



Review

Insight into the confusion over surfactant co-formulants in glyphosate-based herbicides

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ABSTRACT

Glyphosate is the active ingredient in glyphosate-based herbicides (GBHs). Other chemicals in GBHs are presumed as inert by regulatory authorities and are largely ignored in pesticide safety evaluations. We identified the surfactants in a cross-section of GBH formulations and compared their acute toxic effects. The first generation of polyethoxylated amine (POEA) surfactants (POE-tallowamine) in Roundup are markedly more toxic than glyphosate and heightened concerns of risks to human health, especially among heavily-exposed applicators. Beginning in the mid-1990s, first-generation POEAs were progressively replaced by other POEA surfactants, ethoxylated etheramines, which exhibited lower non-target toxic effects. Lingering concern over surfactant toxicity was mitigated at least in part within the European Union by the introduction of propoxylated quaternary ammonium surfactants. This class of POEA surfactants are ~100 times less toxic to aquatic ecosystems and human cells than previous GBH-POEA surfactants. As GBH composition is legally classified as confidential commercial information, confusion concerning the identity and concentrations of co-formulants is common and descriptions of test substances in published studies are often erroneous or incomplete. In order to resolve this confusion, laws requiring disclosure of the chemical composition of pesticide products could be enacted. Research to understand health implications from ingesting these substances is required.

1. General aspects on the composition of pesticides

Pesticides are chemicals, which are used to kill or control living organisms (“pests”) considered to be a nuisance, or an economic or health threat. Commercial pesticide formulations are not single molecules, but mixtures. Some of the compounds used in the formulation of end-use pesticide products are regulated as ‘active’, such as glyphosate in Roundup and other glyphosate-based herbicides (GBHs), while other compounds are categorized as ‘inert’. Such inert ingredients are variously called “co-formulants”, “adjuvants”, or “other ingredients” (EPA, 1997). They are specifically added to increase the efficacy of the active ingredient, and are “inert” only with respect to the pesticide's mode of action targeting a given class of pests.

Under US law, the classification of a pesticide ingredient as either active or inert is governed by the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and its detailed, operating regulations (EPA, 1996). A compound is classified as ‘active’ when it is intentionally added to be toxic to target species. This is the case with glyphosate,

which acts on plants by shutting down the production of essential aromatic amino acids (Schonbrunn et al., 2001). All other ingredients that are added to GBHs to influence the activity or efficacy of glyphosate are defined as inert ingredients (Cox and Sorgan, 2006). Unfortunately, under existing law and policy, regulators generally ignore the innate toxicity of co-formulants and invest little effort in understanding the many ways these substances can alter the environmental fate, metabolism, and/or toxicity of end-use pesticides to a variety of non-target organisms, including humans (Mesnage and Antoniou, 2017; Székács, 2017).

The classification of a compound as ‘inert’ or ‘active’ has serious consequences for pesticide manufacturers and users. It determines the set of tests that have to be performed to assess the toxicity of pesticide ingredients (OECD, 1994; OECD, 2008). In most parts of the world, pesticide toxicity tests for regulatory purposes follow a common set of guidelines and standards set by the Organisation for Economic Co-operation and Development (OECD), in the hope of facilitating cost-effective pesticide regulation, international trade, and limiting the

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number of animals subjected to toxicity testing. OECD guidelines define the scope and protocols of tests, which minimally have to be performed on active ingredients and formulated products (OECD, 1994; OECD, 2008). The results of toxicity tests are used to derive guidance values, which are used in the hope of ensuring that exposure to pesticides does not result in “unacceptable adverse effects on man or the environment,” the core statutory requirement under US pesticide law.

Active ingredients are tested with an array of tissue culture cell assays and laboratory rodent (mouse, rat) bioassays to establish health-based guidance values, for instance an active ingredient's chronic Reference Dose (cRfD) in the US, or acceptable daily intake (ADI) in the EU. Required tests include long-term toxicity and carcinogenicity studies, metabolism studies, and *in vivo* and *in vitro* genotoxicity testing. Pesticide co-formulants considered to be inert are generally exempt from the requirement for establishing a tolerance limit, and for this reason, end-use commercial pesticide formulations escape from being subjected to chronic toxicity testing, despite the fact that the vast majority that have been tested are actually more toxic than the declared active ingredients alone (Mesnage et al., 2015). This was demonstrated for glyphosate on a variety of endpoints such as cytotoxicity (Mesnage et al., 2013; Defarge et al., 2016), oxidative stress (El-Shenawy, 2009; Fan et al., 2013), and genotoxicity (Guilherme et al., 2012; Wozniak et al., 2018). Studies showing that formulated GBHs are more toxic than glyphosate have also been published, covering a large number of species including fish (Folmar et al., 1979; Wan et al., 1989), frogs (Howe et al., 2004; Mann and Bidwell, 1999), crustaceans (Tsui and Chu, 2003), and laboratory animals (Adam et al., 1997; Dedeker et al., 2018).

The identity and concentration of the co-formulants in end-use pesticide products is proffered as confidential business information (CBI) by industry and accepted as such by regulators, and hence is rarely disclosed on product labels or via any publicly accessible source (Weinhold, 2010). Because of the rigor with which CBI protections are enforced, coupled with co-formulant changes made over time in a given brand-name GBH, errors are common in identifying the so called “inert” co-formulant ingredients in GBHs (Mesnage et al., 2015). Little information beyond the concentration of the active ingredient is routinely available on end-use pesticide labels. As a consequence, scientists rarely have access to compositional information, nor descriptions of the structural characteristics of, for example, different POEA surfactants, and how such differences impact toxicity. Identifying such differences, and evaluating their impact on GBH risk profiles, is the focus of this review.

