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Toxicity of formulants and heavy metals in glyphosate-based herbicides and other pesticides

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ABSTRACT

The major pesticides of the world are glyphosate-based herbicides (GBH), and their toxicity is highly debated. To understand their mode of action, the comparative herbicidal and toxicological effects of glyphosate (G) alone and 14 of its formulations were studied in this work, as a model for pesticides. GBH are mixtures of water, with commonly 36–48% G claimed as the active principle. As with other pesticides, 10–20% of GBH consist of chemical formulants. We previously identified these by mass spectrometry and found them to be mainly families of petroleum-based oxidized molecules, such as POEA, and other contaminants. We exposed plants and human cells to the components of formulations, both mixed and separately, and measured toxicity and human cellular endocrine disruption below the direct toxicity experimentally measured threshold. G was only slightly toxic on plants at the recommended dilutions in agriculture, in contrast with the general belief. In the short term, the strong herbicidal and toxic properties of its formulations were exerted by the POEA formulant family alone. The toxic effects and endocrine disrupting properties of the formulations were mostly due to the formulants and not to G. In this work, we also identified by mass spectrometry the heavy metals arsenic, chromium, cobalt, lead and nickel, which are known to be toxic and endocrine disruptors, as contaminants in 22 pesticides, including 11 G-based ones. This could also explain some of the adverse effects of the pesticides. In in vivo chronic regulatory experiments that are used to establish the acceptable daily intakes of pesticides, G or other declared active ingredients in pesticides are assessed alone, without the formulants. Considering these new data, this assessment method appears insufficient to ensure safety. These results, taken together, shed a new light on the toxicity of these major herbicides and of pesticides in general.

1. Introduction

Numerous debates have taken place in scientific and regulatory arenas on the toxicity thresholds of pesticides and the levels of these substances permitted by regulators [1]. Among them, the most used around the world are glyphosate (G)-based, and they are also the most spread on edible genetically modified plants rendered tolerant to G [2]. G has recently been the subject of a controversy between agencies such as the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) and the European Food Safety Agency (EFSA) [3]. The IARC classified G as a probable carcinogen [4], while EFSA did not [5]. According to their detailed reports, this was probably because of the different toxicity profiles of the full formulations and G alone. EFSA is in charge of the assessment of the declared active ingredients of pesticides alone, such as G, which is mainly but not only based on the regulatory studies from the manufacturers. IARC, on the other hand, bases its decisions on epidemiological studies performed after use of the full formulations, among others, as well as animal feeding studies on the formulations and G alone, with the stipulation that all studies considered in the evaluation must be fully available in the public domain.

In order to better understand the mechanisms of action of the pesticide formulations, we tested in this work, as a model, complete GBH formulations on one hand, and their components separately on the other hand, i.e. G and formulants, which are often oxidized petroleum distillates such as families of polyoxyethylenamines (POEA). G is declared to be an active herbicide on plants. In order to calculate the acceptable daily intake (ADI) for regulatory purposes, G alone is tested for toxicity in long-term tests in mammals in vivo. Thus we first tested G alone in plants and human cells at recommended agricultural dilutions.

Abbreviations: G, glyphosate; GBH, glyphosate-based herbicide; R, Roundup; POEA, polyoxyethylenamines (polyethoxylated tallowamine); QAC, quaternary ammonium compounds

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Table 1
Glyphosate-based herbicide (GBH) formulations and others studied in this work.

Name	Class	dAP	g/L	Formulant	Recommended use	dilution
Bayer GC	G-based	G (IPA salt) or indicated	96	1–5% POEA	5.5 L/ha	4.50%
Clinic EV	herbicides		360	11% POEA	3 L/ha	3%
Glyfos			360	9% POEA	12 L/ha	6%
Glyphogan			360	15.5% POEA	3 L/ha	3%
Kapazin			360	C8-10 ethoxylated alcohol (< 2 g/L). Triethylene glycol monobutyl ether (< 2 g/L)	5 L/ha	3%
Medallon			360	10–20% APG (150 g/L)	5 L/ha	3.5%
Pavaprop-G			72	nk	15 L/ha	15%
Radical Tech +			151.4	nk	1.5 L/ha	1.50%
R 3 +			170	nk	14.8 L/ha	14.80%
R Bioforce			360	nk	6 L/ha	6%
R Classic			360	15.5% POEA	7 L/ha	2%
R Express			7.2	nk	250 L/ha	100%
R Ultra			360	16% nk	3.3 L/ha	2 %
R WeatherMax			540	Petroleum distillate/Transorb2	1.67 L/ha	1.67%
R GT +			450	7.5% ethoxylated etheralkylamine	5.6 L/ha	5.60%
Total			360	58.5% nk	6 L/ha	3.5%
APG	GBH adjuvants	nr	31%	Alkyl polyglucosides	nr	nr
Genamin		nr	70%	POEA	5 L/ha	5%
POEA		nr	> 95%	Polyoxyethylenamines	nr	nr
POE-APE		nr	70%	Polyoxyethylene alkyl ether phosphates	nr	nr
QAC		nr	30%	Quaternary ammonium compounds	nr	nr
Lonpar	Other herbicides	2,4-D	150	nk	3 L/ha	2%
Matin		Isoproturon	500	nk	2.4 L/ha	2.40%
Starane		Fluoroxypyr	200	Solvant naphta, alkyl-aryl sulfonates	1.5 L/ha	1.50%
Eyetak	Fongicides	Prochloraze	450	Solvant naphta, xylene, isobutanol	1.33 L/ha	1.33%
Folpan		Folpet	80%	nk	2 kg/ha	1.9%
Maronee		Tebuconazole	250	*N*,*N*-dimethyldecanamide	1 L/ha	1%
Opus		Epoxiconazole	125	Solvant naphta, ethoxylated fatty alcohol	1 L/ha	1%
Pictor		Boscalid	500	nk	0.5/1 kg/ha	0.5%
Teldor		Fenhexamid	50%	nk	1.5 kg/ha	2%
Polysect	Insecticides	Acetamipride	5	1,2-benzisothiazoline-3-one, ethanol	10 mL/L	1%
Pyrinex		Chlorpyriphos	250	nk	2 L/ha	2%

