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*Pesticides, formulants,  
Declared active  
principles*

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November 2017, BfR



# pollution and health

We discovered on baby genes:

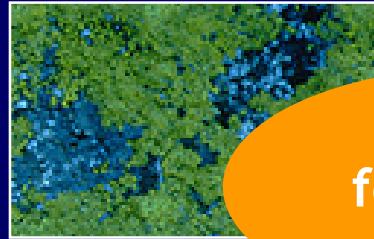
- motor gas residues
- pesticides
- Industrial products  
(dioxins, tobacco additives, formulants, declared active principles...)



# XENOBIOTICS

as spams for life (endocrine and nervous disruptors)

## ENDs



fongicides



Heavy metals,  
nanoparticles,  
plasticizers

herbicides

Roundup



insecticides

Lindane

Medical drugs  
such as synthetic  
estrogens

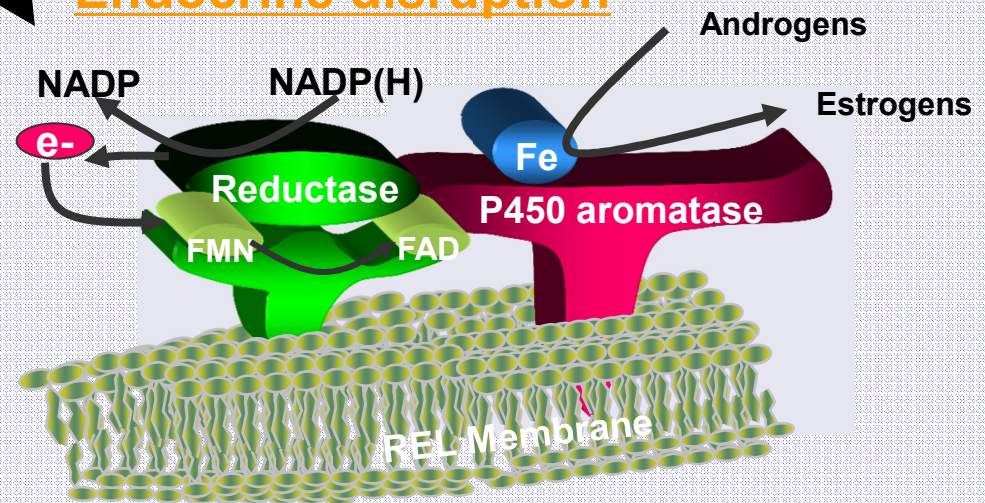
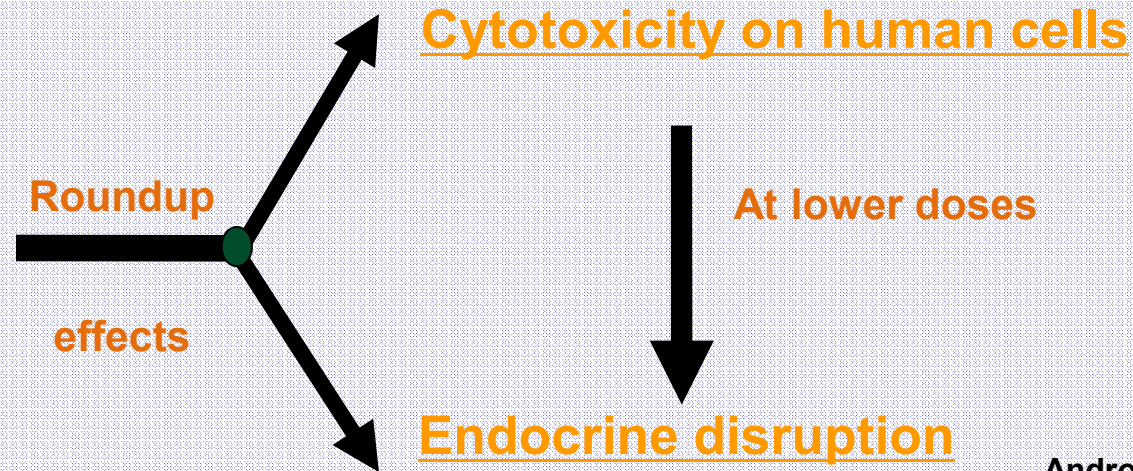


Industrial  
products

Bisphenol A, S

other  
pesticides

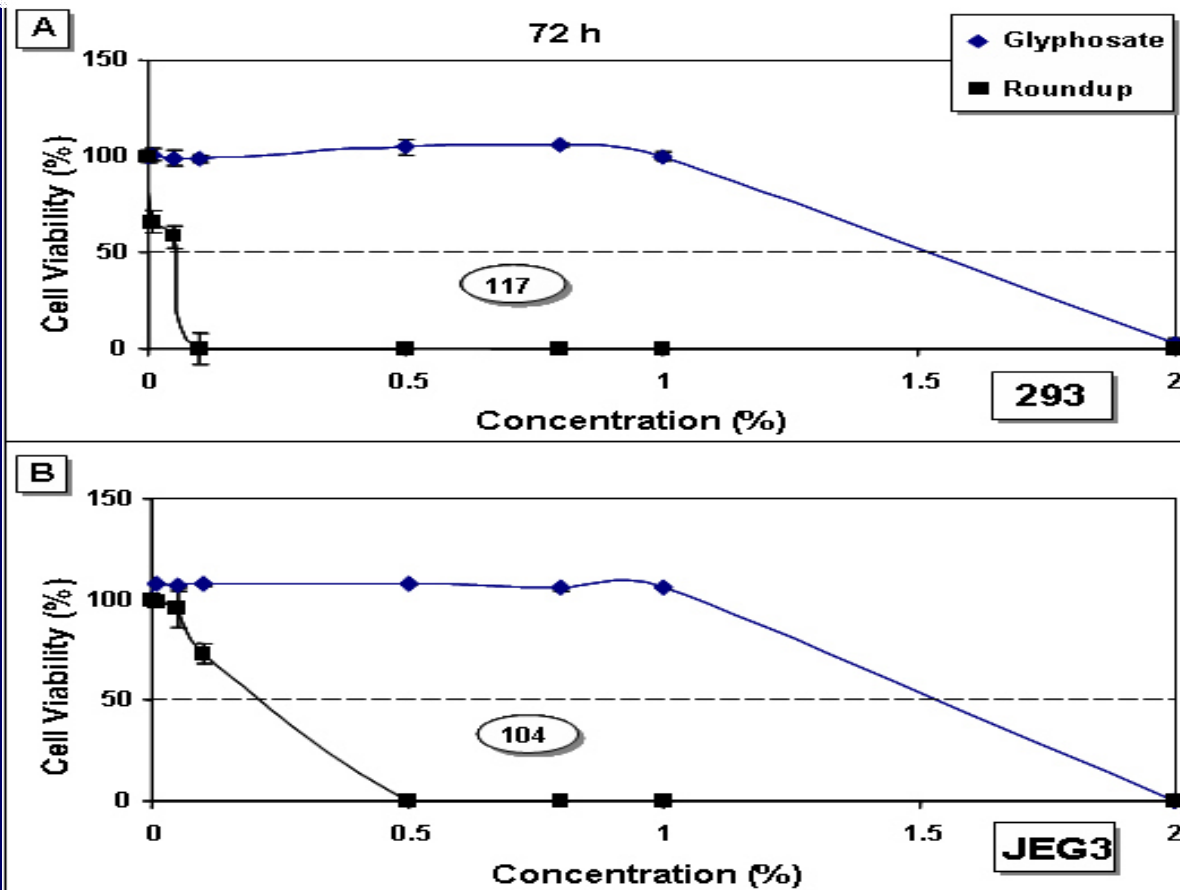
# Roundup in 80% of agricultural GMOs: combined effects of formulants and glyphosate



- Richard et al., EHP, 2005
- Benachour et al., AECT, 2007
- Benachour & Séralini, CRT, 2009
- Gasnier et al., Toxicology, 2009
- Gasnier et al., JOMT, 2010, 2011
- Mesnage et al., JAT 2012
- Clair et al., Tox. in Vitro, 2012
- Mesnage et al., Toxicology 2013... more than 40 papers.



