



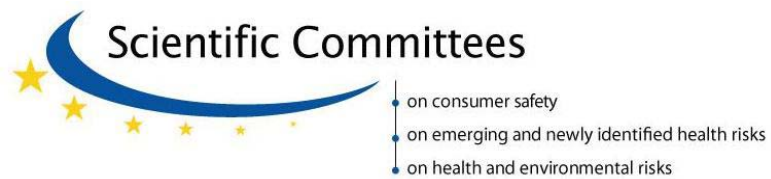
Scientific Committee on Consumer Safety

SCCS

OPINION ON

DIETHYLENE GLYCOL MONOETHYL ETHER

(DEGEE)



The SCCS adopted this opinion at its 8th plenary meeting
of 21 September 2010

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Evaluation Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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1. BACKGROUND

A risk assessment on diethylene glycol monoethyl ether (DEGEE) with the chemical name 2-(2-ethoxyethoxy)ethanol and the INCI-name Ethoxydiglycol was done by a Member State (France). The risk assessment was based mainly upon data from the open scientific literature and on the skin absorption studies performed by industry. This risk assessment led the Member State to put certain restrictions on the use of this substance.

According to the notification to the Commission, DEGEE is currently used in cosmetic products in shampoos (rinse-off) in a concentration up to 5% and in creams (leave-on) in a concentration up to 2%, excluding products intended to be used in the vicinity of the eyes. The notifying Member State concluded that the substance could be considered safe for the consumers, when used in a concentration up to 1.5% in cosmetics product except products for oral hygiene.

As a consequence of the notification, the SCCP was asked to give its opinion on Diethylene glycol monoethyl ether (DEGEE). The first opinion was adopted on 19 December 2006 by the SCCP (SCCP/1044/06) with the following conclusion:

"Based on the information provided, the SCCP is of the opinion that the use of diethylene glycol monoethyl ether (DEGEE) in all cosmetic products, except products for oral hygiene and eye products at a concentrations up to 1.5% does not pose a risk to the health of the consumer, provided that the level of ethylene glycol in DEGEE used is < 0.2%. The opinion relates to the dermal application. It does not include any other cosmetic exposure, such as exposure from possible aerosol/spray products."

In December 2007 an updated risk assessment was submitted by COLIPA¹. In addition to the overall use of DEGEE in a concentration up to 1.5%, the applicant applied for a specific use of DEGEE as a solvent in hair dyes formulations at a concentration up to 7.0% and to support this application, additional percutaneous absorption data was provided.

A second opinion (SCCP/1200/08) on Diethylene glycol monoethyl ether (DEGEE) for its use as solvent in hair dye formulations was adopted on 16 December 2008 with the conclusion that:

"The SCCP is of the opinion that the use of diethylene glycol monoethyl ether (DEGEE) as a solvent in an on-head concentration of up to 7.0% in oxidative hair dye formulations and in an on-head concentration of up to 5.0% in non-oxidative hair dye formulations in addition to the use of DEGEE at concentrations up to 1.5% in all cosmetic products except products for oral hygiene and eye products does not pose a risk to the health of the consumer, provided that the level of ethylene glycol in DEGEE used is < 0.2%."

The opinion relates to the dermal application of cosmetic products only and does not include any other cosmetic exposure, such as exposure from possible aerosol/spray products.

Aggregate exposure to diethylene glycol monoethyl ether (DEGEE) from non-cosmetic sources has not been considered".

In January 2009 an additional submission was presented by EFFCI², providing a subchronic 3-month toxicity study in dogs. Based on this dossier, the applicant considers that DEGEE can be used safely in concentration up to 5.5% in leave-on products and up to 10.0% in rinse-off products.

¹ COLIPA – The European Cosmetic Association

² EFFCI The European Federation for Cosmetic Ingredients

2. TERMS OF REFERENCE

1. *Does the SCCS consider the use of DEGEE as a solvent in cosmetic products in a concentration up to 5.5% in leave-on products and/or in a concentration up to 10% in rinse-off products safe for the consumer, taken into account the provided scientific data?*
2. *Does the SCCS consider that an additional use of the substance DEGEE as solvent in an on-head concentration up 7.0% in oxidative hair dye formulations and in an on-head concentration up 5.0% in non-oxidative hair dye formulations, is safe, taken into account the provided data?*
3. *Does the SCCS have any further scientific concerns with regard to the use of DEGEE?*

3. OPINION

The present Opinion contains the information provided for the previous Opinions on DEGEE adopted by SCCP on 19 December 2006 (SCCP/1044/06) and 16 December 2008 (SCCP/1200/08) with additional data from a recent Submission. References from the new submission are referred to as N Ref.: xx, and are listed separately.

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

IUPAC name: 2-(2-Ethoxyethoxy)ethanol
 INCI name: Ethoxydiglycol

3.1.1.2. Chemical names

Diethylene glycol monoethyl ether, 3,6-Dioxa-1-octanol, Diethylene glycol ethyl ether, Diglycol monoethyl ether, Ethanol, 2,2'-oxybis-, monoethyl ether, Ethyl carbitol, Ethyl diethylene glycol, Ethyl digol,

3.1.1.3. Trade names and abbreviations

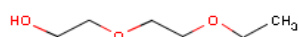
Carbitol, Carbitol solvent, Dioxitol, Dowanol 17, Dowanol DE, Ektasolve DE, Solvolisol, Transcutol CG, Transcutol P, Transcutol HP

DEGEE

3.1.1.4. CAS / EC number

CAS: 111-90-0
 EC: 203-919-7

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: C₆H₁₄O₃

3.1.2. Physical form

Liquid with a mild, pleasant odour; hygroscopic

3.1.3. Molecular weight

Molecular weight: 134.2

3.1.4. Purity, composition and substance codes

The applicant states that from May 1998, the manufacturing process of DEGEE was improved in order to decrease the content in residual impurities.

Transcutol CG: > 99.5% (cosmetics only)
 Transcutol P: > 99.7% (pharmaceutical, topical forms)
 Transcutol HP: > 99.9% (pharmaceutical, other administration routes)

3.1.5. Impurities / accompanying contaminants

Impurities: Ethylene glycol. Commercial products may contain an appreciable amount of ethylene glycol (CAS No. 107-21-1).

Transcutol CG contains < 0.062% (620 ppm) ethylene glycol.

3.1.6. Solubility

In water: Miscible

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: - 0.54 (exp)

3.1.8. Additional physical and chemical specifications

Appearance:	colourless liquid
Melting point:	- 76 °C
Boiling point:	197 – 205 °C
Density:	0.988
Rel. vapour density:	/
Vapour Pressure:	0.19 hPa

Conversion:

1 ppm = 5.58 mg/m³

1 mg/m³ = 0.179 ppm

3.1.9. Stability

Shelf life: At least 3 years of storage under recommended conditions of original hermetically closed container (The product is packed under nitrogen and must be used shortly after opening).

3.2. Function and uses

DEGEE may be prepared from ethylene oxide and 2-ethoxyethanol in the presence of SO₂. It is used in the chemical and paint industries as a solvent for nitro cellulose, resins, and dyes. DEGEE is not used in food or detergent products.

Purified DEGEE (>99%) is used in cosmetics and dermatological preparations and as solvent in some medicine products. Its physical properties make DEGEE useful to solubilise lipophilic and hydrophilic compounds. Moreover DEGEE enhances the percutaneous absorption through the skin and mucosal barriers. It is used in some drugs to enhance absorption.

In its previous opinions (SCCP/1044/06, SCCP/1200/08), the SCCP positively evaluated the use of DEGEE in cosmetic products up to 1.5% and in hair dyes up to 7.0% in oxidative and 5% in non-oxidative formulations, based on the data available at the time. According to the recent application to the Commission, the applicant requested to increase the maximal concentration of DEGEE as a solvent in cosmetic products in a concentration up to 5.5% in leave-on products and up to 10% in rinse-off products, based on new studies provided.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

The acute toxicity after oral administration of DEGEE has been determined in several experiments. The results are summarized in Table 3.1.

Table 3.1: *Acute toxicity after oral administration of DEGEE*

Species	LD ₅₀ (mg/kg bw)	Reference
Mouse	7410	1
Mouse	6580	2
Rat	7410	1
Rat	5400-5500	3
Rat	6000	4
Rat	6310	5
Rat	8690	6
Rat	5540	2
Rat	>5000	7
Guinea pig	3900	1
Rabbit	3600	8

Dogs

Date of report: June 2007
 Guideline/method: /
 Species/strain: Dog/Beagle
 Group size: 1 female
 Test substance: Transcutol® HP (purity: >99.9%)
 Batch: 450449013
 Dose levels: 500, 1000, 1500, 2000 mg/kg bw
 Dose volume: 5 ml/kg bw (500, 1000, 1500 mg/kg bw), 10 ml/kg bw (2000 mg/kg bw)
 Vehicle: Deionized water
 Route: Oral (gavage)
 Exposure period: Single applications on days 0, 3, 6, 9 with increasing amounts of DEGEE
 GLP: Yes
 Study period: 10.10.06 – 07.06.07

Transcutol® HP was examined for its acute toxicity or tolerability in one female Beagle dog. The test substance was dissolved in deionized water and administered as a single dose orally by gavage on study days 0, 3, 6, and 9 followed by a 2-day non-dosing period before the next dose administration. Dose levels were 500, 1000, 1500 and 2000 mg/kg bw. The animal was observed twice daily for mortality and morbidity. Clinical examinations were performed daily. Detailed physical examinations were performed on the days of dosing and 2 days following the final dose administration. Body weight was recorded on the days of dosing (prior to dose administration) and 2 days following the last dose. Food consumption was recorded daily, beginning at least 1 week prior to randomization. This animal was transferred to the stock colony at the end of the study.

The authors reported that there were no test substance-related clinical findings or effects on body weight or food consumption during the escalating-dose phase of the study, where dose levels of 500, 1000, 1500 and 2000 mg/kg bw were administered. They concluded that the

oral (gavage) administration of Transcutol® HP to one female Beagle dog did not result in any test substance-related effects following single oral doses of 500, 1000, 1500 and 2000 mg/kg bw.

N Ref.: 35

Comments

It is noted that the study did only involve 1 dog. The experiment was terminated after 11 days.

Human

In an isolated case report, an alcoholic male (aged 44) drank approximately 300 ml of a liquid containing 47% DEGEE (about 2000 mg/kg). Severe symptoms of central nervous and respiratory injury (dyspnoea) thirst and acidosis occurred. The urine contained albumin. The subject recovered following symptomatic treatment.

Ref.: 9

3.3.1.2. Acute dermal toxicity

The acute toxicity after dermal administration of DEGEE has been determined in several experiments. The results are summarized in Table 3.2.

Table 3.2: *Acute toxicity after dermal administration of DEGEE*

Species	LD ₅₀ (mg/kg bw)	Reference
Mouse	6000	10
Rat	6000	10
Rabbit	8300	10
Rabbit	4200	11
Guinea pig	3200	11

The Hazard Substances Data Bank (HSDB) cites:

.....cosmetic preparations containing more than 5% Carbitol should not be used even for application to small areas of body ...use ... for this purpose may constitute an unexpected hazard, especially if applied to broken skin or in persons with renal disorders.

Ref.: 9

3.3.1.3. Acute inhalation toxicity

LC₅₀ rats = 5240 mg/m³

Ref.: 12

General comment

DEGEE has low acute toxicity by oral, dermal, and inhalation routes.

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

Rabbit

Guideline/method: French authority method (Journal Officiel 21 April 1971) exceeding the requirement of the current OECD 404
 Species/strain: Rabbit/New Zealand White
 Group size: 6 males
 Test substance: 50% aqueous diethylene glycol monoethyl ether (DEGEE)

Opinion on diethylene glycol monoethyl ether (DEGEE)

Batch: not stated
 Concentration: 50% 0.5 g of unchanged test substance
 Route: intact and abraded skin
 Exposure: 24 h
 Observation period: up to 72 h after patch removal
 GLP: No
 Date: April 1974

A 50% aqueous DEGEE solution was investigated for its acute dermal irritation potential in 6 male New Zealand White rabbits on abraded and intact skin. The hair was clipped on the dorsal

area of the trunk one day prior to application. Abraded skin areas were prepared by scarification with a 'vaccinostyle' (3 parallel incisions without damage to the dermis). An amount of 0.5 ml of the test substance was applied to the test site (0.25 ml/cm²) and held in place using a non-irritating tape covered by an occlusive dressing. The exposure lasted 24 h. The animals were examined for erythema/eschar and oedema as well as for other local or systemic signs of toxicity for up to 72 h after patch removal.

The authors reported that no signs of systemic toxicity, no mortality and no signs of irritation were noted during the whole observation period. The mean scores for erythema/eschar and oedema were 0.0 for each animal at each observation time point for the intact as well as for the abraded skin. They concluded that under the condition of this study, the 50% aqueous solution of DEGEE was shown to be non-irritating to the intact or abraded skin of New Zealand White rabbits.

N Ref.: 10

In addition to the study above, information exists on further skin irritation studies performed in rabbits and Guinea pigs. In these studies the tested substance revealed either a slight or no skin irritant effect in the experimental animals. However, it should be noted that only limited information on study methodology, substance characterization or details of results are available.

N Ref.: 48, 51

Human

Guideline: /
 Species/strain: Humans
 Group size: 10 adult volunteers (females)
 Test substance: Transcutol
 Batch: 75412
 Dose level: 0.02 ml (undiluted), 50 mm²
 Route: Skin (occlusive application)
 Exposure period: 48 hours
 Observation: /
 GLP: In compliance
 Date: January 1992

Transcutol was tested for potential irritation on human skin in a primary irritation single patch test as undiluted material. It was applied once at the dose level of about 0.02 ml per volunteer, on a surface of about 50 mm² of skin on the back of 10 volunteers. Transcutol was kept in contact with the skin under an occlusive patch test for 48 hours. This application was performed in parallel and under same condition with patch test alone as "negative" control. Cutaneous macroscopic examinations were performed about 30 min after removal of the patches. Evaluation of the erythematous and oedematous reactions was made according to a given numerical scale. After the removal of the patches, only 1 volunteer showed an erythema of grade 1 out of 4 grades

(i.e. very slight), while all other volunteers showed no erythema. It was concluded that the single epicutaneous application of Transcutol under the experimental conditions used was "well tolerated".

