Regulatory Toxicology and Pharmacology 107 (2019) 104347

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Toxicology and human health risk assessment of polyethoxylated tallow amine surfactant used in glyphosate formulations

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ARTICLE INFO

Keywords:
Roundup
Glyphosate
Polyethoxylated tallow amine
POE-T
POEA
Surfactants
Toxicity
Occupational risk assessment
Food risk assessment
Human health risk assessment

ABSTRACT

Roundup® branded herbicides contain glyphosate, a surfactant system and water. One of the surfactants used is polyethoxylated tallow amine (POE-T). A toxicology dataset has been developed to derive the most representative points of departure for human health risk assessments. Concentrated POE-T was very irritating to skin, corrosive to eyes, and sensitizing to skin. The irritation and sensitization potential of POE-T diminishes significantly upon dilution with water. Repeated dosing of rats with POE-T produced gastrointestinal effects but no systemic effect on organ systems. POE-T was not genotoxic and had no effect on embryo-fetal development or reproduction. The occupational risk assessment of POE- T for the agricultural use of glyphosate products has demonstrated that margins of exposure (MOEs) are 2517 and 100,000 for maximum and geometric mean dermal exposures, respectively. In the food risk assessment for relevant agricultural uses, the range of MOEs for consumption of foods from plant and animal origin were 330 to 2909. MOEs ≥100 are generally considered to be of no toxicological concern. Based on the results of the occupational and food risk assessments, it is concluded that there are no significant human health issues associated with the use of POE-T as a surfactant in glyphosate products.

1. Introduction

Commercially available plant protection products comprise two major components: “active” ingredients and “inert” ingredients. Active ingredients are substances having general or specific action against harmful organisms or pests, and include those with herbicidal, insecticidal, and/or fungicidal activity. Inert ingredients (also known as co-formulants) are added to pesticide products to improve the technical characteristics, efficacy and applicability of the formulation (US EPA, 2007; EC, 2009). They may function as, for example, solvents, fillers, antioxidants, fertilizers, dispersing agents, emulsifiers, dyes, thickeners, anti-clumping agents, wetting agents, and stabilizers. Both active and inert ingredients are reviewed and regulated by government agencies. For example, in the United States (US) only the inert ingredients specifically listed on the United States Environmental Protection Agency's (US EPA) approved list may be used in pesticide products (US EPA, 2013). In the European Union (EU), co-formulants are regulated under the provisions of regulation 1907/2006 (REACH).

Under the pesticide regulations in the US, unless an inert ingredient is considered highly toxic, pesticide manufacturers do not have to disclose the chemical substances, either by name or percentage of composition, that comprise the “inert” ingredients of a pesticide formulation on the label. All that is currently required on the label, at least in the US, is the reporting of the percentage of the active ingredient. Inert ingredients in a pesticide formulation are not required to be listed on the label under any conditions (US EPA, 2007). However, the US EPA could require that the concentration of an inert ingredient in a pesticidal formulation cannot exceed a certain percentage based on a safety assessment.

In the EU, Regulation 1272/2008 on the classification, labeling and packaging of substances and mixtures also applies to plant protection products (EU, 2008). This means that the names of any substance(s) which have resulted in the classification of a pesticide formulation as carcinogenic, mutagenic, toxic for reproduction, very toxic, toxic, harmful, or sensitizing must be mentioned on the label.

The key components of glyphosate herbicides are salts of glyphosate (e.g., isopropylamine, potassium, or ammonium) as the “active” ingredient, and a surfactant system and water as the “inert” ingredients. The surfactant system is added to the formulation to facilitate the uptake of glyphosate by the plant. An example of such a surfactant within

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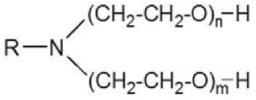
https://doi.org/10.1016/j.yrtph.2019.03.014
Received 30 October 2018; Received in revised form 26 February 2019; Accepted 18 March 2019
Available online 10 May 2019

a surfactant system is polyethoxylated tallow amine, hereafter referred to as POE-T (CAS no 61791-26-2, EINECS no 500-153-8), which belongs to the class of polyethoxylated alkyl amine (POEA) surfactants. POEAs are nonionic surfactants which are used in many consumer products including personal care, home care and lawn and garden. Examples of POEA are polyethoxylated (POE) cocoamine, POE-hydrogenated tallowamine, POE-oleamine, POE-palmitamine, POE-soyamine, POE-rapeseedamine, POE-lauramine, POE-stearamine and POE-tallow amine (POE-T) (Bergfeld et al., 2014).

POE-T is a mixture of congeners with a general molecular structure consisting of a linear aliphatic chain derived from tallow fat (mostly saturated or unsaturated C16 and C18-chains) linked to two polyethoxy chains of variable length (average of 10–15 ethoxy groups (EO) for the two polyethoxy chains combined) with a nitrogen.



R = saturated or unsaturated aliphatic chain

The chain length distribution and degree of unsaturation of the fatty acids in tallow are given in Table 1.

In addition to the extensive toxicology database and reviews available on glyphosate (US EPA, 1993; Williams et al., 2000; WHO/FAO, 2004; Williams et al., 2012; Kier and Kirkland, 2013; Kier, 2015; Greim et al., 2015; EFSA, 2015a,b; Saltmiras et al., 2015; WHO/FAO, 2016; US EPA, 2017; Williams et al., 2016a; Williams et al., 2016b; Solomon, 2016; Brusick et al., 2016; Health Canada PMRA, 2017), the US EPA evaluated the safety of alkyl amine polyalkoxylates (which includes POE-T) and granted them an exemption from the requirement of a tolerance for residues when used as an inert ingredient in pesticide formulations applied to growing crops or to animals (US EPA, 2009). As part of a robust product stewardship program, a core toxicology data set on POE-T was developed and used for the human health risk assessments of POE-T in agricultural applications and in food, and will be presented in this paper.

