 opinion has been largely revised in the update of the collective expertise published in **June 2021**. In this new analysis, based on more recent data, Inserm provides a more in-depth summary of the **available studies reporting the genotoxic potential of glyphosate**. It is specified that studies carried out at high doses, inducing a decrease in cell viability, have been excluded from the analysis.

INSERM'S OPINION (2013, 2021)3

Regarding the data obtained with pure **glyphosate** in vitro, the report mentions **6 comet assays showing positive effects** on various human cells (peripheral blood lymphocyte, oral epithelial, liver and cancer lineage cells). In vivo, data from mammalian models but also from other organisms such as fish or crustaceans were examined. Three studies reporting the induction of micronuclei or chromosomal aberrations by glyphosate in mice and four positive comet assays in fish are mentioned. **In comparison, the tests showing negative effects cited in the report are much less numerous.** Concerning the results of the comet tests, **Inserm summarises** that "*on different experimental models, many results are positive in vitro and in vivo [...] Several in vitro tests observe genotoxic effects at concentrations close to those that can be detected in the environment*". Furthermore, "*With the micronucleus test on vertebrate models, different from the comet test classically used in a large number of studies, a meta-analysis of the literature also concludes that there is a genotoxic effect*".

3. Inserm. Pesticides and health effects: New data. Collection Expertise collective. Montrouge: EDP Sciences, 2021 Carcinogenic Risks to Humans (2015: Lyon, France)

In the end, Inserm concludes that :

> *"The studies showing a lack of genotoxicity of glyphosate appear to be less important in terms of quality and of quantity than those suggesting a positive effect."*

The report also concludes that **glyphosate- based** formulations **show genotoxic effects at lower concentrations than pure glyphosate.** This is due to the surfactants added to the formulations.

Finally, like the IARC in 2015 and during its first assessment in 2013, Inserm in 2021 notes that these genotoxic effects found "*are consistent with the direct or indirect induction of oxidative stress by glyphosate, observed in different species and cellular systems, sometimes at exposure doses compatible with those to which populations may be exposed*".

TO CONCLUDE

The IARC and Inserm agree that glyphosate is genotoxic and that an oxidative stress phenomenon induced by glyphosate is responsible for this genotoxicity.

WHAT ARE THE AGENCIES' VIEWS?

What is the opinion of the regulatory agencies (renewal dossier at European level, RAR 2021) on the genotoxicity of glyphosate?

As we shall see, the conclusion of the glyphosate renewal dossier (RAR) from the four rapporteur member states (France, Netherlands, Sweden and Hungary) is quite different. According to the health agencies of these 4 countries (here called "authorities") glyphosate is not genotoxic. How can this discrepancy be explained? To summarise:

1. The authorities rely exclusively on studies from the industry
2. The authorities ignored the flaws in the studies provided by the industry
3. In vivo data are only available for one type of test and one cell type
4. Data on "non-standard" organisms have not been taken into account
5. Regulatory classification criteria are too restrictive

AND IN DETAIL?

1 / the authorities rely exclusively on studies from the industry

In contrast to the IARC and Inserm, which only took into account data available in the scientific literature. These industrial data respect, as required by the regulations, the OECD guidelines and Good Laboratory Practice (GLP), unlike the academic studies for which these guidelines are not required.
Regarding in vitro data studying clastogenic effects, the RAR considered 5 studies from the industry (4 chromosomal aberration tests and 1 micronucleus test).
All of them reported negative effects and were considered either acceptable or acceptable with reservations and therefore carried significant weight in the final decision.
As required by Regulation 1107/2009, which governs theauthorisation of pesticides at the European level, the RAR also cites publications from the scientific literature. 10 comet tests, 6 micronucleus tests and 3 chromosome aberration tests are mentioned among others.
Of these 19 publications, 16 show positive effects in vitro, mainly in the comet test. However, these studies are considered by the RAR as just "supportive" due to methodological weaknesses described in great detail. **As a result, these 16 in vitro studies showing a genotoxic potential of glyphosate have in the end no weight against the 5 industrial studies.** The authorities write that: *"Overall, the studies published in the literature may indicate positive in vitro effects in comet assays and micronucleus tests to some extent. However, due to inconsistencies in methodology [...] the toxicological relevance of the reported findings is unclear" [...] All studies conducted according to GLP gave negative results. Furthermore, the majority of in vitro chromosome aberration and micronucleus tests were negative.*

In these conclusions, the authorities fail to recall that the studies provided by the industry also contain numerous methodological flaws (see point 2/) and that when looking at all the available data, **6 micronucleus or chromosomal aberration tests are positive against 7 negative.**

Concerning the data obtained in vivo, the industry provided 10 micronucleustests, 2 chromosomal aberration tests and 3 dominant lethal tests, all of which were negative. In addition, 8 studies from the literature are cited, 5 of which show positive effects. **Again, only industry studies that are considered reliable are taken into account** in the decision. The authorities acknowledge the existence of studies showing positive effects but conclude that glyphosate is not genotoxic to rodents.

