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Invited Review

A review of the nonclinical safety of Transcutol®, a highly purified form of diethylene glycol monoethyl ether (DEGEE) used as a pharmaceutical excipient

Dexter W. Sullivan Jr. [a,*], Shayne C. Gad [a], Marjorie Julien [b]

[a] Gad Consulting Services, 102 Woodtrail Lane, Cary, NC 27518, USA
[b] Gattefossé, 36 Chemin de Genas, 69804 Saint-Priest, France

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ABSTRACT

Transcutol® (Diethylene glycol monoethyl ether, DEGEE), CAS # 111-90-0, is commonly used as a vehicle in the formulation or manufacturing process of pharmaceuticals, cosmetics, and food additives. This paper presents unpublished nonclinical safety data using a form of DEGEE which includes a significantly decreased level of impurities, specifically ethylene glycol and diethylene glycol. It also reviews the history of use, regulatory status, and previously published toxicity data for DEGEE. The review supports that DEGEE is well tolerated across animal species and gender with toxicity occurring only at levels well above those intended for human use. At high levels of exposure, the kidney is identified as the critical target organ of DEGEE toxicity. DEGEE is negative for genotoxicity in *in vitro* and *in vivo* studies. Subchronic and chronic toxicity studies produced no reports of preneoplastic changes in organs, but the animal data is insufficient to allow a definitive opinion as to carcinogenicity. *In silico* data suggested that DEGEE is not carcinogenic or genotoxic. Developmental toxicity was seen in rats but only at levels 200 times greater than the estimated oral Permissible Daily Exposure Level of 10 mg/kg/day. The nonclinical data along with the long history of DEGEE use as a vehicle and solvent by multiple routes provide evidence of its safety. Furthermore, the novel data discussed herein provides evidence that toxicity previously associated with high levels of DEGEE in nonclinical studies conducted prior to 1990 could possibly be attributed to the presence of significant amounts of ethylene glycol or other impurities.

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* Corresponding author. Address: 102 Woodtrail Lane, Cary, NC 27518, USA.
E-mail address: dwsullivanjr@gmail.com (D.W. Sullivan Jr.).

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1. Introduction

Transcutol®, purified diethylene glycol monoethyl ether (DEGEE, CAS No. 111-90-0), is an ethylene oxide derivative. Because of its characteristics as a strong solubilizer coupled with its low toxicity, DEGEE has a long history of safe use as a solvent in many products including pharmaceuticals, cosmetics, and food applications. Numerous independent nonclinical studies on the safety of DEGEE are available in the published literature. This paper seeks to evaluate the safety of DEGEE by reviewing the current published literature and adding previously unpublished data performed by Gattefossé to evaluate the safety of the purified compound, Transcutol®, as a pharmaceutical excipient. A brief review of the current uses and regulatory status of Transcutol® are also included.

1.1. Identity and characterization

Diethylene glycol monoethyl ether (DEGEE, CAS No. 111-90-0) is a clear, colorless, hygroscopic liquid with a mild pleasant odor (Fig. 1). It is produced by condensation of ethylene oxide and alcohol, followed by a purification distillation (USP-NF, 2013). DEGEE is soluble in water and miscible in acetone, benzene, chloroform, ethanol (95%), ether, and pyridine. It is partially soluble in vegetable oils and insoluble in mineral oils (Rowe et al., 2012). Table 1 summarizes the physiochemical properties of DEGEE.

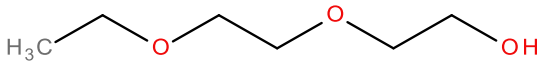

Fig. 1. Chemical structure of diethylene glycol monoethyl ether ($C_6H_{14}O_3$, CAS No. 111-90-0).

Table 1
Physiochemical properties for DEGEE[a].

Property (Unit)	Value
Empirical formula	$C_6H_{14}O_3$
Molecular weight	134.17
Boiling point (°C)	198–201
Freezing point (°C)	−105 to −103
Density (g/cm^3)	0.988
Vapor pressure (mmHg at 20 °C)	0.07–0.12
Relative vapor density (air = 1)	4.6
Flash point (°C)	90–96.1
Octanol/water partition coefficient (log P)	−0.54

[a] Rowe et al. (2012).

DEGEE has a wide variety of uses including pharmaceutical applications, as an indirect food additive for use in food, nutraceutical products and dietary supplements, and in cosmetics. The primary supplier in the US for pharmaceutical grade DEGEE is Gattefossé using the trade name Transcutol® (Osborne, 2011). Prior to 1988, Gattefossé only produced a single grade of DEGEE marketed under the trade name Transcutol® that was 99.5% pure. However, current pharmaceutical grade Transcutol® P (topical route) and Transcutol® HP (oral route) are 99.8% and 99.9% pure, respectively. A cosmetics only grade Transcutol® CG is 99.5% pure. The identified impurities for pharmaceutical- and cosmetic-grade Transcutol®, along with their identified and maximum allowable (for pharmaceutical-grade) levels, are summarized in Table 2. It is important to note that industrial grades of the solvent are at best 98% pure and contain significant levels of ethylene glycol and diethylene glycol as impurities. Toxicology studies prior to the 1990s were typically performed with the industrial grade solvent, with many of the observed adverse effects being attributable to the ethylene glycol impurity (Osborne, 2011). More recent studies with the purified material provide further evidence suggesting the attribution of effects to the impurity.

2. Economic uses in marketed products with human exposure

DEGEE has a long history of use in pharmaceutical applications worldwide in the United States of America, Asia, and Europe. It is an effective solubilizer and is used in oral, topical, transdermal and injectable human and veterinary pharmaceutical products. In recent years it has been widely used as a solvent for topical products on account of three main properties: firstly, it has been shown to solubilize actives that are insoluble in common solvents such as propylene glycol and ethanol. Secondly, it modifies the skin penetration properties of active ingredients allowing different drug delivery outcomes to be obtained including enhanced local absorption, a prolonged release depot effect or systemic absorption for transdermal applications (Osborne, 2011); lastly it provides functionality at concentrations which avoid safety and tolerability issues.

Table 2
Characterization of the impurities or accompanying contaminants for Transcutol®.

Substance	CAS No.	ICH Q3C (ppm)	USP36-NF31 (ppm)	Max level in Transcutol® Grades[a] (ppm)		
				CG	P	HP
Ethylene Glycol	107-21-1	Class 2 (⩽620)	⩽620	⩽620	⩽100	⩽20
Diethylene Glycol	111-46-6	–	⩽150	⩽250	⩽150	⩽50
Ethylene oxide	75-21-8	–	⩽1	⩽1	⩽1	⩽1
2-Methoxyethanol	110-80-5	Class 2 (⩽50)	⩽50	⩽50	⩽50	⩽20
2-Ethoxyethanol	109-86-4	Class 2 (⩽160)	⩽160	⩽160	⩽100	⩽50

ppm: parts per million; CG is the cosmetics only grade Transcutol® and is 99.5% pure; P and HP are pharmaceutical grade Transcutol® and are 99.8% and 99.9% pure, respectively.
[a] *Source:* Gattefossé SAS.