2. Toxicology studies

In total, 23 toxicology studies were conducted to characterize the toxicity of POE-T-based surfactant systems used in glyphosate products, and of glyphosate products themselves and are briefly discussed below (Additional details of the studies can be found in: Supplemental data, Appendix A). An overview of the regulatory compliance of these studies for conduct under either the US EPA or Organization for Economic Co-operation and Development (OECD) Good LaboratoryPractices (GLP) and international test guidelines (TG) of the OECD is given in Tables 2 and 3. The results of the toxicology studies are used for the classification of POE-T and the glyphosate formulations under the Global Harmonized System (GHS) (GHS, 2011) and to derive the most representative points of departure for the human health risk assessments. The aim of the GHS is to harmonize the classification and hazard communication of chemicals. It defines the hazards of chemical products and communicates health and safety information on the labels and safety data sheets and ultimately determining how the product is to be transported, handled and used. The administered dose levels are based on the test substance used in the study (surfactant system or glyphosate formulation). The concentration of POE-T in the surfactant systems that were used for toxicology testing ranged from 70.6 to 78% w/w (Table 2). The concentration of POE-T as a component of glyphosate formulations used for toxicology testing ranged from 0.27 to 11.07% w/w (Table 3). The study end points and/or no observed adverse effect levels (NOAELs) for all test results reported in this paper and for risk assessment purposes are expressed in terms of the POE-T component alone.

Table 1
Distribution of fatty acids in tallow fat (Bergfeld et al., 2014).

Fatty Acid Chain Length	Degree of Unsaturation	% Composition
C14	None	0–6
C16	None	20–37
C18	None	14–21
C16	1	3–9
C18	1	35–46
C18	2	4–10
C18	3	0–3

2.1. Discussion and conclusions of toxicology data

The oral LD50 of a surfactant system containing 78% w/w POE-T in the rat (males and females combined) was estimated to be 1200 mg/kg bw which corresponds to 936 mg/kg bw for POE-T (Birch, 1977). This result warrants classification of POE-T for acute oral toxicity as acute toxicity Category 4 according to GHS with the signal word “Warning” and the hazard statement “Harmful if swallowed”. The dermal LD50 of a surfactant system containing 78% w/w POE-T in the rabbit (4 dose levels, only one animal used per dose level) was estimated to be greater than 1260 mg/kg bw, but less than 2000 mg/kg bw (at which the single animal tested died) which corresponds with a dose range of 983 to 1560 mg/kg bw for POE-T (Birch, 1977). This result warrants classification of POE-T for acute dermal toxicity as acute toxicity Category 4 according to GHS with the signal word “Warning” and the hazard statement “Harmful in contact with skin”.

Two acute inhalations studies were conducted with glyphosate formulations; one was on a concentrate and the other on a ready-to-use. The inhalation LC50 of a concentrated glyphosate formulation containing 11.07% w/w POE-T for a 4-h exposure of rats is 3.05 mg/L for males, 3.33 mg/L for females and 3.18 mg/L for males and females combined (Velasquez, 1983). The inhalation of the concentrated formulation caused severe irritation in the respiratory pathways as indicated by blood congestion in the lungs and nasal and oral discharges in animals dying within 3 days after exposure. The severe irritation of the respiratory tract that was observed after inhalation of the concentrated glyphosate formulation was not observed in a test with a ready-to-use formulation (Dudek, 1987) in which the POE-T concentration of 0.27% w/w represented a 40-fold dilution of the concentrated glyphosate formulation tested by Velasquez (1983). The irritation effects of the respiratory tract seen with the concentrated formulation can be entirely ascribed to the presence of POE-T surfactant. The result of the acute inhalation toxicity test of the concentrated formulation warrants a classification as acute toxicity Category 4 according to GHS with the signal word “Warning” and the hazard statement “Harmful if inhaled”. The ready-to-use formulation, is representative of a diluted spray formulation which is used in the field would not be classified according to GHS criteria. It is important to emphasize that the effects observed in rats after inhalation are not systemic effects.

From the skin irritation studies it can be concluded that the surfactant system containing 78% w/w POE-T produces severe irritation of the skin of rabbits after 24 h of contact (Birch, 1977). These effects can be entirely attributed to POE-T since the other components of the surfactant system (glycols and water) are not irritating to the skin. It is not possible to propose a classification according to the globally harmonized system of classification and labeling of chemicals from this study since the criteria for the classification as irritating to the skin are based on an exposure time of 4 h instead of 24 h. When the primary dermal irritation results after 4 h of skin contact (occlusive dressing) with structurally similar alkyl polyethoxylated surfactants are considered (C12–C14 alkyl chains and 15–20 ethoxy (EO) groups) only a slight irritating effect was observed (HERA, 2009). A glyphosate formulation with a POE-T concentration of 11.07% w/w produced a mild and

Table 2
Concentration of POE-T in the surfactant systems used in the toxicology studies and regulatory compliance.