For the 4 member states that authored the report, the evaluation of all the data is therefore simple: studies from the literature are not taken into account because of their lack of reliability and **all the weight is given to the studies from the industry** that respect the OECD guidelines and Good Laboratory Practice. This aspect is highlighted in the tables summarising all the studies taken into account in the RAR and by the IARC and Inserm, in the appendix to this document.
However, in these simplistic conclusions, the authorities omit several points, including the methodological weaknesses of the industry studies.

AND IN DETAIL?

2 / The authorities ignored the flaws in the studies provided by the industry

Indeed, **industrial studies are not so impeccable**, even if they claim to be OECD and GLP. Exceptionally, the NGO SumOfUs managed to gain access to these data and passed them on to two genotoxicity experts. These experts considered that **the vast majority of these studies were in fact unreliable and did not comply with the requirements issued by the OECD** (4).

Furthermore, in these studies, exposure of bone marrow cells, the cell type studied in the test, to glyphosate was not demonstrated. These facts have also been extensively detailed by Générations Futures in a November 2021 report (5).

4. Armen Nersesyan and Siegfried Knasmueller, Evaluation of the scientific quality of studies concerning genotoxic properties of glyphosate - 2021
5. Evaluation of glyphosate: a seriously biased report! Générations Futures, November 2021

3 / In vivo data are only available for one type of test and one type of cell

The assessment of the in vivo genotoxicity of glyphosate is based exclusively on micronucleus tests, which **are unreliable** and study the effects of the substance on bone marrow cells only and whose exposure has not been demonstrated. However, we have seen that it is important to have results on different types of cells. It should be remembered that in vitro comet tests have shown effects on blood cells, but also on liver and epithelial cells (etc.). However, **no in vivo tests of comets have been provided by the industry**. This weakness had been pointed out by experts mandated by the Anses in 2016 (6):

"While almost all in vivo tests lead to non-statistically significant results, there are no results from in vivo comet tests, which seems to be the most sensitive biological parameter. Therefore, it could be useful to perform an in vivo comet test on the defined target organs (kidney and liver)". However, this opinion has still not led the authorities to request an in vivo test of comets.

6. OPINION of the Agence nationale de sécurité sanitaire de l'alimentation, de l' environnement et du travail relatif à la saisine glyphosate n° 2015 - SA- 0093, 09/02/2016

4 / data on "non-standard" organisms have not been taken into account

While IARC and Inserm analysed studies from academic research on other, so-called "non-standardmodels" (fish in particular), **the authorities simply rejected them**: "*In the face of an extremely large database using standard test systems (bacteria, mammalian cells and mammals), data obtained in non-standard test systems (e.g. plant, insect, worm, fish, etc.) were not taken into account for classification.* However, this approach is more than**questionable given the many**existing publications showing the relevance of models such as fish for the evaluation of genotoxic effects in humans. The use of such models would also make it possible to reduce the number of tests carried out on mammals. The Anses itself supports this position (7): "*The use of animal data outside the rodent model should be discussed in particular for mutagenicity. There would be a huge potential impact on animal testing and this would allow environmental data to be taken into account (e.g. mutagenicity observed in fish), bringing CLP into line with the One Health concept.*

7. Inception impact assessment related to the revision of CLP Regulation - ANSES comment

AND IN DETAIL?

By excluding this type of test from the assessment, many studies available in the literature showing genotoxic effects are in fact immediately rejected.

This increases the weight of negative studies provided by the industry.

5 / Regulatory classification criteria are too restrictive

The final point as to why the authorities consider glyphosate to be non-genotoxic comes from **the classification criteria** themselves. For a substance to be classified and considered genotoxic in Europe, it must meet a number of criteria, described by the European regulation on the classification, labelling and packaging of substances and mixtures (CLP, Classification, Labelling, Packaging).