Ref.: 14

3.3.2.2. Mucous membrane irritation

Rabbit

Guideline/method: Comparable to OECD 405
 Species/strain: Rabbit/New Zealand White
 Group size: 3 males
 Test substance: Transcutol
 Batch: 15809 (purity: 100%)
 Concentration: 0.1 ml of a 30% aqueous solution of Transcutol
 Route: Instillation in the conjunctival sac of the right eye
 Rinsing: No
 Observation period: Up to 72 h after instillation
 GLP: Yes
 Date: June 1996

The potential irritant effect of Transcutol® to the mucous membrane was investigated by instillation of 0.1 ml of a 30% aqueous solution of Transcutol® into the right conjunctival sac of each of three male animals. The left eyes remained untreated and served as controls. Both eyes of the animals were examined within 24 h before application and about 1, 24, 48, 72 h after application. The evaluation and grading of the findings were performed according to the method of Draize (Federal Register 37, p. 8534, 1972).

The treatment resulted in initial conjunctival chemosis of grade 1 in 1/3 rabbits and redness of grade 1 in 2/2 rabbits at the 1 hour reading. Thereafter on at 24, 48 or 72 hours readings no finding was observed in any of the 3 animals.

The authors concluded that Transcutol tested as 30% aqueous solution was slightly and initially irritating to the eyes of 2 New Zealand White rabbits.

N Ref.: 18

Guideline/method: OECD 405
 Species/strain: Rabbit/New Zealand White
 Group size: 3 males
 Test substance: Transcutol
 Batch: 15809 (purity: 100%)
 Concentration: 0.1 ml of Transcutol (undiluted)
 Route: Instillation in the conjunctival sac of the right eye
 Rinsing: No
 Observation period: Up to 72 h after instillation
 GLP: Yes
 Date: June 1996

The potential irritant effect of Transcutol® to the mucous membrane was investigated by instillation of 0.1 ml of neat Transcutol® into the right conjunctival sac of each of three male animals. The left eyes remained untreated and served as controls. Both eyes of the animals were examined within 24 h before application and about 1, 24, 48, 72 h after application. The evaluation and grading of the findings were performed according to the method of Draize (Federal Register 37, p. 8534, 1972).

The treatment resulted in chemosis of grade 1 in 1/3, redness of grade 1 in 3/3 and congestion of grade 1 in 2/3 animals at the 24 hour reading (the period relevant for classification). At the 48 hour reading no chemosis or redness was observed anymore but grade 1 congestion was evident in 2/3 animals. At the 72 hour reading no finding was recorded anymore in any of the animals. Opacity was not recorded at any time in any animal.

The authors concluded that Transcutol tested as neat substance was slightly and transiently irritating to the eyes of New Zealand White rabbits.

N Ref.: 17

Cat

When DEGEE was applied to the eyes of cats, it causes immediate tearing and vigorous rubbing of the eyes, whereas in rabbits the response is less vigorous and the material appears to remain longer in the conjunctival sac. Cats exhibit only slight conjunctival reddening for a day or two, whereas rabbits have been known occasionally to develop conjunctivitis with discharge, iritis, and temporary corneal opacification, with return to normal in a week or two.

Ref.: 18

In addition to the above GLP studies, information exists on further eye irritation studies performed in rabbits and Guinea pigs. In these studies the tested substance revealed either a no, slight or irritant effects on the eyes.

N Ref.: 48, 51

General comment

DEGEE is moderately irritant to the eye.

3.3.3. Skin sensitisation

Guideline:	/
Species/strain:	Humans
Group size:	24 adult volunteers (19 – 38 years old; 18 men and 6 women)
Test substance:	Transcutol
Batch:	/
Dose level:	0.02 ml Transcutol
Epicutaneous induction:	Undiluted Transcutol
Challenge:	Undiluted Transcutol
Route:	Occlusive epicutaneous
Exposure period:	10 days
Observation:	15 days
GLP:	In compliance
Date:	January 1993

The Marzulli and Maibach method was used. 30 volunteers were originally selected. 25 came to the Institute on the day for the first treatment. One male volunteer abandoned the study on the 12th day.

The protocol of the irritation and sensitisation study was allocated into 3 distinct periods.

Induction period. 9 consecutive applications, to the same area, of 0.02 ml, per volunteer, of Transcutol by the occlusive epicutaneous route to the skin of the arm during a 3 week period.

Rest period. 15 days without any application.

Challenge phase. Single application of 0.02 ml Transcutol to the skin of the back.

The cutaneous reaction, control of the primary and cumulative irritations, was evaluated by macroscopic examination of the reactions possibly observed after removal of each patch test

corresponding to the induction period. The cutaneous reaction, control of the sensitisation, was evaluated by macroscopic examination of the reactions possibly noted about 24 and 48 h after removal of the patch test corresponding to the challenge application. These examinations were performed for the 1st, 8th (induction) and 10th (challenge) applications, by comparison to the reaction possibly obtained with a patch test alone (without Transcutol).

It was concluded that no pathological irritation or sensitisation reaction significant to a cutaneous intolerance was noted.

Ref.: 19

General comments

DEGEE has not been demonstrated to cause sensitisation.

The SCCS considers human induction studies as unethical

3.3.4. Dermal / percutaneous absorption

Percutaneous absorption data on rinse-off and leave-on cosmetic products (from Submission for SCCP Opinion in 2006)

Shampoo formulations (rinse-off)

Guideline:	OECD 428
Test substance:	5% and 10% DEGEE in a shampoo considered as a rinse-off reference formulation. ([4- ¹⁴ C] DEGEE 53 mCi/mmole, Specific activity at time of application to the skin 81 – 83 µCi/g of formulation)
Batch:	104-272-053 from ADME BIOANALYSES (30 310 Vergeze, France)
Purity:	98.2%
Dose applied:	5 mg/cm ² of formulation, 279.3 and 529.6 µg/cm ² DEGEE
Skin preparation:	Human skin
Skin temperature:	37°C
Exposure period:	30 min
Donor chamber:	Shampoo formulation containing 5% or 10% DEGEE
Receptor fluid:	Saline phosphate buffer (pH 7.4) containing 15 g/l bovine serum albumin
Skin integrity:	TEWL measurement
GLP:	In compliance
Date:	March 2004

Two different DEGEE concentrations 5 and 10% in a shampoo formulation were applied on human skin during a period of 30 minutes. At this time the skin surface was rinsed off. Then the diffusion was monitored until 24 hours. The receptor fluid (RF) was completely collected after 30 min, 3, 6, 9, 12 hours and replaced by fresh fluid, the last sampling point was 24 hours. At the end of the 24 hr observation period, the different skin layers were separated (horny layer, epidermis (E) and dermis) and analysed for DEGEE remaining. Results are expressed in µg equivalent of DEGEE (µg/cm²) and in percentage of the applied dose for all the compartments analysed (see table 3.3).

Table 3.3: Quantities of DEGEE analysed in the different system compartments for the 2 tested concentrations (5 and 10 %)

	DEGEE 5%		DEGEE 10%	
	$\mu\text{g}/\text{cm}^2$	% of the applied dose	$\mu\text{g}/\text{cm}^2$	% of the applied dose
Washing (W)	194 \pm 4	69 \pm 1	389 \pm 28	73 \pm 5
Receptor fluid (RF)	53.8 \pm 22.3	19.37 \pm 8	89.7 \pm 19.6	16.9 \pm 3.0
Total absorbed (E+D+RF)	60.5 \pm 29.8	21.6 \pm 10.6	92.9 \pm 20.8	17.5 \pm 3.9
Total recovery (%)	91		91	

Ref.: 20

Hydro-Alcoholic Gel Formulation (leave-on)

Guideline:	OECD 428
Test substance:	15% DEGEE in a leave-on hydro-alcoholic gel formulation. ([4- ¹⁴ C] DEGEE 53 mCi/mmole, Specific activity at time of application to the skin 62 – 65 $\mu\text{Ci}/\text{g}$ of formulation)
Batch:	104-272-053 from ADME BIOANALYSES (30 310 Vergeze, France)
Purity:	98.2%
Dose applied:	5 mg/cm ² of formulation, about 831.4 and 859.1 $\mu\text{g}/\text{cm}^2$
Skin preparation:	Human skin
Skin temperature:	37°C
Exposure period:	24 hours
Donor chamber:	Hydro-alcoholic gel formulation containing 15% DEGEE
Receptor fluid:	Saline phosphate buffer (pH 7.4) containing 15 g/l bovine serum albumin
Skin integrity:	TEWL measurement
GLP:	in compliance
Date:	April 2004

A 15% DEGEE leave-on hydro-alcoholic gel formulation was tested in two experiments. The formulation was applied on human skin during a period of 24 hours. The receptor fluid was completely collected after 3, 6, 9, 12 hours and replaced by fresh fluid, the last sampling point was 24 hours. At the end of the 24 hr observation period, the different skin layers were separated (horny layer, epidermis and dermis) and analysed for DEGEE remaining. Results are expressed in μg equivalent of DEGEE ($\mu\text{g}/\text{cm}^2$) and in percentage of the applied dose for all the compartments analysed (see table 3.4).

Table 3.4: Quantities of DEGEE analysed in the different system compartments in two experiments with 15% DEGEE in a leave-on hydro-alcoholic gel formulation

	First experiment		Second experiment	
	$\mu\text{g}/\text{cm}^2$	% of the applied dose	$\mu\text{g}/\text{cm}^2$	% of the applied dose
Washing (W)	6.34 \pm 1.84	0.77 \pm 0.23	7.80 \pm 1.64	0.91 \pm 0.20
Total absorbed (E+D+S+RF)	425 \pm 85	51.0 \pm 9.1	385 \pm 46	44.9 \pm 4.8
Total recovery (%)	52 \pm 9		46 \pm 5	

The percutaneous absorption study was conducted without occlusion. The mass balance of the experiment was low. The low recovery at the end of the 24 hours of diffusion was related to the evaporation of DEGEE from the skin surface. Therefore, the test was repeated under occlusion (by covering the skin with a piece of Parafilm). In the new experiment the total absorbed was 459 $\mu\text{g}/\text{cm}^2$ (51.5%) with a recovery of 92 \pm 6%.

Emulsified formulations (leave-on)

Guideline:	OECD 428
Test substance:	2%, 5%, and 10% DEGEE in Oil in Water emulsion considered as leave-on reference formulations. ($[4-^{14}\text{C}]$ DEGEE 53 mCi/mmol, Specific activity at time of application to the skin 112 – 130 $\mu\text{Ci/g}$ of formulation)
Batch:	104-272-053 from ADME BIOANALYSES (30 310 Vergeze, France)
Purity:	98.2%
Dose applied:	5 mg/cm ² of formulation, 100 – 571 $\mu\text{g/cm}^2$
Skin preparation:	Human skin
Skin temperature:	37 °C
Exposure period:	24 hours
Donor chamber:	Oil in Water emulsions containing 2%, 5% or 10% DEGEE
Receptor fluid:	Saline phosphate buffer (pH 7.4) containing 15 g/l bovine serum albumin
Skin integrity:	TEWL measurement
GLP:	In compliance
Date:	April 2004

Three different DEGEE concentrations 2, 5 and 10% in an Oil in Water emulsion formulation were applied on human skin during a period of 24 hour. The receptor fluid was completely collected after 3, 6, 9, 12 hours and replaced by fresh fluid, the last sampling point was 24 hours. At the end of the 24 hr observation period, the different skin layers were separated (horny layer, epidermis and dermis) and analysed for DEGEE remaining. Results are expressed in μg equivalent of DEGEE ($\mu\text{g/cm}^2$) and in percentage of the applied dose for all the compartments analysed (see table 3.5 and 3.6).

First experiment

Table 3.5: Quantities of DEGEE analysed in the different system compartments for the 3 tested concentrations (2, 5 and 10 %)

	DEGEE 2%		DEGEE 5%		DEGEE 10%	
	$\mu\text{g/cm}^2$	% of the applied dose	$\mu\text{g/cm}^2$	% of the applied dose	$\mu\text{g/cm}^2$	% of the applied dose
Washing (W)	0.87 ± 0.36	0.87 ± 0.36	1.56 ± 0.67	0.63 ± 0.29	1.82 ± 0.89	0.35 ± 0.18
Total absorbed (E+D+RF)	43.7 ± 7.0	43.2 ± 4.3	140 ± 28	56.1 ± 12.5	267 ± 43	50.4 ± 7.3
Total recovery (%)	44 ± 4		57 ± 12		51 ± 7	

Second experiment

Table 3.6: Quantities of DEGEE analysed in the different system compartments for the 3 tested concentrations (2, 5 and 10%)

	DEGEE 2%		DEGEE 5%		DEGEE 10%	
	$\mu\text{g/cm}^2$	% of the applied dose	$\mu\text{g/cm}^2$	% of the applied dose	$\mu\text{g/cm}^2$	% of the applied dose
Washing (W)	0.98 ± 1.25	0.85 ± 1.08	1.05 ± 0.37	0.36 ± 0.13	1.32 ± 0.37	0.24 ± 0.11
Total absorbed (E+D+RF)	52.7 ± 7.0	45.6 ± 4.8	128 ± 22	44.4 ± 5.1	294 ± 32	51.6 ± 3.3
Total recovery (%)	46 ± 4		45 ± 5		52 ± 3	

The percutaneous absorption study was conducted without occlusion. The mass balance of the experiment was low. The low recovery at the end of the 24 hours of diffusion was related to the evaporation of DEGEE from the skin surface. Therefore, the test was repeated under occlusion (by covering the skin with a piece of Parafilm). In the new experiment the total absorbed was, 2%: 59.5 $\mu\text{g}/\text{cm}^2$ (55.9%) with a recovery of 85 \pm 14%, 5%: 167 $\mu\text{g}/\text{cm}^2$ (63.8%) with a recovery of 92 \pm 3%, 10%: 319 $\mu\text{g}/\text{cm}^2$ (56.4%) with a recovery of 93 \pm 5%.