**Roundup is up to 100,000 times more toxic than glyphosate alone according to time of exposure – on more than 10 fresh cells and cell lines**



Benachour  
et al., 2007

**If it is not glyphosate alone, what is the compound responsible for toxicity?**

# Glyphosate is never used alone

- Monsanto declared it as the active ingredient ??? of Roundup : NO -
- It is the **declared active ingredient**
- We have discovered the toxicity of hidden compounds in its formulations (1,000 times more toxic than G alone)



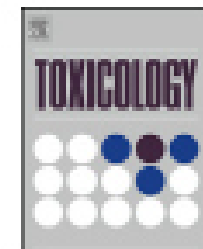




Contents lists available at SciVerse ScienceDirect

## Toxicology

journal homepage: [www.elsevier.com/locate/toxicol](http://www.elsevier.com/locate/toxicol)



# Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity

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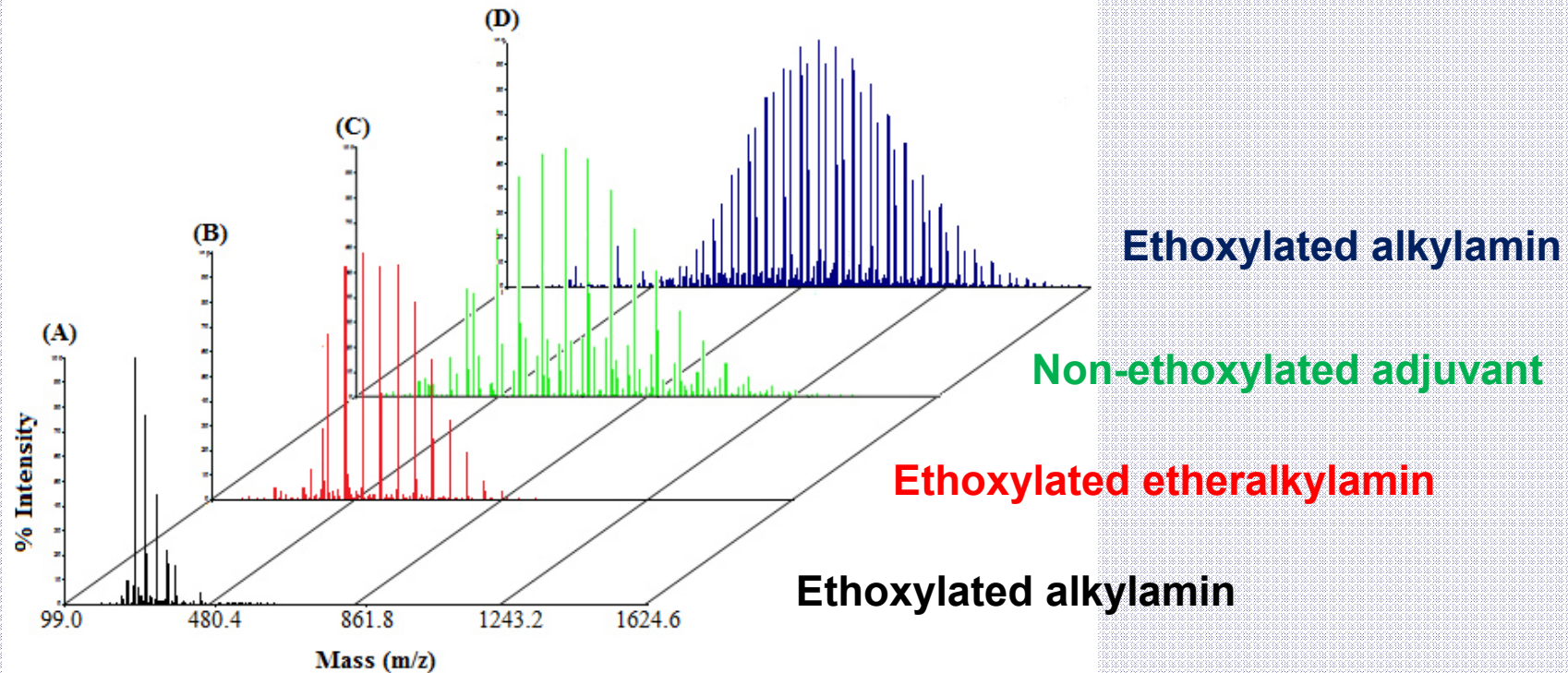
Available online xxx

### ABSTRACT

Pesticides are always used in formulations as mixtures of an active principle with adjuvants. Glyphosate, the active ingredient of the major pesticide in the world, is an herbicide supposed to be specific on plant metabolism. Its adjuvants are generally considered as inert diluents. Since side effects for all these compounds have been claimed, we studied potential active principles for toxicity on human cells for 9 glyphosate-based formulations. For this we detailed their compositions and toxicities, and as controls

**EFSA and BfR could work  
on non-declared active substances?**

# Analysis of the chemical composition of formulants



**Analysis by mass spectrometry MALDI-TOF MS/MS of 9 glyphosate-based herbicide formulations and their constituents**

**Different formulants are present in different formulations**



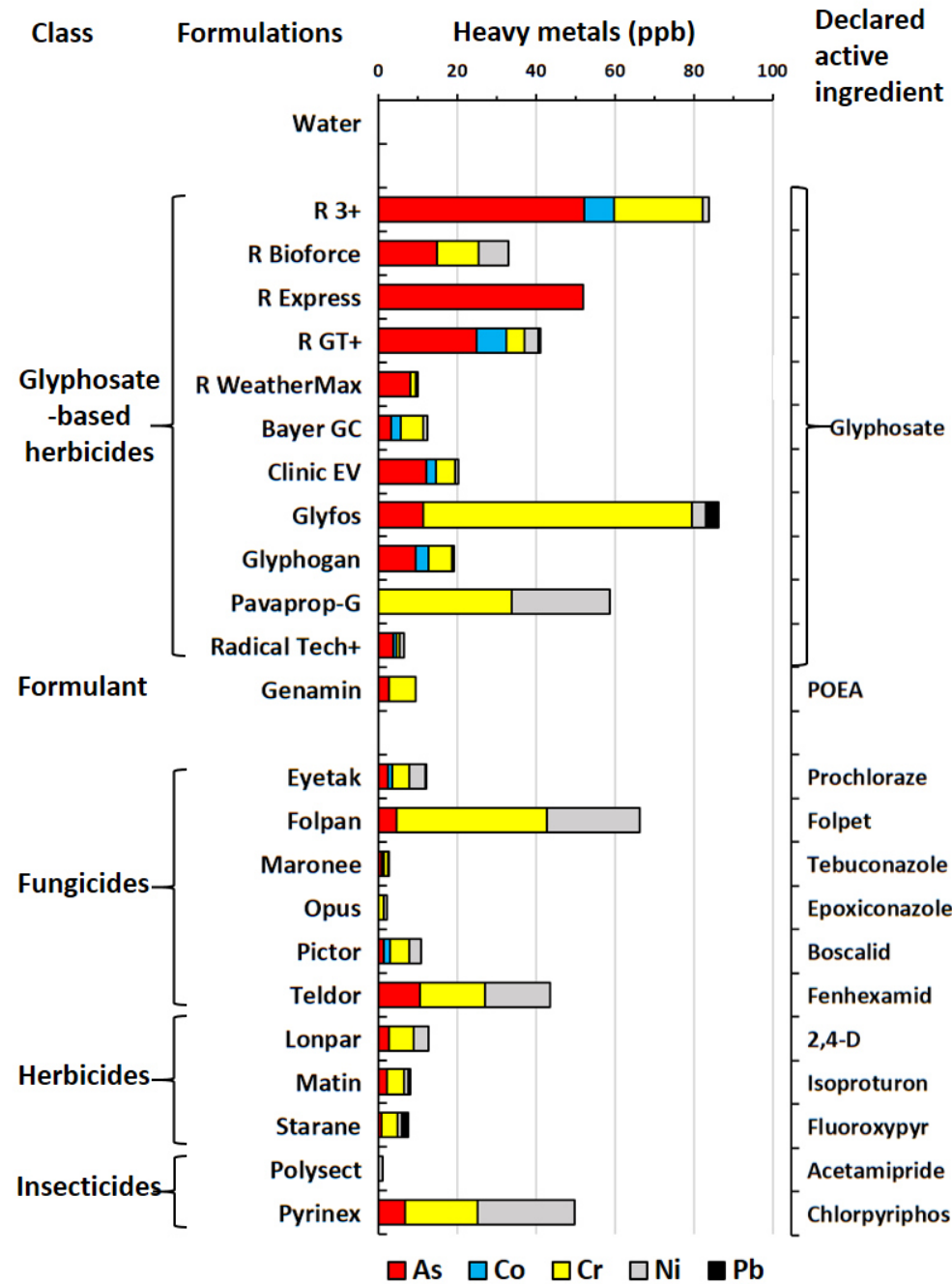
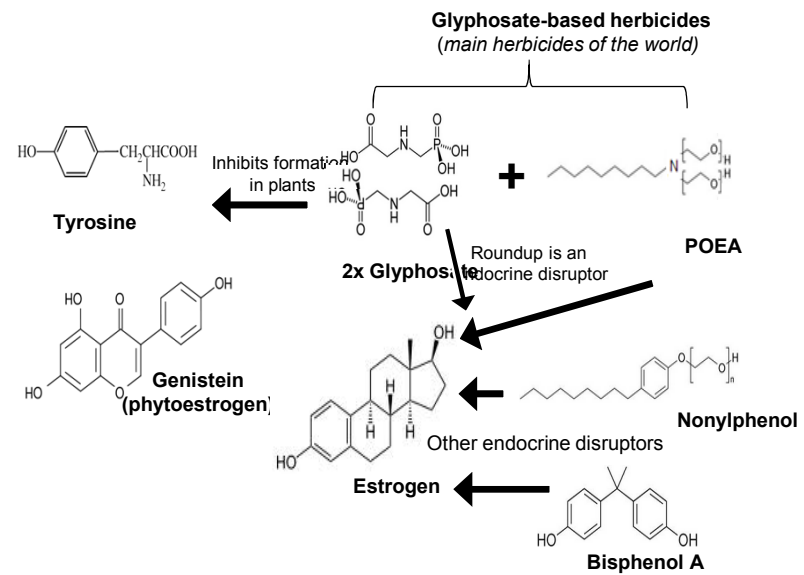