According to the CLP, only the mutagenic character at the germ cell level is retained for a substance to be classified and considered as genotoxic. Indeed, the permanent and transmissible character to the following generations is the most important criterion for the classification.
Depending on the level of evidence available, 3 categories are distinguished:

Hazard category	Data needed for classification
Category 1A	Positive results from human **epidemiological studies**
Category 1B	Positive **in vivo** mammalian **germ cell mutagenicity** tests Or Positive in vivo mammalian somatic cell mutagenicity tests + evidence that the substance can induce germ cell mutations (e.g. demonstration that the substance or its metabolites are capable of interacting with germ cell genetic material) Or Tests that have shown mutagenic effects on human germ cells, without evidence of transmission of these mutations to offspring, e.g. increased frequency of aneuploidy in sperm of exposed males
Category 2	Positive **in vivo mutagenicity** tests **on** mammalian **somatic cells** Or Positive in vivo **genotoxicity** tests **on somatic cells, + positive in vitro mutagenicity results**

AND IN DETAIL?

According to these criteria, data obtained on somatic cells (other than germ cells) are not sufficient to classify a substance in category 1, which is synonymous with exclusion and non-authorisation for pesticides.
Also, **genotoxicity tests, such as the comet test, are only considered as "indicator tests" for mutagenicity and allow for a maximum classification in category 2**, if positive effects are shown in vivo. In vitro genotoxicity tests, on the other hand, carry almost no weight. **The comet assay, although it appears to be the most sensitive and relevant test for assessing the genotoxicity of glyphosate**, and is widely used by academic researchers, **is therefore of very limited weight in the classification.**
Comparing these classification criteria with the available data on glyphosate considered acceptable by the authorities, it can be **seen that the types of tests carried out cannot lead to a category 1 classification, due to the lack of data on germ cells.** Indeed, although this is the main criterion, only 3 germ cell studies, dating back more than 30 years (1980, 1982 and 1992) and all judged to be complementary only because of methodological flaws, are available in the dossier.

Simply because of these criteria, it is therefore very difficult for a substance to be classified as category 1 genotoxic because germ cell tests are not carried out as a first line of defence, are not requested by the authorities and are therefore most often absent from the dossiers.
As proof that these classification criteria are problematic, the Anses requested their revision during the public consultation organised on the occasion of the revision of the CLP regulation. The objective is to allow classification in category 1B even if no data on germ cells are available: "*classification criteria should be refined in order to identify Muta. 1B substances in the absence of specific data on genotoxicity on the gonads*" (8).

8. Inception impact assessment related to the revision of CLP Regulation - ANSES comment

CONCLUSION

The controversy over the genotoxicity of glyphosate is emblematic of the flaws in a system based exclusively on OECD and GLP-compliant studies. **Although numerous studies show that glyphosate causes genotoxic and mutagenic effects these have been ignored by the authorities.** The same applies to mechanistic studies showing an oxidative stress effect of glyphosate. However, the repetition of these positive studies and the conclusions of internationally recognised research agencies should have given the authorities a warning signal to re-evaluate the reliability of the studies provided by the industry and to request additional studies, in particular on tests allowing the cells studied to be varied or tests on germ cells, which are necessary for classification in category 1. Instead, the authorities simply repeated the arguments already put forward by the German authorities in charge of drafting the renewal dossier in 2016, arguments largely taken from the dossier submitted by the industry itself.

"This biased assessment of genotoxicity is highly reflected in the assessment of the carcinogenicity of glyphosate."

Indeed, **it is increasingly acknowledged that the process of tumour genesis involves changes at the genetic level.** The recognition of genotoxicity would be an additional argument for classifying glyphosate as a human carcinogen.