Toxicology test	Concentration of POE-T in the surfactant systems used (% w/w)	Reference	Compliance	
			GLP	OECD TG
Acute oral toxicity, rat	78	Birch (1977)	*No*	*Similar**
Acute dermal toxicity, rabbit	78	Birch (1977)	*No*	*No*
Skin irritation, rabbit	78	Birch (1977)	*No*	*No*
Eye irritation, rabbit	78	Birch (1977)	*No*	*Yes*
Skin sensitization, guinea pig	75	Blasczak (1987a)	*Yes*	*Similar**
Ames	71.9	Stegeman and Li (1990)	*Yes*	*Similar**
Mouse micronucleus	71.9	Stegeman and Kier (1998)	*Yes*	*Similar**
4-week oral toxicity, rat	70.6	Ogrowsky (1989)	*Yes*	*No*
13-week oral toxicity, rat	71.9	Stout (1990)	*Yes*	*Yes*
14-week oral toxicity, dog	78	Fillmore (1973)	*No*	*Similar**
Developmental oral toxicity, rat	71.9	Holson (1990)	*Yes*	*Similar**
2-gen reproduction oral toxicity, rat	71.9	Knapp (2007)	*Yes*	*Yes*
4-week oral toxicity + reprotoxicity screen, rat	71.9	Knapp (2008)	*Yes*	*Limit test*

*Not completely compliant, but method considered sufficient to reliably assess toxicity.

transient dermal irritation after 4 h of contact. Based on the mean scores for erythema and edema (0.64 and 0.03, respectively) calculated for 2 application sites per animal, all animals and observation times 24, 48 and 72 h this formulation would not be classified as irritating to the skin according to GHS (Blaszcak, 1988). A glyphosate herbicide formulation with a POE-T concentration of 4.8% w/w left in contact with the skin for 4 h showed barely perceptible erythema that resolved within 72 h after application (Auletta, 1985a) Based on the mean scores for erythema and edema (0.19 and 0.00, respectively) calculated for 2 application sites per animal, all animals and observation times 24, 48 and 72 h this formulation would not be classified as irritating to the skin according to GHS. A ready to use glyphosate formulation containing 0.27% w/w POE-T produced mild and transient erythema that resolved within 48 h (Blaszcak, 1987b). Based on the mean scores for erythema and edema (0.03 and 0.00, respectively) calculated for 2 application sites per animal, all animals and observation times 24, 48 and 72 h this formulation would not be classified as irritating to the skin according to GHS. In conclusion, formulations containing 11.07% w/w POE-T or less should not be classified as irritating to the skin.

From the eye irritation studies it can be concluded that the surfactant system containing 78% w/w POE- T produces corrosion in the eyes of rabbits (Birch, 1977). Because these effects can be entirely attributed to the POE-T present in the surfactant system these results warrant classification of POE-T as a Category 1 according to GHS with the signal word “Danger” and the hazard statement “Causes serious eye damage”. Due to the irreversible and delayed effects in the cornea (opacity, ulceration and pannus) of rabbits at 28 days after instillation, a concentrated glyphosate formulation with a POE-T concentration of 11.07% w/w should be classified in Category 1 according to GHS with the signal word “Danger” and the hazard statement “Causes serious eye damage” (Blaszcak and Auletta, 1994). A glyphosate formulation with 4.8% w/w POE-T showed much less irritation and all animals tested were free of ocular irritation within 7–14 days (Auletta, 1985b). Based on the mean scores for conjunctival erythema and edema and corneal opacity (1.33, 1.17 and 0.11, respectively) calculated over all rabbits and 24, 48 and 72 h this formulation would not be classified as irritating to eyes according to GHS. A ready-to-use glyphosate formulation containing 0.27% w/w POE-T produced slight conjunctival irritation, corneal opacity and iridial changes (Blaszcak, 1987c). These mild effects were all reversible within 48 h after instillation. Based on the mean scores for conjunctival erythema and edema and corneal opacity (0.17, 0.00, and 0.00, respectively) calculated over all rabbits and 24, 48 and 72 h this formulation would not be classified as irritating to eyes according to GHS. In conclusion, formulations containing 4.8% w/w POE-T or less should not be classified as irritant to the eyes.

The skin sensitization test with a surfactant system containing 75% w/w POE-T carried out according to the method of Buehler produced some skin irritation at rechallenge with an incidence (6 of 10 animals after 24 h, 7 of 10 animals after 48 h) that is sufficient for classification as a Category 1 skin sensitizer, sub-category 1B according to GHS. Subcategory 1B is assigned when the incidence of the irritation response in the Buehler assay at challenge or rechallenge is greater than 15% when the concentration of the test item is greater than 20% of the concentration at topical induction. (Blaszcak, 1987a). The signal word “Warning” and the hazard statement “May cause an allergic skin reaction” are of application in this case. Because the other components of this surfactant system (glycols and water) are not skin sensitizers it is clear that POE-T should be classified in the same way. When a glyphosate formulation with a POE-T concentration of 11.07% w/w was tested in the 9-induction Buehler assay no dermal reactions were

Table 3
Concentration of POE-T in glyphosate formulations used in toxicology studies and regulatory compliance.

Toxicology test	Concentration of POE-T in formulation as tested (% w/w)	Reference	Compliance	
			GLP	OECD
Acute inhalation toxicity, rat	11.07	Velasquez (1983a)	No	Yes
Acute inhalation toxicity, rat	0.27	Dudek (1987)	No	Limit test
Skin irritation, rabbit	11.07	Blaszcak (1988)	Yes	Yes
Skin irritation, rabbit	4.8	Auletta (1985)	Yes	Yes
Skin irritation, rabbit	0.27	Blaszcak (1987b)	Yes	Yes
Eye irritation, rabbit	11.07	Blaszcak and Auletta (1994)	Yes	Yes
Eye irritation, rabbit	4.8	Auletta (1985)	Yes	Yes
Eye irritation, rabbit	0.27	Blaszcak (1987c)	Yes	Yes
Skin sensitization, guinea pig	11.07	Auletta (1983)	No**	Similar*
4-week inhalation toxicity rat	3.69	Velasquez (1983b)	No	Similar*

*Not completely compliant, but method considered sufficient to reliably assess toxicity. **Study conducted prior GLP but good scientific practices were followed.

observed at challenge so that no classification as a skin sensitizer is warranted (Auletta, 1983). Repeated insult patch tests of a hairstyling formulation containing 1% w/w polyethoxylated cocoamine (15 EO groups), a member the POEA family of surfactants, in 212 subjects and an adult sunscreen formulation containing 2.9% w/w polyethoxylated cocoamine (15 EO groups) in 201 subjects were negative. No photoallergic (116 subjects) or other phototoxic effects (22 subjects) were found in the skin after exposure to an adult sunscreen containing 2.9% w/w polyethoxylated cocoamine (15 EO groups) (Bergfeld et al., 2014). In conclusion, formulations containing 11.07% w/w POE-T or less should not be classified as sensitizing to the skin.