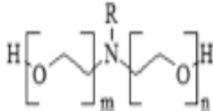
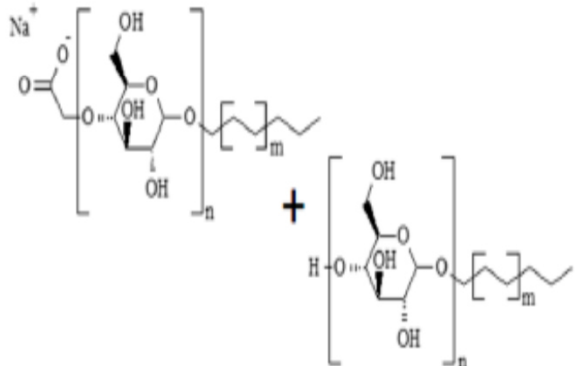
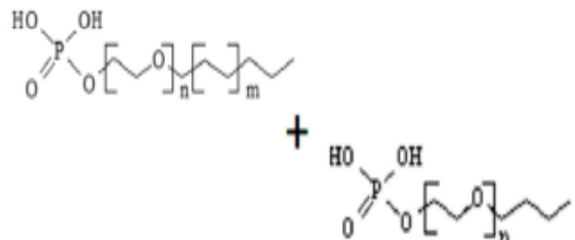

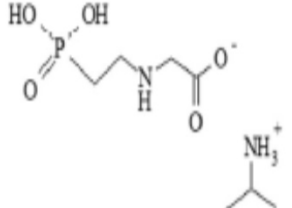
Figure 2. Various heavy metals in formulations of pesticides at their recommended dilutions

# glyphosate and Roundup formulants





	Products	Trade Name (Manufacturer, Country)	Declared Active Ingredient (dAI)	dAI (%)	Present in	(ppm)	NOEC	LOEC	LC50
Co-formulants	POEA	Emulson AG GPE 3SS (Lamberti, Ita)	Polyethoxylated tallow amine	100	Roundup Classic, Glyphos		3.0	3.5	3.9
	POEA/F	Emulson AG GPE 3/SSM (Lamberti, Ita)	Polyethoxylated tallow amine	70	Roundup Classic, Glyphos		4.0	4.5	4.7
	QAC	Emulson AG CB 30 (Lamberti, Ita)	Quaternary ammonium compound	30	other herbicides		35	50	58
	POE-APE	Rolfen Bio (Lamberti, Ita)	POE alkyl phosphate ether	70	other herbicides		150	200	222
	APG	Plantapon LGC (The Soap kitchen, UK)	Alkyl polyglucoside	28.5-34.0	Medallon Premium		200	400	421
			G salt of (g/L)	G (g/L)	Co-formulants (%)				
Formulations	RWMAX	Roundup WeatherMAX (Monsanto, Can)	Potassium (660)	540	Petroleum distillate /Transorb2		60	70	71
	Glyphos	Glyphos (Cheminova, Hun)	IPA (486)	360	9% POEA		75	85	86
	R Classic	Roundup Classic (Monsanto, Hun)	IPA (486)	360	15,5% POEA		75	80	89
	Kapazin	Kapazin (Arysta, Hun)	IPA (486)	360	C8-10 ethoxylated alcohol (<2 g/L), Triethylene glycol monobutyl ether (<2 g/L)		75	85	128
	Total	Total (Sinon Corporation, Hun)	IPA (486)	360	58,5% unknown surfactant		100	125	130
	Medallon	Medallon Premium (Syngenta, Hun)	diammonium (433)	360	10%-20% APG (150 g/L)		500	600	1268
	dAI	G	Glyphosate isopropyl ammonium (Hun)	IPA (486)	360			3100	4600

Chemical Structure	CAS RN *	Chemical Class of Substance Group/Substance Name
	61791-26-2	polyethoxylated tallowamine (POEA) ( $R = C_{14}-C_{18}$ ) ( $n + m = 2-28$ )
	383178-66-3 + 110615-47-9	alkyl polyglucosides (APG) ( $n < 3$ , $m = 3-6$ )
	68130-47-2 + 50769-39-6	polyoxyethylene alkyl ether phosphates (POE-APE) ( $n = 6-10$ , $m = 2-3$ )
	66455-29-6	quaternary ammonium compound (QAC)
<b>Active Ingredient</b>		
	386411-94-0	isopropylamine salt of glyphosate

\* Chemical Abstracts Registry Number.



Article

# Co-Formulants in Glyphosate-Based Herbicides Disrupt Aromatase Activity in Human Cells below Toxic Levels

Nicolas Defarge <sup>1,3</sup>, Eszter Takács <sup>2</sup>, Verónica Laura Lozano <sup>1</sup>, Robin Mesnage <sup>1,3</sup>,  
Joël Spiroux de Vendômois <sup>3</sup>, Gilles-Eric Séralini <sup>1,3,\*</sup> and András Székács <sup>2</sup>

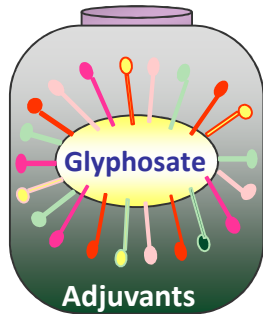
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- <sup>2</sup> Agro-Environmental Research Institute, National Agricultural Research and Innovation Centre, H-1022, Herman Ottó u. 15, Budapest, Hungary; e.takacs@efri.hu (E.T.); a.sekacs@efri.hu (A.S.)
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**Abstract:** Pesticide formulations contain declared active ingredients and co-formulants presented as inert and confidential compounds. We tested the endocrine disruption of co-formulants in six

All ingredients apart the declared active principle, even toxic ones,  
 are kept secret and are considered as inerts: **this is wrong**  
 We studied more than ten formulations on human and rat cells



Some « inerts » in glyphosate-based herbicides:

3-Iodo-2-propynyl butyl carbamate

Thyroid damages

**ARSENIC +  
 PETROLEUM  
 BURNED  
 RESIDUES**

N-Nitrosoglyphosate

Carcinogenic

1,4-dioxane

Alkylamine polyethoxylated

Cytotoxicity, possible genotoxicity

Methyl p-hydroxybenzoate

Genetic Damages

+  
**FORMALDEHYDE  
 + BPA...**

5-Chloro-2-methyl 3(2H)-iso-thiazolone



**“Active principle” does not mean that it is the most toxic in the mixture for non-target species**



# Is is true for other pesticides?

We have tested the toxicity of 9 pesticides (insecticides, fongicides, herbicides), in comparison to their declared active principle

Hindawi Publishing Corporation  
BioMed Research International  
Volume 2014, Article ID 179691, 8 pages  
<http://dx.doi.org/10.1155/2014/179691>