Ref.: 22

Comment (1)

Three well-conducted *in vitro* studies on percutaneous absorption through human skin are available in relation to the use in cosmetic products. In a study of a shampoo formulation (rinse-off) with a contact time of 30 min, 21.6 \pm 10.6% was absorbed using a shampoo with 5% DEGEE (total recovery 91%). With 10% DEGEE 17.5 \pm 3.9% was absorbed (total recovery 91%). In the second study with a hydro-alcoholic formulation (leave-on) containing 15% DEGEE 51 \pm 9.1% was absorbed. The total recovery was however, only 52 \pm 9%. The low recovery was due to evaporation as the recovery increased to 92 \pm 6% when performed under occlusion (total absorption 51.5%). The third study involved emulsified formulations (leave-on) containing 2, 5, and 10% DEGEE. The total absorption was 43.2 \pm 4.3, 56 \pm 12.5, and 50.4 \pm 7.3%, respectively in the first experiment and 45.6 \pm 4.8, 44 \pm 5.1, and 51.6 \pm 3.3%, respectively in the second experiment. The total recovery was only between 44 and 53%. When performed under occlusion the recoveries were >90%. The absorption with 2% DEGEE was 55.9%.

Percutaneous absorption data on hair colorant product usage (from Submission for SCCP opinion of 2008)

Hair colorant formulations

Oxidative hair formulations

Guideline:	OECD 428
Test substance:	4%, 7% and 14% DEGEE in an oxidative hair colorant before mixing with a placebo developer (without hydrogen peroxide) (1:1, w/w).
Batch:	[¹⁴ C]-DEGEE (GTS24740), batch no. 212507-MC0692-14-1, was supplied by Charles River Laboratories, UK. (373 $\mu\text{Ci}/\text{mg}$)
Purity:	97.6%
Dose applied:	20 mg/cm^2 of formulation, 1400, 700, and 400 $\mu\text{g}/\text{cm}^2$ DEGEE
Skin preparation:	Human skin. Five samples of full-thickness human skin (3 abdomen and 2 breast)
Skin temperature:	32°C
Exposure period:	30 min
Donor chamber:	Oxidative hair colorant mixed with placebo developer. Total DEGEE concentration 2, 3.5, and 7%. 12 chambers used at each concentration
Receptor fluid:	Phosphate buffered saline (pH 7.4 at 25°C) containing polyoxyethylene 20-oleyl ether (PEG, ca 4%, w/v) and sodium azide (ca 0.01%, w/v)
Skin integrity:	TEWL measurement
Recovery:	100.2%
GLP:	in compliance
Experimental period:	24 August 2007 – 18 October 2007

Three oxidative hair formulations with final concentrations of [¹⁴C]-DEGEE were 7%, 3.5% and 2% were prepared by mixing an oxidative hair colorant with placebo developer (without

hydrogen peroxide). The formulations were applied on human skin during a period of 30 minutes.

Non-oxidative formulations

Guideline:	OECD 428
Test substance:	1%, 3%, and 5% DEGEE in a non-oxidative hair colorant base containing no dye materials (typical semi-permanent hair dye formulation)
Batch:	[¹⁴ C]-DEGEE (GTS24740), batch no. 212507-MC0692-14-1, was supplied by Charles River Laboratories, UK. (373 µCi/mg)
Purity:	97.6%
Dose applied:	20 mg/cm ² of formulation, 1000, 600, and 200 µg/cm ² DEGEE
Skin preparation:	Human skin. Five samples of full-thickness human skin (3 abdomen and 2 breast)
Skin temperature:	32°C
Exposure period:	30 min
Donor chamber:	Non-oxidative hair colorant mixed base containing no dye materials. Total DEGEE concentration 1%, 3%, and 5%. 12 chambers used at each concentration
Receptor fluid:	Phosphate buffered saline (pH 7.4 at 25°C) containing polyoxyethylene 20-oleyl ether (PEG, ca 4%, w/v) and sodium azide (ca 0.01%, w/v)
Skin integrity:	TEWL measurement
Recovery:	100.6%
GLP:	in compliance
Experimental period:	24 August 2007 – 18 October 2007

Three typical semi-permanent hair dye formulation containing final concentrations of [¹⁴C]-DEGEE, 5%, 3% and 1% were used. The formulations were applied on human skin during a period of 30 minutes.

General procedure

After 30 minutes the skin surface was rinsed off. Then the diffusion was monitored until 24 hours. Receptor fluid was collected in 30 min fractions from 0 to 1 h post dose and hourly fractions from 1 to 6 h post dose and then in 2 hourly fractions from 6 to 24 h post dose. All receptor fluid samples were mixed with scintillation fluid (10 mL) and analysed by liquid scintillation counting. At the end of the 24 hr observation period, the stratum corneum was removed with 20 successive tape strips. The results with 7% DEGEE in the oxidative formulations and with 5% DEGEE in the non-oxidative formulation are summarized in Table 3.7 and Table 3.8, respectively.

Table 3.7: Dermal absorption obtained with 3 different concentration of DEGEE in oxidative formulations

DEGEE concentration in final mixed formulation % [#]	Amount collected in receptor fluid after 24hrs µg/cm ² *	Amount left in epidermis/dermis after 24 hours µg/cm ² *	Systemically Available Level µg/cm ² * (%)
7	33.42 ± 14.70	0.77 ± 0.5	34.18 ± 14.99 (2.4 ± 1.1)
3.5	13.51 ± 5.88	0.25 ± 0.09	13.76 ± 5.92 (2.0 ± 0.8)
2	8.2 ± 3.33	0.17 ± 0.08	8.37 ± 3.36 (2.1 ± 0.8)

[#] corresponds to on-head level following 1:1 mixing with developer solution

* values provided as mean ± standard deviation

Table 3.8: *Dermal absorption obtained with 3 different concentration of DEGEE in non-oxidative formulations*

DEGEE concentration %	Amount collected in receptor fluid after 24hrs $\mu\text{g}/\text{cm}^2$*	Amount left in epidermis/dermis after 24 hours $\mu\text{g}/\text{cm}^2$*	Systemically Available Level $\mu\text{g}/\text{cm}^2$* (%)
5	9.59 \pm 3.69	0.29 \pm 0.17	9.89 \pm 3.75 (0.9 \pm 0.4)
3	8.22 \pm 2.26	0.12 \pm 0.04	8.33 \pm 2.28 (1.4 \pm 0.4)
1	3.56 \pm 2.11	0.11 \pm 0.06	3.67 \pm 2.15 (1.8 \pm 1.1)

* values provided as mean \pm standard deviation

Ref.: 49

Comment (2)

Two *in vitro* studies on percutaneous absorption through human skin are available for the intended use in hair dye product. The contact time was 30 min in both studies. $34.18 \pm 14.99 \mu\text{g}/\text{cm}^2$ ($2.4 \pm 1.1\%$) was absorbed in the study with the oxidative hair colorant formulation using 7% DEGEE (total recovery 100%) and $9.89 \pm 3.75 \mu\text{g}/\text{cm}^2$ ($0.9 \pm 0.4\%$) was absorbed with the non-oxidative hair colorant formulation using 5% DEGEE (total recovery 100%). It is noted that no oxidative agent (hydrogen peroxide) was present in the oxidative hair colorant.

General comment on dermal absorption studies

Table 3.11 summarizes the dermal absorption studies performed with DEGEE. A dermal absorption of the order of 50% after 24 h exposure was reported in all studies submitted for the Opinion in 2006 (21, 22). In one experiment with 30 min contact time the dermal absorption was about 20% with shampoo formulations (20). This latter study may be compared with the new experiments with hair dye formulations, where a dermal absorption of 1 – 2% was reported (49). The SCCS finds it difficult to explain the large difference between these results since the concentrations of DEGEE in the formulations used were in the same range in both studies. One factor that may contribute to the difference is that 5 mg/cm² of the formulations was applied to the filter in the first experiments while 20 mg/cm² was used in the experiments with the hair dye formulations.

The applicant applies for the use of 5.5% DEGEE in leave-on cosmetic products. Two experiments relevant for this use with 10 cells each have been performed. In the first experiment the mean absorption was $56.1 \pm 12.5\%$ and in the second experiment $44.4 \pm 5.1\%$. If the two studies are considered together (altogether 20 cells), the mean absorption will be $50.0 \pm 11.0\%$ with an upper 95% confidence value of 55.1% (22). The study carried out with occlusion is not satisfactory reported with standard deviations

Conclusions

In the MOS calculation for use of 5.5% DEGEE in leave-on cosmetic products SCCS will use $(56.1 + 12.5)$ 68.6%. This is considered a conservative estimate as the upper 95% confidence value when using both experiments together (20 cells) is 55.1%. Moreover, the number 68.6% is higher than the absorption reported with occlusion.

For the use of 10% DEGEE in rinse-off product, SCCS will use $(17.5 + 3.9)$ 21.4% (See comment I).

In the MOS calculation for the hair dyes, SCCS will in the case of oxidative hair dyes use $(34.2 + 2 \times 15.0)$ 64.2 $\mu\text{g}/\text{cm}^2$ and for the non-oxidative hair colorant formulation $(9.9 + 2 \times 3.8)$ 17.5 $\mu\text{g}/\text{cm}^2$. The addition of 2SD has been made due to the large difference found with the hair dye formulations compared to the other absorption studies. No explanation is available to account for the large difference (See also General comment on dermal absorption studies).

Table 3.11: Summary of dermal absorption studies with DEGEE

Formulation	Incubation time	Concentration		Dermal absorption		Recovery %
		%	µg/cm ²	µg/cm ²	%	
Shampoo (rinse-off) (20)	30 min	5	279	60.5 ± 22.3	21.6 ± 10.6	91
		10	530	92.9 ± 20.8	17.5 ± 3.9	91
Hydro-alcoholic gel (leave-on) (21)	24 h	15	831	425 ± 85	51.0 ± 9.1	52
		15	859	385 ± 46	44.9 ± 4.8	46
Repeated under occlusion (21)	24 h	15	Ca 890	459	51.5	92
Emulsified formulations (leave-on) First exp. (22)	24 h	2	Ca 100	43.7 ± 7.0	43.2 ± 4.3	44
		5	Ca 285	140 ± 28	56.1 ± 12.5	57
		10	Ca 570	267 ± 43	50.4 ± 7.3	51
Second exp. (22)	24 h	2	Ca 100	52.7 ± 7.0	45.6 ± 4.8	46
		5	Ca 285	128 ± 22	44.4 ± 5.1	45
		10	Ca 570	294 ± 32	51.6 ± 3.3	52
Repeated under occlusion (22)	24 h	2	Ca 100	59.5	55.9	85
		5	Ca 285	167	63.8	92
		10	Ca 570	319	56.4	93
Hair dye, oxidative formulations (49)	30 min	2	400	8.4 ± 3.4	2.1 ± 0.8	100
		3.5	700	13.8 ± 5.9	2.0 ± 0.8	100
		7	1400	34.2 ± 15.0	2.4 ± 1.1	100
Hair dye, non-oxidative formulations (49)	30 min	1	200	3.7 ± 2.2	1.8 ± 1.1	100
		3	600	8.3 ± 2.3	1.4 ± 0.4	100
		5	1000	9.9 ± 3.8	0.9 ± 0.4	100

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated dose (28 days) oral / dermal / inhalation toxicity

Oral

Dogs

Guideline: /
 Species/strain: Dog/Beagle
 Group size: 2 females per group
 Test substance: Transcutol HP
 Batch: 450449013 (purity: > 99.9%)
 Dose levels: 0, 500, 1000, 2000 mg/kg bw
 Dose volume: 10 ml/kg bw
 Vehicle: Deionized water
 Route: Oral (gavage)
 Exposure period: 7 days
 Exposure frequency: Daily
 Recovery period: None
 GLP: Yes
 Date: June 2007

Transcutol HP was examined in a 7-day oral (gavage) toxicity range-finding study in female Beagle dogs to characterize the tolerability and to aid the selection for the following subchronic toxicity study. The test substance dissolved in deionized water was administered orally by gavage once daily for 7 consecutive days at dose levels of 500, 1000 and 2000 mg/kg bw, while the concurrent control group received the vehicle (deionized water). Each group consisted of 2 females.

The animals were observed twice daily for mortality and morbidity. Clinical examinations were performed daily. Detailed physical examinations were performed approximately

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weekly. Individual body weights were recorded on study day 0 and at the time of the scheduled necropsy. Food consumption was recorded daily, beginning at least 1 week prior to randomization. Clinical pathology evaluations were performed prior to the initiation of dose administration and on study day 7. Complete necropsies were performed on all animals, and selected organs were weighed at the scheduled necropsy and selected tissues were examined microscopically from all animals.

There was no premature mortality, no treatment-related clinical finding or effect on haematology parameters or organ weight, as well as no substance-induced macroscopic or microscopic finding. Although a slight body weight loss (<3% compared to study day 0) was noted at 2000 mg/kg bw from study day 0 to 6, which correlated to slightly lower individual food consumption, these findings were not conclusively test substance-related or considered adverse due to the small magnitude of change. Slightly impaired clinical pathology parameter occurred in form of lower 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 but due to low animal number could not be clearly associated to the treatment regiment.

