



Toxic compounds in herbicides without glyphosate

Gilles-Eric Seralini^{a,*}, Gerald Jungers^b

^a University of Caen Normandy, Network on Risks, Quality and Sustainable Environment, France

^b Spark-Vie, France

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ABSTRACT

Glyphosate has been banned in some herbicidal formulations. We analyse for the first time 14 marketed products in Europe where glyphosate was replaced by acetic, pelargonic, caprylic or capric acids, or even benzalkonium chloride, to be supposedly less toxic. 35 heavy metals, 16 polycyclic aromatic hydrocarbons (PAHs), and essential minerals were tested by specific mass spectrometry associated with gas chromatography or inductively coupled plasma methods in the formulations. Essential minerals do not reach toxic levels, but heavy metals are found at levels up to 39 mg/L, depending on the product, and include silicon, arsenic, lead, iron, nickel, and titanium. Their presence at up to several hundred times the admissible levels in water may be due to nanoparticles embedding pesticides. PAHs reach levels of 32–2430 µg/L in 12 of the 14 samples; for instance, the carcinogen benzo(A)pyrene was detected. It was found to be present at up to several thousand times above the norm in water, as was benzo(A)anthracene. These compounds did not add significant herbicidal effects. Low levels of glyphosate were detected in 2 samples. These variable levels of undeclared toxic chemicals violate European Union rules on pesticides and may have health and environmental consequences, especially when exposure is long-term.

1. Introduction

We previously demonstrated the toxicity of some glyphosate-based herbicidal formulations (GBH) *in vitro*, not only in cell cultures (Richard et al., 2005) but also *in vivo* on mammals over the short term (Cassault-Meyer et al., 2014) and in chronic long-term studies at ppb doses in the diet (Seralini et al., 2013, 2014). This was a focus for an intense debate (Duke, 2018; Portier and Clausen, 2017; Tarazona et al., 2017; Davoren and Schiestl, 2018), but it was more recently confirmed and explained that it was induced by compounds of the formulations other than glyphosate (Lopes et al., 2018; Hao et al., 2019). We conducted *in vivo* transcriptomic, proteomic and metabolomic studies (Mesnage et al., 2015a, 2017). *In vitro*, oxidative stress, mitochondrial dysfunction, apoptosis, membrane disruption (Benachour et al., 2009), estrogen and androgen receptors blockade as well as aromatase disruption (Richard et al., 2005; Gasnier et al., 2009) were demonstrated in hepatic, kidney, placental and embryonic human cells. *In vivo*, liver, kidneys, and hormone-dependent organs such as mammary glands and thyroid were affected by these cellular and endocrine disruptions.

Proteins disturbed were involved in organonitrogen metabolism and fatty acid β -oxidation. Proteome disturbances reflected peroxisomal proliferation, steatosis, and necrosis. Metabolomic studies confirmed lipotoxic conditions and oxidative stress (Mesnage et al., 2017). *In vivo* at the transcriptomic level, alterations in gene expression indicated fibrosis, necrosis, phospholipidosis, mitochondrial membrane dysfunction and ischemia (Mesnage et al., 2015a). These findings confirm the histological and electron microscopic observations, as well as blood and urine biochemistry (Seralini et al., 2014).

All these effects were caused by long-term consumption of formulations from 0.1 ppb, in which we identified by mass spectrometry oxidized petroleum residues and toxic heavy metals (Mesnage et al., 2013; Defarge et al., 2018). The effects (including endocrine disruption) were not caused by glyphosate alone at the same level (Defarge et al., 2016). We originally demonstrated the differential toxicity between glyphosate and its formulations marketed as Roundup, the most widely used herbicide in the world (Richard et al., 2005). We found that Roundup contained formulants that were 1000 times more toxic, and at a lower threshold more endocrine disrupting than glyphosate, but these

Abbreviations: AMPA, aminomethyl phosphonic acid; G, glyphosate; GBH, glyphosate-based herbicides; GC, gas chromatography; HPLC, High performance liquid chromatography; ICP, Inductively coupled plasma method; MS, mass spectrometry; PAH, polycyclic aromatic hydrocarbons; ppb, part per billion.

* Corresponding author.

E-mail address: gilles-eric.seralini@unicaen.fr (G.-E. Seralini).