*Research Article*

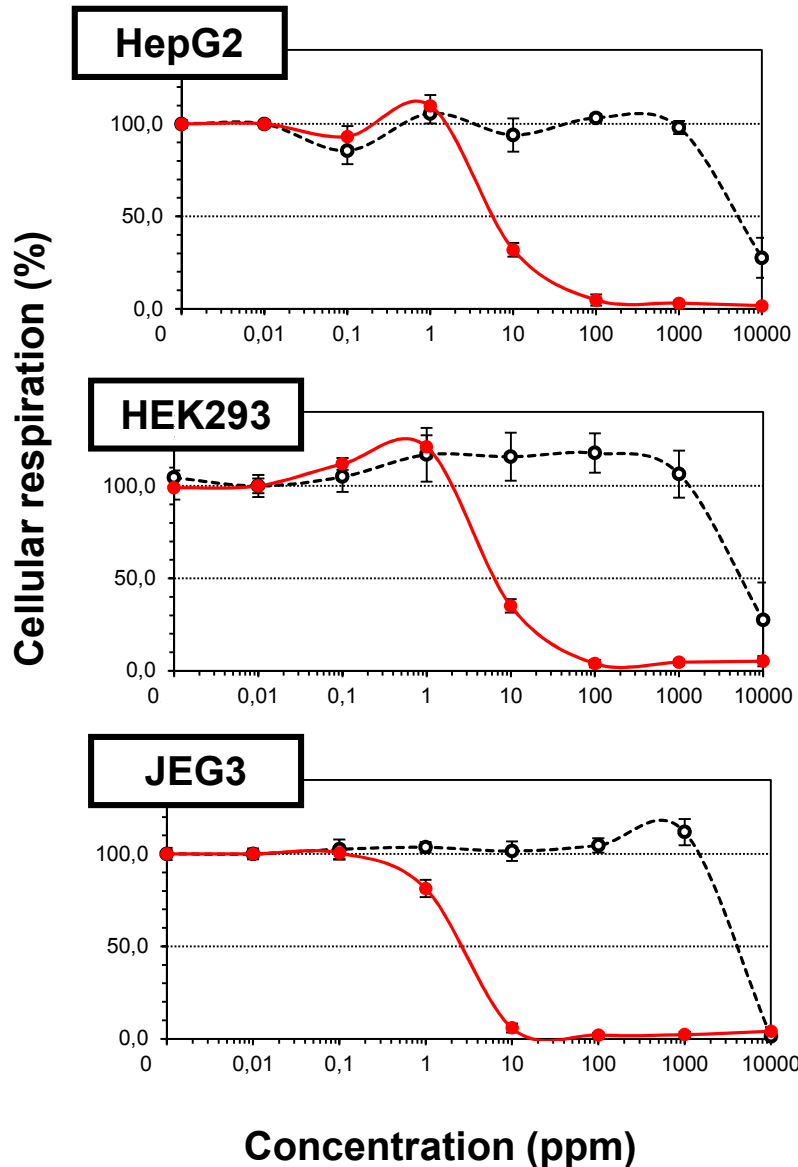
## Major Pesticides Are More Toxic to Human Cells Than Their Declared Active Principles

Robin Mesnage,<sup>1</sup> Nicolas Defarge,<sup>1</sup> Joël Spiroux de Vendômois,<sup>2</sup> and Gilles-Eric Séralini<sup>1</sup>

	Pesticide class	Active Principle	Formulation
Herbicides	Phosphonoglycine	<b>Glyphosate</b>	Roundup GT+ (450 g/L)
	Urea	<b>Isoproturon</b>	Matin EL (500 g/L)
	Synthetic auxin	<b>Fluroxypyr (ester 1-methylheptyl)</b>	Starane 200 (200 g/L)
Insecticides	Carbamate	<b>Pirimicarb</b>	Pirimor G (50%)
	Neonicotinoid	<b>Imidacloprid</b>	Confidor (200g/l)
	Neonicotinoid	<b>Acetamiprid</b>	Polysect Ultra (5g/L)
Fungicides	Triazole	<b>Tebuconazole</b>	Maronee (250 g/L)
	Triazole	<b>Epoxinazole</b>	Opus (125 g/L)
	Imidazole	<b>Prochloraz</b>	Eyetak (450 g/L)

# Fongicide : Maronee (250 g/L Tebuconazole)

Active principle: dotted line  
Formulation : red line



One of the most used triazole fungicides, applied on field crops, fruit trees, vegetables, and grass areas



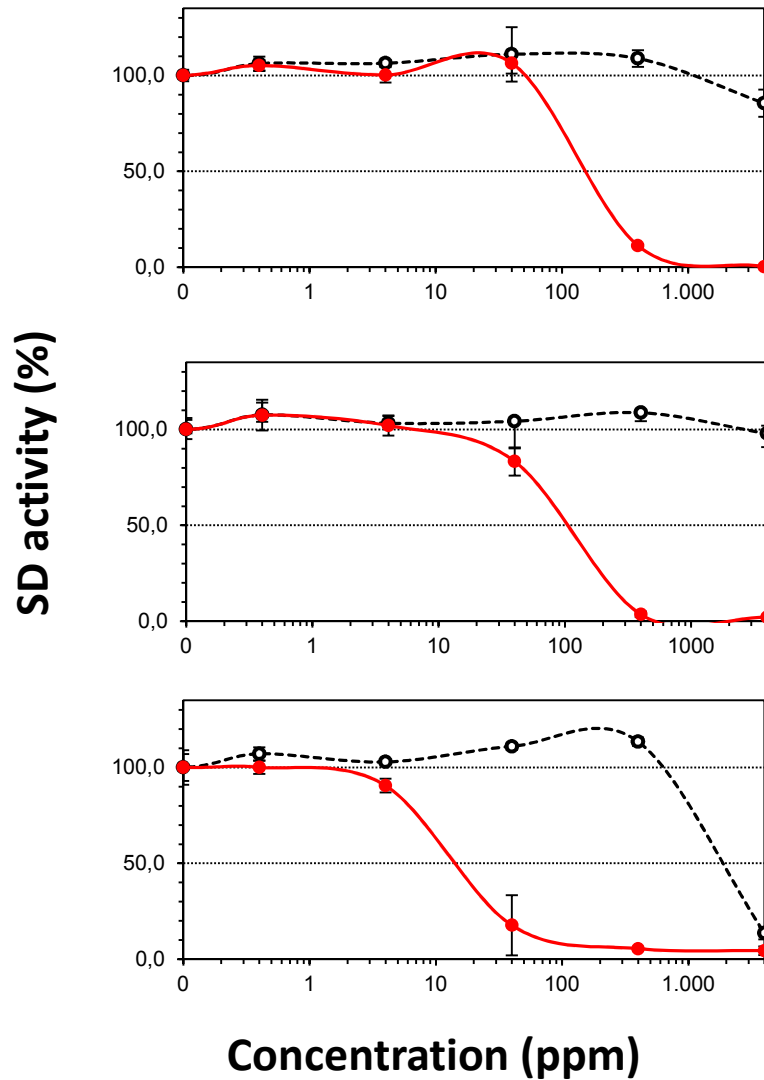
**~ 1056 times more toxic than its active principle**

Overall, fungicides were the most toxic from doses 300-600 times lower than agricultural dilutions, with very similar profiles in all cell types.

Herbicide: **Starane** (200g/L Fluoxypyr)

Active principle: dotted line

Formulation : solid line



HepG2

Used as an alternative to known toxic pesticides on non-crop areas but also for agricultural use on wheat, barley, corn, and oat.



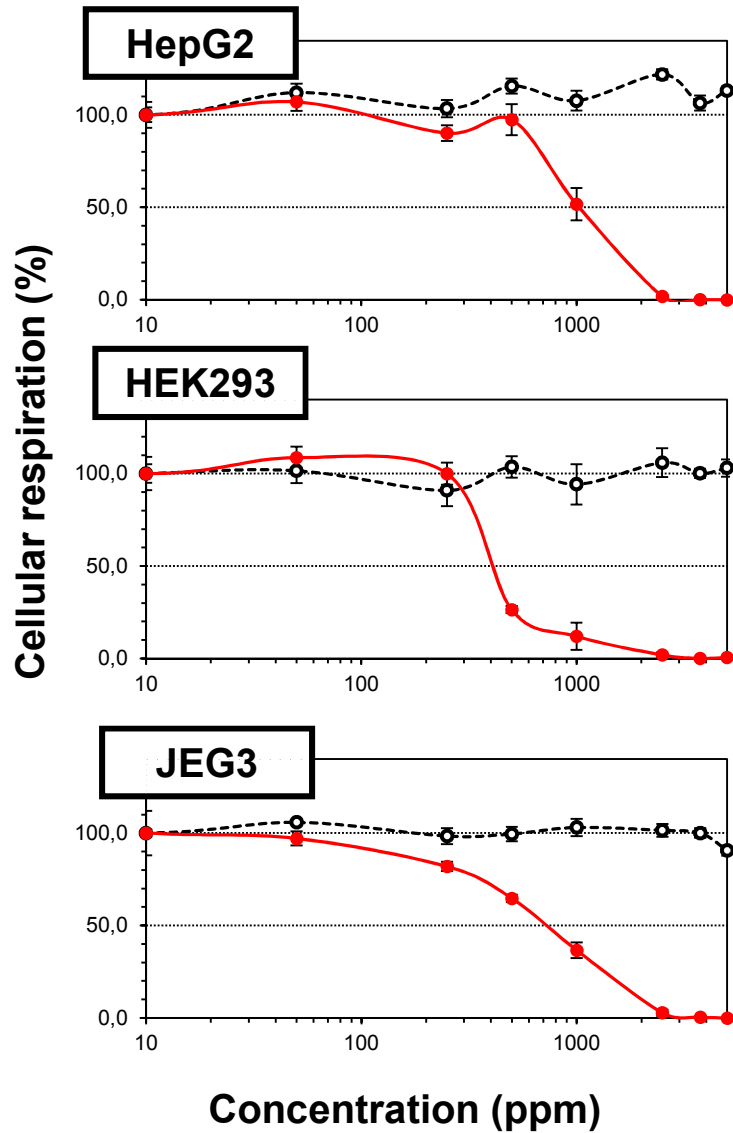
HEK293

JEG3

**A lot more toxic than its declared active principle**

# Insecticide : Confidor (200 g/L Imidacloprid)

Active principle: dotted line  
Formulation : solid line



The major neonicotinoid,  
the bestselling insecticides  
worldwide



**A lot more toxic than its  
declared active principle**



# 8 formulations out of 9 were up to 1000 times more toxic than their supposed active principles

Active principles	Formulations	Declared adjuvants
Glyphosate	Roundup GT+	Ethoxylated etheralkylamine
Isoproturon	Matin EL	Unknown
Fluroxypyr (ester 1-methylheptyl)	Starane 200	Solvent naphtha; alkyl-aryl sulfonates
Pirimicarb	Pirimor G	Docusate sodium; benzenesulfonic acid
Imidacloprid	Confidor	1-Methyl-2-pyrrolidinone
Acetamiprid	Polysect Ultra	1,2-Benzisothiazoline-3-one; ethanol
Tebuconazole	Maronee	N,N-Dimethyldecanamide
Epoxiconazole	Opus	Solvent naphtha; fatty alcohol ethoxylated
Prochloraz	Eyetak	Solvent naphtha; xylene; isobutanol