The authors concluded that the maximum tolerated dose (MTD) of Transcutol HP administered orally to female Beagle dogs for 7 consecutive days was not achieved as all dosage levels appeared to be well tolerated. Dosage levels of 400, 1000 and 2000 mg/kg bw/day were selected for the 13-week dog study.

N Ref.: 35

Cats

Kidney damage (2 mid doses) and treatment-related mortality (highest dose) were reported in cats treated orally with DEGEE (300, 500, 1000, 4900 mg/kg bw/day) for up to 52 days.

Ref.: 23

Rats

Rats receiving DEGEE in drinking water for 30 days showed reductions in food intake, growth and unspecified micro-pathological changes at all dose levels above approximately 490 mg/kg bw/day.

Ref.: 24

DermalRabbits

Kidney damage and treatment-related mortality were reported in rabbits following dermal application of DEGEE for 30 days.

Ref.: 10

Guideline:	/
Species/strain:	Young adult New Zealand albino rabbits
Group size:	5 males and 5 females
Test substance:	Transcutol
Batch:	Lot No. 96933
Purity:	100%
Dose levels:	0, 100, 300, and 1000 mg/kg bw/day
Route:	Dermal (semi-occlusive)
Exposures:	28 days for a period of 6 h each day
GLP:	In compliance
Date:	January 1995

New Zealand rabbits in groups of 5 males and 5 females, received 0, 100, 300, and 1000 mg/kg bw/day for 28 days. Transcutol were applied dermally and allowed to remain in

contact with the skin for a period of 6 h each day. The test site was covered with one 4 x 6 inch 6-ply gauze pad. The animals were observed for signs of toxicity and mortality each day. Blood was collected from all animals on day 1 and at termination for haematology and blood chemistry evaluation. Complete necropsies were performed on all rabbits.

All treated animals survived and gained weight. Apart from several instances of transient soft faeces during the study, all animals appeared active and healthy. There were no signs of gross toxicity, adverse pharmacologic effects or abnormal behaviour. Animals from the exposed groups exhibited barely perceptible erythema and/or oedema and desquamation. The incidence of irritation increased with increasing dose level. Gross necropsy revealed small black masses on the ovaries of 3 females from group 1 (control), 3 (300 mg/kg bw/day), and 4 (1000 mg/kg bw/day). In the affected female from group 3, it was also noted that the left kidney was small in size, tan in colour and had small black masses on its surface. Additionally in group 4, the kidneys of 2 males were either mottled tan or irregularly shaped.

The study authors concluded that Transcutol is not toxic when applied dermally and allowed to remain in contact with the skin for 6h/d for 28 days at dose levels up to 1000 mg/kg bw/day.

Ref.: 25

Inhalation

Rats

SD rats were exposed to 0, 16, 50, and 200 ppm (16, 49, or 200 mg/m³) DEGEE (nose-only) for 6 hours/day, 5 days/week for 4 weeks. There were no signs of systemic intoxication, but there were histopathological changes indicative of mild non-specific irritation in the upper respiratory tract at the mid- and high-exposure levels.

Ref.: 26

Mice, rats, guinea pigs, rabbits, and cats

Daily exposure of mice, rats, guinea pigs, rabbits, and cats to an atmosphere saturated with DEGEE for 12 days was reported not to cause adverse effect.

Ref.: 27

3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity

Oral

Dogs

Guideline/method:	Subchronic oral toxicity study according to pharmaceutical guideline (FDA) exceeding the methodology of the OECD 408 testing guideline
Species/strain:	Dog/Beagle
Group size:	6 males and 6 females in the control and high dose groups, 4 males and 4 females in the low and mid dose groups
Test substance:	Transcutol HP
Batch:	450449013(purity: > 99.9%)
Dose levels:	0, 400, 1000, 2000/1500 mg/kg bw
Vehicle:	Deionized water
Application volume:	5 ml/kg bw
Route:	Oral (gavage)
Exposure period:	13 weeks
Exposure frequency:	Daily
Recovery period:	None
GLP:	Yes
Date:	23.01.07 – 26.07.07

Study design

The subchronic toxicity of Transcutol HP with special emphasis on possible renal effects was examined in a 13-week oral toxicity study in male and female Beagle dogs. The selected animals were approximately 5 to 6 months old at the initiation of dose administration; body weights ranged from 6.3 kg to 9.1 kg for the males and 5.3 kg to 7.9 kg for the females. In addition, the toxicokinetic profile of the parent compounds (i.e. diethylene glycol monoethyl ether (DEGEE) and the metabolite (ethoxyethoxyacetic acid (EEAA)) was investigated. The test substance dissolved in deionized water was administered to groups of male and female Beagle dogs orally by gavage once daily for 13 consecutive weeks. The initial dosage levels were 400, 1000 and 2000 mg/kg bw and a concurrent control group received the vehicle. The dosing volume was 5 ml/kg bw for all groups.

The animals were observed twice daily for mortality and morbidity. Clinical examinations were performed daily, and detailed physical examinations were performed weekly. Individual body weights were recorded weekly. Food consumption was recorded daily and reported weekly. Clinical pathology evaluations were performed and renal function parameters were calculated prior to the initiation of dose administration (study week -1) and during study week 13. Blood samples for toxicokinetic evaluation (DEGEE and EEAA) were collected from all animals at 30 minutes, 1, 2, 4, 8 and 24 hours after dose administration on study days 0 and 86. Additionally, urine samples for toxicokinetic evaluation (DEGEE and EEAA) were collected from all animals for approximately 24 hours on study days 0 and 86. Ophthalmic examinations were performed during study weeks -1 and 12. Electrocardiograms and heart rate were recorded during study weeks -2 and 12. Complete necropsies were performed on all animals, and selected organs were weighed at the scheduled necropsy. Selected tissues were examined microscopically from all animals.

Results

Two males in the 2000 mg/kg bw group had to be euthanized in extremis on study day 7 and one female of this group on study day 15. Prior to cessation of dosing and/or on the day of euthanasia, these 3 animals were noted with severe clinical observations including a marked decrease in food consumption, as well as pronounced body weight losses from initiation to the end of dose administration. The most probable cause of morbidity, based on histologic findings, was moderate to severe renal tubular degeneration. Due to the early deaths of 2 males and 1 female, the high dose was lowered to 1500 mg/kg bw from study day 21 for the remaining males and study day 20 for remaining females. All other animals survived to the scheduled necropsy.

Body weight effects were noted for females in the 1000 mg/kg/day group and the 2000/1500 mg/kg/day group. Following reduction of high dose to 1500 mg/kg/day weekly body weight changes and cumulative body weight gains in the 2000/1500 mg/kg/day group females continued to be slightly lower than the control group for the duration of the study, which resulted in a 13.3% lower final body weight at study week 13 compared to the control group. In addition, slightly lower weekly body weight gains and cumulative body changes were noted in the 1000 mg/kg/day female group over the course of the study when compared to the control group, resulting in a 10.0% lower final body weight from the control group. Body weights in the 400 mg/kg/day groups, 1000 mg/kg/day group males and 2000/1500 mg/kg/day group males were similar to the control group values throughout the study.

Slight differences from controls in isolated clinical pathology parameters (haematology [MCV and MCH], serum chemistry [alkaline phosphatase, albumin, A/G ratio, chloride, bicarbonate] and urine chemistry [specific gravity, osmolality, pH and electrolyte balance]) were observed in all groups at study week 13. These differences from controls were relatively small and were considered to represent residual effects of a regenerative response in the case of the red blood indices or compensatory/adaptive mechanisms in the case of slightly elevated alkaline phosphatase and/or urine differences in urine parameters to

eliminate the test substance. The animals euthanized in extremis revealed gross pathologically enlarged and discoloured kidneys and dark red areas in gastro-intestinal tract of both sexes with microscopic correlates of renal tubular degeneration in the kidney; ulceration or erosion in the oesophagus, stomach and duodenum and haemorrhage and muscle degeneration in the ileum. The liver weights were increased in the 1000 mg/kg bw group females and the 2000/1500 mg/kg bw animals of both sexes at the scheduled necropsy. However, there were no histological changes which correlated to the increase in liver weights. Therefore, the liver weight increases can be considered as an adaptive response related to the metabolism of the test article and not as an adverse effect. There were no treatment-related microscopic findings in any organ of any group for the animals surviving to the scheduled necropsy, and the histological alterations in the unscheduled death animals at 2000 mg/kg bw were considered reversible, since there was no histological evidence of injury in the animals continued to be dosed at 1500 mg/kg bw.

All animals receiving oral doses of Transcutol HP were systemically exposed to DEGEE and EEAA. The systemic exposure (AUC and C_{max}) to DEGEE and EEAA in male and female dogs generally increased with increasing dosage. Exposure to DEGEE increased more than proportionally to the increase in Transcutol HP dosage in terms of AUC, while it increased proportionally in terms of C_{max} regardless of evaluation day or gender. Exposure to EEAA appeared to increase less than proportionally to the increase in Transcutol HP dosage in terms of C_{max}, but proportionally in terms of AUC. For male and female dogs, systemic exposure to DEGEE and EEAA typically increased from Day 0 to Day 86 at 400 and 1000 mg/kg/day, and decreased from Day 0 to Day 86 at 2000/1500 mg/kg/day. The ratios of AUC_{0-t} for the metabolite, EEAA, to the parent drug, DEGEE, ranged from less than 1 to 2.7, but were typically about 1 to 2 regardless of dosage level. Generally less than 5% of DEGEE dose was eliminated unchanged in the urine over a 24 hour period, whereas about half of the dose was eliminated in the urine over the same period as EEAA. The respective percentages of dose eliminated in the urine as the parent drug or the metabolite were similar on Day 0 and Day 86. The ratios of metabolite/parent excreted in urine over 24 hours post-dosing decreased with increasing dosage on both evaluation days, suggesting saturation of the metabolism of the parent and/or of the elimination of the metabolite.

Conclusion

The study authors concluded that 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 primary histological alteration contributing to the morbidity for these dogs was severe renal tubular degeneration in the kidney. Slightly lower terminal body weights were noted in the 1000 and 2000/1500 mg/kg bw females, but with no correlating decrease in food consumption. Slight, non-adverse findings in clinical pathology parameters were noted in the 400, 1000 and/or 2000/1500 mg/kg bw groups and slightly increased liver weights were observed at 1000 mg/kg bw females and at 2000/1500 mg/kg bw in both sexes. They were considered as an adaptive response rather than a sign of toxicity. Based on the results of this study, the no-observed-adverse-effect level (NOAEL) for oral (gavage) administration of Transcutol HP for 13 weeks was considered to be at least 1000 mg/kg bw.

N Ref.: 34

Comment

SCCS notes that the absolute and relative liver weight in the 1000 mg/kg bw/day female group is increased by 11.7% (non-significant) and 22.7% ($p = 0.01$), respectively. Alkaline phosphatase was significantly increased both among males ($p = 0.01$) and females ($p = 0.05$) in the 1000 mg/kg bw/day groups as well as in the high dose groups ($p = 0.01$). Urine sodium and chloride was significantly decreased among males in all groups ($p = 0.01$), but not among females. Urine creatinine was significantly decreased among all groups of males ($p = 0.01$) and in the 1000 mg/kg bw/day and the 2000/1500 mg/kg bw/day groups of females ($p = 0.01$). The SCCS considers 400 mg/kg bw/day to be the NOAEL of this repeated dose study.

Rats

A six week oral gavage study was conducted in which groups of 10 male Sprague Dawley rats were administered doses of 1340, 2680, and 5360 mg/kg bw/day DEGEE. At the highest dose, four animals died before study termination and 3 were terminated moribund. Seven animals had bloody urine at various times throughout the study. Several other haematological and clinical chemistry signs were observed. At the intermediate dose, one animal died before study termination. Lethargy was noted during the first week of treatment. There were no significant effects of treatment with the intermediate dose on haematology or clinical chemistries. The relative liver, heart, and kidney weights (but not absolute weights of these organs) were increased with respect to control. Pathological changes included hyperkeratosis of the stomach (2/10), and splenic congestion (1/9). No effects were noted at the lowest dose; therefore the NOAEL was established as 1340 mg/kg bw.

N Ref.: 6, 48

Guideline: /
 Species/strain: Wistar rats (SPF-derived)
 Group size: 12 males and 12 females
 Test substance: DEGEE
 Batch: /
 Purity: Contain 0.4% ethylene glycol
 Dose levels: 0, 0.25, 1.0, and 5% DEGEE in the diet
 Route: Oral in diet
 Exposures: 90 days
 GLP: /

Wistar rats, groups of 12 males and 12 females, received diet containing 0, 0.25, 1.0, and 5.0% DEGEE for 13 weeks. The growth of male and female rats which was significantly retarded at the 5% level was associated with fall in food consumption. No haematological changes were seen at any dietary level. The raised levels of urinary glutamic-oxaloacetic transaminase which occurred in both sexes at the 5% level indicated impaired renal function. This effect was more pronounced in males which also showed a high degree of proteinuria. At the 5% level, increases were observed in the relative weights of the kidney in both sexes and of the testes. It was concluded that the NOAEL corresponded to 1% DEGEE in the diet or about 800 mg/kg bw/day.

Ref.: 28

Guideline: /
 Species/strain: CFE rats (SPF-derived)
 Group size: 15 males and 15 females
 Test substance: DEGEE
 Batch: /
 Purity: < 0.4% ethylene glycol
 Dose levels: 0, 0.5, and 5% DEGEE in the diet; Intake, males 0, 570-260, and 5450-2710 mg/kg bw/day, females 0, 470-350, and 5000-3560 mg/kg bw/day
 Route: Oral in diet
 Exposures: 90 days
 GLP: /

CFE rats, groups of 15 males and females, received 0, 0.5, and 5.0% (about 250 and 2500 mg/kg bw/day) DEGEE in the diet for 13 weeks. At both levels of treatment the rats appeared healthy and there were no deaths. The growth rate was reduced at the highest level of DEGEE. At terminal haematological examination there was a slight anaemia in male rats in the high dose group. The relative kidney weight was significantly

increased in the high dose group (14% male, 16% females). Histological examination showed hydropic degeneration of the proximal renal tubules. The males were more affected than the females. It is concluded that NOAEL is about 250 mg/kg bw/day.