Table 3
DEGEE use as a vehicle in nonclinical studies.

Species	Route	Duration	Dose	Comments
Cat	Intravenous	1 month	DEGEE 2 mL/kg	Well tolerated, no evidence of hemolysis or hematotoxicity
Rabbit	Dermal	Skin irritation	Transcutol®5 mL over 2 cm^2 area	50%; Non-irritant
		28 days	Transcutol® 0, 300, 1000, 3000 mg/kg/day	Undiluted; NOEL > 1000 mg/kg/day
	Ocular	Eye irritation	Transcutol® 0.1 mL	30%; Slight irritation
		Eye irritation	Transcutol® 0.1 mL	Undiluted; Slight irritation
Rat	Oral	90 days	DEGEE 0%, 0.25%, 1% and 5%	NOEL is 1%
		Acute	Transcutol® 5.0 g/kg	LD 50 > 5000 mg/kg
		Fertility and embryo toxicity range-finding study	DEGEE 500, 1000, 2000, 4000 mg/kg/day	NOEL > 500 mg/kg/day
Mouse	Oral	Acute	DEGEE	6.6 g/kg tested toxic
		Chronic (12 months)	DEGEE	NOEL: 850–1000 mg/kg
Dog	Oral	90 days	DEGEE	NOAEL: 1500 mg/kg/day

Source: Gad et al. (2006).

In topical products DEGEE is often used in an aqueous gel. ACZONE, the 5% dapsone gel for the treatment of acne was the first prescription drug product containing DEGEE as Transcutol® approved by the FDA (Osborne, 2011). Transcutol® has also been formulated in solutions, ointments and creams (emulsions and microemulsions) for the delivery of hormones, anti-inflammatory, anti-fungal, anesthetic, analgesic and antiseptic agents in prescription products approved in numerous countries around the world (USFDA CDER, 2013; Gattefossé SAS).

In Europe, Transcutol® HP (the high purity DEGEE) has been used in a number of oral prescription drugs including the oral drop product 'Lysanxia', and the oral solutions 'Pilosuryl' and 'Urosiphon' as well as a sublingual solution 'Natispray'. In emerging Asia Pacific countries, notably South Korea, Transcutol® is used in soft gelatin capsules in approved antiviral, anti-inflammatory, and immune suppressant medicines (source Gattefossé SAS).

Historically, Transcutol® has been used in injectable products, although its use in marketed human medicines remains limited to a few examples. In 1977 it was used in an intravenous injectable (IV) product 'Trombovar' approved in Europe for the treatment of varicose and spider veins in the leg; this product is no longer available. More recently, it has been formulated in an IV and IM injection of sodium diclofenac and an alpha beta-arteether intramuscular (IM) injection for the treatment of severe/cerebral malaria approved in India (source Gattefossé SAS).

The aforementioned uses of high purity DEGEE in the form of Transcutol® are associated with human medicine. Veterinary medicines require the same level of purity of excipients as human medicines, and as such, Transcutol® is also widely used in veterinary applications including topical solutions, sprays and spot-on's, often containing anti-parasitic agents which are formulated for transdermal delivery (source Gattefossé SAS). It is also used in injectable veterinary products including the anti-inflammatory SC and IM product 'Tolfedine' and a 'Vitamin E' IV injection (Strickley, 2004).

DEGEE is used as an indirect food additive for use in food, nutraceutical products and dietary supplements. The safety of use of this substance in such applications has been evaluated and is largely confirmed by many years of use.

DEGEE has a long history of use in cosmetic and personal care applications. Currently, it can be found in over 740 cosmetic products including eye makeup, fragrances, nail preparations, sunless tanning products, hair coloring products, and skin care preparations (Elder, 1985; Osborne, 2011). The safety of use of this substance in such applications has been evaluated and is largely confirmed by many years of use (CIR Expert Panel, 2006; Elder, 1985; Osborne, 2011).

DEGEE is used as a vehicle for use in *in vivo* nonclinical safety assessment studies and in clinical products. Gad et al. (2006) conducted a data mining project to determine the safe dosing level of drug delivery vehicles for *in vivo* animal studies. The results included information on 65 different vehicles and 9 animal species. The use of Transcutol® as a vehicle was reported for five species of animals and across four routes of exposure as shown in Table 3.

3. Regulatory status

The United States Food and Drug Administration (Center for Drug Evaluation and Research (CDER)) maintains an Inactive Ingredients Database (USFDA CDER, 2013). This database provides a partial listing of excipients being used in authorized medicinal products in the USA. This information can be used by industry as an aid in developing drug products. Once an inactive ingredient has appeared in an approved drug product for a particular route of administration, the inactive ingredient is considered qualified at the approved level and may require a less extensive review the next time it is included in a new drug product. For example, after an inactive ingredient has been approved for a specific dosage form and potency, a sponsor could consider it safe for use in a similar manner for a similar type of product. DEGEE is listed in the FDA Inactive Ingredient Database for topical use in a gel (25% maximum potency), transdermal use in a gel (5% maximum potency) and for use in a transdermal patch (maximum potency not reported). It is important to note that the approved maximum potency is not a limit for inactive ingredients, as higher levels may be approved with justification, but merely lists the amount of such ingredients that are currently approved for use in drug products.

Similarly, DEGEE is listed in the Australian Register of Therapeutic Goods (Australian Therapeutic Goods Administration, 2013) which includes all therapeutic goods, including medicines and medical devices, approved for use in Australia. Health Canada (2013) maintains a repository of approved medicinal and non-medicinal ingredients approved for use in Canada. DEGEE is listed in the Canadian natural health products ingredients database.

US FDA has approved DEGEE as an inactive ingredient for use as a component of adhesives for use in packaging, transporting, or holding food (21 CFR 175.105). US FDA has also approved DEGEE for use as a component of paper and paperboard in contact with dry food (21 CFR 176.180) and as a sanitizing agent for food-processing equipment and utensils (21 CFR 178.1010). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) have evaluated the use of DEGEE in food. The JECFA concluded that an Acceptable Daily Intake (ADI) for DEGEE could not be established due to the absence of adequate long-term (chronic/carcinogenicity) feeding studies in rats and mice and the absence of adequate data indicating that human intake of DEGEE from food are sufficiently low (JECFA, 1995).