**Known hidden toxic active principle**  
(Mesnage et al., 2013)

**High toxicity to larval bees**  
(Zhu et al., 2014)  
**Developmental toxicant in rats**  
(Saillenfait, 2002)

**Developmental effects in rodents**  
(McKee et al., 1990)

**associated with cardiac and central nervous system diseases in humans**  
(Langman et al., 1984)

**developmental toxicant in rodents**  
(US EPA)

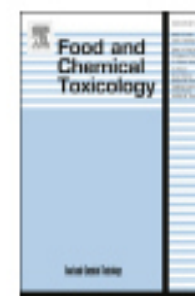
**Differential toxicities between the supposed active principles and their formulations appear to be a general property of pesticide toxicology.**



Contents lists available at ScienceDirect

## Food and Chemical Toxicology

journal homepage: [www.elsevier.com/locate/foodchemtox](http://www.elsevier.com/locate/foodchemtox)



### Review

# Potential toxic effects of glyphosate and its commercial formulations below regulatory limits



R. Mesnage <sup>a, b, 1</sup>, N. Defarge <sup>a, b</sup>, J. Spiroux de Vendômois <sup>b</sup>, G.E. Séralini <sup>a, b, \*</sup>

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### ABSTRACT

Glyphosate-based herbicides (GlyBH), including Roundup, are the most widely used pesticides worldwide. Their uses have increased exponentially since their introduction on the market. Residue levels in food or water, as well as human exposures, are escalating. We have reviewed the toxic effects of GlyBH measured below regulatory limits by evaluating the published literature and regulatory reports. We reveal a coherent body of evidence indicating that GlyBH could be toxic below the regulatory lowest observed adverse effect level for chronic toxic effects. It includes teratogenic, tumorigenic and hep-





**Formulants are the real ACTIVE  
INGREDIENTS**

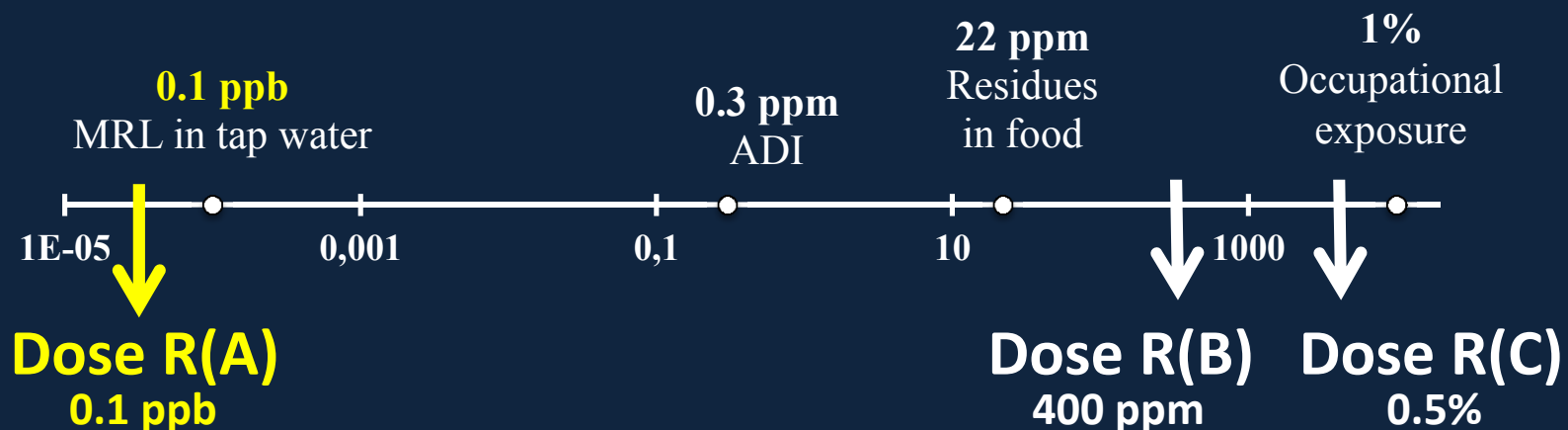
**2 MISTAKES POSSIBLE :**

**DECLARATION OF WRONG ACTIVE  
INGREDIENT (1)**

**DECLARATION OF REAL ACTIVE  
INGREDIENTS AS INERTS AND  
CONFIDENTIAL (2)**



- The first **most detailed life-long** rodent (rat) feeding study investigating possible toxic effects rising from:
- A Roundup-tolerant **GM maize** (NK603), treated or not with Roundup (11, 22 and 33%),
- A complete commercial formulation of a **Roundup herbicide** (0.1 ppb; 400 ppm and 0.5% in water)



RESEARCH

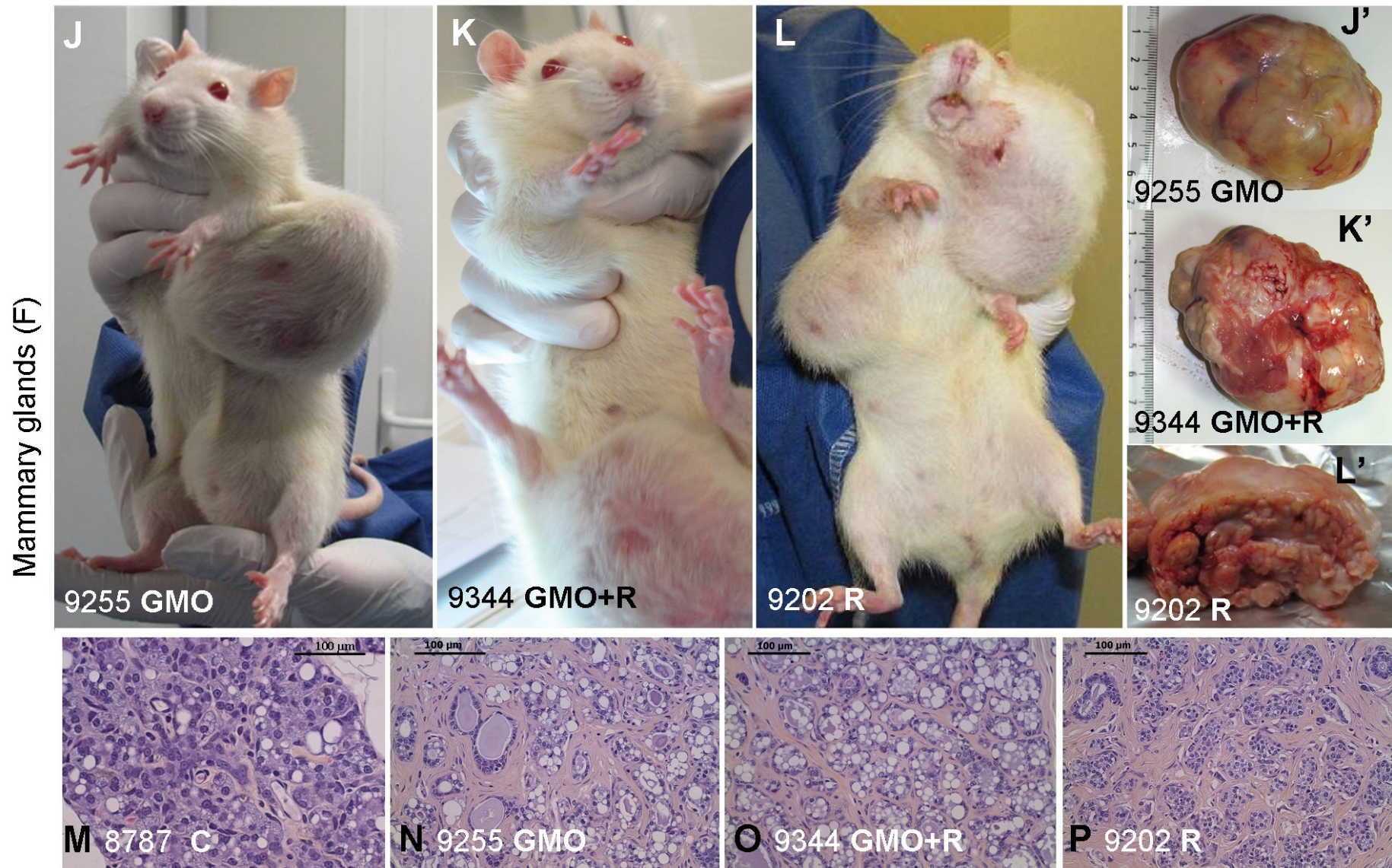
Open Access

# Republished study: long-term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize

Gilles-Eric Séralini<sup>1\*</sup>, Emilie Clair<sup>1</sup>, Robin Mesnage<sup>1</sup>, Steeve Gress<sup>1</sup>, Nicolas Defarge<sup>1</sup>, Manuela Malatesta<sup>2</sup>, Didier Hennequin<sup>3</sup> and Joël Spiroux de Vendômois<sup>1</sup>