Ref.: 29

Comment

A 90 day rat subchronic gavage study (ref.: 30) has been evaluated by the French authorities (this study has not been submitted to SCCS). The French authorities stated:

"Among the studies submitted to the experts of AFSSAPS, a 90 day subchronic oral (gavage) toxicity study in rats with DEGEE at the unique dose of 180 mg/kg bw/d. Toxicological endpoints measured during the study included clinical observations, body weights, feed consumption, ophthalmology, clinical chemistry (including methemoglobin analysis), haematology, urinalysis, necropsy, organ weights, and histopathology. A toxicokinetic analysis were also performed and the results showed that DEGEE was rapidly absorbed after oral administration to rats and even if the oral bioavailability of DEGEE could not be determined in this study, it was clear that oral administration of DEGEE resulted in a significant systemic exposure to the compound for up to 8 hours after each exposure in male and female rats. No significant toxicity were observed after DEGEE treatment at the single dose level tested. Therefore, the NOAEL for oral DEGEE treatment is 180 mg/kg bw/d. This value is the one used in the calculation of the safety margin, but a new NOAEL may be chosen if new reliable data are submitted to the experts"

Mice

Guideline:	/
Species/strain:	CD-1 mice
Group size:	20 males and 20 females
Test substance:	DEGEE
Batch:	/
Purity:	< 0.4% ethylene glycol
Dose levels:	0, 0.2, 0.6, 1.8, and 5.4% DEGEE in the diet; Intake, males 0, 370-270, 1020-800, 3240-2540, and 9930-6980 mg/kg bw/day, females 0, 380-320, 1100-820, 4600-3660, and 12880-9080 mg/kg bw/day
Route:	Oral in diet
Exposures:	90 days
GLP:	/

CD-1 mice, groups of 20 males and 20 females, received 0, 0.2, 0.6, 1.8, and 5.4% DEGEE in the diet for 13 weeks. 10 of the 20 males at the high dose died between week 5 and 12. The growth rate was reduced at the highest level of DEGEE. The relative kidney weight was significantly increased in the high dose group (16% male, 18% females) and next high dose among males (16%). Histological examination showed hydropic degeneration of the proximal renal tubules. It is concluded that NOAEL is about 850-1000 mg/kg bw/day.

Ref.: 29

Pigs

Guideline:	/
Species/strain:	White pigs
Group size:	3 males and 3 females
Test substance:	DEGEE
Batch:	/

Opinion on diethylene glycol monoethyl ether (DEGEE)

Purity: < 0.4% ethylene glycol
Dose levels: 0, 167, 500, and 1500 mg/kg bw/day DEGEE, top dose decreased to 1000 mg/kg bw/day after 3 weeks
Route: Oral in diet
Exposures: 90 days
GLP: /
Date: 1967

White pigs, groups of 3 males and 3 females (6 weeks old), received 0, 167, 500, and 1500 mg/kg bw/day DEGEE (top dose decreased to 1000 mg/kg bw/day after 3 weeks) in the diet for 13 weeks. 1 male and 2 females at the highest dose were killed between week 2 and 3. These pigs were lethargic for the terminal 4-5 days and became comatose with a slow laboured respiration during the last 24 h. The body weights were not reduced during the treatment and increased from about 10 kg to 35 kg during the 13-week treatment. There was a slight anaemia in male pigs at the highest dose. The killed pigs had a more severe anaemia associated with a reduced haematocrit and erythrocyte count. The absolute and relative kidney weight was increased in the high dose group. Histological examination showed hydropic degeneration of the proximal renal tubules at the highest level of treatment and at 500 mg/kg bw/day (in one of two female pigs). It is concluded that NOAEL was 167 mg/kg bw/day.

Ref.: 29

DermalRabbits

Rabbits receiving dermal treatments (not further specified) of DEGEE at dose levels of 0.1, 0.3, 1.0 and 3.0 ml/kg bw 5 times per weeks over a period of 90 days. The animals revealed no effects on growth, mortality, haematology, clinical chemistry or gross pathology at dose level up to 0.3 ml/kg bw (corresponding to about 300 mg/kg bw) A treatment related histopathological effect was seen in the kidneys of the animals at 1000 and 3000 mg/kg bw.

N Ref: 6, 48, 51

Ref.: 31

InhalationRats

Continuous DEGEE inhalation exposure of rats at 0.27, 1 or 4.5 ppm (1, 5, 25 mg/m³) for 4 months followed by a recovery period resulted in changes in blood cell (anaemia) and chemistry profiles as well as CNS effects. Changes in the functional state of the nervous system were claimed during both the treatment and the recovery periods in rats exposed to 5 mg/m³ or more, but narcosis was not observed. Analysis of blood samples was said to reveal indications of anaemia 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 killed before the end of the treatment period. However, it is not clear which groups were affected; but the authors stated that "the findings were confined mainly to rats receiving 5 mg/m³ or more". No conclusions were drawn from this study due to the nature of exposure as well as the limited reporting of the study.

N Ref.: 6, 48, 51

Ref.: 32

3.3.5.3. Chronic (> 12 months) toxicity**Oral****Rats**

In a 2-year dietary study with rats, employing limited pathological examination, rats were exposed for 2 years on a diet containing 2.16% of purified DEGEE. This is probably equivalent to slightly more than 1.0 g/kg/day. The only adverse effects noted were a few oxalate crystals in a kidney of one animal, slight liver damage, and some interstitial oedema in the testes. Since the quality of the material tested was not established, the possibility of the crystals being caused by the presence of small amounts of ethylene glycol in the test sample cannot be overlooked.

Ref.: 33

Comment

The above study was published in 1942 and is not considered relevant for the risk assessment of currently used DEGEE.

Albino rats receiving two grades of DEGEE through three generations (F0, F1 and F2) during a 2-year period. Each group contained 8 rats of each sex. One grade contained less than 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% (10, 40, 200 and 950 mg/kg bw/day). F I and F 2 generations received the same dosage levels as the parents, and all survivors were killed off 718 days from the start of the test. The sample that contained 29.5% ethylene glycol was considerably more toxic than the purer grade. The "toxic" group constituted 16 rats showing severe injury, notably kidney damage or bladder concretions; the animals comprised 39% of animals receiving 950 mg/kg bw and 11% of animals receiving 200 mg/kg bw DEGEE with 29.5% ethylene glycol and 7% of animal receiving 950 mg/kg bw and none with 200 mg/kg bw DEGEE with less than 0.2% ethylene glycol. It was concluded that the maximum safe dose of the impure material was 10 mg/kg bw/day whereas it was about 200 mg/kg bw/day for the purer sample.

Ref.: 34

Rats and mice

In an incomplete study DEGEE caused no apparent adverse effects when presented at 1% concentration in the drinking water to rats or mice for up to 23 months.

Ref.: 1

Ferrets

Ferrets showed no adverse treatment related effects following dietary feeding with DEGEE at concentrations ranging from 490 to 2960 mg mg/kg bw/day for 9 months.

Ref.: 37

General comment

Table 3.11: Summary on oral repeated toxicity

Study	Species	Sex	Effects	Critical doses	Ref
90 day	Mice: CD-1	m + f	5.4% in diet: 10 males died 1.8% in diet: Relative kidney weight significantly increased among males. 0.6% in diet: No effects recorded. Corresponds to 850 – 1000 mg/kg bw/day	NOAEL = 850 – 1000 mg/kg bw/day	29
6-week	Rat: Sprague Dawley	M	5360 mg/kg: 4 rat died. 2680 mg/kg: 1 rat died. Relative liver, heart, and kidney weights increased. 1340 mg/kg: No effects noted	NOAEL = 1340 mg/kg bw/day	N-6, 48
90 day	Rat: Wistar	m + f	5% in diet: Growth significantly retarded. 1% in diet: No effect recorded. Corresponds to about 800 mg/kg bw/day	NOAEL = 800 mg/kg bw/day	28
90 day	Rat: CFE	m + f	5% in diet: Growth rate reduced. Slight anaemi in males. Relative kidney weigh significantly increased among both males and females. 0.5% in diet: No effects recorded. Corresponded to about 250 mg/kg bw/day	NOAEL = 250 mg/kg bw/day	29
90 day	Rat		180 mg/kg: Study evaluated by French authorities (AFSSAPS in 2005 concluded that no adverse health effects were observed at the single dose tested	NOAEL = 180 mg/kg bw/day (communication from AFSSAPS)	--
90 day	Pigs: White	m + f	1500/1000 mg/kg: 3 pigs had to be killed 500 mg/kg: Hydropic degeneration of the proximal renal tubules in one of two females. 167 mg/kg: No effects recorded	NOAEL = 167 mg/kg bw/day	29
90 day	Dog: Beagle	m + f	2000/1500 mg/kg: Due to deaths, dose reduced to 1500 mg/kg. 1000 mg/kg: 11.7% (non-significant) and 22.7% (p= 0.01) in absolute and relative liver weight in females. Alkaline phosphatase significantly increased both among males and females. 400 mg/kg: Urine sodium and chloride and urine creatinine significantly decreased among males but not among females.	NOAEL = 400 mg/kg bw/day	N-34
2 year	Rat: Albino	m + f	Two samples, one containing 29.5% and one less than 0.2% ethylene glycol. Results with the latter is given. 950 mg/kg: 7% with pathological kidney changes. 200 mg/kg: No pathological kidney changes.	NOAEL = 200 mg/kg bw/day	34

The toxicity of DEGEE has been examined in oral repeated dose studies with mice, rats, pigs and dogs. A NOAEL of 850 – 1000 mg/kg bw/day was found in one study with mice based on increased relative kidney weight. Five studies have been carried out with rats. The observed NOAELs varied from 180 to 1340 mg/kg bw/day. The lowest value was based on a one dose study with no effect reported. In a two year and a 90 day rat study, NOAELs of 200 - 250 mg/kg bw/day was recorded based on pathological kidney changes at 5 to 10 times higher doses. In a 90 day pig study, kidney damage was observed at 500 mg/kg bw/day and a NOAEL of 167 mg/kg bw/day was derived.

In the previous opinions on DEGEE (SCCP/1044/06, SCCP/1200/08) a NOAEL of 200 mg/kg bw/day based on an albino 2 year oral study from 1964 was used in the calculation of MOS.

However, a newly submitted 13 week dog study, in contrast to the previously available studies, was performed conforming to GLP and according to modern guidelines and with high purity DEGEE (purity >99.9%). In this study, liver changes were observed at 1000 mg/kg bw/day. Based on the results of this study the SCCS considered the NOAEL for repeated dose toxicity to be 400 mg/kg bw/day, which will be used in the calculation of MOS.

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

DEGEE displayed a weak mutagenic activity at high concentrations in some tested *Salmonella typhimurium* strains (TA1535, TA1537, TA1538) and in *Saccharomyces cerevisiae* (D7)

Ref.: 1

Guideline:	OECD 471
Species/strains:	<i>Salmonella typhimurium</i> TA98, TA100, TA102, TA1535, and TA1537
Test substance:	Transcutol P
Batch:	9833703 (Purity > 99.7%)
Replicates:	Two independent experiments in triplicate
Concentrations:	Experiment 1: 0 (control), 52, 164, 512, 1600, and 5000 µg/plate (±S9) Experiment 2: 0 (control), 492, 878, 1568, 2800, and 5000 µg/plate (±S9)
Solvent:	Water
Positive Controls:	- S9-mix: TA 98: 2-nitrofluorene, 5.0 µg/plate TA 100, TA 1535: sodium azide, 10.0 µg/plate TA 102: t-butyl hydroperoxide, 100 µg/ml TA1537: 9-aminoacridine, 50 µg/plate +S9-mix: all strains: 2-aminoanthracene, 5.0 µg/plate
GLP:	In compliance
Date:	February 1999

Transcutol P was tested for mutagenicity in the reverse mutation assay on bacteria with and without metabolic activation (S9 mix prepared from Aroclor 1254 induced male Sprague-Dawley rat liver) according to the pre-incubation and plate incorporation assays. The *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537 were exposed to the test substance (dissolved in water) at concentrations ranging from 52 – 5000 µg/plate. For control purposes, the solvent (water) and positive controls (2-nitrofluorene, sodium azide, 9-aminoacridine, t-butyl hydroperoxide, and 2-aminoanthracene) were also investigated.

No bacteriotoxicity and no precipitation occurred up to the highest tested concentrations. The test substance did not induce an increase in revertant colony numbers in the bacterial strains at any concentration tested in the presence or absence of metabolic activation. The sensitivity and validity of the test system used was demonstrated by the expected induction of a significantly increased number of revertants with the positive controls.

The authors concluded that Transcutol P did not induce gene mutations by base pair changes or frame shifts in the genome of the bacterial strains used either in the presence or absence of S9-mix. Thus, it was shown to be non-mutagenic in this bacterial gene mutation test.