DEGEE has been evaluated by the Cosmetic Ingredient Review (CIR) expert panel (CIR Expert Panel, 2006; Elder, 1985). The panel noted that DEGEE was used in 80 different cosmetic preparations in 1981 (0.1 to greater than 50%) with the largest uses found in hair dyes and colors as well as skin cleansing creams, lotions, liquids, and pads. By 2002, DEGEE was used in 622 preparations at concentrations ranging from 0.0004% to 80%. The panel concluded that based on the available data DEGEE is safe as presently used in cosmetics (2006; Elder, 1985). The Scientific Committee on Consumer Safety (SCCS, 2013) issued an opinion on the safety of DEGEE in cosmetic products. The SCCS concluded that DEGEE in cosmetic products (excluding oral hygiene and eye products) does not pose a risk to consumer health at concentrations up to 10% in rinse-off products, up to 7.0% in hair dye formulation and up to 2.6%, pro in all other cosmetic products provided that the level of ethylene glycol in DEGEE used is <0.1%.

4. Safety evaluation

A number of toxicity studies have been conducted with DEGEE by multiple routes of administration in a variety of species for a period of up to two years. Additionally, Gattefossé has completed a full battery of additional studies on DEGEE (as Transcutol®), including those evaluating toxicokinetics, local tolerance, skin sensitization, reproductive effects, teratogenicity, genotoxicity, and systemic toxicity. The new data for DEGEE generated within Gattefossé, in combination with the preexisting data, are presented here and serve to provide a dataset sufficient for determining the safety of DEGEE in humans.

4.1. Toxicokinetics

The absorption of DEGEE *in vitro* has been evaluated in human abdominal, whole skin. The rate of absorption was 0.125 ± 0.103 mg/cm^2/h, the permeability constant was 1.32×10^{-4} cm/h, and the damage ratio (a measure of integrity of the skin) was 1.20 ± 2.62. Among numerous glycol ethers tested in this study, including ethylene glycol monomethyl, monoethyl and monobutyl ethers, and DEGEE and diethylene glycol butyl ether, DEGEE had the lowest damage ratio and the second lowest permeability constant and rate of absorption, suggesting a decreased absorption rate with increasing molecular weights of glycol ethers (Dugard et al., 1984).

Unlike monoethylene glycol ethers, diethylene glycol ethers (including DEGEE) are poor substrates for alcohol dehydrogenase and expected to be good substrates for cytochrome P-450 based on experiments that measured induction of P-450. In an *in vitro* system using equine liver alcohol dehydrogenase, the V_{max} (μmol) K_m (μM) and V_{max}/K_m were 6.94, 6.31×10^{-2}, and 0.11, respectively (Miller, 1987). In an adult human volunteer (sex and age not reported) given a single oral dose of 11.2 mmol DEGEE, approximately 68% of the dose was recovered in the urine as (2-ethoxyethoxy) acetic acid within 12 h (Kamerling et al., 1977).

A number of studies have been completed in which multiple parameters were measured to further evaluate the toxicokinetic profile of purified DEGEE as Transcutol®. In an *in vitro* study performed to determine the metabolism profile of DEGEE (as Transcutol®) and ethylene glycol monoethyl ether (EGEE) formed by rat and human hepatocytes, EGEE was readily metabolized by both rat and human hepatocytes to ethoxy acetic acid (EAA) and ethylene glycol (EG), and the rat liver cells metabolized EGEE at a higher rate than human liver cells. However, contrasting results were seen with DEGEE including slow metabolism by rat hepatocytes to several different unidentified metabolite peaks that accounted for approximately 1–17% of the total radioactivity. DEGEE was not significantly metabolized by human hepatocytes (Gattefossé, 2001a).

In vivo, the absorption, distribution and excretion of DEGEE (as Transcutol®) was investigated comparably in Sprague–Dawley and BDIX rats after a single oral or intravenous dose of 20 mg ^{14}C-DEGEE/kg bw each. The GLP-compliant study was performed according to internal laboratory methodology comparable to OECD 417. Rapid excretion of radioactivity occurred in the urine, regardless of sex and route of administration. The maximum plasma concentration of the radioactivity was observed 0.25 h following intravenous injection, while after oral administration it was observed at 0.25–0.50 h post dose. The plasma half-life corresponded to 37–84 h with measurable concentrations observed in most of the tissues 168 h following administration. The absolute bioavailability of the radioactivity was very high (79–95%). The distribution of radioactivity in tissues was characterized by high concentrations detected in pituitary, thyroid, adrenals and bone marrow with regards to the concentrations observed in blood/plasma (100–1000 times less) at the same sampling time. The radioactivity levels in tissues was significantly decreased at 48 h. No biologically relevant differences were observed between the two rat strains (Gattefossé, 2002a).

In studies evaluating the metabolic fate and excretion of DEGEE (as Transcutol®) results indicated that following a single oral administration, the large majority (90%) of the administrated radioactivity was rapidly excreted (within the first 24 h) in the urine and ^{14}C-DEGEE was intensively metabolized as Ethoxyethoxyacetic acid (83%) and Diethylene glycol (5.4%) with only 3% of the urinary excreted radioactivity corresponding to unchanged compound. In plasma, only Ethoxyethoxyacetic acid and unchanged ^{14}C-DEGEE were detected, which was consistent with urinary results. The GLP-compliant study was performed according to internal laboratory methodology comparable to OECD 417 (Gattefossé, 2003).

4.2. Local tissue tolerance (skin, eye, intravenous, and mucosal irritation, sensitization, hematocompatibility, and parenteral irritation)

The current published data has shown that DEGEE is not a skin irritant in rabbits even after prolonged and repeated contact under normal study conditions while being only slightly irritating to rabbit skin with the use of an occlusive wrap (Cragg, 2012; Rowe, 1947; Krasavage and Terhaar, 1981). In rabbits, ocular administration of 500 mg DEGEE has produced moderate irritation

Table 4
DEGEE acute toxicity summary (external publications).

Route	Species	Effect
Oral	Rabbit	LD_{50} = 3620 mg/kg
	Mouse	LD_{50} = 7250 mg/kg
	Rat	LD_{50} = 7500 mg/kg
	Guinea Pig	LD_{50} = 3000 mg/kg
Intravenous	Cat	LD_{Lo} = 1000 mg/kg
	Dog	LD_{50} = 3000 mg/kg
	Rat	LD_{50} = 4000 mg/kg
	Mouse	LD_{50} = 4300 mg/kg
	Rabbit	LD_{50} = 2500 mg/kg
Intraperitoneal	Rat	LD_{50} = 6300 mg/kg
	Mouse	LD_{50} = 3900 mg/kg
		LD_{50} = 2300 mg/kg
Subcutaneous	Rat	LD_{50} = 6000 mg/kg
	Mouse	LD_{50} = 5500 mg/kg
	Rabbit	LD_{50} = 2000 mg/kg

Source: Leadscope Portal Dataset, Version 3.1.2-1, Accessed March 18, 2014.