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formulants were declared as inert by the manufacturer and their identity was kept confidential by regulatory bodies. Therefore the scientific debate was centred mostly on glyphosate alone (Bai and Ogbourne, 2016; de Araujo et al., 2016; Van Bruggen et al., 2018), even if numerous authors used glyphosate formulations but interpreted the results as glyphosate effects (Mesnage et al., 2015b). None of the above-mentioned cytologic and pathological effects seen from Roundup exposure were observed in studies using glyphosate alone at the ppb level, but in studies using the formulations, all these effects were documented. However there is still a need to measure multi-generational effects of these herbicides (Landrigan and Belpoggi, 2018).

Due to the world debate on health, food and environmental toxicology, glyphosate was replaced in formulations for private use only in some countries, mostly by pelargonic and acetic acid that were declared in 2019. Thus we carefully undertook an analysis of the formulations in which these new products were dissolved, to check the potential toxicity in water and food of the new residues. We focused mainly on petroleum (PAHs) and numerous heavy metals with an extensive study on this issue. These products are still in some instances called Roundup, but the marketing has been changed to imply that the herbicides are more natural, since they no longer contain glyphosate.

2. Materials and methods

2.1. Materials

Out of 16 glyphosate-free labelled herbicides available on the French market for gardeners in 2019, plus one marketed in Germany and 3 in Poland, 14 samples were selected for their authorization numbers, manufacturer or distribution company, or references of lots and dates of manufacturing. All were carefully numbered A-N and their respective contents, in terms of declared active principles, were listed (Table 1). To ensure the accuracy and reproducibility of the data, in particular for adequate replications, standard deviations and coefficients of variations, all measurements were performed in laboratories accredited by COFRAC, the French accreditation body.

2.2. ICP-MS for essential minerals and metals

Inductively coupled plasma mass spectrometry (ICP-MS) was used according to the norm NF IN ISO 17292-2. For essential minerals, calcium, magnesium, phosphorus, potassium, and sodium were detected; for metals or metalloids, other minerals, aluminum, antimony, arsenic, barium, beryllium, bismuth, cadmium, chromium, cobalt, copper, iron, gallium, germanium, hafnium, indium, lithium, lead, manganese, molybdenum, nickel, niobium, palladium, rhenium, selenium, silicon,

silver, strontium, tantalum, tellurium, thallium, tin, titanium, tungsten, vanadium, zinc, and zirconium were assayed (see Table 2). Mineralization of water was adapted from the norm NF EN ISO 15587-2; the mineralization was performed by evaporation with addition of nitric acid. A self-control matrix followed the mineralization protocol. The analysis of a range blank and the calibration range were accomplished; this was controlled by an independent standard with the acceptance criterion range of $\pm 10\%$. Other independent standards were analyzed every 10–15 samples with an acceptance criterion of $\pm 20\%$; at the end of the measurements, low-end standards were analyzed.

2.3. HPLC and fluorescence detections for glyphosate and AMPA

High performance liquid chromatography (HPLC) followed by final stack derivatization and fluorescence detection (F22, all method DIN 38407-22) was performed for G and its main metabolite aminomethyl phosphonic acid (AMPA).

2.4. GC-MS for PAH

The international normalized method DIN 38407-39 was applied, including gas chromatography and mass spectrometry (GC-MS) for PAH measures. First, PAHs in water were extracted with cyclohexane. The extract was concentrated by evaporation. The PAHs were then separated by gas chromatography on capillary columns with suitable stationary separation phases and identified and quantified by mass spectrometry. The products assayed were acenaphthene, acenaphthylene, anthracene,

Table 2

All compounds assayed in pesticides. Measurements were performed according to accredited norms in the European Union for ICP-MS (essential minerals, metals), GC-MS (PAHs), or HPLC (G, AMPA).

Metals: Aluminium, Antimony, Arsenic, Baryum, Beryllium, Bismuth, Cadmium, Chromium, Cobalt, Gallium, Germanium, Hafnium, Indium, Iron, Lead, Lithium, Mercury, Molybdenum, Nickel, Niobium, Palladium, Rhenium, Selenium, Silicon, Silver, Strontium, Tantalum, Thallium, Tin, Titanium, Tellurium, Tungsten, Vanadium, Zinc, Zirconium.

Life salts: Calcium, Copper, Magnesium, Manganese, Phosphorus, Potassium, Sodium.

Polycyclic aromatic hydrocarbons: Acenaphthene, Acenaphthylene, Anthracene, Benzo (A)-anthracene, Dibenzo (A,H)Anthracene, Benzo(A)Pyrene, Benzo(B) Fluoranthene, Benzo (G,H,I)Perylene, Benzo(K)Fluoranthene, Chrysene, Fluoranthene, Fluorene, Indeno (1,2,3-C,D)Pyrene, Naphtalene, Phenanthrene, Pyrene.