The genotoxicity tests *in vitro* (Ames assay) and *in vivo* (mouse micronucleus assay) have clearly indicated that POE-T, does not produce gene mutations, chromosomal aberrations or aneuploidy (Stegeman and Li, 1990; Stegeman and Kier, 1998). In conclusion, POE-T or formulations containing POE-T should not be classified as mutagens.

A number of short term repeat dose toxicology studies were conducted in rats and dogs to assess dose-response relationships and were used determine a point of departure (POD) for the human occupational and food risk assessment. A surfactant system containing 71.9% w/w POE-T was administered to groups of 10 male and 10 female Sprague-Dawley rats for 13 weeks at corrected mean dietary concentration levels of 0, 420, 1300 and 3700 mg/kg diet corresponding with daily doses of approximately 27.7, 86.4 and 239.1 mg/kg bw/day in males and 33.5, 107.1 and 292.4 mg/kg bw/day in females respectively (Stout, 1990). The NOAEL was considered to be 420 mg/kg diet in males and females corresponding with 27.7 and 33.5 mg/kg bw/day for males and females, respectively. These NOAELs are not based on systemic toxicity as was observed in the highest dose tested that exceeded the Maximum Tolerated Dose (MTD) but are based on local gastrointestinal irritation as indicated by hypertrophy and/or vacuolation of histiocytes in the lamina propria of the jejunum and ileum accompanied by histiocytosis and accumulation of macrophage aggregates in the mesenteric lymph nodes. These are signs of irritation of the gastro-intestinal tract produced by POE-T which correlate well with the soft stools and the significant decrease in food consumption, body weight (19% and 18% decrease in males and females, respectively) and cumulative body weight change (31% and 35% decrease in males and females, respectively) in the high dose animals. Because no specific systemic target organ toxicity was observed in this study no classification for this endpoint is warranted. The same is true for the 28-day inhalation toxicity study in Sprague-Dawley rats which the aerosol was prepared from a dilution of a concentrated glyphosate formulation (Velasquez, 1983b). Apart from an increase in the incidence of minimal signs of local irritation in the respiratory passages (subacute inflammation of the nasal turbinates and perivascular lymphoid infiltrates in the lungs) only in the females of the high exposure group, no systemic effects were seen. The mean analytical atmospheric concentrations were 0, 0.05, 0.16 and 0.36 mg/L of air expressed as the undiluted glyphosate formulation. The no observed adverse effect concentration (NOAEC) in this study was considered to be 0.36 mg test substance/L of air.

A surfactant system was administered orally in gelatin capsules to male and female dogs at increasing doses during the first 4 weeks of the study and then maintained at 0, 30, 60 or 90 mg/kg bw/day for the final 10 weeks. Changes in the clinical condition of dogs treated with a surfactant system containing 78% w/w POE-T for 14 weeks at the mid and high dose levels included signs that are indicative of gastrointestinal irritation (Fillmore, 1973). Although no specific target organ toxicity was identified in this study, it was not possible to derive a NOAEL from this assay since body weights were significantly reduced at all dose levels tested. Since no specific systemic target organ toxicity was observed in this study no classification for this endpoint is warranted.

In the embryo-fetal development toxicity study in Sprague-Dawley rats exposed orally to a surfactant system containing 71.9% w/w POE-T at doses of 0, 15, 100 and 300 mg/kg bw maternal toxicity signs indicative of gastrointestinal irritation were noted in the mid- and high-dose level groups (Holson, 1990). No changes were found in the gestational and embryo-fetal developmental parameters. The NOAELs for maternal and developmental toxicity were 15 and 300 mg/kg bw/day, respectively. This study clearly demonstrated that POE-T does not produce embryo-fetal development toxicity even at dose levels with marked maternal toxicity and no classification is warranted for this endpoint.

In the 2-generation reproduction toxicity study with a surfactant system containing 71.9% w/w POE-T Sprague-Dawley rats were exposed to dietary concentrations of 0, 100, 300 and 1000 mg/kg diet, no effects were observed on survival and clinical condition, reproductive performance, body weight and food consumption, organ weights and macroscopic and microscopic morphology of the F0 and F1 parental generations, developmental landmarks, estrous cyclicity, spermatogenic endpoints and testosterone and thyroid hormone levels of the F1 generation, the clinical condition and body weight of the F1 and F2 litters and litter viability and postnatal survival of the F2 litters (Knapp, 2007). A non-statistically significant decrease in the mean number of pups born and live litter size was noted in the high-dose group which was due to 3 F0 dams with atypically small litters. No similar effects were seen in the F2 litters. The absence of any effect on litter parameters was confirmed in a combined repeated dose toxicity/developmental toxicity screening study in Sprague-Dawley rats treated at the same dietary level (1000 mg/kg diet) (Knapp, 2008). Therefore, POE-T should not be considered as toxic to reproduction and should not be classified for this endpoint.