We provide here clarification of the composition of several glyphosate formulations based on information available from the scientific literature, patents, industry publications, or material safety data sheets. The aim of this review is not to provide a comprehensive overview of the toxicology and other data supporting GBH human-health risk assessments, which can be found elsewhere (Mesnage et al., 2015; Myers et al., 2016; Székács and Darvas, 2018; Benbrook, 2019).

The biodistribution of surfactants in crop plants following application of a pesticide depends of the type of co-formulant present in the commercial product. The many radiocarbon-labelled co-formulant studies conducted over the years show mixed results. Depending on the co-formulant tested and the plants used in the investigation, in some cases these adjuvants show little movement from the area of contact on the plant's surface, whereas in other cases there is penetration into the sprayed plants (de Ruiter et al., 2004). For example, rapid uptake of poly(oxyethylene)-based surfactants occurs when applied to wheat (Anderson and Girling, 1983). Furthermore, as adjuvants can be degraded upon application and uptake, the toxicity of adjuvant-breakdown products also needs to be taken into account. Importantly, co-formulants in commercial pesticide formulations are highly complex mixtures that have evolved and changed over the years. Thus, it is not possible to extrapolate the results obtained from experiments conducted, in some instances, decades ago, to all possible components in present-day pesticide (herbicide, insecticide, fungicide, nematocide,

etc) formulations.

The regulatory position of treating pesticide co-formulants as inert also results in these substances not being officially monitored for their presence either in the environment, food and feed, or in animal and human consumers. Thus to date, there is little information regarding the levels of co-formulants, some of which are surely present in food-stuffs and in human populations. However, surveys in several states in the American mid-west where glyphosate-tolerant, genetically modified crops such as soy and corn are grown on a vast scale, have found POE-tallowamine to be ubiquitously present in agricultural fields (Tush and Meyer, 2016) and beyond in watercourses (Tush et al., 2018). Furthermore, the use of GBHs as pre-harvest desiccants, especially on cereal crops such as oats and wheat, results in grain being harvested shortly (i.e., within 2 weeks) after application of the herbicide. This makes it likely that both active ingredient (glyphosate) and co-formulants will carry over into the food supply.

In support of this outcome are the findings from surveys for glyphosate residues in cereal foodstuffs, especially those based on oats. Employing the services of government certified analytical labs, US-based non-government organisations have reported residues up to 2.7 mg/kg (EWG report, 2018), whilst analyses conducted by the UK government have detected up to 1.8 mg/kg (UK Defra, 2016). Although the long term health implications of consuming food contaminated with glyphosate residues at this level is currently unknown, these observations suggest that there is a very good probability that commercial pesticide co-formulants are entering the environment and food supply, along with their respective active ingredient, with as yet unknown health consequences to consumers. There is a need to conduct surveys for co-formulant residues in the foodchain, as well as in livestock and humans to determine the daily intake and body burden of these compounds, and explore resultant impacts on animal and human health.

2. Examples of confusion over test substances

Many authors refer to “glyphosate” in published papers, when they actually tested a formulated product (for example, see Cavusoglu et al., 2011; George et al., 2010). “Glyphosate” technical is typically 95% and 98.3% pure glyphosate acid (EFSA, 2015a), and comes in at least three major forms: glyphosate-isopropylammonium, glyphosate-potassium, glyphosate-monoammonium. At any given time over more than 30 years of GBH use, there have been 100 to 150 significantly different formulations on the market in various regions; over 2000 formulated GBH products were registered in Europe in 2012 alone (Glyphosate Task Force, 2012).

Some authors state that they tested a formulated GBH, but do not provide a product name, making it impossible to determine the possible impacts of co-formulants (Chan et al., 2007; Hokanson et al., 2007; Sivikova and Dianovsky, 2006). Very few authors provide clear information regarding where and when a glyphosate or GBH-test substance was procured and manufactured, introducing a further source of uncertainty since co-formulants in a given brand name product can vary geographically and over time, with no warning or indication on product labels, in material safety data sheets (MSDS), or other publicly available sources of information.

Cytotoxicity of Roundup formulations can vary by up to 100-fold, even if commercial products contain the same form and concentration of glyphosate, but different co-formulants (Mesnage et al., 2013). Although reviewers and journal editors are generally aware of the importance of clarity in describing the composition of test substances, confusion still arises from the lack of information on GBH labels and the secrecy surrounding end-use product formulations.

Reporting errors are common and can have serious consequences on the evaluation of risks associated to GBH exposure. In the study of Guilherme and colleagues, which addressed potential genotoxicity of Roundup ingredients, the authors concluded that glyphosate and its co-formulant surfactants had an antagonistic interaction because the

genotoxicity of the pesticide formulation was inferior to the toxicity of the sum of the individual effects of MON 0818 (the presumed surfactant) and glyphosate (Guilherme et al., 2012). However, it is likely that these authors erroneously concluded that the Roundup formulation tested in their study contained MON 0818. In their investigation they tested a “Roundup Ultra containing POEA (16%) as surfactant”. However, the Roundup Ultra declared in Portugal (MON 52276) does not contain POEA, but rather instead a propoxylated quaternary ammonium surfactant (Monsanto, 2019).

In many cases, authors only report the trade name without reporting the country where the formulation was sourced. It is important to note that a formulation called Roundup Ultra can contain different co-formulants depending on the country in which it is sold. The Roundup Ultra sold in Ireland is MON 76473, while it is MON 79351 in Greece, MON 52276 in Germany, and MON 77360 in the US. Information on GBH formulations across regions is available on the websites of manufacturers (Monsanto, 2019).

Companies use a double nomenclature: a brand name, which does not reflect the formulation composition (i.e., Roundup Ultra), and an internal classification number, which is unique for a given formulation (MON number in the case of Monsanto brand-name products, or Monsanto-licensed GBHs). This dual approach gives companies the opportunity to make changes in the composition of formulations without changing the end-use product trade name and marketing efforts and materials. Only regulators in countries where a given GBH is registered and sold would have information about changes in the formulation of a given brand name product.