Herbicides: Glyphosate, AMPA.

Table 1

Ten different samples used in this work and their declared active principles available on the French market for gardeners in 2019, plus one German and 3 Polish herbicides used for comparisons. The % of each declared active principle on the label is indicated.

Sample	Herbicide name	Declared active principle	%	Authorization	Holder
A	Roundup Speed Evergreen Monsanto	Acetic acid	6	2130153	Monsanto technology LLC
B	Herbatak Contact Fertiligene Scotts		6	2130153	SCOTTS France SAS
C	Biocontrôle Starnet Jardin d'Eden Jade	Pelargonic acid	51.9	2170243 n°CAS 112-05-0	JADE
D	Herbatak Express garden Scotts Jade		51.9	2170243	JADE
E	Clairland Herbistop Compo		24.3	2140121	COMPO France SAS
F	Clairland express Herbistop spray Compo		3.1	2160115	COMPO France SAS
G	Solabiol Beloukha Garden SBM		51.9	2170243	JADE
H	Neudorff Finalsan W.Neudorff		18.8	2170355 CAS 112-05-0	W.Neudorff GmbH KG
I	Roundup Evergreen Belchim Germany		51.92	Nr 008529-62	Belchim Crop Protection
J	Target Poland		71.7	MRiRW nrR-140/2017	Target SA
K	Compo Poland		24.26	MRiRW nrR-34/2016	Compo Poland
L	Solabiol Herbiclean SBM	Caprylic and Capric Acids	3	2140167	SBM Life Science SAS
M	Solabiol Herbiclean SBM		3	2140167	SBM Life Science SAS
N	Bros Poland	Benzalkonium Chloride	1.25	2JFA-40VN-G008 BJWH	Bross Sp. Zo.o.sp.k

benzo(A)anthracene, benzo(A)pyrene, benzo(B)fluoranthene, benzo (G, H,I)perylene, benzo(K)fluoranthene, chrysene, dibenzo (A,H)anthracene, fluoranthene, fluorene, indeno (1,2,3-C,D)pyrene, naphthalene, phenanthrene, and pyrene (Table 2).

2.5. Herbicidal activity

Six squares of 14 cm² of *Poa annua* L. in vegetative state (10–20 blades of grass/cm²) were treated once in triplicate, simultaneously in spring in a field in Normandy. These products are for gardens to remove weeds. Herbicides were diluted as per the manufacturers' instructions. To optimize the effects in a short time and on a small surface, we poured homogeneously 50 ml on each square. Their active principles are used alone similarly as controls: i.e. acetic acid 6%, and pelargonic acid at the same final intermediate concentration as declared in samples E and K, i. e. 24%. Since pure pelargonic acid was dissolved first with 70% alcohol which reached a final 3% concentration of alcohol in the dilution, negative controls were water and water with 3% alcohol. At 3 h and 24 h after treatments, the grass was observed (see results); then the remaining plants were carefully cut and weighed. The effects on soil were observed after 3, 4 and 6 weeks.

3. Results

Following glyphosate prohibition for private use, we obtained a representative sampling of new herbicides declared as glyphosate-free, marketed in European Union since 2019 (Table 1). Some are still called Roundup, which previously contained glyphosate, but are now composed of new declared active principles – mainly pelargonic acid for around 65% of the available products, in which it was found to be present in highly variable concentrations (3.1–72%). It is present in pelargoniums but for herbicidal use is chemically synthesized. The next most common new declared active principle was acetic acid; it is present in vinegar at around the same concentration (6%). Less frequently, the declared active ingredients were caprylic and capric acids. These are respectively octanoic and decanoic acids. They are found in some plants like coconut and in goat's milk but can also be chemically synthesized. In one instance, benzalkonium chloride was present, in a product sourced from Poland. This is a mixture of a benzyl structure with carbon chains of different lengths; it is known as a detergent with antiseptic or biocide activity provoking cell membrane disruptions and is also used in petroleum. Some of these compounds are sold by Bayer, which has acquired Monsanto, historically the main marketer of glyphosate-based herbicides. However, the products used for this research were marketed by different companies, also indicated in Table 1. All compounds measured are listed in Table 2 and the results detailed below.

3.1. Essential minerals concentrations

Essential minerals concentrations were highly variable in the herbicides tested, from <5 to 497 mg/L (Table 3). Only manganese was below detectable levels; copper was quantifiable only in one instance (Table 4). Potassium was by far the element found at the highest levels, reaching 0.3–0.4 g/L. Sodium was the element found at the second highest level, at half the level of potassium, followed by calcium,

phosphorus, and low levels of magnesium.