The surfactant system NOAEL for reproductive and developmental toxicity in this study was considered to be 300 mg/kg diet corresponding with 16.6 and 14.9 mg/kg bw/day for F_0 and F_1 males, respectively, and 19.5 and 18.9 mg/kg bw/day for F_0 and F_1 females, respectively.

In the combined repeated dose toxicity/developmental toxicity screening study a surfactant system containing 71.9% w/w POE-T was only tested at one dietary concentration 1000 mg/kg diet (Knapp, 2008). In this study, no substance related effects were observed on systemic toxicity, reproductive effects or effects on pup survival or morphology. This confirms that POE-T should not be considered toxic to reproduction and should not be classified for this endpoint.

An overview of the NOAELs that can be derived from this dataset for purposes of human health risk assessments and adjusted for POE-T content are given in Table 4.

The NOAELs in the 13-week oral toxicity study in rats (Stout, 1990) were based on local gastrointestinal irritation and not systemic toxicity as was observed at the higher doses. Taking the concentration of POE-T in the surfactant system used in this study into account (71.9% w/w) the NOAELS of POE-T were 19.9 and 24.1 mg/kg bw/day for males and females, respectively. The NOAEL for developmental toxicity (Holson,

Table 4
NOAELs for a surfactant system containing 71.9% w/w POE-T and adjusted for POE-T content.

Study	Species	Gender	Generation	NOAEL (mg/kg bw/day)	
				Surfactant system	POE-T
13-week oral (dietary) toxicity (Stout, 1990)	Rat	Males		27.7	19.9
		Females		33.5	24.1
Developmental oral (gavage) toxicity (Holson, 1990)	Rat	Females		300	215.7
Reproduction oral (dietary) toxicity screen (Knapp, 2007)	Rat	Males	F_0	16.6	11.9
			F_1	14.9	10.7
		Females	F_0	19.5	14.0
			F_1	18.9	13.6

1990) was the highest dose tested at 300 mg/kg bw/day and taking into consideration the concentration of POE-T in the surfactant system used in this study the NOAEL of POE-T was 215.7 mg/kg bw. The reproductive and developmental NOAELs provided in Table 4 are from the mid-dose groups and were based on the reduction of the number of pups born and live litter size in the F_1 generation in the high-dose, a result that was not confirmed in the F_2 generation in the same study (Knapp, 2007) and in the repeated dose toxicity/reproductive toxicity screening study performed at the same dietary level (1000 mg/kg diet, Knapp, 2008). The NOAELs for reproductive and developmental toxicity of POE-T that can be derived from the Knapp (2008) study taking into consideration the of POE-T in the surfactant system are 11.9 and 10.7 mg/kg bw/day for F_0 and F_1 males, respectively and 14.0 and 13.6 mg/kg bw/day for F_0 and F_1 females, respectively. Since the NOAELs for reproductive toxicity are based on an effect that was not confirmed in the F2 generation of the same study and in a separate study at the same dose level preference is given to the NOAEL from the 13-week oral toxicity study as the point of departure for the risk assessment of repeated exposure to POE-T. Since the effects found (gastrointestinal irritation) are not gender specific the NOAELs from males and females can be averaged resulting in a NOAEL of 22 mg/kg bw/day.

In summary, surfactant molecules, when presented in high enough concentrations, can disrupt the structure and function of cell membranes. As observed in the studies on the POE-T surfactant system this can result in irritation of the skin, corrosion of the eyes, and irritation of the mucous membranes of the respiratory and gastrointestinal tracts leading to decreased food consumption and body weight gain. As observed in the studies with the formulated products, the irritation and sensitization potential of POE-T diminishes rapidly upon dilution of the concentrated glyphosate formulation with water but even more dramatically in the ready-to-use products and can impact the GHS classification.

3. Risk assessment

3.1. Occupational risk assessment

POE-T is not one single substance but a complex mixture of more than 100 alkylamine congeners with different molecular weights and differences in the structure of their lipophilic (number of carbon atoms, saturated and unsaturated bonds) and hydrophilic (number of ethoxy (EO) groups) chains (Table 1). Given this complexity, monitoring of exposure associated with the use of these surfactant systems based on POE-T is very difficult.

A conservative approach to address this issue for glyphosate products is to monitor exposure to glyphosate and then extrapolate the systemic dose of glyphosate to the systemic dose of POE-T. By doing this, the physicochemical and pharmacokinetic properties of glyphosate and POE-T have to be taken into consideration. This pragmatic approach is applied here to evaluate the risk associated with exposure to POE-T via the use of glyphosate herbicides in agriculture.

Acquavella et al. (2004) published a large-scale biomonitoring study where glyphosate was measured in 24-h urine samples of farmers, their spouses, and their children over a period of 3 days following one day of application of a glyphosate formulation in the field. Because glyphosate exposure during application occurs primarily via the dermal route (Williams et al., 2000), glyphosate is not metabolized once absorbed and is only excreted in urine (US EPA, 2017), the best way to monitor exposure is to measure glyphosate excretion in urine. Overall, 40% of the urine samples from farmers had glyphosate concentrations that were lower than the limit of detection of the analytical method employed (1 μg/L) even for field applications of up to 100 acres. For the calculation of the systemic dose, the amount of glyphosate excreted in urine over the period of 3 days following application was adjusted for incomplete excretion at the last day by estimating each individual's excretion rate based on an open, single-compartment pharmacokinetic model for days 0–3, and determining the amount not yet excreted. The systemic dose was also corrected for incomplete urinary excretion once systemically available. The latter correction was based on a 95% recovery of glyphosate in urine of monkeys after intravenous administration (Wester et al., 1991). The corrected amount of glyphosate excreted in urine was then divided by each individual's body weight to obtain the systemic dose per kg bw. The maximum daily systemic (absorbed) doses of glyphosate associated with the application of glyphosate products in agriculture were estimated to be 0.004, 0.00004 and 0.0008 mg/kg bw for these farmers, their spouses, and their children, respectively. The geometric mean of the absorbed dose for farmers after one day of application was 0.0001 mg/kg bw. Since farmers apply glyphosate products only a few times per year with several weeks between one-day applications, the occupational risk assessment for farmers should be based on a single day of exposure.