This practice can mislead just pesticide users and the scientific community. This misinformation happens because regulators only have access to confidential statements of the formula for a brand-name product sold in their country or jurisdiction, and do not have any means of knowing if a product with the same name sold elsewhere contains the identical mix and levels of co-formulants. As a consequence of this lack of information on test substances across different studies, it is not surprising that some results differ in laboratories around the world from research purportedly using the same assay on the same brand-name GBH.

3. Structural characteristics of POEA surfactants

The major co-formulants in Roundup and other GBH herbicides are surfactants. They generally account for 5–15% of concentrated products by weight; glyphosate technical in one of its salt forms typically accounts for 40%–60% of concentrated products, with water making up the balance of the product (EFSA, 2015a). Concentrated products, as sold to end-users, must be diluted during the mixing/loading process to different concentrations of glyphosate technical per litre, depending on the intended use and application equipment used. The most common surfactants in GBH formulations over the last 40 years have been ethoxylated amines (EFSA, 2015b), which are also called ‘POEAs.’ Here, we provide an explanation of the structural characteristics of Roundup and GBH surfactants.

The acronym ‘POEA’ is widely used in reference to a broad class of surfactants employed in many formulated pesticides, drugs, and other compounds. POEA stands for ‘PolyOxyEthylene Amine’. POEA is not a single molecule, but a class of surfactants with common structural features (van Os, 1997). A polyoxyethylene amine surfactant can be represented by the general formula $R-N-(CH_2CH_2O)_xH$, where ‘R-N’ is an amine, and ‘ $(CH_2CH_2O)_x$ ’ is a chain made up of an assemblage of ethylene oxide molecules (and hence, a polyoxyethylene amine) (Fig. 1).

The ‘amine’ in polyoxyethylene amine is generally a lipid-based amine. They are synthesized by reacting fatty acids with ammonia. If the fatty acids are from animal fat (tallow), the fatty amine will typically be referred to as a tallow amine (EFSA, 2005). If the fatty acids are from coconut oil, the resulting fatty amine is designated as a coco

amine. The polyoxyethylene in the general formula for POEA is an oligomer of ethylene oxide. Chemicals that fall within the POE category are also sometimes called polyethylene oxide (PEO), or polyethylene glycol (PEG). In the formulation of pesticide, ethylene oxide is added to fatty amines to produce polyoxyethylene amines. The successive addition of ethylene oxide molecules to the nitrogen atom of the tallow amine produces a polyoxyethylene tallow amine, a major surfactant from the POEA family of compounds (Fig. 1).

When ethylene is used to synthesize surfactants, it is reacted with other compounds such as ethers, fatty acids, or amines, in a process called ethoxylation. The goal is to produce surfactants that can be used in a wide range of products such as pesticides, cosmetics, drugs, or cleaning products. When an amine compound is used, the reaction product becomes a “polyoxyethylene amine” (POEA) mixture, containing amines that become linked to oligomers of ethylene units. In turn, the ethylene units can have different lengths, leading to the great diversity of compounds within the POEA family (van Os, 1997). Ethoxylation is also sometimes called alkoxylation. Alkoxylation is a more general term that includes the polymerisation of epoxyde units (e.g., ethylene oxide) with a different number of carbon atoms (ethylene oxide has two carbons). In some cases, surfactants are propoxylated. Propoxylation is similar to ethoxylation, and involves the addition of propylene oxide monomers instead of ethylene oxide.

Ethylene oxide is a widely used disinfectant and sterilizing agent. Most global production is used as an intermediary in the production of other chemicals (antifreeze, polyesters, surfactants). Global production of ethylene oxide in 2000 was estimated to be 15 million tons per year (Ullmann and Bohnet, 2003). Approximately 13% of this ethylene oxide is used to produce surfactants. Ethylene oxide is commonly used in synthesizing POEA pesticide surfactants, because the length of the ethylene oxide oligomer can be easily changed to modify the detergent properties of the resulting formulation (van Os, 1997).

Acidic or basic catalysts are then mixed with ethylene oxide in order to, in effect, open the ethylene oxide molecules and bring them together to form a POE. Commonly used catalysts include sodium hydroxide, potassium hydroxide, benzoyl chloride, toluenesulfonyl chloride, triisopropylsilane, dichloromethane, or trifluoroacetic acid (French et al., 2009). A variety of chemical engineering processes can be used to produce POEA, which is one reason why impurities and their concentrations can vary widely from one manufacturing process to another.

POEAs are not pure chemicals, but mixtures (Fig. 2). The successive addition of ethylene oxide molecules to form POE does not occur at the same speed for each molecule in the melange (van Os, 1997). When the chemical reaction is stopped, the POE chains produced by the reaction have different lengths. Low molecular-weight POEs are typically colourless and odourless viscous liquids, whereas long-chain polymers are solid. Surfactants with this basic structure are also known as ‘ethoxylated amine surfactants’.

4. Polyoxyethylene tallowamine: the first generation of POEA surfactants

The first generation of glyphosate-based herbicides sold in the 1970s and 1980s predominantly contained polyoxyethylene tallowamine surfactants typically derived from animal fat. Tallow sources ranged from fat products destined for human consumption to industry intermediates used in the manufacturing of surfactants (EFSA, 2005). Tallow amines are 99% primary, straight-chain alkyl amines (mostly C16 and C18, with 40–50% being unsaturated). The first Roundup formulation commercialised by Monsanto (MON 2139) contained the surfactant MON 0818 that has an average of 15 ethylene oxide units (Figs. 1 and 2).