## Abstract

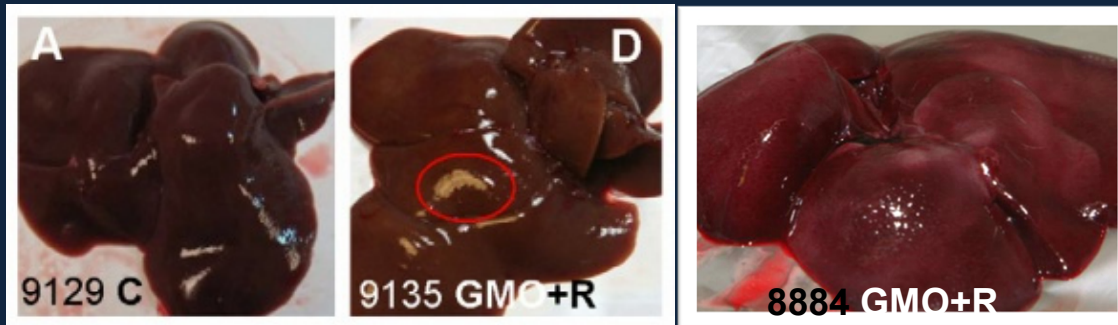
**Background:** The health effects of a Roundup-tolerant NK603 genetically modified (GM) maize (from 11% in the diet), cultivated with or without Roundup application and Roundup alone (from 0.1 ppb of the full pesticide containing glyphosate and adjuvants) in drinking water, were evaluated for 2 years in rats. This study constitutes a



**Fig. 3.** Anatomopathological observations in rats fed GMO treated or not by Roundup, and effects of Roundup alone. Macroscopic and microscopic photographs show male livers (A-E) and left kidneys (F-I'), female mammary glands (J-P) and pituitaries (Q-T), according to Table 2. The number of each animal and its treatment is specified. Macroscopic pale spots (D) and microscopic necrotic foci in liver (C clear-cell focus, E basophilic focus with atypia), and marked or severe chronic progressive nephropathies, are illustrated. In females, mammary tumors (J,J',N adenocarcinoma and K,K',L,L',O,P fibroadenomas) and pituitary adenomas (R-T) are shown and compared to controls (C after the rat number).



# Males died mostly from pathologies in **liver** and **kidneys**

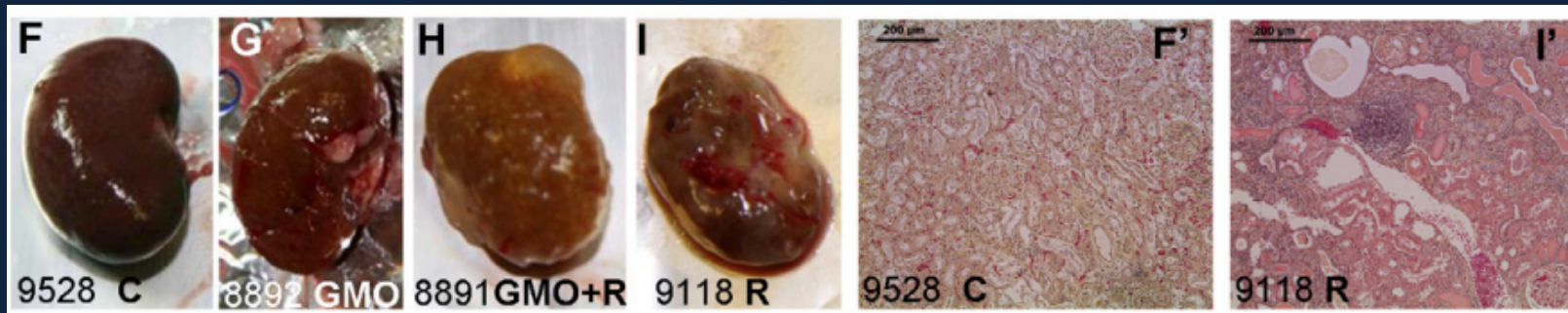


Control

Treated

Liver : congestions,  
macroscopic spots,  
necrotic foci

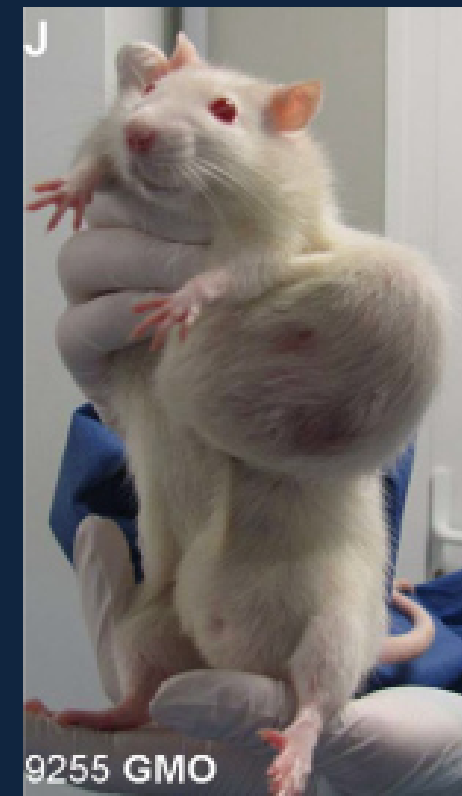
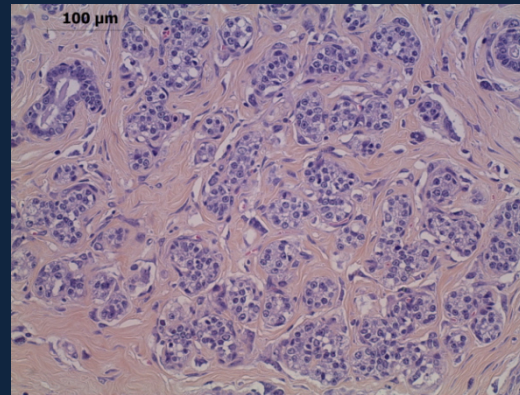
**Kidney:** chronic progressive nephropathies  
more severe and earlier than in controls



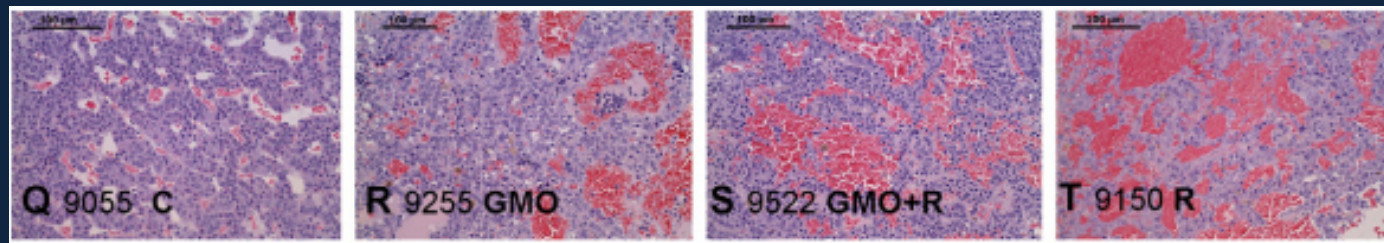


# Females died mostly from mammary tumors

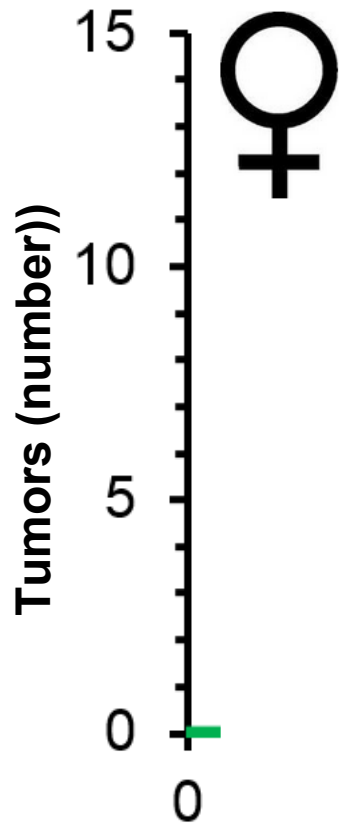
*93% tumors are in mammary glands*



# ...and pituitary dysfunctions



# Rats have more tumors when they drink water with 0.1 ppb of Roundup



Mammary fibroadenoma



RESEARCH

Open Access



# Transcriptome profile analysis reflects rat liver and kidney damage following chronic ultra-low dose Roundup exposure

Robin Mesnage<sup>1</sup>, Matthew Arno<sup>2</sup>, Manuela Costanzo<sup>3</sup>, Manuela Malatesta<sup>3</sup>, Gilles-Eric Séralini<sup>4</sup> and Michael N. Antoniou<sup>1\*</sup>