Ref.: 37

3.3.6.2 Mutagenicity/Genotoxicity <i>in vivo</i>
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Unscheduled DNA assay in rat liver

Guideline:	Comparable to OECD 486
Species/strain:	Rat/Wistar
Group size:	Range-finding study: 3 male and 3 female rats per group
Main study:	5 male rats per dose group/sacrifice time
Test substance:	Transcutol® (purity: >99%)
Batch:	9600544
Dose levels:	Range-finding study: 0, 2000 mg/kg bw UDS: 0, 800, 2000 mg/kg bw
Exposure:	Single application
Route:	Oral (gavage)
Application volume:	10 ml/kg body weight
Vehicle:	Purified water for Transcutol® and DMN, corn oil for 2-AAF,
Sacrifice Times:	Range-finding assays: 48 hours after application UDS: 2 – 4 hours and 12 – 14 hours after application
Positive control:	2-Acetamidofluorene (2-AAF): 75 mg/kg bw (for the 12-14 h experiments); Dimethylnitrosamine (DMN): 10 mg/kg bw (for the 2-4 h experiments)
GLP:	In compliance
Date:	August 1996

Transcutol was assessed for its potential to induce DNA damage and repair in the *in vivo/in vitro* UDS test using rat hepatocytes. In the dose range finding phase, each 3 male Wistar rats received doses of 2000 mg/kg bw to determine the maximum tolerated dose to be used in the definitive UDS study. The dosing volume was 10 ml/kg bw. All rats were observed for 4 days for clinical signs of toxicity. Following the last observation the animals were sacrificed and not further examined.

In the UDS test, 5 male Wistar rats received a single oral administration of Transcutol at 800 and 2000 mg/kg bw. Five male rats were used as controls receiving the vehicle (purified water) while 5 positive control rats received a single oral application of either 10 mg DMN/kg bw (2-4 h experiments) or 75 mg 2-AAF/kg bw (12-14 h experiments). Dosing was carried out in an interval of 2 hours in each 3 and 2 rats per dose group. All animals were observed for clinical signs of intoxication. The animals were exposed for 2 – 4 hours or 12 – 14 hours. After the exposure periods, the animals were sacrificed and liver perfusion was carried out in 3/5 rats per dose group. From each 3/5 animals at least two primary hepatocyte cultures were established and exposed for 4 hours to ³H-thymidine, which is incorporated into the DNA, if UDS occurs. Following the ³H -thymidine exposure period, cells were washed and mounted on cover slips, coated and stored in darkness for 14 days at refrigerator temperature. Thereafter, the slides were developed at room temperature, fixed and stained. In total, six slides from each animal were prepared. The net nuclear grain counts were determined by counting 2/3 slides per animal and 50 cells per slide (100 nuclei in total/animal). Appropriate reference mutagens (DMN and 2-AAF) were used as positive controls.

In the range-finding segment, the animals did not show any mortalities or clinical findings. In the UDS study, no significant increases in the group mean or net nuclear grain (NG) count for animals treated with Transcutol were observed at any dose level or at harvest time.

The group mean NG count for the vehicle control was within historical control values, while the positive controls induced a significant increase in NG count demonstrating the sensitivity and validity of the test system.

The authors concluded that Transcutol did not induce DNA-damage, i.e. no increased repair synthesis in hepatocytes of treated rats under the experimental conditions reported.

N Ref.: 19

In a micronucleus assay with Swiss CD-1 mice, the test material was administered i.p. to 2 groups of 4 male mice in two subsequent daily doses of 2 ml/kg (1980 mg/kg bw). Two groups of 4 male mice (positive controls) were treated with 100 mg/kg benzoapyrene (BP) dissolved in DMSO. One group of 4 rats (control) was left untreated. One group of animals treated with test material and BP was killed at 48 hours, and the other was killed at 72 hours. Negative controls were killed at 72 hours. Bone marrow smears were made and stained with Giemsa. One thousand polychromatic erythrocytes (PCE) from each animal were scored for micronuclei. The PCE/normochromatic erythrocyte ratio (NCE) was also scored to evaluate any toxic effect. The tested material had no effect on the number of NCE, ratio of PCE/NCE or on the number of micronuclei. The number of NCE was increased by treatment with BP at 72 hours and the number of micronuclei observed in animals treated with BP was increased at both time points showing the sensitivity of the assay.

Ref.: 1

Comment

The study is from 1986 and is poorly reported. Therefore the reliability is considered as limited.

General comment

In one purely reported study from 1986, DEGEE displayed a weak mutagenic activity at high concentrations in some tested *Salmonella typhimurium* strains (TA1535, TA1537, TA1538) and in *Saccharomyces cerevisiae* (D7) while no mutagenic activity were reported in another Salmonella test performed according to GLP. DEGEE did not induce unscheduled DNA synthesis (UDS) test in primary rat hepatocytes *in vivo* after exposure of rats up to 2000 mg/kg bw by gavage. In one poorly reported study from 1986, DEGEE did not induce micronuclei in CD-1 mouse bone marrow following 2 daily i.p. injections at 1980 mg/kg bw.

3.3.7. Carcinogenicity

No adequate data available

3.3.8. Reproductive toxicity

Oral route

Mice

Guideline:	/
Species/strain:	Swiss CD-1 mice
Group size:	50 pregnant mice
Test substance:	DEGEE
Batch:	/
Purity:	>99%
Dose levels:	5500 mg/kg bw/day
Route:	Oral, gavage
Exposures:	Pregnant mice, days 7 through 14 of gestation
GLP:	In compliance

Fifty mated CD1 mice were orally administered DEGEE (>99% purity) by gavage at 5500 mg/kg/day (calculated LD₁₀ based on a non-pregnant mouse pilot study) in corn oil from GD7-14 (GD1=vaginal sperm plug), then allowed to litter and to rear pups to PND3. 14% of the dams died, maternal weight gain was reduced and, of 33 surviving pregnant females, there were 32 viable litters (97%) compared with 100% control litter viability. No external

malformations were seen, pup survival to PND was unaffected and no other indication of specific developmental toxicity was found.

Ref.: 39, 40

Guideline: /
 Species/strain: CD-1 outbred Swiss albino mice
 Group size: 20 males and 20 females; control group 40 males and 40 females
 Test substance: DEGEE
 Batch: /
 Purity: >99%
 Dose levels: 0, 0.25, 1.25, and 2.5% (440, 2200, and 4400 mg/kg bw/d)
 Route: Oral, in drinking water
 Exposures: See below
 GLP: /

Continuous breeding

During the first 7 days of treatment (prematuring exposure) the sexes were housed separately. Subsequently, females and males from the same dose group were paired and cohabited for 98 days while being continuously exposed to DEGEE. The pairs were then separated and exposed for further 3 weeks. The animals received DEGEE in drinking water at concentrations of 0, 0.25, 1.25, and 2.5% (440, 2200, and 4400 mg/kg bw/d). During the 119 day period, different reproduction parameters were recorded. There was a small significant decrease in the mean body weights of the males during weeks 1 and 5 in the high dose group. DEGEE had only minimal effects on fertility or reproductive performance.

Offspring assessment. The F₁ generation from the final litters was reared and continuously treated with 0 or 2.5% DEGEE (4400 mg/kg bw/day) and at 74±10 days of age paired with nonsiblings from the same dose group. A significant decrease (34%) in motile sperm from de cauda epididymis in males exposed to 2.5% DEGEE was seen. In addition the relative liver weights were increased (16% in males and 10% in females).

Ref.: 41, 42

Rats

Prenatal developmental toxicity study in rats

Guideline: Comparable to OECD 414
 Species/strain: Rat/Sprague-Dawley
 Group size: 25 pregnant females per group
 Test substance: Transcutol HP (purity: 99.98%)
 Batch No: D 4089
 Dose level: 0, 300, 1000, 2000 mg/kg bw
 Vehicle: Sterile water
 Application volume: 2 or 4 ml/kg bw (0 and 2000 mg/kg bw or 300 and 1000 mg/kg bw, respectively)
 Route: Oral (gavage)
 Exposure period: Day 6 – 17 post coitum
 GLP: In compliance
 Date: June 2002

The prenatal developmental toxicity was investigated in Sprague-Dawley rats. The test substance was administered to 25 mated female rats per group by gavage as an aqueous solution in sterile water at doses of 300, 1000 and 2000 mg/kg bw on day 6 through day 17 post coitum (p.c.). The control group, consisting of 25 females, was dosed with the vehicle (sterile water) in parallel. At terminal sacrifice 24 - 25 females/group had implantation sites and were considered as pregnant. The dams were examined for clinical condition and

reaction to treatment at least once daily. Body weights were reported for days 0, 6, 11, 15, 18 and 20 of gestation. Food consumption was calculated for the periods (days) 0 to 6, 6 to 11, 11 to 15, 15 to 18 and 18 to 20 during gestation. All females were killed on day 20 of gestation for examination of their uterine contents including examination of the placenta. At necropsy the females were examined macroscopically and all foetuses were weighed, sexed and examined for external abnormalities. Half of the foetuses were examined internally prior to processing for skeletal examination. The remaining foetuses were preserved for fixed-visceral examination by the modified Wilson-Barrow technique.

Findings in the dams:

There was no mortality or treatment-related effects on clinical condition in any of the groups. Maternal body weight gain and food consumption were statistically significantly reduced in the

2000 mg/kg bw group during the first five days of treatment. No effect on body weight/body weight gain or food consumption was observed at 300 and 1000 mg/kg bw. Necropsy examination of the adult females did not reveal any treatment-related abnormalities.

Reproduction data of dams:

Pregnancy was confirmed for 24-25 rats/group. One dam of the mid dose group had a single early resorption and no live foetuses. The mean number of uterine implantation was slightly lower in the 300 mg/kg bw group due to an incidental increase in pre-implantation loss as this period was prior to the start of treatment. Pre-implantation data were comparable in the other treated and control groups. No substance-related differences were seen with regard to conception rate, mean number of corpora lutea, or placental weights.

Examination of foetuses:

There were no adverse influences of treatment on embryo-foetal survival. Mean foetal weights and sex ratio were comparable in all groups. There were no foetal malformations in any group and no effect on foetal morphology was noted with regards to external or visceral findings. The study authors reported that there was a minor effect on the foetal skeleton in form of an increase in the incidence of foetuses with reduced ossification, principally of the cranial bones, in the 1000 and 2000 mg/kg bw groups. These effects were considered by the study authors as an indication of a retarded skeletal development but clearly no indication of teratogenicity.

Conclusion

The authors concluded that the oral administration of Transcutol to pregnant Sprague-Dawley rats from implantation to day 17 of gestation resulted in maternal toxicity at 2000 mg/kg bw in form of retarded body weight gain and reduced food consumption. Gestation was not affected at any dose level.

Prenatal developmental toxicity occurred at 2000 mg/kg bw in form of minor skeletal findings predominantly in form of a clear and statistically significant increase in the incidence of reduced ossification of cranial bones as an indication of transiently retarded development. There was also some variation in the spontaneous increased incidence of delayed ossification in some cranial bones at 1000 mg/kg bw but these were partly not dose-related.

The authors concluded that the NOAEL for maternal toxicity as well as for prenatal developmental toxicity was 1000 mg/kg bw. There was no indication of teratogenicity up to the highest dose level tested and therefore the respective NOAEL was >2000 mg/kg bw.

N Ref.: 26

Comment

The SCCS concluded in the previous opinions on DEGEE (SCCP/1044/06, SCCP/1200/08) a NOAEL of 300 mg/kg bw/day for embryo-foetal toxicity. However, in the recent submission,

additional argumentation for the NOAEL of 1000 mg/kg bw/day was provided and the SCCS considers 1000 mg/kg bw/day being the NOAEL for maternal and embryo-foetal toxicity. It was concluded that there was no indication of teratogenicity at any dose level used in the study.

Fertility and general reproductive performance in rats

Guideline:	Elements comparable to OECD 415
Species/strain:	Rat/Sprague-Dawley
Group size:	24 males and 24 females per group
Test substance:	Transcutol HP (purity: 99.9%)
Batch No:	0025005
Dose level:	0, 300, 1000, 2000 mg/kg bw
Vehicle:	Sterile water
Application volume:	2 or 4 ml/kg bw (0 and 2000 mg/kg bw or 300 and 1000 mg/kg bw, respectively)
Route:	Oral (gavage)
Exposure period:	Males: 63 days before mating, throughout mating and up to one day prior to necropsy Females: 14: days before mating, throughout mating until day 7 of gestation
GLP:	In compliance
Date:	November 2001

Transcutol was investigated for its effects on fertility and general reproductive performance in and female Sprague-Dawley rats. Three groups each consisting of 24 male and 24 female rats received Transcutol by the oral route (gavage) at dose levels of 300, 1000 and 2000 mg/kg bw) for a pre-mating period (63 days for males, 14 days for females) and during mating. Treatment continued until day 7 of gestation for the females and up to the day before necropsy for the males. A similar group of rats received the vehicle (sterile water) only over the same periods and served as a control.

Clinical condition and reaction to treatment were recorded daily. Body weights of males were recorded twice weekly. Body weights of females were recorded twice weekly during pre-mating and mating periods (only pre-mating data are reported) and on days 0, 4, 8 and 13 of gestation. Food consumption was measured weekly during the pre-mating period and for the periods (days) 0 to 8 and 8 to 13 of gestation. All surviving females were killed, where possible, on day 13 of gestation for examination of their uterine contents, including examination of the placentae. At necropsy, all animals were examined macroscopically and the uterine status, number of corpora lutea and numbers and type of uterine implantations were determined for females. Testes and epididymides were weighed and used for automated sperm analysis. The kidneys of all rats and the ovaries of all females were weighed. Selected organs were fixed and preserved for all animals and histopathological examinations were performed for control and high dose males. Similar examinations were performed for any male animal of the low and mid dose group that had abnormalities associated with the sperm analysis.

Results

There was no treatment-related effect in any of the groups on gonadal function, fertility and reproductive performance in any group. The predominant finding was related to a reduction in body weight gain and transient clinical findings following test substance application (gavage) at 1000 and 2000 mg/kg bw/day. During the premating period these signs included salivation and subdued behaviour and were mainly restricted to the males of the mid and high dose groups, while in females, these signs could only be noted at 2000 mg/kg bw. A body weight reduction was mostly apparent in males at 2000 mg/kg bw, with females less affected. Food consumption was not impaired in any treated group.