Ethoxylated alkylamine surfactants have problematic toxicological properties that began to be observed in the late 1970s. The surfactant POE 15 tallow amine used in MON 0818 is 5–50 times more toxic than

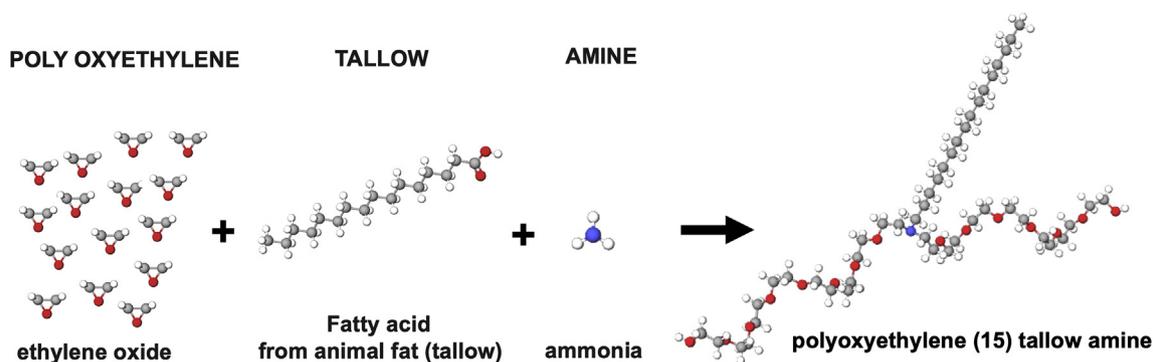


Fig. 1. Summary of polyoxyethylene (15) tallow amine synthesis. First, ammonia is reacted with animal fat extracts (tallow) to produce a tallow amine. Then, the tallow amine is ethoxylated to form a polyoxyethylene tallow amine. This representation of the different chemicals used to synthesize one molecule of polyoxyethylene (15) tallow amine is a simplification. In reality, tallow is composed of a melange of fatty acids having different chain lengths: the example presented has a 16 carbon atom chain length while the fatty acids present in the tallow mixtures have between 12 and 18 carbon atoms. In addition, the number of ethylene oxide molecules added to the different fatty amines varies due to the reaction occurring at different rates for each molecule. Thus, the POEA surfactants resulting from these chemical reactions are a mixture, and not a single compound. Red spheres, oxygen atoms; white spheres, hydrogen atoms; blue spheres, nitrogen atoms; grey spheres, carbon atoms. Figure created using MolView v2.4 (molview.org). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

glyphosate to mammalian species or aquatic organisms (Table 1). Studies showing that the surfactant POE 15 tallow amine was more toxic than glyphosate in animals were published decades ago (Folmar et al., 1979). In a series of experiments on four aquatic invertebrates and four fish species, the toxic effects of designated inert ingredients were similar to those of the Roundup formulation. The formulation MON 2139 (original Roundup) was 10 to 40-times more toxic than glyphosate in different fish species (Folmar et al., 1979; Wan et al., 1989). MON 2139 was also 10 to 50-times more toxic than glyphosate in frogs (Howe et al., 2004; Mann and Bidwell, 1999) and crustaceans (Tsui and Chu, 2003). The involvement of ethoxylated alkylamine surfactants in the toxic effects of original Roundup in humans, rather than glyphosate alone, was first described in 1988 (Sawada et al., 1988).

Although most surfactants share common physical and chemical properties, and serve similar functions in formulated pesticides, the nature and severity of their toxic effects and environmental fate vary widely across organisms and ecosystems (Tsui and Chu, 2003). The acute toxicity of POEA surfactants follows general rules (Talmage, 1994). The hydrophobicity of the molecule increases with the length of the alkyl chain; long chain fatty acids are more likely to penetrate cell membranes and disturb their structure. As a consequence, toxicity tends to increase in step with the length of alkyl chain lengths. The toxicity of ethoxylated surfactants tends to decrease when chemical groups (e.g., methyl groups) are attached to alkyl chains due to resulting impairment of penetration through cell membranes. In contrast, longer, water soluble chains (e.g., ethylene oxide chains) tend to be less toxic, because they interact less efficiently with cell membranes. This is the case for human cell toxicity (Mesnage et al., 2013; Defarge et al., 2016), as well as toxicity to many organisms. In fish and aquatic invertebrates, surfactants can emulsify the mucus on gill surfaces (Talmage, 1994). The gill membrane surface may also be penetrated by the hydrophobic portions of surfactant molecules. As a consequence, respiration is impaired and the animal dies of suffocation. Longer-chain, ethoxylated surfactants are less fat soluble, which hinders emulsification and/or penetration into gill membranes (Talmage, 1994).

POEA produced with alkyl amines rather than from animal fats have comparable structural and functional properties, for example polyethoxylated coco amine (from coconut oil) or polyethoxylated soya amine (from soy). The chemical structure of the molecules comprising these POEA mixtures are typically comparable, regardless of the origin of the amine (vegetal or animal fat).