Table 5
Acute toxicity studies conducted with Transcutol® (previously unpublished data from studies conducted by Gattefossé).

Study type/duration	Route	Species	Test article	Results/conclusion
Acute toxicity	Oral (gavage)	Rat	Transcutol® Pure (undiluted) Dose levels: 5000 mg/kg	$LD50_{(oral)}$ > 5000 mg/kg
Acute toxicity (Dose escalating)	Oral (gavage)	Dog	Transcutol® Pure (undiluted) Dose levels: 500, 1000, 1500, 2000 mg/kg	$MTD_{(oral)}$ > 2000 mg/kg
Acute toxicity (Dose escalating)	IV bolus (tail vein)	Mouse	Transcutol® Vehicle: Physiological saline solution M: 25, 50, 100, 200, 400, 800, 1600, 6400, 3200 and 4800 mg/kg F: 25, 50, 100, 200, 400, 800, 1600, 8000, 6400, 4800 and 3200 mg/kg	$MTD_{(IV)}$: 3200 mg/kg

(Cragg, 2012; Union Carbide Corporation, 1968). When used as in vaginal and nasal gels and emulsions in rabbits with repeat doses, it has not shown itself to be an irritant (Mourtas et al., 2010; Elshafeey et al., 2009).

GLP-compliant primary irritation single patch and a repeat insult patch tests in human performed by Gattefossé (1992, 1993) showed that undiluted DEGEE (as Transcutol®) applied under occlusive conditions was well tolerated and did not lead to any classifiable primary or cumulative skin irritation. A skin irritation study in rabbits, using an older, less pure form of DEGEE (as Transcutol®), which was not performed under GLP conditions but exceeded the current guideline requirements (OECD 404) in respect to animal numbers and can be considered as scientifically valid, showed that a 50% aqueous solution was not a skin irritant (Gattefossé, 1974). Guideline-conforming (OECD 405, EEC 92/69) eye irritation studies in rabbits performed under GLP conditions, revealed only a slight irritant effect to the eyes, when tested neat or as 30% aqueous solution (Gattefossé, 1996a). However, as the observed findings were only slight and transient in nature, and were not sufficient to be considered an eye irritant according to EU classification criteria (mean score of 2.00 for acute ocular irritation), it is concluded that DEGEE (as Transcutol®) is not an eye irritant.

Intravenous administration of 1 mL/kg or less of aqueous solutions containing concentrations of 5% or less is not hemolytic. *In vitro* hemolysis studies of a range of excipients showed no hemolysis caused by DEGEE (as Transcutol®) at concentrations up to 80 µl/ml (Aparicio et al., 2005.) Intramuscular injection of 30% oily solution and 50% aqueous solutions of DEGEE (as Transcutol®) causes moderate but reversible irritation. Microemulsions containing DEGEE (as Transcutol®) have been shown to not be irritating to veins when given intravenously (He et al., 2010).

4.3. Acute toxicity studies

Both external publications (see Table 4) and studies conducted by Gattefossé (see Table 5) indicate that the acute toxicity of DEGEE after oral, intraperitoneal, intravenous, and subcutaneous application can be regarded as very low in all species investigated. The LD_{50} values for acute toxicity were generally much higher than 2000 mg/kg bw, and the available LC_{50} value for acute inhalation was >5 mg/L (i.e. 5.24 mg/L).

4.4. Repeat-dose toxicity studies

4.4.1. Previously published oral data

A six week study was conducted in which groups of 10 male Sprague–Dawley rats were administered DEGEE by oral gavage at doses of 1340, 2680, and 5360 mg/kg/day. In the high dose group, four animals died before study termination and 3 were terminated moribund. Seven animals had bloody urine at various times throughout the study. Several other hematological and clinical chemistry signs were observed. One death also occurred at the intermediate dose prior to study termination. Lethargy was noted during the first week of treatment. However, there were no significant effects of treatment with the intermediate dose on hematology or clinical chemistries. Increased organ weights seen include the relative liver, heart, and kidney weights (but not absolute weights of these organs) with respect to control. Microscopic changes included hyperkeratosis of the stomach (2/10), and splenic congestion (1/9). Because no effects were seen at the lowest dose, the NOAEL was established as 1340 mg/kg/day (European Chemicals Bureau, 2000; OECD, 2005).

In a further study, groups of 15 male and female CFE rats were fed DEGEE at doses 250 and 2500 mg/kg bw (0.5% and 5.0% in the diet, respectively) for 90 days. Effects observed at the high dose included reductions in growth rate and food consumption as well as the average male final body weight. Decreased hemoglobin concentration of high dose males was seen at 90 days and the hemoglobin concentration and red blood cell count were decreased in females at 45 days. In high dose males and females, oxalate crystals in urine were observed. Increased relative kidney weights were seen in high dose males and females and the spleen and thyroid of high dose females were increased. Advanced intracellular edema (hydropic degeneration) of the kidney was reported in 6 high dose males and 1 high dose female. Calcification of the renal cortex was reported in three high dose males and 1 high dose female. Based on these effects, the NOAEL was determined to be 250 mg/kg bw (Gaunt et al., 1968).

Groups of 12 male and 12 female Wistar rats received diet containing 0%, 0.25%, 1.0%, and 5.0% DEGEE for 13 weeks. Decreased growth of male and female rats, which was associated with a reduction in food consumption, was seen in high-dose rats. No hematological changes were seen in any dose group. Males and females given 5% test material had elevated urinary glutamic-oxaloacetic transaminase and kidney weights compared to controls. High dose males also had proteinuria. Hydropic degeneration was seen in the kidneys of two high dose males and one high dose female. Slight to moderate fatty changes in the liver were seen in most high dose animals (incidences not provided). Because no treatment-related effects were seen in 0.25% or 1.0% dose groups, the NOAEL in this study was 1.0% in the diet corresponding to about 800 mg/kg bw (Hall et al., 1966).

Wistar rats were exposed orally to a blend of Labrasol, Labrafil, and Transcutol® (L/L/T) at dose levels of 0, 5, 10, or 20 mL/kg/day (approximately 0, 1000, 2000, and 4000 mg/kg/day Transcutol®) for four weeks to evaluate the safety of the formulation for use in *in vivo* non-clinical safety assessment studies for poor water soluble drugs. The blend was well tolerated at 5 mL/kg/day. In the mid-dose group, changes in appearance and behavior were seen. Lethality occurred in one animal at 20 mL/kg/day. In addition, renal and hepatic effects were also seen at 20 mL/kg/day. The authors

concluded that 5 mL/kg/day of the blend (including approximately 1000 mg/kg/day Transcutol®) was acceptable for use as a vehicle for poorly water soluble drugs in Wistar rat toxicity studies (Delongeas et al., 2010).