ANNEX 1

IN VITRO DATA

	Type of cells analysed	Type of tests	IARC 2015	Inserm 2021	RAR 2021
+ Studies showing genotoxic effects	Human cells	Comet test (10 studies)	Monroy et al., 2005 Mladinic et al., 2009a Manas et al., 2009 Koller et al., 2012 Alvarez-Moya et al., 2014	Manas et al., 2009 Koller et al., 2012 Kwiatkowska et al., 2017 Townsend et al., 2017 Kasuba et al., 2017 Wozniak et al., 2018	Monroy et al., 2005 Koller et al., 2012 (résultat équivoque) Alvarez-Moya et al., 2014 Kwiatkowska et al., 2017 Townsend et al., 2017 Kasuba et al., 2017 Suarez-Larios et al., 2017 Wozniak et al., 2018 De Almeida et al., 2018
		Micronucleus (3)			Koller et al., 2012 Santovito et al., 2018 Kasuba et al., 2017 (résultat équivoque)
		Abb. Chrom.* (1)	[2]		Santovito et al., 2018
		ECS** (1)	Bolognesi et al., 1997		Bolognesi et al., 1997
	Mammalian cells	Micronucleus (1)	Roustan et al., 2014	Roustan et al., 2014	Roustan et al., 2014 Bolognesi et al., 1997
		Abb. Chrom.(1)	Lioi et al., 1998		Lioi et al., 1998
		ECS Test (1)	Lioi et al., 1998		Lioi et al., 1998
	Fish cells	Test des comètes (1)	Alvarez-Moya et al., 2014		

Total: 18 studies showing positive in vitro effects / 0 considered in RAR decision

0 Studies showing no effects	Fish cells	Micronucleus (2)	Mladinic et al., 2009b		**1 industry study** Mladinic et al., 2009b
		Abb. Chrom (3)	Manas et al., 2009		2 industry studies Manas et al., 2009
		Comet test (1)	[2]		Nagy et al., 2019
	Mammalian cells	Abb. Chrom. (2)			2 industry studies
		UDS Test *** (2)	Li et Long, 1988	Li et Long, 1988 Rossberger, 1994	

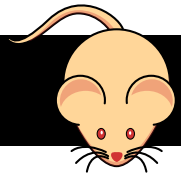
Total: 10 studies showing no in vitro effects / 5 industry studies considered in the RAR decision

* Chromosomal aberrations ** Sister chromatid exchange test *** Unscheduled DNA synthesis (UDS) test on mammalian hepatocytes

Criterion of reliability of studies used by authorities in the RAR :
Acceptable study; **Acceptable study with restrictions**; **"Additional" study**; **Not acceptable study**

ANNEX 2

IN VIVO DATA



	Type of cells analysed	Type of tests	CIRC 2015	Inserm 2021	RAR 2021
+ Studies showing genetoxic effects	Rodents	Comet tests (2)	Bolognesi et al., 1997	Bolognesi et al., 1997	Bolognesi et al., 1997 Manas et al., 2013
		Micronucleus test (3 + 1 meta-analysis)	Bolognesi et al., 1997 Manas et al., 2009	Bolognesi et al., 1997 Manas et al., 2009 Ghisi et al., 2016 (méta-analyse)	Bolognesi et al., 1997 Manas et al., 2009 Ilyushina et al., 2018b (positivité assimilée à la présence de formaldehyde)
		Other study (DNA adducts) (1)	[2]		Peluso et al., 1998
	Fish	Comet tests (4)	Moreno et al., 2014 Guilherme et al., 2012 Lopes et al., 2014 Alvarez-Moya et al., 2014	Guilherme et al., 2012 Alvarez-Moya et al., 2014	
	Crustaceans	Comet tests (1)		Hong et al., 2017	
	Plants	Comet tests (2)	Alvarez-Moya et al., 2011	Alvarez-Moya et al., 2011 Lioi et al., 1998	
		Abb. Chromosomal (2)	Frescura et al., 2013 Siddiqui et al., 2012	Frescura et al., 2013	

Total: 15 studies showing positive effects in vivo (including 6 in rodents) / 0 taken into account in the RAR decision

0 Studies showing no effects	Rodents	Micronucleus test (13)	Rank et al., 1993	Rank et al., 1993	**4 industry studies** 4 industry studies 2 industry studies (equivocal result) Ilyushina et al., 2018a Rank et al., 1993 Chruscielska et al., 2000
		Abb. Chrom (3)	Li et Long, 1988	Li et Long, 1988	2 industry studies
		Dominant lethal test (germ cell) (4)	EPA, 1980	EPA, 1980	3 industry studies
	Plants	Micronucleus test(1)	De Marco et al., 1992	De Marco et al., 1992	
		Abb. Chrom (1)	Rank et al., 1993	Rank et al., 1993	
	Oysters	Comet Test (1)	Akcha et al., 2012		

Total : 23 studies showing no in vivo effects (of which 20 in rodents) / 10 taken into account in the RAR decision

* Chromosomal aberrations ** Sister chromatid exchange test *** Unscheduled DNA synthesis (UDS) test on mammalian hepatocytes

To go further, read our report on the biases in the evaluation of glyphosate

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