The acute, ocular toxicity of glyphosate-based herbicides sold in the 1970s and 1980s was identified as an unexpectedly serious worker-safety concern by the California Department of Pesticide Regulation

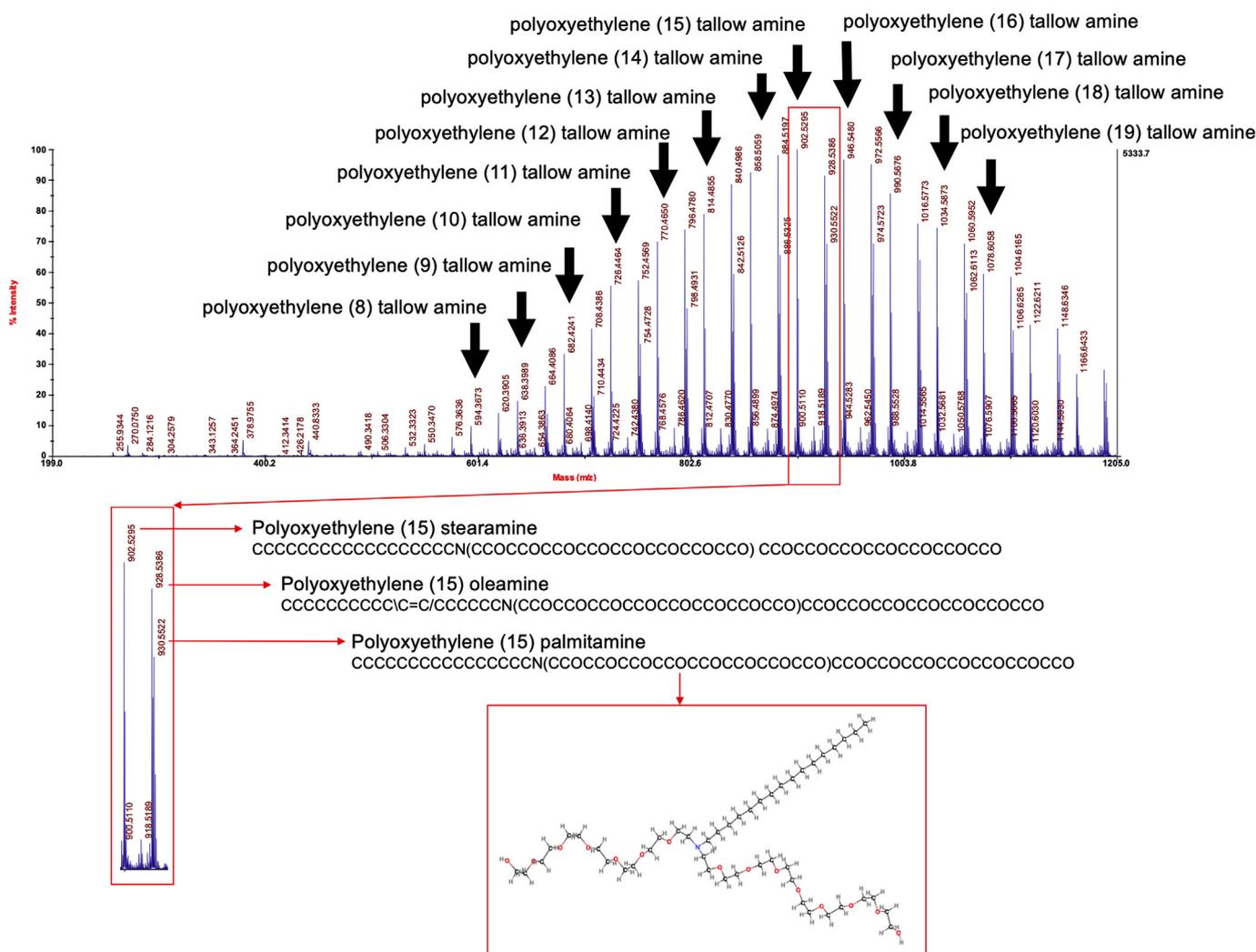
(Blondel, 1986). Data collected between 1981 and 1985 showed that the top two causes of illness after occupational pesticide incidents were eye (50%) and skin (35%) injuries. Glyphosate ranked third among all pesticides as a cause of occupational illness in California. In response, Monsanto introduced formulations including POEA surfactants in conjunction with compounds known to lessen eye irritation (US Patent 5683958A, 1990). This is the case with Roundup Pro, a formulation that contains a phosphate-ester neutralized polyethoxylated tallow amine surfactant (US Forest Service, 1997). Roundup Pro (MON 77360) is, as a result, classified as a FIFRA category III eye irritant (slightly irritating), while original Roundup (MON 2139) is a FIFRA category II eye irritant (moderately irritating).

This change in co-formulants has reduced eye irritation risk and episodes. However, these surfactants have not completely eliminated concerns arising from the heightened toxicity of POEA surfactants across multiple non-target organisms. For instance, the acute toxicity profiles of Roundup Pro (MON 77360) and Roundup (MON 2139) are comparable on bluegill sunfish (LD50 of 7.3 mg/L and 5.8 mg/L, respectively), rainbow trout (LD50 of 5.4 mg/L and 8.2 mg/L, respectively) and water fleas (LD50 of 11 mg/L for both) (Fig. 3).

5. Etheramines replace alkylamines in POEA surfactants

Although the acronym POEA is mostly used to designate the polyoxyethylene (15) tallow amine used in the first Roundup formulations, different types of amines can be, and have been ethoxylated to produce POEA surfactants incorporated in Roundup products (Fig. 4). For instance, etheramines were introduced as an alternative to alkylamines in order to improve the herbicidal profiles of glyphosate-based herbicides. Etheramines render unnecessary the addition of the anti-gelling agent polyethylene glycol in ethoxylated alkylamines, which tend to form a stiff gel when water is added. This gelling action had to somehow be counteracted. In addition, formulations including etheramine surfactants can have a higher glyphosate-acid equivalent loading compared to prior formulations with comparable uses and efficacy. A given volume bought by an end-user covers more acres, while providing superior herbicidal efficacy comparable to formulations containing ethoxylated alkylamine surfactants (US Patent 5750468A, 1995).