Conclusion

The authors concluded that the oral administration of Transcutol within the fertility and general reproductive performance study in female Sprague-Dawley rats, showed that all doses levels, up to 2000 mg/kg bw/day, were well tolerated, although minor effects on clinical condition and body weight were observed at the higher dose levels (mainly in males). There were no effects of the test article on gonadal function, fertility and reproductive performance in any group. Finally, the no observed adverse effects level (NOAEL) for fertility and general reproductive performance was 2000 mg/kg bw, while the NOAEL for systemic toxicity was 1000 mg/kg bw in male and female Sprague-Dawley rats under the condition of this study.

N Ref.: 23

Dermal routeRats

Guideline:	/
Species/strain:	Sprague-Dawley rats
Group size:	13 rats, control 17 rats
Test substance:	DEGEE
Batch:	Lot 792796
Purity:	/
Dose levels:	0.35 ml x 4 per day from GD 7 – 16
Route:	Skin
Exposures:	10 days
GLP:	/

DEGEE was applied to the skin (unoccluded) of 13 pregnant SD rats to investigate its potential for developmental toxicity. Four doses each 2.5 hours apart of 350 mg DEGEE (total daily dose of 1400 mg, 5600 mg/kg bw/day) were applied daily to shaved interscapular skin of rats on GD 7 – 16 (GD0 = sperm positive). Extragestational weight gain in the DEGEE rats was significantly less than in the water controls. Thus, DEGEE caused a slight maternal toxicity. No embryotoxic, foetotoxic, or teratogenic effects were, however, detected with DEGEE treatment at the concentration of approximately 5600 mg/kg bw/day.

Ref.: 45

Comment

No clear conclusion can be drawn from the findings of this study since DEGEE was applied to the skin without occlusion, which would potentially enable evaporative loss from the site of application.

InhalationRats

Sprague-Dawley rats, a group of 20 pregnant female rats were exposed to 0, 102 ppm DEGEE for 7 h/day from day 7 to day 15 of gestation. The animals were killed for necropsy at day 20. No selective developmental toxicity was seen under the treatment conditions. It was concluded that DEGEE was not a developmental toxicant.

Ref.: 46

General conclusion on reproductive toxicity

DEGEE has low toxicity on reproductive performance and development. Evidence of embryo-foetal toxicity was restricted to minor skeletal findings which principally included an increase

in the incidence of reduced ossification of cranial bones. These minor skeleton findings were not considered to be indicative of a teratogenic potential and was not considered an adverse effect on the developing fetuses. In a rat study the dose of 1000 mg/kg bw/day was considered a NOAEL for maternal and embryo-foetal toxicity.

3.3.9. Toxicokinetics

3.3.9. 1. *In vitro* metabolism

Guideline:	/
Test system:	Hepatocytes from rats and human
Test substance:	Non-labelled: Transcutol HP (DEGEE), ethylene glycol mono ethyl ether (EGEE) Labelled: [¹⁴ C]-Diethylene glycol monoethyl ether ([¹⁴ C]-DEGEE), [¹⁴ C]-Ethylene glycol monoethyl ether ([¹⁴ C]-EGEE)
Batch:	Non-labelled: DEGEE: 0025005, EGEE: 049H1248 Labelled: [¹⁴ C]-DEGEE: 209-201-053 (radiochemical purity: 100%), [¹⁴ C]-EGEE: 209-209-053 (radiochemical purity: 97%, both supplied by Moravek Biochemicals Inc., USA)
Concentrations:	DEGEE: 0, 15, 150, 1500 µM EGEE: 150 µM
Analysis:	Reverse phase HPLC
GLP:	/
Date:	June 2001

The objective of this study was to determine the *in vitro* metabolism profile of diethylene glycol monoethyl ether (DEGEE = Transcutol HP) and ethylene glycol mono ethyl ether (EGEE) formed by rat and human hepatocytes. The rate and extent of formation of major metabolites was used to make predictions concerning the metabolism of DEGEE and EGEE by rats compared to humans.

Rat hepatocytes were isolated from 2 different rat livers, R1 and R2. Human hepatocytes were also isolated from two different human liver specimens, H1 and H2. Hepatocyte suspensions were incubated with 15, 150, and 1500 µM [¹⁴C]-DEGEE or 150 µM [¹⁴C]-EGEE. Incubation medium aliquots were removed at 0, 1, and 4 h after the addition of [¹⁴C]-DEGEE or [¹⁴C]-EGEE. Control incubations without hepatocytes were included with the first experiment using 1500 µM [¹⁴C]-DEGEE or 150 µM [¹⁴C]-EGEE. Incubation medium aliquots were removed from the controls at 0 and 4 h. Samples from the ¹⁴C-DEGEE incubations were analyzed for DEGEE, EGEE, and ethoxyacetic acid (EAA), and samples from the [¹⁴C]-EGEE incubation were analyzed for EGEE and EAA. HPLC separation with detection by an in-line radiochemical detector was used for sample analysis. The hepatocyte preparations used in this study exhibited 7-ethoxycoumarin O-deethylation activity, a cytochrome P450 associated activity.

Results

Total ECOD activity for the rat hepatocytes was 17630 and 21549 pmol 7-hydroxycoumarin/mg protein/hr, for R1 and R2, respectively. The total ECOD activity of the human hepatocyte preparations was 5384 and 5528 7-hydroxycoumarin/mg protein/h, for H1 and H2, respectively. At the end of the 4 h incubation period, over 88% of the radioactivity was associated with DEGEE in rat hepatocyte incubations with 150 and 1500 µM [¹⁴C]-DEGEE and 70% of the radioactivity was [¹⁴C]-DEGEE in cells incubated with the lowest concentration (15 µM [¹⁴C]-DEGEE). The rest of the radioactivity was associated with several peaks that were not identified. Rat hepatocyte incubations with [¹⁴C]-EGEE (150 µM) for 4 h contained 9.65% of the radioactivity as Unknown 1 (ethylene glycol; EG), 52.18% as EAA, and 38.18% of the radioactivity as EGEE. EGEE was metabolized by rat hepatocytes in the current study similarly to previous studies. Approximately 98 to 99% of the radioactivity remained as [¹⁴C]-DEGEE in the H1 and H2 hepatocyte preparations. When

H1 hepatocytes were incubated for 4 h with 150 µM [¹⁴C]-EGEE, 25.37% of the radioactivity identified as Unknown 1 (EG), 69.50% was identified as EAA, and 5.14% was EGEE. H2 hepatocytes were less active in the metabolism of EGEE than the H1 preparation and the metabolite profile included 7.66% of the radioactivity as EG, 17.57% as EAA, and 74.77% as EGEE. The results obtained with H2 were comparable to those reported in an earlier study.

Conclusion

The authors concluded that 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, in agreement with published *in vitro* metabolism data. In contrast, DEGEE was slowly metabolized by rat hepatocytes to several different unidentified metabolite peaks that accounted for approximately 1-17% of the total radioactivity. Human hepatocytes did not metabolize DEGEE significantly.

N Ref.: 21

3.3.9. 2. *In vivo* toxicokinetics or metabolism

An anecdotal report of rabbits treated orally or by s.c. injection indicated degradation of DEGEE and elimination in the urine as glucuronic conjugates.

Ref.: 47

DEGEE given orally to an adult human at a dose of about 20 mg/kg bw resulted in formation of 2-(2-ethoxyethoxy)acetic acid as a major (68% of the dose) metabolite in the urine.

Ref.: 48

Guideline:	Comparable to OECD 417
Species/strain:	Rat/Sprague-Dawley and BDIX
Group size:	Sprague-Dawley rats: 3 males and 3 females/time point (blood and plasma kinetics) 3 males and 3 females/time point (balance of excretion) 3 males and 3 females/time point (tissue distribution, oral route) BDIX Rats: 3 males and 3 females/time point (blood and plasma kinetics) 3 males and 3 females/time point (tissue distribution, oral route)
Test substance:	Non-labelled: Transcutol® HP (DEGEE) Labelled: [¹⁴ C]-Diethylene glycol monoethyl ether ([¹⁴ C]-DEGEE)
Batch:	Non-labelled: DEGEE: 0025005 Labelled: [¹⁴ C]-DEGEE: 104-272-053 (radiochemical purity: 100%, supplied by Moravek Biochemicals Inc., USA)
Dose level:	Oral and intravenous: 20 mg/kg bw (50 µCi/kg bw)
Exposure:	Single application
Route:	Oral (gavage) and intravenous
Application volume:	Oral: 5 ml/kg bw, intravenous: 2 ml/kg bw
Vehicle:	Oral: water for injection
Intravenous:	Physiological saline solution (0.9% NaCl)
Sampling time-points:	Sprague-Dawley rats: 0.25 up to 168 hours after oral or intravenous application BDIX rats: 0.25 up to 6 hours after oral or intravenous application
GLP:	In compliance
Date:	March 2002

The absorption, distribution and excretion of Transcutol® HP was investigated comparably in male and female Sprague-Dawley or BDIX rats after a single oral (gavage) administration or intravenous injection at a dose level of 20 mg [¹⁴C]-DEGEE /kg bw each.

Results and conclusion

After administration of 20 mg/kg of [¹⁴C]-Diethylene glycol monoethyl ether in male and female Sprague Dawley rats, the radioactivity was rapidly excreted in urine, irrespectively on sex and route of administration (85 % to 90 % within 24 hours post dose).

After intravenous injection, the maximum plasma concentration of the radioactivity was observed 0.25 hours post dose and the plasma concentrations corresponded to about 32-35 mg eq/kg. The maximum plasma concentration of the radioactivity after oral administration was observed 0.25 - 0.50 hours post dose and the maximum concentrations corresponded to about 23-27 mg eq/kg. The plasma half-life corresponded to 37 to 84 hours and measurable concentrations were observed in almost of the tissues 168 hours post dose. The absolute bioavailability of the radioactivity is very high (79 – 95 %). The tissue distribution of the radioactivity was characterised by high concentrations observed in pituitary, thyroid, adrenals and bone marrow with regards to the concentrations observed in blood / plasma (100 to 1000 times less) at the same sampling time. The radioactivity measured in tissues was significantly decreased at 48 hours. No biologically relevant difference has been observed with BDIX rats.

N Ref.: 25

Guideline:	Comparable to OECD 417
Species/strain:	Rat/Sprague-Dawley
Group size:	4 male rats in total (2 for plasma samples, 0.75 h; 2 for plasma (24 h), urine and faeces samples
Test substance:	Non-labelled: Transcutol HP (DEGEE) Labelled: [¹⁴ C]-Diethylene glycol monoethyl ether ([¹⁴ C]-DEGEE)
Batch:	Non-labelled: DEGEE: 0025005 Labelled: [¹⁴ C]-DEGEE: 104-272-053 (radiochemical purity: 100% supplied by Moravek Biochemicals Inc., USA)
Dose level:	1000 mg/kg bw including 1.85 MBq (50 µCi) of [¹⁴ C]-DEGEE
Exposure:	Single application
Route:	Oral (gavage)
Application volume:	5 ml/kg bw
Vehicle:	Water for injection
Sampling time-points:	Blood: 0.75 and 24 h Urine: pre-dose, 0 – 8 h, 8 – 24 h Feces: pre-dose, 0 – 24 h
Analysis:	Liquid scintillation counting
GLP:	/
Data:	September 2003

The metabolic fate and excretion of Transcutol HP was investigated in 4 male Sprague-Dawley rats after a single oral administration of 1000 mg [¹⁴C]-DEGEE /kg bw by gavage. Blood samples were collected at 0.75 h and at 24 h. Urine samples were collected before administration and between 0 – 8 hours and 8 – 24 hours and faeces were sampled prior to treatment and during 0 – 24 hours..

Results and conclusion

After administration of [¹⁴C]-DEGEE, 90% of the administrated radioactivity was excreted in the urine within the first 24 hours. [¹⁴C]-DEGEE was intensively metabolised, only 3% of the urinary excreted radioactivity correspond to unchanged compound. The two major urinary metabolites were identified as Ethoxyethoxyacetic acid and Diethylene glycol, which represented 83% and 5.4% of the excreted urinary radioactivity, respectively. In plasma, only Ethoxyethoxyacetic acid and unchanged [¹⁴C]-DEGEE were detected, which was consistent with urinary results.

N Ref.: 27

Guideline:	Comparable to OECD 417
Species/strain:	Rat/Sprague-Dawley
Group size:	30 male rats in total
Test substance:	Transcutol HP (DEGEE)
Batch:	D 4089
Dose levels:	20, 100 mg/kg bw
Exposure:	Single application
Route:	Oral (gavage)
Application volume:	5 ml/kg bw
Vehicle:	Water for injection
Sampling time-points:	Blood: 0.5, 1, 3, 6, 24 h Urine: pre-dose, 0 – 8 h, 8 – 24 h
Analysis:	LC/MS/MS.
GLP:	In compliance
Date:	December 2003

The metabolic fate at a doses of Transcutol® HP was investigated in 30 male Sprague-Dawley rats after a single oral administration of 20 mg/kg bw (15 males) or 100 mg/kg bw (15 males) by gavage. Blood samples were collected at 0.5, 1, 3, 6 and at 24 h. Urinary samples were collected before administration and between 0 – 8 h and 8 – 24 h. The validated analytical method applied consisted in LC/MS/MS analysis after protein precipitation.

Results and conclusion

The results obtained confirmed the presence of unchanged DEGEE and ethoxyethoxy acetic acid as major metabolites for the 0.5 hour plasma sampling times. However after 3 hours the difference observed between the total radioactivity (N Ref.: 27) and the specific analysis show a difference probably due to others metabolites. In urine, the amount recovered by the analysis of ethoxyethoxyacetic acid was low: about 17% of the administrated dose for the rats treated with 20 mg/kg of DEGEE and about 40% of the administrated dose for the rats treated with 100 mg/kg of DEGEE. As no satisfactory result was observed for the recovery of ethoxyethoxy acetic acid in plasma after precipitation of protein, it was not possible to conclude about the presence or not of this metabolite. In urine, a discrepancy was observed between these results and radioactivity study mentioned above (N Ref.: 27)

N Ref.: 28

Conclusion on toxicokinetics and metabolism by the applicant

An *in vitro* metabolism study to determine the metabolism profile of Transcutol showed that DEGEE was slowly metabolized by rat hepatocytes to several different unidentified metabolite peaks that accounted for approximately 1-17% of the total radioactivity. Human hepatocytes did not metabolize DEGEE significantly.