3.2. Metals

Out of the 35 metals investigated, only 8 were detected in the mg/L range (Table 4), except arsenic. Silicon was by far the metal present at the highest levels, with a maximum of 36 mg/L (in H); in general there was 7 times less nickel; then titanium, iron, copper, lead, zinc, and silver were present at levels less than 10 mg/L. The herbicide with the highest level of heavy metals was C with 39 mg/L; it was also the only one containing titanium. Arsenic was specifically detected in the µg/L range from 6.33 to 20.9 in half of the pesticides (see summary Table 6).

3.3. PAHs

In total, 16 PAHs were detected in the herbicides investigated, from well-known markers of PAH to reference carcinogens. They were <10 µg/L only in 2 samples, but then reached 28.1–2430 µg/L in the 12 others (Table 5). The herbicide with the highest level of PAH presence was K, with fluorene reaching 2100 µg/L. Herbicides E and K had a maximal dose of PAHs of over 2 mg/L and were from the same company in France and Poland, called Compo, with different authorization numbers. The class of PAHs found at the second highest levels were phenanthrene, naphthalene, and chrysene, which were detected at around a 10 times lower level (see column Total A-N, Table 5). Others include the reference carcinogen benzo(A)pyrene, which reached quantifiable levels in a third of the new herbicides. Acenaphthylene, acenaphthene, benzo(A)anthracene, fluoranthene and pyrene constituted the third sub-group, measuring between 100 and 300 µg/L in total.

3.4. Totals and main compounds in pesticides

To facilitate comparisons, Table 6 summarizes the totals of metals, PAHs and essential minerals and presents the detailed results by pesticide for arsenic, but also G and its metabolite AMPA. This is because arsenic is measured on a different scale from other metals. 7 pesticides out of 14 contained quantifiable levels of arsenic. G and AMPA were also not declared but were surprisingly found in 2 pesticides at low levels in comparison to their previous uses in herbicides for which they are the declared active ingredient (around 400 g/L). Even so, the 2 pesticides had a difference of more than 21 times in G + AMPA. However, they had the same authorization number (L and M) and thus should contain the same composition of all chemical products. In both cases, caprylic and capric acids were the only chemicals declared. For C and E also, 2 different lots of the same authorization number were measured, but the results were different in each case (see Raw Data). There was no obvious correlation between essential minerals and metals or PAHs.

3.5. Toxicities for metals and PAHs

A simple observation can be made of the maximal levels of metals observed in the herbicide formulations of this study, in comparison to the norms of toxicities (Table 7A). The so-called toxicity thresholds in water are indicated according to at least one national or international agency (AFSSA, ANSES, EPA, INERIS, NIH, WHO), but are classical

Table 3
Essential minerals found in pesticides in mg/L.

Salts mg/L	A	B	C	D	E	F	G	H	I	J	K	L	M	N	Total A-N
calcium	70.8	63	12.7	<5	11.8	<5	9.8	9	<5	<5	<5	<5	<5	<5	177.1
magnesium	5.1	5	<5	<0.5	<5	<0.5	<0.5	<0.5	<5	<5	<5	<0.5	<0.5	<0.5	10.1
phosphorus	<10	<10	26.2	29.5	16.35	<10	23.4	<2.5	26.05	<10	17.75	<2.5	<2.5	<2.5	139.25
potassium	14.6	15	389.05	376.5	44.6	<10	420.7	<2.5	307.5	83.5	41	<2.5	<2.5	<2.5	1692.45
sodium	175.3	107	54.2	91	103.6	31.9	10.7	10	27.7	8.45	105	<5	<5	161	885.85
Total	265.8	190	482.15	497	176.35	31.9	464.6	19	361.25	91.95	163.75	<5	<5	161	2904.75

Table 4**Minerals, metals, metalloids found in pesticides in mg/L. Arsenic is indicated in Table 6 in µg/L.**