An etheramine is an ether linked to an amine (e.g., $\text{CH}_3\text{-CH}_2\text{-O-CH}_2\text{-CH}_2\text{-CH}_2\text{-NH}_2$). The number of oxygen atoms in an etheramine chain can vary from 1 to 10 (US Patent 5750468A, 1995). Different etheramines are used to prepare glyphosate-based herbicides. An etheramine is used, for example, in the surfactant MON 59117, which is included in the commercial formulation MON 79545 (BVL,



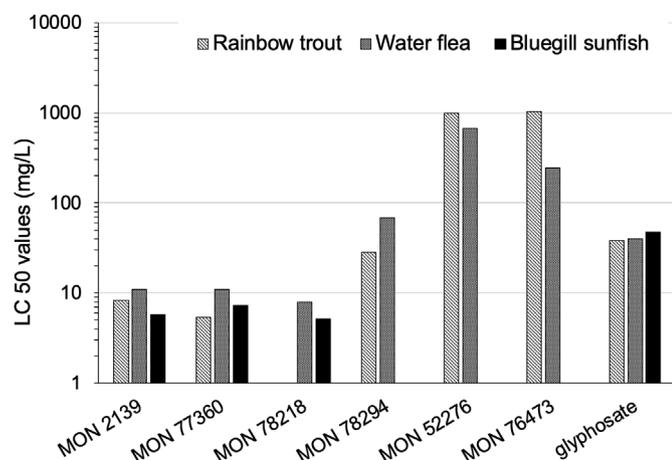


Fig. 3. Acute toxic effects of Roundup formulations containing different generations of surfactants in comparison to glyphosate. The formulations containing polyethoxylated amine (POEA) surfactants (MON 2139, MON 77360, MON 78218, MON 78294) are more toxic than more recent formulations containing non-POEA surfactants. Note that toxicity data is not available for all species. LC50 values are expressed in mg of formulated products per litre. Concentration of surfactants and glyphosate range from 6 to 15% and 35–65%, respectively. Data extracted from formulations MSDS (Monsanto, 2019) or from EFSA evaluation (EFSA, 2015a).

ethoxylated surfactant polyoxyethylene (15) tallow amine (Table 1) are comparable on rainbow trout (LD50 of 0.16 mg/L and 0.65 mg/L, respectively). However, all Roundup brands do not contain POEA. Other

surfactants have different toxicity profiles, as described in the following sections.

6. Non-POEA surfactants

GBH formulations containing ethoxylated surfactants are progressively being phased out in Europe and replaced by a new generation of surfactants, but curiously, not in the US. A major surfactant used in EU Roundup formulations is a propoxylated quaternary ammonium surfactant known as Dodigen 4022 (in the formulated product MON 52276) (EU Patent 0568635B1, 1991; Metruccio et al., 2017). It is included in the representative, formulated product used for the EU's registration review, and sold under different trade names in Europe (Roundup Pro Biactive in Ireland, Roundup Extra 360 in France, Roundup BioFlow in Italy, Roundup Ultra in Belgium, or Roundup Bio in Finland). The use of a propoxylated quaternary ammonium surfactant considerably reduces the toxicity of Roundup formulations to many organisms. The acute toxicity of MON 52276 is much lower than MON 2139 in rainbow trout (LD50 of 989 mg/L and 8.2 mg/L, respectively) and water fleas (LD50 of 676 mg/L and 11 mg/L, respectively) (data from MSDS). Tests on human cells found that MON 52276 was 50-times less cytotoxic than the etheramine-based formulation Roundup GT plus (Mesnage et al., 2013). In addition, The quaternary ammonium surfactant Emulson AG CB 30 (CAS No: 66455-29-6) has been demonstrated to be approximately 10-times less toxic than polyethoxylated tallow amine surfactants (Defarge et al., 2016).

Glyphosate-based herbicides recently introduced in the European Market also include the UK marketed formulations Roundup ProBio (MON 76473) and Roundup Flex (MON 79351) and Roundup Optima+ (MON 76476) (Monsanto, 2019). These GBHs contain a blend of