Based on the pharmacokinetic data of structurally similar alkyl polyethoxylates (HERA, 2009) with an alkyl chain length of 14–18 carbon atoms and a polyethoxy chain of 10 EO groups, it is expected that POE-T once absorbed is rapidly eliminated from the body within 72 h. This means that farmers exposed to POE-T following a one-day application of a glyphosate product should have eliminated the surfactant completely before being exposed to POE-T from the next application. For the occupational risk assessment of farmers, the maximum systemic dose as well as the geometric mean of the dose are used.

For the extrapolation from systemic exposure to glyphosate to systemic exposure to POE-T, it is assumed that:

1) The systemic dose of POE-T is primarily the result of dermal absorption as the most relevant route of exposure for pesticide applicators is dermal exposure (van Hemmen et al., 1995; Lebailly et al., 2009). This is consistent with the observation that exposure to an aerosol of diluted glyphosate products through inhalation is minimal for overall mixer/loader/applicator exposure Williams et al. (2000),
2) The dermal absorption of glyphosate as its isopropylamine salt from a representative glyphosate formulation is a maximum of 1% (EFSA, 2015a),
3) For the dermal uptake of POE-T from a glyphosate product a default value of 5% is taken based on models used in the cosmetic and detergent industry (EPA, 2009),
4) The rate of permeation of POE-T through clothing is comparable to that of glyphosate, and
5) The glyphosate:POE-T ratio is approximately 2.8:1, which represents the highest expected level of POE-T relative to glyphosate.

The calculation of the systemic dose for POE-T in farmers after one day of application is done according to the following steps:

1) Calculation of the external dermal dose of glyphosate based on the systemic dose derived from the urinary excretion measured in biomonitoring studies (Acquavella et al., 2004): (0.004 mg/kg bw/day) × (1/1%) = 0.4 mg/kg bw/day for the maximum exposure, and (0.0001 mg/kg bw/day) × (1/1%) = 0.01 mg/kg bw/day for the geometric mean.
2) Calculation of the external dermal dose of POE-T: (0.4 mg/kg bw/day) × (1/2.8) = 0.143 mg/kg bw/day for the maximum exposure, and (0.01 mg/kg bw/day) × (1/2.8) = 0.0036 mg/kg bw/day for the geometric mean.
3) Calculation of the internal (systemic) dose of POE-T after one day of exposure: (0.143 mg/kg bw/day) × (5%) = 0.00715 mg/kg bw/day for the maximum exposure, and (0.0036 mg/kg bw/day) × (5%) = 0.00018 mg/kg bw/day for the geometric mean.

To evaluate the risk to human health after one day of exposure in the field, the systemic NOAEL for POE-T from the most reliable toxicology study is taken and compared against the systemic exposure of

POE-T. This ratio is referred to as the margin of exposure (MOE) (US EPA, 2012). The only short-term exposure (4 weeks or less) toxicology study available (Velasquez, 1983) was not selected for use in this occupational risk assessment because it was not conducted according to GLP, was not fully compliant with OECD test guidelines and the only effects observed were signs of local irritation in the respiratory system with no evidence of systemic toxicity. Therefore, the study of choice is the 13-week oral toxicology study in rats from Stout (1990) as the systemic dose of POE-T is primarily associated with dermal exposure since the contribution of exposure to an aerosol of diluted glyphosate products through inhalation is minimal for overall mixer/loader/applicator exposure (Williams et al., 2000). The average NOAEL for POE-T derived from this study is 22 mg/kg bw/day. Since it was found that the oral absorption of alkyl polyethoxylate surfactants with an alkyl chain length of 14–18 carbon atoms and a number of EO groups of 10 was at least 80% of the dose administered (HERA, 2009), 80% can be taken to calculate the average systemic NOAEL in the rat: (22 mg/kg bw/day) × (80%) = 18 mg/kg bw/day.

The MOEs obtained are 2517 for the maximum exposure and 100,000 for the geometric mean of exposure to POE-T after one day of application of a glyphosate product. A MOE of ≥100 is generally considered to be of no toxicological concern. This is a very conservative estimate since the NOAEL from a representative short-term toxicology study (2 weeks or less) would have been much larger. Moreover, it needs to be emphasized that the NOAEL of the oral 13-week toxicology study was based on effects ensuing from local irritation of POE-T in the gastro-intestinal tract and not on systemic target organ effects. Significant effects on food consumption and body weight were observed at the high dose which exceeded the MTD and was approximately 10X higher than the NOAEL used in these risk assessments. The local irritation of the gastrointestinal tract is characteristic for oral administration of surfactants and does not apply to dermal exposure. Taking all of this into consideration it can be concluded that POE-T based surfactant systems do not pose a health risk to humans when used according to regulatory labels for use in agriculture.