Groups of 20 male and 20 female CD-mice were fed a diet with levels of 0%, 0.2%, 0.6%, 1.8%, or 5.4% (approximately 300, 900, 2700 and 8100 mg/kg bw) DEGEE in food for 90 days. Six of 20 males fed 5.4% died with signs of advanced intracellular edema (hydropic degeneration) of the kidney. Increased relative kidney weights were seen in males treated with 1.8% or 5.4% and females treated with 5.4%. Relative liver and heart weights and absolute brain weight of high dose males were also increased. In the high dose group treatment was associated with a decrease in red blood cells (males), liver cell enlargement (8 males and 5 females), protein inclusions in the lumen of the bladder (8 males), and submucosal inflammatory cell infiltration of the bladder (3 males). Tubular degeneration and atrophy was noted in 13 high dose males and 8 females (compared with 6 control males and 3 control females). Because no treatment related effects were seen at 0.6% (approximately 850–1000 mg/kg bw/day), this level was identified as the NOAEL (Gaunt et al., 1968).

In another study conducted by Gaunt et al. (1968), pigs (strain not specified) were fed DEGEE in the diet at doses of 0, 167, 500, or 1500 mg/kg/day for 90 days. The 1500 mg/kg/day dose was reduced to 1000 mg/kg bw/day after 21 days due to severe toxicity. Three pigs given 1500 mg/kg bw for 14–21 days died or were killed with symptoms of uremia. Histopathological effects observed included hydropic degeneration of liver and proximal tubules of the kidney in half of the females treated with 500 mg/kg/day and all animals treated with 1000–1500 mg/kg/day. Increased relative kidney weight was seen in males treated with 1000–1500 mg/kg/day. Decreased red blood cell counts were observed in males at the highest dose. Based on these effects, the NOAEL was determined to be 167 mg/kg/day.

In a 2-year dietary study, employing limited histopathological examination, 10 rats were fed a diet containing 2.16% of purified DEGEE corresponding to an estimated dose of 1000 mg/kg/day. The only adverse effects reported were a few oxalate crystals in a kidney of one animal, slight liver damage and some interstitial edema in the testes (Morris et al., 1942). With regards to current requirements and modern standards, the 2-year study conducted by Morris et al. (1942) is inadequate as documentation is poor, too few animals were tested and the types of tissues examined were limited. Therefore, the determination of a NOAEL or LOAEL and an assessment as to tumorigenicity were precluded.

Groups each of 8 weanling albino rats per sex were exposed to two grades of DEGEE in the drinking water through three generations (F0, F1 and F2) during a 2-year period. One grade contained 0.2% ethylene glycol and the other 29.5% ethylene glycol. The drinking water levels were 0%, 0.01%, 0.04%, 0.2%, and 1% (corresponding to about 0, 10, 40, 200 and 950 mg/kg/day). F1 and F2 generations received the same dosage levels as the parents, and all survivors were euthanized 718 days from the start of the test. The duration of exposure for the different rat generations was not reported. Based on the obtained results, the authors concluded that the grade of DEGEE containing 29.5% ethylene glycol was considerably more toxic than the purer grade containing 0.2% ethylene glycol. For the "toxic" group, 16 rats (8 males and 8 females) showed severe injury, notably kidney damage or bladder concretions. The authors concluded that the maximum safe dose of the material containing a large amount of ethylene glycol was 10 mg/kg, whereas it was about 200 mg/kg for the purer sample (Smyth et al., 1964). As with the 2-year study conducted by Morris et al. (1942), no NOAEL or LOAEL is determined due to poor documentation, a small number of animals, and examination of a limited number of tissues.

4.4.2. Previously published inhalation studies

In a 28-day nose only inhalation study, groups of five male and five female Sprague–Dawley rats were exposed to 0, 16, 49, or 200 ppm (0, 90, 270, 1100 mg/m^3) DEGEE for 6 h/day, 5 days/week. No systemic effects were observed for any dose group. However, mild local irritation of the larynx and nasal turbinates were found in some rats (numbers were not stated). In the small ventral cartilage of the larynx, focal necrosis was observed in 2/5 or 3/5 males inhaling 270 or 1100 mg/m^3, respectively. In this study, 1100 mg/m^3 was higher than the maximum concentration at which only vapor was present. The NOAEL from this study for systemic toxicity was determined to be 1100 mg/m^3 (Hardy et al., 1997).

No adverse effects were seen mice, rats, guinea pigs, rabbits, and cats during daily exposure to an atmosphere saturated with DEGEE for 12 days (SCCP, 2006).

SCCP (2006, 2013) reviewed a study in which rats were exposed continuously to 0.2, 1 or 4 ppm (1, 5, or 25 mg/m^3) DEGEE. The original study was not available. According to the review, the study was poorly reported by the initial authors (Krotov et al., 1981) and is provided here as supporting information regarding inhalation. Changes in the functional state of the nervous system (without narcosis) were reported during both the treatment and the recovery periods in rats exposed to $\geqslant$5 mg/m^3. Analysis of blood samples was said to reveal indications of anemia and changes in the differential white blood cell count and in the concentrations of urea, lactic acid and pyruvic acid. Increased liver weight was noted in animals sacrificed prior to the end of the treatment period. While it is unclear which groups were affected, SCCP (2013) reported that the findings were confined mainly to rats receiving $\geqslant$5 mg/m^3. The reviewers further noted that because the continuous nature of the exposure is unlikely to reflect true exposure situations and the limited reporting of the study, no conclusions were drawn from this study.

4.4.3. Previously published intramuscular data

According to a review by the European Chemicals Bureau (2000), no treatment-related effects were seen in rabbits following intramuscular administration of DEGEE at dose levels of 0, 0.62, 0.82, or 1.6 mL/kg/day for 14 days. No clinical signs were seen and no treatment related effects were seen for hemoglobin concentrations, erythrocytes, leukocytes, platelets, or reticulocytes. Therefore the NOAEL for this study was determined by the reviewers to be 1.6 mL/kg/day (equivalent to 1582 mg/kg/day). The original study performed by Hanzlik et al. (1947) was not available.

4.4.4. Previously unpublished studies conducted by Gattefossé

The repeat-dose toxicity of DEGEE (as Transcutol®) has been investigated by Gattefossé in subacute (7-day, Gattefossé, 2007a) and subchronic (13-week, Gattefossé, 2007b) oral dog studies using characterized test material. The 13-week study was conducted under GLP conditions and exceeding OECD 408 guidelines. The 7-day dog study was a range finding study and was not performed according to GLP conditions. In general, the observed toxicity was low across both studies (see Table 6).