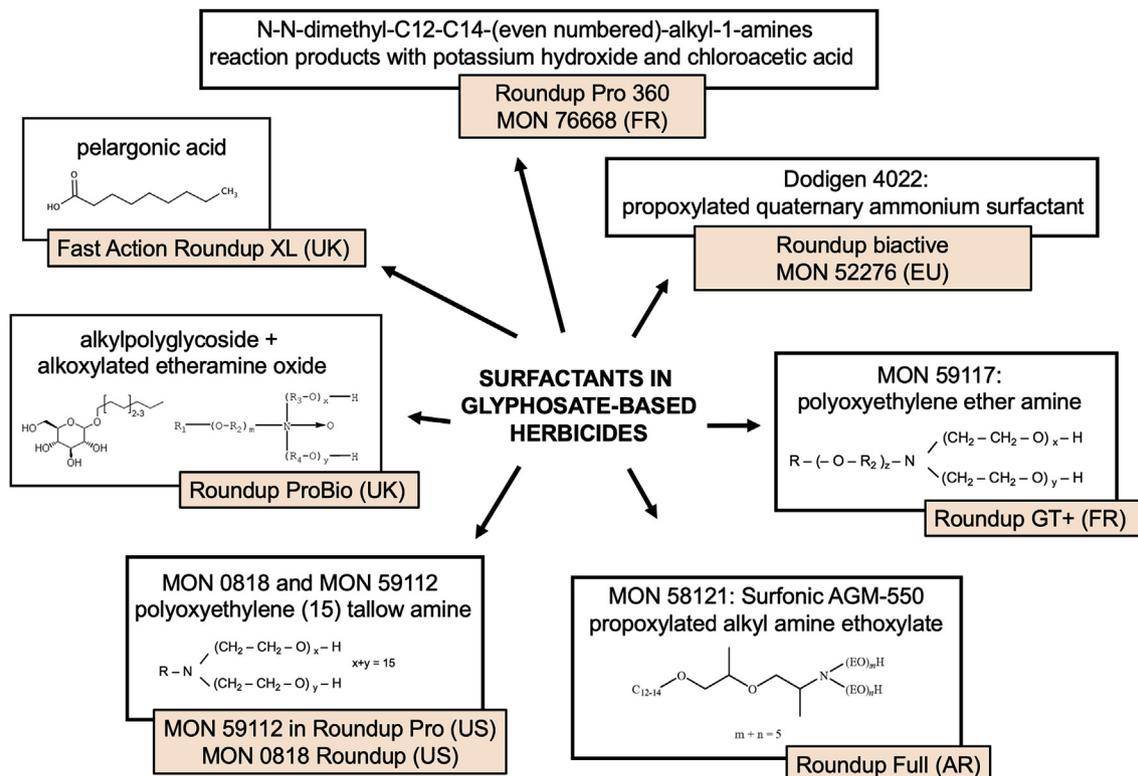


Fig. 4. Overview of surfactants forming part of the composition of glyphosate-based herbicides. The structure of the most common glyphosate-based herbicide surfactants are presented to the best of our knowledge. Note that this cannot be considered as a comprehensive list of co-formulant composition. This overview is limited by the restrictions on disclosure of pesticide confidential commercial statements of formulas. Examples of common Roundup formulations used across the world (United Kingdom, UK; United States, US; France, FR; Argentina, AR; European Union, EU) are associated with each of these surfactants. Structures were obtained from patents (EU Patent 0568635B1, 1991; US Patent 5683958A, 1990; US Patent 5750468A, 1995; US Patent 9743663B2, 2017; US Patent, 20100113274A1, 2010; US Patent, 20100234228A1., 2000), material safety data sheet databases (Monsanto, 2019), regulatory documents (Conseil d'Etat, 2012), or from experimental investigations of pesticide composition (Mesnage et al., 2013).

alkylpolyglycoside (cas 68515-73-1) and nitroaryl (cas 226563-63-9). Nitroaryl refers to an alkoxyethyl etheramine oxide co-surfactant called TOMAH AO-17-7 (US Patent, 20100113274A1, 2010). Some formulations also include non-alkoxyethylated surfactants. This is the case of Fast Action Roundup XL (MON 76610) that contains ‘pelargonic and related fatty acids’ (cas 112-05-0) (US Patent 9743663B2, 2017). The Roundup formulation MON 76668 contains ‘N-N-dimethyl-C12-C14-(even numbered)-alkyl-1-amines, reaction products with potassium hydroxide and chloroacetic acid’ (European Community number 939-682-8). Little information is available on the mammalian toxicity of these products. A comparative cytotoxicity study of surfactants concluded that alkyl polyglycosides were the least toxic compounds, followed by polyethoxylated alkyl phosphate ethers, quaternary ammonium surfactants, with polyethoxylated tallow amines being the most toxic to human cell lines (Defarge et al., 2016). Ecotoxicological profiles seem favourable. For instance, MON 52276 neither shows the ‘aquatic toxicity’ nor the ‘eye irritancy’.

7. Discussion, conclusion and recommendations

Because GBH composition is considered as CBI, most studies do not, indeed are unable to report information sufficient for other researchers to match test substances, dose rates, and reproduce results, or deepen understanding of mechanisms explaining toxic impacts. A GBH is defined by a reference name (e.g., MON 2139) and a country-specific product name (e.g., Roundup). The MON reference number is generally either not known or reported by authors of published scientific studies.

Formulated GBHs with the same product name from different countries may not be the same mixture of chemicals, nor the same as the brand-name product bought previously, or in the future. This practice causes experiment reproducibility problems within the scientific community. It also contributes to uncertainty in interpreting the results of toxicity assays run with GBHs. We recommend the publication of a minimum quantity of information to foster reproducibility across toxicity studies. Material and methods sections of research papers should include the trade name, the country of origin, and dates of manufacture, as well as the MON number (if a variant of Monsanto's Roundup is used). Information of this type is available on the websites of manufacturers (e.g., see Monsanto, 2019). These minimum, compositional data should be added to scientific journal instructions to authors. Because product names are not unique and can refer to different formulations, we suggest that MON reference numbers could be added to product labels.

As soon as practical, policy-makers should forge a consensus on, and put in place new pesticide-product labelling requirements that include a mandatory, accurate description of the full chemical composition of formulated pesticides. The toxicity of co-formulants should be fully acknowledged and misleading terms such as ‘inerts’ should be avoided. There are compelling reasons to make this change for the benefit of scientists and risk assessors. Significant progress in reducing the adverse public health and environmental impacts of pesticide use will be stymied as long as product composition is kept in the dark.

We identified some surfactants included in Roundup formulations and compared their acute toxic effects on human cell lines, as well as their effects on aquatic organisms for which formulated product toxicity information is available. Ethoxylated alkylamines, the first generation of GBH surfactants, have the highest acute toxic effects. LC50s for non-target organisms are often below 10 mg/L. The study of (Guilherme et al., 2012) demonstrated that the POEA surfactant MON 0818 likely contributes to the genotoxicity of Roundup formulations. The possibility that the heightened genotoxicity of some formulated products is due to the surfactants in a product's composition is cautiously acknowledged by the European Food Safety Agency (EFSA), but is as yet not taken seriously by the US EPA (Benbrook, 2019; EPA, 2016). In 2015, EFSA was asked by the European Commission to review the toxicity of ethoxylated tallowamines (POE-tallowamine). They

concluded that “*POE-tallowamine is clearly more toxic than glyphosate when tested in glyphosate-based formulations*” and that “*there is evidence that products which contain cytotoxic tallowamine (or other) surfactants might produce, mostly in very high concentrations, DNA damage*” (EFSA, 2015b).