3.2. Food risk assessment

For the food risk assessment of POE-T a refined risk assessment has been applied according to methods used by international agencies for the calculation of the daily exposure to pesticide residues in food of plant and animal origin, as described below. While POE-T residues have not been measured directly, they can be estimated based on the following:

1) Uptake and translocation studies on surfactants related to POE-T have shown that these surfactants do not translocate to any significant degree within a crop following application (Sherrick et al., 1986; Smith and Foy, 1966). Potential exposure to POE-T should therefore be driven by surface residues following direct application of the glyphosate formulation to crop commodities.
2) Glyphosate uses that can result in direct surface residues on the crop commodity shortly before harvest are considered relevant for POE-T dietary exposure. These are also the uses that typically result in the highest glyphosate residues.

In this food risk assessment two scenarios of glyphosate use are considered yielding the highest possible residues of POE-T. Residues resulting from other agricultural uses of glyphosate products are expected to be much lower. The first scenario is a preharvest application made one to two weeks prior to harvest, in cereal grains, pulses and oilseeds. In this scenario, the field is sprayed after the crop is mature, and surface residues of glyphosate and POE-T are expected to be present in food and feed commodities. The other scenario is spot treatment or pasture renovation in alfalfa and pasture grass. The high pesticide residues that are possible from this type of application would not be expected frequently in grass used for feed, as they would only be seen following uniform application to the pasture during renovation. The residue data are derived from Joint Meeting on Pesticide Residues (JMPR) reviews of pesticide residues in food and feed. Data for cereal grains, straws and fodders, oilseed rape, sunflower, soya bean, peas, beans, and grass and alfalfa forages and hays were taken from the JMPR 2005 report (WHO/FAO, 2005), and lentil residue data were taken from the JMPR 2011 report (WHO/FAO, 2011). The reported values were used for pesticide residues of directly consumed food commodities in the exposure assessment, and for determination of the livestock dietary burden.

For the risk assessment of POE-T residues in foods the following assumptions were made:

1) Only uses were considered resulting in direct application to the crop commodity,
2) Glyphosate residue data are from samples taken immediately or shortly after application for the calculation of POE-T residues,
3) POE-T was present at the same ratio to glyphosate in the crops as in the formulation,
4) The concentration of total glyphosate residues (glyphosate plus the metabolite aminomethylphosphonic acid (AMPA), expressed as glyphosate) present in the samples did not change significantly in the short time between application and harvest, and thus these data represent a reasonable estimate of POE-T residues in the crop commodities.

The estimation of residues in commodities of animal origin for inclusion in the dietary exposure assessment consisted of several steps, as outlined below:

1) POE-T residues were calculated for the cereal grains and straw, oilseeds and grass using the Supervised Trial Median Residue (STMR) residue data (as total glyphosate and AMPA residues, expressed as glyphosate) from the 2005 and 2011 JMPR reviews of pesticide residues in food and feed (Appendix B, Supplemental Table B. 1). Livestock dietary burdens for POE-T were calculated from the relevant feed commodities using the European Food Safety Authority (EFSA) calculator (*Animal model 2015a.xls*) as referenced in EFSA guidance on estimating animal intakes (EFSA, 2015b), which uses percentages of each commodity used in livestock diets based on the feedstuff tables reported in the OECD Guidance 64/32 and OECD guidance 73 (OECD, 2009; OECD, 2013) (Appendix B, Supplemental Table B. 2). The resulting calculated dietary burdens were expressed as dry weight.
2) Transfer factors (TF, the ratio of residues in animal feed to the resulting residues in a commodity of animal origin) for commodities of animal origin were estimated using a model described in the literature (MacLachlan and Bhula, 2008; US EPA, 2005) (Appendix B, Supplemental Table B. 3). The model correlates TFs with the log Kow of the compound. The model determines TFs for cattle fat and offal, milk from cattle, and poultry fat and eggs.
3) POE-T residues in commodities of animal origin were calculated from the livestock dietary burdens and the estimated TFs (Appendix B, Supplemental Table B.4). For meat, it was assumed that the transfer is due to bioconcentration into the fat, and so the TFs for meat are based on the fat content of the meat (EC, 2001) and the fat TF. TFs for cattle were used as surrogates for other animals such as swine and sheep.
4) The overall consumer dietary exposure to POE-T from both direct consumption of crop commodities and from consumption of animal commodities was assessed. The assessment was conducted using the World Health Organization (WHO) Template for the Evaluation of Chronic Exposure (IEDI), 17 cluster diet version 02, 6 October 2014 (final).

Table 5
Summary of human dietary exposure.

GEMS cluster number	Total intake (μg/person)	Body weight per region (kg)	Total intake (mg/kg bw/day)
G01	1690	60	0.0282
G02	3265	60	0.0544
G03	454	60	0.0076
G04	2111	60	0.0352
G05	1279	60	0.0213
G06	1603	60	0.0267
G07	3642	60	0.0607
G08	3190	60	0.0532
G09	1103	55	0.0201
G10	3313	60	0.0552
G11	4004	60	0.0667
G12	1961	60	0.0327
G13	1087	60	0.0181
G14	594	60	0.0099
G15	3590	60	0.0598
G16	619	60	0.0103
G17	1411	60	0.0235

For this exercise, a glyphosate:POE-T ratio of 2.8:1 is used which represents the highest expected level of POE-T relative to glyphosate.

Following the process outlined above for calculating chronic exposure to POE-T, the International Estimated Daily Intake (IEDI) values across the 17 GEMS (Global Environment Monitoring System) cluster diets ranged from 0.0667 to 0.0076 mg/kg bw/day and are presented in Table 5.

The total intakes in Table 5 were calculated using the Template for the Evaluation of Chronic Exposure (IEDI), 17 cluster diet version 02, 6 October 2014, final (WHO, 2014).