Metals mg/L	A	B	C	D	E	F	G	H	I	J	K	L	M	N	TOTAL A-N
copper	<5	<0.005	6.8	<0.25	<5	<5	<5	<5	<5	<0.25	<0.25	<0.25	<0.5	<0.25	6.8
iron	<5	<5	7.7	<0.5	<5	<5	<5	<5	<5	<0.5	<0.5	<0.5	<0.5	<0.5	7.7
lead	2	<0.01	2	<0.5	1	<0.5	1	<0.5	<10	<0.5	<5	<0.5	<0.5	<0.5	6.00
nickel	<5	<0.005	<5	<5	<0.25	<0.25	<0.25	<0.25	<5	13.82	<0.25	<0.25	<0.25	<0.25	13.82
silicon	12.1	11	8.8	<2.5	<2.5	7	6.8	36	6.9	<5	<5	<2.5	<2.5	5.35	93.95
silver	<5	<0.01	1	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	1.00
titanium	<5	<0.005	7.8	<0.25	<5	<5	<5	<5	<5	<0.25	<0.25	<0.25	<0.25	<0.25	7.8
zinc	<5	<5	4.9	<0.5	<5	<1	<5	<5	<5	<0.5	<0.5	<1	<1	<0.5	4.9
Total	14,1	11	39	<5	1	7	7.8	36	6.9	13.82	<5	<2.5	<2.5	5.35	141.97

Table 5**PAHs found in pesticides in µg/L.**

PAH µg/L	A	B	C	D	E	F	G	H	I	J	K	L	M	N	Total A-N
Acenaphthene	<5	<5	88	<2	79.5	<10	<2	<5	nm	nm	nm	<10	<10	3	170.5
Acenaphthylene	<5	<5	<15	<2	210	<10	<2	<5	<2	<2	10	<10	<10	<3	220
Anthracene	2.9	<5	20	<2	26.5	<10	<2	<5	<2	6	11	<10	<10	<3	66.4
benzo(A)anthracene	<5	<5	<15	2.5	37	11	4	<5	<2	11	67	<10	<10	<3	132.5
dibenzo (A,H)anthracene	<5	<5	<15	<2	33	<10	<2	<5	<2	<2	<10	<10	<10	<3	33
benzo(A)pyrene	6.4	<5	<15	2.3	30.5	<10	<2	<5	<2	<2	17	<10	<10	<3	56.2
benzo(B)fluoranthene	<5	<5	<15	9.3	24.5	<10	6	<5	<2	16	22	<10	<10	<3	77.8
benzo (G,H,I)perylene	<5	<5	<15	<2	29.5	<10	<2	<5	<2	<2	12	<10	<10	<3	41.5
benzo(k)fluoranthene	<5	<5	<15	<2	<15	<10	<2	<5	<2	9.1	<10	<10	<10	<3	9.1
Chrysene	5.6	<5	<15	9.6	279.5	10	4.7	<5	<2	11	28	<10	<10	<3	348.4
Fluoranthene	11	7.1	<15	14	38	20	5.5	<5	8.9	3.8	55	<10	<10	<3	163.3
Fluorene	2.2	5	660	3.3	1127	<10	3.3	<5	12	21	2100	<10	<10	<3	3933.8
indeno (1,2,3-C,D)pyrene	<5	<5	<15	<2	39.5	<10	<2	<5	<2	<2	11	<10	<10	<3	50.5
Naphthalene	2.1	<5	24	<2	230.5	<10	<2	25	<2	<2	<10	<10	<10	69	350.6
Phenanthrene	16	10	76	30	62	26	5.3	8.4	16	49	71	<10	<10	6	375.7
Pyrene	8.7	6	<15	4	33	17	2.9	<5	6.1	<2	26	<10	<10	<3	103.7
Total PAH	54.9	28.1	868	75	2280	84	31.7	33.4	43	126.9	2430	<10	<10	78	6133

Table 6**Summary of main compounds and comparisons in pesticides. nm: not measured. For As, the norm M0-309 was also applied.**

Table:Main compounds						
Samples	Declared actives principles	Metals mg/L	Arsenic µg/L	PAH µg/L	Glyphosate + AMPA µg/l	Salts mg/L
A	Acetic	14.1	6.33	54.9	<50	265.8
B	Acid	11	<5	28.1	nm	190
C		39	16.69	868	<500	482.15
D		<5	<5	75	<50	497
E		1	7.21	2280	<500	176.35
F	Pelargonic	7	<5	84	nm	125.25
G	Acid	7.8	20.9	31.7	nm	464.6
H		36	16.3	33.4	nm	19
I		6.9	6.68	43	nm	361.25
J		13.82	<5	126.9	<50	91.95
K		<5	<5	2430	<500	163.75
L	capric and	<2.5	5.13	<10	86	<5
M	caprylic acids	<2.5	<5	<10	1850	<5
N	benzalkonium chloride	5.35	<5	78	<500	161

values. This reveals that As reached two times the norm, Zn 16 times, Fe 38 times, and then Ag, Cu, Pb, Ni and Ti respectively reach 100, 170, 200, 1382, and 7800 times the admitted threshold; as a result these metals can be considered the most toxic in the present study.