Ethoxylated alkylamines have been replaced by ethoxylated etheramines in Europe, which has improved product performance, ease of mixing and stability in tank mixes, and compatibility with GMO crops. Etheramine surfactants could trigger risks comparable to those arising from typical GBH formulations containing tallowamine surfactants. Propoxylated quaternary ammonium surfactants introduced in EU formulations in the last decade or so are better alternatives, since they are around 100-times less toxic to human cells and aquatic organisms.

Pesticide applicators never spray glyphosate alone, but instead Roundup or another GBH. Thus, the safety of Roundup cannot be accurately assessed by referring just to toxicity studies performed with glyphosate alone. Future studies will hopefully clarify, as well, whether the different co-formulants added to GBHs have a role in the genotoxicity and carcinogenicity of glyphosate-based herbicides.

The reduction of surface tension caused by the addition of a surfactant explains the phenomenon allowing glyphosate, which is water soluble, to penetrate lipid-based plant-cell walls and membranes. The action of surfactants is similar on skin. Surfactants are added to cosmetics or drugs to increase the penetration of active agents in the formulations (Sarpotdar and Zatz, 1986; Shokri et al., 2001).

The dermal absorption factor was changed in the most recent peer review of the risk assessment of glyphosate in Europe, because MON 2139 is no longer used within the EU. The representative formulation chosen by EU risk assessors was MON 52276. Glyphosate dermal absorption in the EU is now purportedly representative of the MON 52276 formulation, and has been adjusted downward from 3% to 1% for the concentrated product. A comparable change has been made in the US EPA's glyphosate human-health risk assessment (EPA, 2017). In the course of litigation over the possible contributions of Roundup to non-Hodgkin lymphoma in the US (Zhang et al., 2019), questions have been raised about the dermal penetration studies undertaken by Monsanto in support of this reduction (Benbrook, 2019; Centner et al., 2019). Although MON 52276 was used as a reference formulation in EU risk assessments, many other formulations are sold in Europe containing a vast array of co-formulants with potential to alter dermal penetration rates.

Differences in surfactant composition could also influence the possible carcinogenic potential via impurities unique to specific manufacturing processes. POEA mixtures can be contaminated by carcinogenic impurities such as 1,4-dioxane and ethylene oxide. In a 1993 study, ethylene oxide was found at levels ranging from 30 to 394 ppm (parts per million) in 5 of 18 POEA surfactant samples (Sasaki et al., 1993). These impurities are formed when ethylene oxide is added to lipid-soluble substances such as fatty amines. The most frequent impurities are polyethylene glycols, which are formed when the ethylene oxide molecules react together, instead of reacting with a fatty amine. However, the levels of contaminating ethylene oxide found in POEA preparations may not be of any toxicological significance as exposure to this compound can arise from other uses of ethylene oxide-based surfactants. For example, cosmetic products, can contain as much as 1 ppm of ethylene oxide (Kreuzer, 1992). Furthermore, such exposures may be of little concern in comparison to the quantity of ethylene oxide that is produced in the human body (Filser et al., 1994). Other impurities such as dioxane, dioxolane, acetals, and acetaldehyde are also produced during the manufacture of POEA (Schwartz, 1969). Of concern is there is little information available on the dioxane content in pesticide formulations. A concentration of 73 ppm of 1,4-dioxane was found in an analysis of surfactants made of polyethylene oxide (Fuh et al., 2005). Documents from the United States Department of Agriculture described that 1,4-dioxane was found at an upper limit of 300 ppm in Roundup formulations, and that it was subsequently reduced to 23 ppm (USDA,

1997).

Based on herbicide physical and chemical properties, the various scenarios within which herbicides are used, and results from surveys of levels in the environment and foodstuffs, we conclude that GBH co-formulant surfactants could be present in the food and feed supply and may be altering risk profiles following exposures. The same concern applies to some other pesticides, e.g. the neonicotinoid insecticides (Mullin et al., 2016). This is the case not only for food and animal feedstuffs derived from glyphosate-tolerant genetically modified crops, but also cereals such as oats and wheat, some of which are subjected to pre-harvest desiccation with GBHs.

Given the potential toxicity that can arise from ingestion of co-formulants, we recommend that food and feed be assessed for its content of these pesticide components in addition to the stated active ingredients. Furthermore, farm animals and human populations need to be monitored for their daily intake of co-formulants and research undertaken in controlled laboratory animal feeding studies to determine the health implications of the levels of residues that are detected.

In conclusion, our review reveals a compelling need for full transparency on the composition of pesticides on product labels, MSDSs, and all sources of technical information on formulated pesticides. The confusion on surfactant content works to obscure consensus on first, the contribution of co-formulants to GBH toxicity, and second, differences in the toxicity of GBHs in contrast to technical glyphosate. Toxicity profiles in the literature are generally more consistent when the composition of the formulations is accurately reported and properly taken into account. In addition, the knowledge gap regarding exposure, daily intake and health consequences of co-formulants for farm animals and humans needs to be speedily addressed. A first-step forward is for laws and regulations to change to require public disclosure of 100% of a pesticide product's composition.

Competing interests

CB has served since September 2016 as an expert witness for plaintiffs in US litigation involving the impact of glyphosate-based herbicides on non-Hodgkin lymphoma (HNL). His research undertaken in the course of answering questions directed to him in the NHL litigation has focused to a large extent on differences in the toxicity of glyphosate technical and GBHs. He has served as an expert witness in other pesticide-related litigation and cases addressing whether foods containing genetically engineered ingredients can be labelled as “natural” without misleading consumers. RM has served as a consultant on glyphosate risk assessment issues as part of litigation in the US over glyphosate health effects. For both CB and RM, lawyers involved in the above-mentioned cases had no role in the decision to write this paper, nor in reviewing or commenting on its content. MNA declares that he has no financial or non-financial competing interests.

Transparency document

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