In GBH, G is present as a salt of isopropyl ammonium (IPA), except in Medallon (di-ammonium salt) and Roundup WeatherMax (potassium salt). dAP declared active principle, G glyphosate, nk not known because undeclared, nr not relevant. The recommended uses and pesticide dilutions according to the manufacturer instructions are indicated.

No observable adverse effect was measured. Then we tested the full formulations and some formulants alone in similar dilutions. Both exhibited full herbicidal and cytotoxic activities, without any G.

As endocrine disruption in mammals was proposed for G, a semi-irreversible inhibitor of aromatase [6,7], we also compared the effect on aromatase inhibition below direct toxic levels G, its formulations and formulants. Again, the formulants and formulations demonstrated more endocrine disruptive effects than G.

Finally, we tested if other elements that could participate in toxic or endocrine effects were present in the formulations. Unexpectedly, arsenic (As), cobalt (Co), chromium (Cr), nickel (Ni) and lead (Pb) were present in numerous pesticide formulations, at levels well above admissible ones in water. We discuss why it appears erroneous to calculate the ADIs based on only one chemical of the formulations used in agriculture or gardens.

2. Materials and methods

2.1. Chemicals

GBH formulations and others studied in this work (Table 1) were on the market (approval numbers in parentheses) in France unless otherwise indicated: Bayer GC (Bayer Garden Cambridge UK, 05873567), Clinic EV (Nufarm, 9900039), Glyfos (Cheminova, 9100154), Glyphogan (Adama, 9100537), Kapazin (Arysta, 02.5/12062-2/2010, Hungary), Medallon Premium (Syngenta, 02.5/10506-2/2010, Hungary), Pavaprop-G (Bayer, 9500572), Radical Tech + (BHS, 2090044), Roundup Bioforce (Monsanto, 9900451), Roundup Classic (Monsanto, 02.5/915/2/2010, Hungary), Roundup Express (Monsanto, 201321), Roundup Grands Travaux plus (GT +, Monsanto, 2020448), Roundup WeatherMax (Monsanto, 27487, Canada) and Total (Sinon Corporation, 02.5/12059-2/2010, Hungary).

G (*N*-phosphonomethyl glycine, G, CAS 1071-83-6) was tested in two forms: G alone (Sigma–Aldrich, Saint Quentin Fallavier, France) or its isopropyl ammonium salt (G-IPA, 386411-94-0, Lamberti, Abizzate, Italy).

Common GBH formulants were: polyoxyethylenamines (POEA) with an average ethoxylation of 15 carbons (POE-15, CAS 61791-26-2, Emulson AG GPE 3SS, Lamberti), and formulated POEA (Genamin T200, Monsanto, 8500170, containing 70% of POE-15); POE-APE, a mixture of alkyl(C8-10) polyoxyethylene ether phosphates (68130-47-2) and polyoxyethylene alkyl ether phosphate (50769-39-6), known as Rolfen Bio (from Lamberti) alkyl polyglucoside (APG, 383178-66-3/110615-47-9, Plantapon LGC, The Soap Kitchen, Torrington, UK); and quaternary ammonium compounds (QAC, 66455-29-6, Emulson AG CB 30, Lamberti).

Three non-GBH (Table 1) were also analyzed: Lonpar (Dow Agrosciences, 8200538), Matin (Tradi Agri, 2020328) and Starane (Dow Agrosciences, 8400600), as well as 6 fungicides: Eyetak (Barclay Chemical, 9400555), Folpan (Makheshiam Agan, 9300143); Maronee (Bayer, 2000420), Opus (BASF, 9200018), Pictor (BASF, 2050075), Teldor (Bayer, 9800244), and 2 insecticides: Polysect ultra SL (Scotts, 2080018) and Pyrinex (Adama, 9900104).

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was obtained from Sigma–Aldrich. It was prepared as a 5 mg/mL stock solution in phosphate- buffered saline, filtered through a 0.22 mm filter before use, and diluted to 1 mg/mL in a serum-free medium. 4-androstene-3,17-dione and formestane (4-hydroxyandrost-4-ene-3,17-dione, CGP-32349) were also obtained from DM Labo (Caen, France). [1β-3H] androstenedione (specific activity, 25.3 Ci/mmol; 958.3 GBq/mmol) was purchased from DuPont-New England Nuclear (Les Ulis, France). Ultima-Gold LLT was from Perkin-Elmer.