## Abstract

**Background:** Glyphosate-based herbicides (GBH) are the major pesticides used worldwide. Converging evidence suggests that GBH, such as Roundup, pose a particular health risk to liver and kidneys although low environmentally relevant doses have not been examined. To address this issue, a 2-year study in rats administering 0.1 ppb Roundup (50 ng/L glyphosate equivalent) via drinking water (giving a daily intake of 4 ng/kg bw/day of glyphosate) was conducted. A marked increased incidence of anatomorphological and blood/urine biochemical changes was indicative of liver and kidney structure and functional pathology. In order to confirm these findings we have

# SCIENTIFIC REPORTS



OPEN

## Multionics reveal non-alcoholic fatty liver disease in rats following chronic exposure to an ultra-low dose of Roundup herbicide

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Accepted: 22 November 2016  
Published: 09 January 2017



Robin Mesnage<sup>1</sup>, George Renney<sup>2</sup>, Gilles-Eric Séralini<sup>3</sup>, Malcolm Ward<sup>2</sup> & Michael N. Antoniou<sup>1</sup>

The impairment of liver function by low environmentally relevant doses of glyphosate-based herbicides (GBH) is still a debatable and unresolved matter. Previously we have shown that rats administered for 2 years with 0.1 ppb (50 ng/L, glyphosate equivalent dilution: 4 ng/kg body weight/day daily intake) of a





RESEARCH ARTICLE

# Laboratory Rodent Diets Contain Toxic Levels of Environmental Contaminants: Implications for Regulatory Tests

Robin Mesnage<sup>1,2</sup>, Nicolas Defarge<sup>1,2</sup>, Louis-Marie Rocque<sup>2</sup>, Joël Spiroux de Vendômois<sup>2</sup>, Gilles-Eric Séralini<sup>1,2\*</sup>

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 These authors contributed equally to this work.

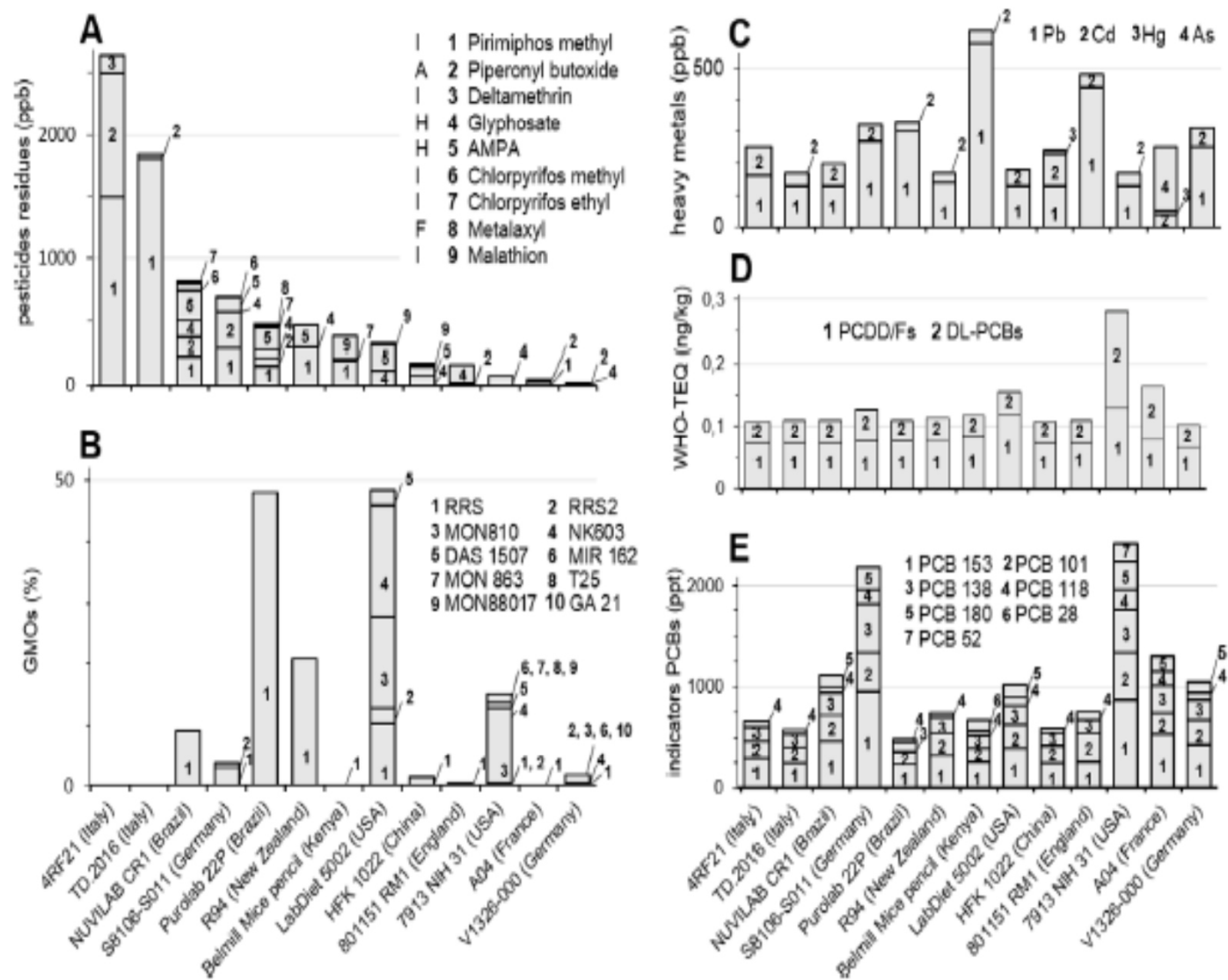
 Current address: Gene Expression and Therapy Group, King's College London, Faculty of Life Sciences & Medicine, Department of Medical and Molecular Genetics, 8th Floor, Tower Wing, Guy's Hospital, Great Maze Pond, London, SE1 9RT, United Kingdom

\* [gilles-eric.seralini@unicaen.fr](mailto:gilles-eric.seralini@unicaen.fr)