For PAHs, since all the compounds measured, except for anthracene, exceeded the toxicity threshold, and because most of them are considered carcinogens, a column was added showing the ratio between the maximal levels found in this study in the samples indicated and the toxicity thresholds (Table 7B). We observed that benzo(A) pyrene and benzo(A) anthracene were present at levels around 3–7 thousand times higher than their respective norms; a majority reached important levels, according to this criterion.

3.6. Herbicidal effects

Fig. 1A shows the herbicidal effects after 3 or 24 h provoked by 4 compounds: since acetic and pelargonic acids are the compounds present at the highest levels, the herbicides containing these were tested once in comparison to their declared active principles alone at similar concentrations. Water was used as a control. The results clearly showed that all products and their declared active principles were highly toxic for plants and were similarly toxic 3 h after the spreading. Acetic acid (6%) and pelargonic acid (24%), also known as nonanoic acid, are respectively white vinegar or pelargonium extracts and were thus diluted in water. They proved as efficient in herbicidal activity as the

Table 7A
Comparisons of maximal levels of metals found in pesticides and norms for toxicities. Max: maximum level observed in this study in the samples indicated in the right column; Toxicity: toxicity threshold determined by at least one national or international agency including AFSSA, ANSES, EPA, INERIS, NIH, WHO or scientific reviews in a few cases cited in discussion; K: carcinogen; K?: probable or possible carcinogen; nd: not determined.

Metals	Max	Toxicity	Sample
Arsenic µg/L	20.9	10	G
Mercury µg/L	<300	0.1	A-N
Other metals	mg/L	µg/kg	
Aluminum	<5	30, K	A-N
Antimony	<20	5	A, C, I
Barium	<5	0.7, K?	A-N
Beryllium	<10	0.2, K	A-N
Bismuth	<10	10	A-N
Cadmium	<2	2, K	A-N
Chromium	<5	50	A-N
Cobalt	<5	1.8, K?	A-N
Copper	6.8	40	C
Gallium	<10	nd	A-N
Germanium	<10	5800	A-N
Hafnium	<10	1.2E5	A-N
Indium	<10	3	A-N
Iron	7.7	200	C
Lead	2.00	10	A, C
Lithium	<10	2	A-N
Molybdenum	<10	5	A-N
Nickel	13.82	10	J
Niobium	<10	40 000	A-N
Palladium	<2.5	1000, K?	A-N
Rhenium	<10	nd	A-N
Selenium	<5	10	A-N
Silicon	36	nd	H
Silver	1.00	10	C
Strontium	<5	600, K	A-N
Tantalum	<10	nd	A-N
Thallium	<20	0.07	A-N
Tin	<20	1	A-N
Titanium	7.80	1	C
Tellurium	<20	3.750E6, K	A-N
Tungsten	<10	8	A-N
Vanadium	<5	9	A-N
Zinc	4.90	300, K	C
Zirconium	<10	0.08, K	A-N

Table 7B
Comparisons of maximal levels of PAHs found in pesticides and norms for toxicities. Max: maximum level observed in this study in the samples indicated; Toxicity: toxicity threshold for the product determined by at least one national or international agency including AFSSA, ANSES, EPA, INERIS, NIH, WHO. K: carcinogen; K?: probable or possible carcinogen; max/norm ratio of the maximal level to the toxicity threshold.

PAH	Max µg/L	Toxicity µg/kg	Samples	max/norm
Acenaphthene	88	60	C	1.46
Acenaphthylene	210	10	E	21
Anthracene	26.5	40	E	0.66
Benzo (A)anthracene	67	0.01, K	K	6700
Dibenzo (A,H)anthracene	33	0.5, K	E	66
Benzo (A)pyrene	30.5	0.01, K	E	3050
Benzo(B)fluoranthene	24.5	0.1, K	E	245
Benzo (G,H,I)perylene	29.5	0.1, K	E	295
Benzo(K)fluoranthene	9.1	1, K	J	9.1
Chrysene	279.5	50, K	E	5.59
Fluoranthene	55	0.1, K	K	550
Fluorene	2100	40, K	K	52.5
Indeno (1,2,3-C,D)pyrene	39.5	0.2, K	E	197.5
Naphtalene	230.5	40, K	E	5.76
Phenanthrene	76	20, K?	C	3.8
Pyrene	33	30, K?	E	1.1

formulated herbicides containing PAHs and heavy metals after 3 h; truly little living grass was remaining on the soil after 24 h (Fig. 1B). Then the grass was cut and weighed. No difference was observed between treatments: almost all *Poa annua* L. was killed (>90%) in comparison to controls. After 21 days, the grass has re-grown only on the water-treated squares. Acetic acid prevented subsequent re-growth better than pelarmonic acid. Therefore the herbicides do not need the toxic PAHs and metal residues to exercise their herbicidal activity.