In the 7-day oral (gavage) toxicity range-finding study in female Beagle dogs (2 animals per dose group), DEGEE (as Transcutol®) was dissolved in deionized water and administered once daily at dose levels of 500, 1000 and 2000 mg/kg bw. The concurrent control group received the vehicle (deionized water). No mortality occurred during the study. No treatment-related clinical findings, hematological findings, organ weight changes, or substance induced macroscopic or microscopic findings were observed.

Table 6
Repeat-dose toxicity studies conducted with DEGEE (previously unpublished data from studies conducted by Gattefossé).

Study type/duration	Route	Species	Test article	Results/conclusion
Subacute 7-day study (DRF)	Oral (Gavage)	Dog	Transcutol® Dose levels: 0, 500, 1000 and 2000 mg/kg/day	$MTD_{(oral)}$ > 2000 mg/kg/day
Subchronic 13-week study	Oral (Gavage)	Dog	Transcutol® Dose levels: 0, 400, 1000, 2000/1500 mg/kg/day	$NOAEL_{(oral)}$ = 1000 mg/kg/day

Table 7
Renal lesions in male and female beagle dogs administered Transcutol® orally by gavage once daily for 13 weeks.

	0 mg/kg/day	400 mg/kg/day	1000 mg/kg/day	1500 mg/kg/day[a]
Males				
Unscheduled deaths				
Crystals				
Present	0/0	0/0	0/0	2/2
Tubular degeneration				
Moderate	0/0	0/0	0/0	1/2
Severe	0/0	0/0	0/0	1/2
Scheduled deaths				
Unremarkable	5/6	3/4	4/4	3/4
Basophilic tubules				
Minimal	0/6	1/4	0/4	1/4
Fibrosis				
Severe	1/6	0/4	0/4	0/4
Females				
Unscheduled death				
Crystals				
Present	0/0	0/0	0/0	1/1
Tubular degeneration				
Severe	0/0	0/0	0/0	1/1
Scheduled deaths				
Unremarkable	5/6	4/4	3/4	5/5
Basophilic tubules				
Minimal	1/6	0/4	0/4	0/5
Infiltrate, lymphocyte				
Minimal	0/6	0/4	1/4	0/5

[a] Animals received 2000 mg/kg/day on Days 0–6 and 11–15 for males and Days 0–14 for females. Because of unscheduled deaths occurring at 2000 mg/kg/day, dosing was suspended on Days 7–10, 16–20 for males and Days 15–19 for females. Dosage was lowered to 1500 mg/kg/day and dosing resumed on Days 21 and 20 for males and females, respectively.

A slight decrease in body weight (<3% compared to study day 0) was seen at 2000 mg/kg bw from study day 0 to 6, which correlated to slightly lower individual food consumption, but was not conclusively test substance-related or considered adverse due to the small magnitude of change. Slightly impaired clinical chemistry parameters reported included decreased serum potassium, higher urine sodium, potassium and chloride excretions and higher urine volume in the 2000 mg/kg bw group females on study day 7. However, due to the small number of animals, the clinical chemistry changes could not be clearly associated to the treatment regimen. Because DEGEE was well tolerated at all dose levels, a maximum tolerated dose (MTD) of DEGEE (as Transcutol®) for oral administration to female Beagle dogs for 7 consecutive days was not achieved. Potential changes induced by the test article at the highest dose levels of 2000 mg/kg bw included only lower serum potassium and higher urine sodium, potassium and chloride excretions and higher urine volume (Gattefossé, 2007a).

In the subsequent subchronic (13-week) oral toxicity study in beagle dogs, initial dosage levels of 400, 1000 and 2000 mg/kg bw of DEGEE (as Transcutol®) were investigated. Because the initial high dose of 2000 mg/kg bw was severely toxic and resulted in premature mortality for 2 males and 1 female within the first 2 weeks, the high dose was reduced to 1500 mg/kg bw. Prior to the euthanization of these 3 animals, clinical observations included hypoactivity, thinness, diarrhea, decreased activity, impaired equilibrium, excessive salivation, body cool to touch, and emesis. Decreased food consumption and body weight losses were also reported for these animals. Histological examination revealed the morbidity for these dogs was primarily due to severe renal tubular degeneration in the kidney (see Table 7). After reduction to 1500 mg/kg bw no further deaths occurred and no animals of the other groups died prematurely (Gattefossé, 2007b).

Slightly decreased terminal body weights were seen in the 1000 and 2000/1500 mg/kg bw females with no correlating decrease in food consumption. No adverse findings in clinical pathology parameters were seen in any dose group. Slightly increased liver weights were observed at 1000 mg/kg bw in females and at 2000/1500 mg/kg bw in both sexes but were considered as an adaptive response rather than a sign of toxicity (Gattefossé, 2007b).

Histopathology revealed no organ-specific microscopic finding at any dosage level for the animals surviving to the scheduled necropsy. Since there was no histological effects in the animals where exposure was reduced from 2000 mg/kg bw to 1500 mg/kg bw, the histological changes in the unscheduled deaths in animals treated with 2000 mg/kg bw were considered reversible. Furthermore, the 1500 mg/kg bw dose level was well tolerated while being administered for 10 consecutive weeks. Thus, the NOAEL for oral administration of DEGEE (as Transcutol®) for 13 weeks was at least 1000 mg/kg bw, the highest dosage level administered consecutively for the entire duration of the study (Gattefossé, 2007b).

4.5. Reproductive and developmental toxicity

4.5.1. External data

In a developmental toxicity screening assay, 50 mated CD1 mice were administered 5500 mg/kg/day DEGEE (>99% purity) in corn

Table 8
Reproductive/developmental studies conducted with Transcutol® (previously unpublished data from studies conducted by Gattefossé).

Study type	Route	Species	Test article	Results/conclusion
Fertility study (Segment I)	Oral (Gavage)	Rat	Transcutol®	$NOAEL_{(oral)}$: 2000 mg/kg/day
			Vehicle/control: sterile water	No effects on gonodal function, fertility and reproductive performance for dose levels up to 2000 mg/mg/day
			Dose levels: 0, 300, 1000, 2000 mg/kg/day	
Embryo/fetal development study (Segment II)	Oral (Gavage)	Rat	Transcutol®	$NOAEL_{(dev,\ mat)}$: 1000 mg/kg/day
			Vehicle/control: sterile water	No indication of teratogenicity for dose levels up to 2000 mg/kg/day
			Dose levels: 0, 300, 1000, 2000 mg/kg/day	

oil orally by gavage from gestation day (GD) 7–14. The mice were allowed to litter and to rear pups to post natal day (PND) 3. Fourteen percent maternal mortality was seen following exposure. Maternal weight gain was decreased. Viable litters were seen in 32 of 33 surviving pregnant females (97%) compared with 100% control litter viability. No external malformations were seen and pup survival was unaffected. Treatment with 5500 mg/kg bw DEGEE was not associated with any developmental toxicity (Schuler et al., 1984).