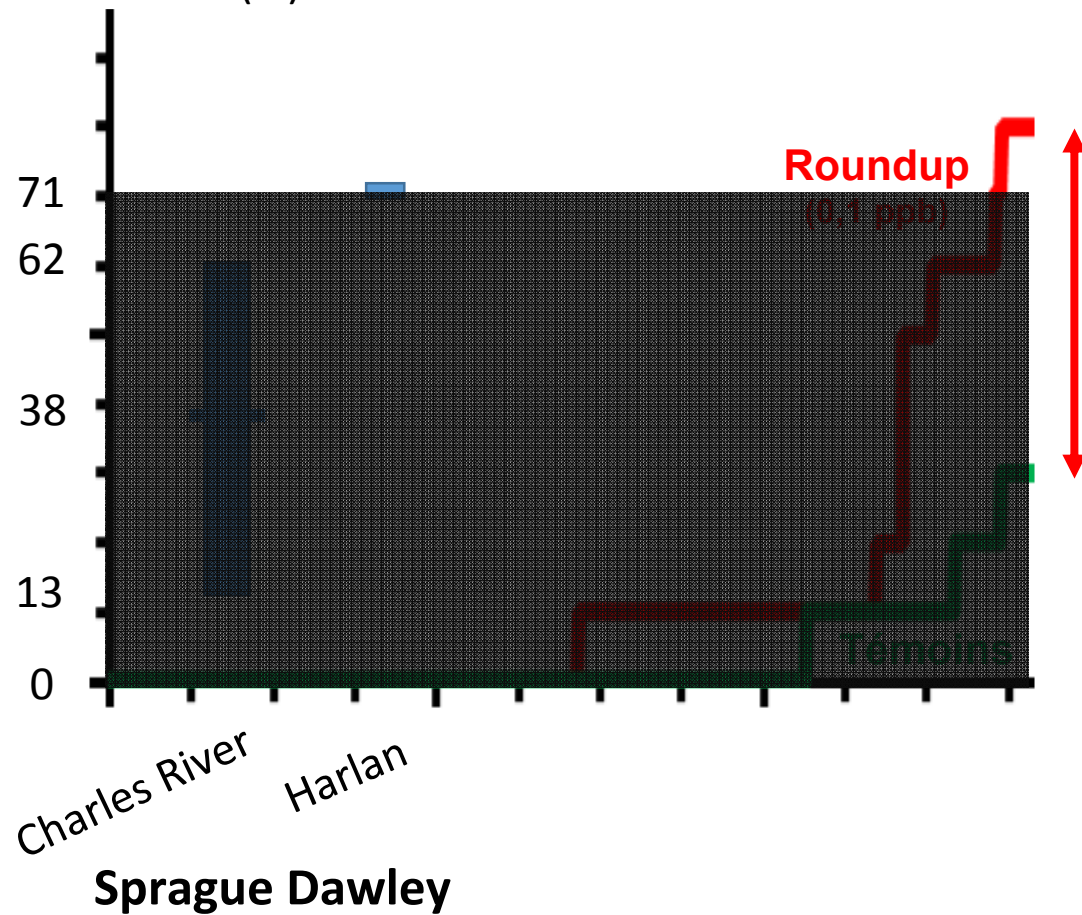


**Fig 2. Environmental contaminants in 13 rodent diets used worldwide.** Countries indicate the feed manufacturing locations, which can differ from the cultivations. (A) 262 pesticides have been measured in ppb ( $\mu\text{g}/\text{kg}$ ) by multi-residue GC-MS and/or LC-MS/MS. F Fungicide, H Herbicide, I Insecticide. (B)

# Spontaneous tumors in the long term ?

Des tumeurs spontanées à long terme ?

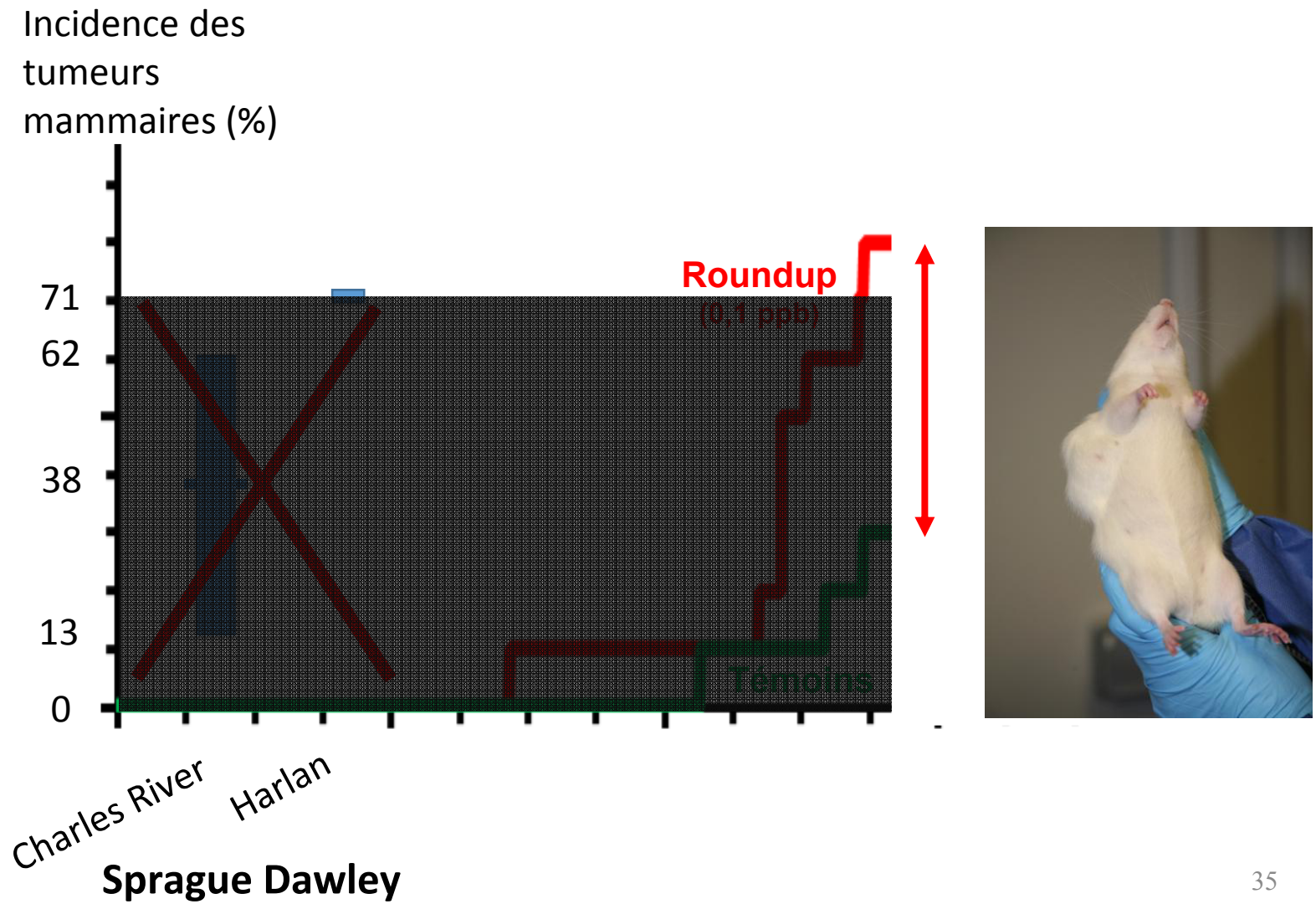
Incidence des tumeurs mammaires (%)





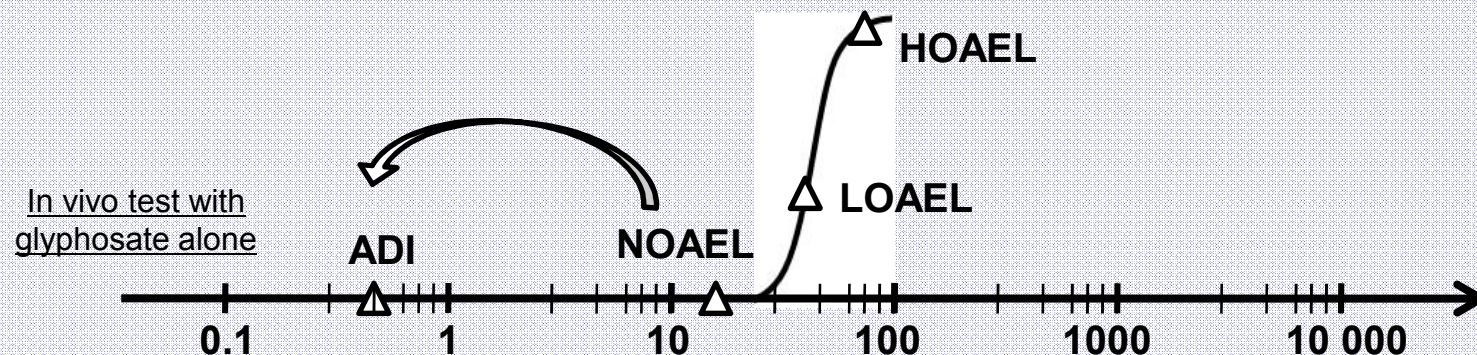
# Relevance

- WRONG HISTORICAL DATA**



## These results heavily question regulatory thresholds

Acceptable Daily Intakes (ADIs) are calculated from chronic rat tests with the declared active principle of pesticides alone. This neglects toxicities of formulants which are wrongly considered as inert!



**ADIs and other deduced regulatory values are underestimated (x1,000) by neglecting the effects of formulants**

COMMENTARY

Open Access

# Conflicts of interests, confidentiality and censorship in health risk assessment: the example of an herbicide and a GMO

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## Abstract

We have studied the long-term toxicity of a Roundup-tolerant GM maize (NK603) and a whole Roundup pesticide formulation at environmentally relevant levels from 0.1 ppb. Our study was first published in *Food and Chemical Toxicology* (FCT) on 19 September, 2012. The first wave of criticisms arrived within a week, mostly from plant biologists without experience in toxicology. We answered all these criticisms. The debate then encompassed scientific arguments

# TODAY, CONCLUSION

- Confidential formulations for scientists = confusion between active principle and inerts
- Confidential raw data for blood analyses after long term pesticide treatments with the declared active principle alone, in regulatory tests
- This is flawed science with inconclusive results
- We need full transparency for the scientific community
- ADIs should be divided by 1,000