4. Discussion

It was important for this study to have access to a representative sampling of all new herbicides declared as G-free and available on the market for private use since 2019 (Table 1). Before that time, G-based herbicides were the most common pesticides (US EPA, 2017). An intense scientific, legal, political, and social debate throughout the world has centred on G toxicity (Bai and Ogbourne, 2016) because this compound was the declared active ingredient in the herbicide formulations by the manufacturers, even if other substances in the commercialized formulations are more toxic (Benachour and Seralini, 2009). G in isolation has thus been the substance regulated over the long term by environmental and health agencies since its first use in 1974 (Duke, 2018). The same reasoning has been applied since the Second World War for all pesticides: only the declared chemical alone is tested over the long term; long-term toxicity studies of formulated pesticides are not compulsory. In fact, G has never been used alone in fields but only in formulations, which are mixtures containing in general around 40% G (Mesnage et al., 2015b). However, in many marine and terrestrial ecosystems the formulations have been demonstrated to be much more toxic than their declared active principles (Marc et al., 2002; Mesnage et al., 2015b). The remaining 60% of the products in formulations are generally declared as safe, inert, and confidential, and as adjuvants allowing the active principle's cell penetration and/or stability. For instance, they are claimed to help spraying. Their own toxicity in the environment and the food chain has been rarely debated. Between 2013 and 2018, we demonstrated by mass spectrometry the presence of poly-ethoxylated tallowamines such as chemical detergents, other petroleum-derived residues, and 5 heavy metals in G-based herbicide formulations (Mesnage et al., 2013; Defarge et al., 2018). These present their own long-term toxicities to plants and the ecosystem, including to mammals (Seralini et al., 2014) and humans (Love et al., 2011; IARC, 2017).

Then the question arose of the content of the formulations of G-free new formulated products. We screened in total 35 heavy metals, 16 polycyclic aromatic hydrocarbons (PAHs), and essential minerals (Table 2). These were tested by specific mass spectrometry associated with gas chromatography or inductively coupled plasma methods in the formulations.

The essential minerals (Table 3) did not reach toxic levels. These K, Na, P, Ca, Mg ions do not appear to be present in herbicide formulations at levels that could kill plants, and they could even stimulate growth (Nath et al., 2016; Raddatz et al., 2020).

It is a different situation for non-nutritive, potentially toxic metals. Ti, Ni, Pb, Ag, Fe and As appear to be the most toxic over the long term, for humans and environment in general, as they were detected well over the admissible level in drinkable water. For instance, Pb (Vorvolakos et al., 2016), Ni (Das et al., 2018), and Ti (Fage et al., 2016) were found at several hundred to a thousand times the chronic toxicity levels; and this could well intoxicate gardeners, soils, and the environment. Metalloids or metals like Si, Ni, Ti, Fe and Pb, five of the elements detected at the highest levels, are increasingly commonly used as nanoparticles in pesticides, to accelerate cell penetration and toxicity (Baker et al., 2017). Due to their stability and new chemical properties, they could also play this negative role in the ecosystem (Jaiswal et al., 2018) and human health, including in the endocrine (Rana, 2014) and nervous systems (Zeng et al., 2016). Since these are not declared nor assessed for long-term toxicity, they could cause new environmental problems. Many