Developmental toxicity was also investigated in groups of 15 and 20 Sprague–Dawley rats exposed to DEGEE (98–99.5% purity) by whole body inhalation at concentrations of 0 or 102 ppm, respectively, for 7 h/day on GD 7–15. Females were weighed and euthanized on Day 20. The uterus was removed and the numbers of resorption sites (early, middle or late), and live fetus numbers and sex, fetal weight, external malformations, visceral malformations (two-thirds of the fetuses), and skeletal defects (remaining one third of fetuses) were determined. No maternal or fetal toxicity was reported including no effect of treatment on the number of pregnancies, implants/dam, litters with resorptions, live fetuses, live fetuses/litter, live fetal weight, or the number of litters with abnormal fetuses, number of fetuses with visceral or skeletal malformations or variations. Exposure to 102 ppm DEGEE was not associated with developmental toxicity in rats (Nelson et al., 1984).

4.5.2. Previously unpublished studies conducted by Gattefossé

In addition to the previously conducted reproductive and developmental studies summarized above, Gattefossé has conducted a segment I reproductive study (Gattefossé, 2001b) and a segment II developmental study (Gattefossé, 2002b) in rats. The GLP-compliant studies were conducted according to ICH S5(R2) guidelines using characterized and analyzed test material (see Table 8).

In female Sprague–Dawley rats, oral administration of up to 2000 mg/kg/day DEGEE (as Transcutol®) was well tolerated within the fertility and general reproductive performance study. However, in males minor effects on clinical condition and body weight were observed at > 1000 mg/kg/day. There were no test article related effects on gonadal function or fertility and reproductive performance in any group. The NOAEL for fertility and general reproductive performance was 2000 mg/kg/day, while the NOAEL for systemic toxicity was 1000 mg/kg/day (Gattefossé, 2001b).

In the prenatal developmental toxicity study, the oral administration of DEGEE (as Transcutol®) to pregnant Sprague–Dawley rats from implantation to GD 17 resulted in decreased maternal body weight gain and decreased maternal food consumption at 2000 mg/kg/day. Gestation was not affected at any dose level. Prenatal developmental toxicity was seen at 2000 mg/kg/day in the form of minor skeletal findings (reduced cranial bone ossification as an indication of transiently retarded development). There was no specific pattern of substance-related teratogenicity at any dose level. The overall NOAEL for maternal toxicity as well as for prenatal developmental toxicity was 1000 mg/kg/day (Gattefossé, 2002b).

4.6. Genotoxicity studies

In previously published data, *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 showed no mutagenic results, when tested in the range of 3–20 mg/plate with and without metabolic activation (OECD, 2005). However, there exists also a poorly reported study, where DEGEE of unknown purity was tested in *S. typhimurium* strains TA97, TA100, TA102, TA1535, TA1537, TA1538 with and without metabolic activation at concentrations of 0, 0.01, 0.1 and 1 ml/plate (Berte et al., 1986). The authors concluded that DEGEE was weakly positive for mutagenicity in *S. typhimurium* strains TA1535, TA537 and TA1538 and negative for strains TA97, TA100, and TA102, with and without activation. Furthermore, DEGEE was negative for mutagenicity in *Saccharomyces cerevisiae* D7 yeasts and did not induce micronuclei in CD-1 mice *in vivo* (Berte et al., 1986).

In previously unpublished studies, DEGEE (as Transcutol®) was tested for mutagenicity/genotoxicity in a range of validated and/or scientifically reasonable GLP studies *in vitro* and *in vivo* (see Table 9). No genotoxic/mutagenic potential was noted in reliable

Table 9
Genotoxicity/mutagenicity studies conducted with DEGEE.

Study type/duration	Dose	Species/strains	Results/conclusion
In vitro			
Ames test (With and without activation)	Transcutol® 52–5000 μg/plate	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	Negative; not mutagenic
In vivo			
DNA damage and repair (UDS: Unscheduled DNA synthesis in mammal), Oral Route	Transcutol® 800, 2000 mg/kg	Rat/Wistar	Negative; not genotoxic for dose levels up to 2000 mg/kg/day
Mammalian Erythrocyte Micronucleus Test[a]	DEGEE 2 mL/kg	Mouse	Negative. Not clastogenic at 2 mL/kg

[a] Berte et al. (1986).

bacterial gene mutation assays *in vitro* with *Salmonella typhimurium* in the presence or absence of metabolic activation (Gattefossé 1999). The GLP-compliant bacterial gene mutation assays were conducted in conformance with OECD 471, EEC92/69 guidelines. *In vivo,* DEGEE (as Transcutol®) did not possess a mutagenic/genotoxic potential covering two independent endpoints. No indication of clastogenicity was observed in a GLP-compliant (OECD 486) limited micronucleus tests in mice after intraperitoneal injection and no increased repair synthesis as measure of DNA damage in the hepatocytes of the treated rats was observed (Gattefossé, 1996b). Based on the weight of the evidence, DEGEE can be considered to be of no genotoxic/mutagenic risk to humans.

4.7. Carcinogenicity

Limited data exists concerning the carcinogenic effects of DEGEE. In a 2-year rat dietary feeding study of 2.16% (approximately 1000 mg/kg bw/day) to 10 rats, DEGEE induced toxicity to the liver and kidney but no tumor data was reported (Morris et al., 1942). A 718-day drinking water study in groups of 8 Wistar rats/sex exposed to up to 950 mg/kg bw indicated that no treatment-related increase in tumors was seen. However, individual tumor data were not provided (Smyth et al., 1964). Both studies suffer from inadequacies including poor documentation, too few animals tested and limited tissue examinations. However from the full battery of studies conducted for DEGEE, including genotoxicity and mutagenicity studies, as well as the long history of safe use in humans, there are no indications that DEGEE is carcinogenic. Therefore, no carcinogenic effects are expected in association with DEGEE exposure.

Because the available data are insufficient to allow a definitive opinion as to the carcinogenicity of DEGEE, an *in silico* method was employed to evaluate the toxicity of DEGEE. The potential carcinogenicity, as well as genotoxicity and mutagenicity, for DEGEE was evaluated using a SAR analysis of the structure by means of Derek 3.0.1, Nexus 1.5.0 (Lhasa Ltd.), a well-recognized expert rule-based system for the prediction of toxicity (Dobo et al., 2012; Sutter et al., 2013). No alerts of potential carcinogenicity or genotoxicity (including mutagenicity in bacteria) were indicated for DEGEE, providing supportive evidence of a lack of carcinogenic effects in association with exposure to DEGEE.