When the average NOAEL (i.e., combined male-female NOAEL of 22 mg/kg/day) of POE-T from the most representative repeated dose oral toxicology study in the rat (Stout, 1990) i.e., 22 mg/kg bw/day is compared against the highest (GEMS cluster diet 11) and the lowest (GEMS cluster diet 03) daily total intake values (Table 5), the margins of exposure (MOEs) range from 330 to 2909. A MOE of ≥100 is generally considered to be of no toxicological concern. This is a very conservative estimate since the residue data considered are from samples taken immediately or shortly after application and it is assumed that the concentration of total glyphosate (glyphosate and AMPA) and POE-T residues present in the samples did not change significantly during the time that elapsed between application and harvest and final consumption. Removal of POE-T during cleaning and processing of the foods was also not taken into account. For the calculation of the MOE, no correction was made for differences in absorption of POE-T from the gastrointestinal tract between rats and humans since no such differences were observed for alkyl polyethoxylates (Drotman, 1980).

4. Discussion and conclusions

When present in sufficiently high concentrations, surfactant molecules disrupt the structure and function of cell membranes leading to biological effects such as irritation of the skin, corrosion of the eyes, and irritation of the mucous membranes of the respiratory and gastrointestinal tracts. The severest of effects were observed in studies conducted with the neat surfactant system, containing > 70% w/w POE-T. These local effects decrease rapidly in severity upon dilution with water as observed with the tests on formulations. Effects such as decreased food consumption and decreased body weight gain are secondary to gastrointestinal irritation and/or palatability of the diet admixed with surfactants.

Beside their use in herbicide formulations POEAs are also used as in consumer products (Bergfeld et al., 2014). The most extensively used nonionic surfactants with a molecular structure closely related to that of POE-T are the aliphatic alkyl polyethoxylates (also referred to as alcohol polyethoxylates). These surfactants are composed of a long-chain fatty alcohol (hydrophobic moiety) connected, via an ether linkage, to one or more ethoxylate (EO) groups (hydrophilic moiety). Alkyl polyethoxylate commercial blends are complex mixtures of homologues with an even number of 12–18 alkyl carbon atoms or with an even-odd linear and 2-alkyl substituted alkyl chains with 11–15 carbon atoms. The average number of ethoxylate groups is 5–15 (Talmage, 1994; HERA, 2009).

Alkyl polyethoxylates are widely used and are present in consumer products (regular laundry detergents, concentrated laundry detergents, fabric conditioners, laundry additives, hand dishwashing detergents, machine dishwashing detergents, surface cleaners and toilet cleaners), cosmetics (shampoos, face washes and similar cosmetic cleaning preparations) and pharmaceuticals (emulsifying agents for water-in-oil and oil-in-water emulsions, stabilization of micro-emulsions and multiple emulsions, solubilizing agents for essential oils, perfumery chemicals, vitamin oils, and drugs of low water solubility, antidusting agents for powders, wetting and dispersing agents for coarse-particle liquid dispersions, as gelling and foaming agents and coating agents to provide hydrophilicity to polymeric nanoparticles. Some alkyl polyethoxylates are suitable for use in injectable formulations as a solubilizer or dispersant) (Rowe et al., 2009).

From the comparison of POE-T with alkyl polyethoxylates posessing comparable alkyl and ethoxylate groups (Talmage, 1994; HERA, 2009), it can be concluded that, apart from the skin sensitization detected in the neat surfactant system but not in a less concentrated form such as in glyphosate products, both classes of surfactants have similar toxicity profiles for acute toxicity, skin and eye irritation. As is the case with alkyl polyethoxylates, POE-T based surfactant systems were not found to target any specific organ system after repeated oral or inhalation exposures beside local irritation of the respiratory and gastrointestinal tracts. POE-T does not produce embryo-fetal development toxicity, has no effect on reproductive performance up to dietary concentrations of 1000 mg/kg diet, corresponding with 47.5 and 68.3 mg/kg bw/day for males and females, respectively, and has not been found to be genotoxic. In the safety assessment report from the Cosmetic Ingredient Review (CIR) expert panel (Bergfeld et al., 2014) POE-T with 7–30 EO groups were included in the list of ingredients that are safe to be used in cosmetics in the present practices and concentrations when formulated to be non-irritating.

For the occupational risk assessment of POE-T in the application of glyphosate products in agriculture, systemic (absorbed) exposure data are used. These have been obtained from monitoring of glyphosate in 24-h urine samples from farmers exposed for one day during mixing/loading/spraying collected up to 3 days after application. When the average systemic NOAEL from a reliable GLP and guideline compliant 13-week oral toxicity study in the rat (Stout, 1990) is compared against the maximum systemic exposure and the geometric mean of systemic exposure, the MOEs are 2517 and 100,000, respectively.

A food risk assessment with consumption of crops and animal commodities based on livestock diet including cereals, pulses, oilseeds, grass and alfalfa treated with a glyphosate product within a few weeks before harvest and considering the IEDI of the 17 GEMS cluster diets, revealed a MOE range from 330 to 2909. A MOE of ≥100 is generally considered to be of no toxicological concern. Based on the results of the occupational and food risk assessments, it is concluded that there are no significant human health issues associated with the use of POE-T as an inert in glyphosate products.

Declaration of interest

The employment affiliation of the authors is shown on the cover page. Dr. Mark Martens was a former employee of the Monsanto Company and currently employed with the consulting firm MMTA bvba. Marian Bleeke, Donna Farmer and Vincent Leopold are all employed by Bayer Crop Sciences Division.

Acknowledgements

The authors wish to acknowledge Kimberly Hodge-Bell, William Heydens, Michael Koch, Mitch Kurtzweil, David Saltmiras and, Milica Vogt for their thoughtful review of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yrtph.2019.03.014.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.yrtph.2019.03.014.

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