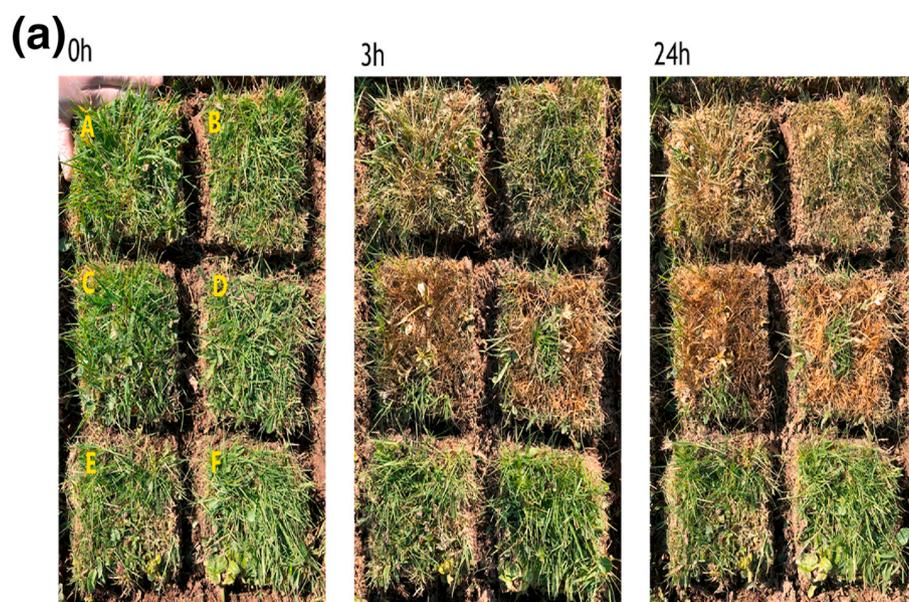


Fig. 1A. Time effects of herbicides and of their declared active principles. Immediately at treatment (0 h), and 3 or 24 h after, *Poa annua* L. was observed. The treatments are: A Roundup (sample A) at recommended dilution containing 6% acetic acid, B acetic acid 6% in water, C Clairland (sample E) containing 24% pelargonic acid, D pelargonic acid 24% dissolved in water with ethanol first (3% final), E water, F water with 3% ethanol.

(b)



Fig. 1B. Grass weight (g) after 24h on the surfaces treated for 24h. The treatments were studied in triplicate.

of the gardeners to whom these new herbicides are targeted could think these are natural due to the commercial claims made for acetic and pelargonic acids by the retailers having special formations for this communication. Private vegetable gardens will be sprayed, and all the compounds described in this study can enter the food chain. The variability in herbicide compositions concerning these elements and PAHs evidences the differential production methods used for pesticide manufacture.

PAHs are well established carcinogens, like the detected benzo(A) pyrene, and their hidden presence at levels up to the mg/ml range appears abnormal in these garden products for consumers. Their presence in petroleum residues at fluctuating concentrations may indicate that pesticide formulations come from petroleum wastes. In conjunction with heavy metals, a synergic toxicity is possible. They could form many hazardous metabolites. Moreover, all these compounds form toxic mixtures having possible combined effects on the long term, for instance significant perturbations in blood and tissue redox profiles

(Fountoucidou et al., 2019), inducing several diseases. Even very low doses of toxics below regulatory limits should be measured for real exposure assessments (Tsatsakis et al., 2016).

To check if any of these chemicals add significant short-term efficacy in herbicidal properties, we compared the effects of the formulated pesticides on *Poa annua* L. to the effects of their declared active principles at the same concentrations. This plant is one of the most common, that may be considered as a weed in gardens. These herbicides are designed for gardeners. People can remove it between edible plants in vegetable gardens, and thus can contaminate the food chain. We noticed no detectable difference, for instance, between acetic acid at 6% in vinegar and acetic acid at 6% in formulated pesticides; the first was being sold as vinegar at considerably cheaper prices in the same supermarkets, with obviously less environmental impact due to its biodegradability. The use of acetic and pelargonic acids alone as herbicides could be recommended, which would avoid toxic formulants present in commercial formulations.

However, all of the detected PAHs and heavy metals could be used as biocides by themselves over the long term; but they have not been declared, in violation of the conclusion of the Court of Justice of the European Union (CJUE, 2019). This could have also been concluded from our previous studies with G-based herbicides (Defarge et al., 2016, 2018), and the policy of pesticide production has apparently not changed with G removal.

Traces of G were even found at different levels in two formulated pesticide products with the same authorization number. In another case the authorization numbers were the same but different contaminants were found. This shows that the methods of formulation manufacturing are not controlled or reproducible; and this impacts the feasibility of scientific assessment of the long-term toxicity of formulated pesticides in general.

In conclusion, all the toxic compounds discovered in this study may have potential interactive effects such as synergism, antagonism or additivity in environment or living organisms, where they are known to bioaccumulate. They can even be toxic by themselves. This is because the complete formulation is employed in the field, and not only the declared active principle which to date is studied alone for long term toxicity purposes. Future work on pesticide toxicity for regulatory assessments should include the study of full commercial formulations

including declared and undeclared ingredients.

Credit author statement

Professor Seralini was the main investigator and supervised the work; Gerald Jungers helped with technical assistance. We acknowledge the Network on Risks, Quality and Sustainable Environment of the MRSH in the University of Caen Normandy for structural support.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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