5. Discussion

The available literature shows that DEGEE has a long history of safe use in pharmaceuticals, food additives, and cosmetic and personal care products. Toxic effects reported in humans are limited with central nervous and respiratory injury, thirst, acidosis, and albumin in the urine reported in an individual following accidental ingestion of 300 mL of liquid that contained 47% DEGEE and less than 0.2% methanol (Cragg, 2012).

In addition, DEGEE has been extensively studied in animals of both genders and is well tolerated with toxicity only occurring at levels well above those in products intended for human use. With the purified form provided as Transcutol®, toxicity concerns reported in the past (e.g. attributed to the presence of significant amounts of impurities, most likely ethylene glycol) for less pure forms of DEGEE have not been replicated in more recent studies performed using purified DEGEE (as Transcutol®). For ethylene glycol the kidney has been clearly identified as the target organ of toxicity. The formation of calcium oxalate crystals, and possibly other metabolic products, in the renal tubules following exposure to ethylene glycol leads to oliguria, acute tubular necrosis, and ultimately renal failure (Cavender, 2012; Devlin and Schwartz, 2014). These effects are similar to those seen in nonclinical studies performed with less pure forms of DEGEE and provide supporting evidence that toxicity seen in these studies is likely due to the presence of significant amounts of ethylene glycol as an impurity.

The pharmacokinetics for DEGEE indicates that it is well absorbed, up to 52% via the dermal route. Following absorption, DEGEE is rapidly and largely metabolized to ethoxyethoxyacetic acid (83%) with a much smaller amount (5.4%) being metabolized to diethylene glycol. The majority of the metabolites, and a small amount of the unchanged compound, are excreted in the urine within the first 24 h following exposure.

In animals, observed acute oral, IV, and dermal toxicity generally occurred only at extremely high doses, greater than 2000 mg/kg bw. DEGEE is not a skin, mucous membrane, vein or eye irritant. It is negative for genotoxicity both *in vivo* and *in vitro*. Transcutol® did not cause reproductive toxicity in rats at doses up to 2000 mg/kg bw. Developmental toxicity was noted following exposure to high doses of DEGEE (as Transcutol®). Following exposure of maternal rats to 2000 mg/kg DEGEE (as Transcutol®) from implantation to GD 17, minor skeletal findings in the form of reduced ossification of cranial bones were noted in fetuses. In IV formulations, levels of Transcutol® up to 5% in formulations are not hemolytic.

Repeat-dose subchronic studies were conducted in a number of species including rats, mice, pigs, and dogs. DEGEE was generally well tolerated across species at doses up to 1000 mg/kg. Gaunt et al. (1968) conducted studies in mice, rats, and pigs and noted effects in pigs at a lower dose than seen with other species. In pigs dosed with 500 or 1000–1500 mg/kg/day DEGEE in the diet for 90 days, hydropic degeneration of the liver and proximal tubules of the kidney were seen as were increased relative kidney weight and decreased red blood cell counts at 1000–1500 mg/kg/day DEGEE (Gaunt et al., 1968). However, given the time at which this study was conducted, it is likely that the effects seen in the pigs were due to the presence of significant amounts of the impurity ethylene glycol. Although the purity of DEGEE was not stated for this study, it was reported to contain up to 0.4% ethylene glycol. The previously unpublished study conducted by Gattefossé (presented here) in dogs and using a more purified version of DEGEE provides a better assessment of the toxicity of purified DEGEE without ethylene glycol present. Initially, the highest dose administered orally by gavage to Beagle dogs was 2000 mg/kg bw. However, within the first 2 weeks of daily exposure, 2/6 males (Day 7) and 1/6 female (Day 15) from the high-dose group were euthanized in extremis. Prior to euthanization, clinical observations were noted in these 3 animals and included hypoactivity, thinness, diarrhea, decreased activity, impaired equilibrium, excessive salivation, body cool to touch, and emesis. In addition, both males and the single female showed decreased food consumption and body weight losses from the beginning of the study until the day of euthanasia. Kidney histopathology data are summarized in Table 7 and for animals with unscheduled deaths revealed moderate to severe renal tubular degeneration, which was determined to be the most probable cause of morbidity and is consistent with the target organ toxicity for DEGEE seen in other studies at high doses. A decrease of the highest dose from 2000 to 1500 mg/kg on Day 20–21 resulted in no treatment-related clinical or histological effects for the remaining part of the study, indicating a recovery for the surviving animals. Although the chronic/carcinogenic nonclinical data are limited, carcinogenic effects are not expected to be associated with exposure to DEGEE. The structure-based *in silico* assessment performed for DEGEE did not provide any positive carcinogenic or genotoxic predictions for DEGEE, and therefore, provides supporting evidence that DEGEE is not a carcinogen. While both genders were evaluated in the systemic toxicity studies, no difference in sensitivity to toxicity or in target organs was seen.

In conclusion, the nonclinical study data summarized above in combination with a long history of DEGEE use in food, pharmaceutical,

and personal care products provide evidence for the safety of DEGEE in humans. Toxicity reported in older (all but the most recent) studies for DEGEE were most likely due to a significant amount of impurities, specifically ethylene glycol. The presence of a significant amount of diethylene glycol as a metabolite in fate and excretion studies suggests that the differences in toxicity between impure and purified DEGEE is not likely due to diethylene glycol impurity. Recent advances in the formulation process for Transcutol® have reduced impurities to less than 0.1% for pharmaceutical-grade Transcutol®. Herein we have reviewed the existing published data for DEGEE as well as presented previously unpublished data performed by Gattefossé using pharmaceutical-grade DEGEE (as Transcutol®). Using a NOAEL of 1000 mg/kg/day obtained from the 13-week study in Beagle dogs and a safety factor of 100 we derive a conservative safe exposure level in humans or Permissible Daily Exposure (PDE) of 10 mg/kg/day (or 600 mg/day in a 60 kg human) for Transcutol® by the oral route. Similarly, using the Intramuscular NOAEL of 1582 mg/kg/day obtained from the 14-day study in rabbits and a safety factor of 300 we derive a human PDE of 5.3 mg/kg/day (318 mg/day in a 60 kg human) for Transcutol® by the intravenous route. Because of the conservative nature of the PDE, i.e., the calculations assume that Transcutol® will be ingested (or injected) daily over a lifetime, this does not constitute a maximum safe level, as higher exposures may be safe when used for a shorter duration. Safe levels for the use of Transcutol® as an excipient should be evaluated on a per drug product basis and take into account factors such as route and duration of exposure.

Conflict of Interest

This work was supported by Gattefossé. However, the views presented in this paper are strictly those of the authors and do not necessarily reflect the positions of the sponsor.

Transparency Document

The Transparency document associated with this article can be found in the online version.

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