In vivo, the absorption, distribution and excretion of Transcutol® was investigated comparably in two strains of rats after a single oral or intravenous dose of 20 mg [¹⁴C]-DEGEE /kg bw each. It was demonstrated that the radioactivity was rapidly excreted in urine, irrespectively of sex and route of administration. After intravenous injection, the maximum plasma concentration of the radioactivity was observed 0.25 hours post dose, while after oral administration it was observed at 0.25 - 0.50 hours post dose. The plasma half-life corresponded to 37 to 84 hours and measurable concentrations were observed in almost of the tissues 168 hours post dose. The absolute bioavailability of the radioactivity is very high (79 – 95%). The tissue distribution of the radioactivity was characterised by high concentrations observed in pituitary, thyroid, adrenals and bone marrow with regards to the concentrations observed in blood / plasma (100 to 1000 times less) at the same sampling time. The radioactivity measured in tissues was significantly decreased at 48 hours. No biologically relevant differences were observed within both strains of rats. In studies on the metabolic fate and excretion of Transcutol it could be shown that after a single oral administration, 90% of the administered radioactivity was excreted in the urine within the first 24 hours and [¹⁴C]-DEGEE was intensively metabolised as only 3% of the urinary

excreted radioactivity correspond to unchanged compound. The two major urinary metabolites were identified as ethoxyethoxyacetic acid and diethylene glycol, which represented 83% and 5.4% of the excreted urinary radioactivity, respectively. In plasma, only ethoxyethoxyacetic acid and unchanged [¹⁴C]-DEGEE were detected.

3.3.10. Photo-induced toxicity

No data submitted

3.3.11. Human data

See sections 3.3.2 and 3.3.3

3.3.12. Special investigations

Local tolerance study in the rabbit

Guideline:	/
Species/strain:	Rabbit/New Zealand White
Group size:	Each 3 animals of either sex per test substance
Test substance:	Transcutol either 30% in olive oil or 50% in water
Batch:	Not stated
Dose/Concentration:	1 ml of 30% oily solution or 50% aqueous solution
Route:	Intramuscular
Exposure:	24 h prior to sacrifice and 48 h prior to sacrifice
GLP:	/
Date:	April 1979

Transcutol was investigated for its local tolerance after a single intramuscular injection in rabbits. It was tested either as 30% oily solution or as 50% aqueous solution. The treatment area was shaved and disinfected prior to injection. Each 3 animals received 1 ml of the test solution into the left muscular muscles at 24 hours prior to sacrifice or an injection of 1 ml of the test solution into the right lumbar muscles followed by 1 ml of physiological serum (9% NaCl) in front of the previous injection at 48 hours prior to sacrifice. The animals were sacrificed by intravenous injection of pentobarbital and the skin of the injection sites were investigated and scored for signs of irritation on a scale of 5 grades. The muscle was investigated by histopathology.

Result and conclusion

Both formulations caused macroscopically irritation at the application sites, which were slightly more pronounced after single injection of the oily solution of Transcutol. Histopathology confirmed the local irritation. Thus, the 30% oily solution and the 50% aqueous solution of Transcutol® were shown to be moderately irritant in this local tolerance study in rabbits.

N Ref.: 11, 12

3.3.13. Safety evaluation (including calculation of the MoS)

The margin of safety (MoS) has been calculated according to the use of DEGEE as given by the applicant. Separate calculations have been performed for the use of DEGEE in hair dye formulations.

Use of 5.5% DEGEE in leave-on products and 10% DEGEE in rinse-off products

Table 3.12: Calculation of the daily exposure to cosmetics using SCCP Notes of Guidance data

Product type	Amount of substance applied	Frequency of application	Retention factor ³	Leave-on/ Rinse-off	Daily exposure calculated (g/day)
Hair care					
Shampoo	8.0 g	1 / day	0.01	Rinse-off	0.08
Hair conditioner	14.0 g	0.28 / day	0.01	Rinse-off	0.04
Hair styling products	5.0 g	2 / day	0.1	Leave-on	NA ⁴
Oxidation or permanent hair dyes	100 ml	1 / month (30 min.)	0.1	Rinse-off	Not calculated ⁵
Semi-permanent hair dyes (and lotions)	35 ml	1 / week (20 min.)	0.1	Rinse-off	Not calculated
Bathing, showering					
Shower gel	5.0 g	2 / day	0.01	Rinse-off	0.10
Skin care					
Face cream	0.8 g	2 / day	1.0	Leave-on	1.6
General purpose cream	1.2 g	2 / day	1.0	Leave-on	2.4
Body lotion	8.0 g	1 / day	1.0	Leave-on	8.0
Make-up and nail care					
Make-up remover	2.5 g	2 / day	0.1	Leave-on	0.5
Eye make-up	0.01 g	2 / day	1.0	Leave-on	NA
Mascara	0.025 g	1 / day	1.0	Leave-on	NA
Eyeliners	0.005 g	1 / day	1.0	Leave-on	NA
Lipstick, lip salve	0.01 g	4 / day	1.0	Leave-on	NA
Deodorant					
Deodorant stick / roller	0.5 g	1.0 / day	1.0	Leave-on	0.5
Oral hygiene					
Toothpaste (adult)	1.4 g	2.0 / day	0.17	Rinse-off	NA
Mouthwash	10.0 g	3.0 / day	0.10	Rinse-off	NA
TOTAL DAILY EXPOSURE (g/day)				Rinse-off	0.22
TOTAL DAILY EXPOSURE (g/day)				Leave-on	13.00

³The retention factor was introduced by the SCCNFP to take into account rinsing off and dilution of finished products by application on wet skin or hair (e.g. shower gels, shampoos, ...) [SCCNFP/0321/00]

⁴ Not applicable since these products do not contain DEGEE according to the applicant

⁵ MOS calculations performed separately

CALCULATION OF THE MARGIN OF SAFETY**Diethylene glycol monoethyl ether (DEGEE)**

The safety calculation is only considering dermal exposure.

NOAEL based on kidney damage in a 13 week study with dogs was 400 mg/kg bw/day

The applicant wants to use DEGEE in a concentration up to 5.5% in leave-on products and in a concentration up to 10% in rinse-off products.

Leave-on products

A dermal absorption of (56.1 + 12.5) 68.6% is used in the MOS calculations.

Exposure 13 g/day, 5.5% DEGEE		=	715 mg/day
Maximum absorption through the skin	715 x 68.6/100	=	490.5 mg/day
Typical body weight of human		=	60 kg
Systemic exposure dose (SED)	490.5 /60	=	8.2 mg/kg bw

Margin of Safety	NOAEL / SED	=	49
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Rinse-off products

A dermal absorption of (17.5 + 3.9) 21.4% is used in the MOS calculations.

Exposure 0.22 g/day, 10% DEGEE		=	22 mg/day
Maximum absorption through the skin	22 x 21.4/100	=	4.7 mg/day
Typical body weight of human		=	60 kg
Systemic exposure dose (SED)	4.7 /60	=	0.08 mg/kg bw

Margin of Safety	NOAEL / SED	=	5000
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Total exposure	490.5 +4.7	=	495.2 mg/day
Systemic exposure dose (SED)	495.2/60	=	8.25 mg/kg bw

Margin of Safety	NOAEL / SED	=	48
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The previously assessed use of 1.5% DEGEE in all cosmetic products (SED 1.97mg/kg bw) would result in a MoS of 203 when compared to the NOAEL of 400 derived from a newly provided study. The use of 10% DEGEE in rinse-off products does not significantly lower this MoS and is not of concern. However, a MOS of 48 is not considered to give sufficient protection in relation to the combined use of DEGEE in the intended concentrations of 5.5% in leave-on and 10% in rinse-off cosmetic products.

USE of DEGEE as solvent in an on-head concentration up 7.0% in oxidative hair dye formulations

A dermal absorption of $(34.2 + 2 \times 15.0)$ $64.2 \mu\text{g}/\text{cm}^2$ is used in the MOS calculations.

Maximum absorption through the skin	A ($\mu\text{g}/\text{cm}^2$)	=	64.2 $\mu\text{g}/\text{cm}^2$
Skin Area surface	SAS (cm^2)	=	580 cm^2
Dermal absorption per treatment	SAS x A x 0.001	=	37.2 mg
Typical body weight of human		=	60 kg
Systemic exposure dose (SED)	SAS x A x 0.001/60	=	0.62 mg/kg bw
No observed adverse effect level	NOAEL	=	400 mg/kg bw

Margin of Safety	NOAEL / SED	=	645
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A MOS of 645 gives sufficient protection in relation to the use of DEGEE as a solvent in oxidative hair dye formulations.

USE of DEGEE as solvent in an on-head concentration up 5.0% in non-oxidative hair dye formulations

A dermal absorption of $(9.9 + 2 \times 3.8)$ $17.5 \mu\text{g}/\text{cm}^2$ is used in the MOS calculations.

Maximum absorption through the skin	A ($\mu\text{g}/\text{cm}^2$)	=	17.5 $\mu\text{g}/\text{cm}^2$
Skin Area surface	SAS (cm^2)	=	580 cm^2
Dermal absorption per treatment	SAS x A x 0.001	=	10.2 mg
Typical body weight of human		=	60 kg
Systemic exposure dose (SED)	SAS x A x 0.001/60	=	0.17 mg/kg bw
No observed adverse effect level	NOAEL	=	400 mg/kg bw

Margin of Safety	NOAEL / SED	=	2353
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A MOS of 2353 gives sufficient protection in relation to the use of DEGEE as a solvent in oxidative hair dye formulations.

Although the consumer may be exposed to DEGEE both from its use in cosmetics in general and in hair dyes formulations, the SCCS considers the proposed uses safe for the consumer taking into consideration the use pattern of hair dyes.

3.3.14. Discussion

The safety has only been evaluated for dermal exposure. The exposure due to possible evaporation of DEGEE, as observed in various experiments, has not been considered.

The stability of DEGEE in preparations is not reported. The physico-chemical characterisation and purity of the substance is not reported in several of the old studies. Commercial products of DEGEE may contain an appreciable amount of ethylene glycol.

Acute toxicity

The acute toxicity of DEGEE after oral or dermal application as well as inhalation can be regarded as very low in all species investigated. The LD50 values for acute oral and acute dermal toxicity were generally much higher than 2000 mg/kg bw and the available LC50 value for acute inhalation was $>5000 \text{ mg}/\text{m}^3$ (i.e. $>5 \text{ mg}/\text{l}$).

Irritation /sensitization

DEGEE is not irritant to the skin. DEGEE was found to be moderately irritant to the eye. DEGEE has not been demonstrated to cause sensitization.

Dermal absorption

Use in concentrations up to 10% in rinse-off cosmetic products

Well-conducted *in vitro* studies on percutaneous absorption through human skin are available for rinse-off product. In a study of a shampoo formulation (rinse-off) with a contact time of 30 min, $21.6 \pm 10.6\%$ was absorbed using a shampoo with 5% DEGEE (total recovery 91%). With 10% DEGEE $17.5 \pm 3.9\%$ was absorbed (total recovery 91%). In the MOS calculation of rinse-off products $17.5 + 3.9\% = 21.4\%$ (mean + SD; $113.7 \mu\text{g}/\text{cm}^2$) was used.

Use in concentrations up to 5.5% in leave-on cosmetic products

In a hydro-alcoholic formulation (leave-on) containing 15% DEGEE $51 \pm 9.1\%$ was absorbed. The total recovery was however, only $52 \pm 9\%$. The low recovery was due to evaporation as the recovery increased to $92 \pm 6\%$ when performed under occlusion (total absorption 51.5%). With emulsified formulations (leave-on) containing 2, 5, and 10% DEGEE, two experiments were performed at all three concentrations. No occlusion was used and the recovery was only between 44 and 57%. The mean absorption in the six experiments varied from 43.2% to 56.1%.

Two experiments with 10 cells each have been performed with 5% DEGEE. In the first experiment the mean absorption was $56.1 \pm 12.5\%$ and in the second experiment it was $44.4 \pm 5.1\%$. In the MOS calculation for use of 5.5% DEGEE in leave-on cosmetic products SCCS will use $(56.1 + 12.5) 68.6\%$. This is considered a conservative estimate as the mean absorption when calculated on the basis of all 20 cells was $50.0 \pm 11.0\%$ with an upper 95% confidence value of 55.1%.

Use as solvent in an on-head concentration up 7.0% in oxidative hair dye formulations and in an on-head concentration up 5.0% in non-oxidative hair dye formulations

Two *in vitro* studies on percutaneous absorption through human skin are available. In both studies a contact time of 30 min were used. The final DEGEE concentrations were 2, 3.5, and 7% in the case of the oxidative formulations and 1, 3 and 5% in the case of the non-oxidative formulations. The systemically available levels for the relevant concentrations used were $34.2 \pm 15.0 \mu\text{g}/\text{cm}^2$ ($2.4 \pm 1.1\%$) for the oxidative formulation and $9.9 \pm 3.8 \mu\text{g}/\text{cm}^2$ ($0.9 \pm 0.4\%$) for the non-oxidative formulation. The total recovery was 100%. In the MOS calculation $64.2 \mu\text{g}/\text{cm}^2$ (mean + 2SD) is used for the oxidative hair dye formulation and $17.5 \mu\text{g}/\text{cm}^2$ (mean + 2SD) for the non-oxidative formulation.

The large difference in the dermal absorption reported in the three first study and the two second studies with hair dye formulations is noted. The cause for the large difference has not been resolved.

Repeated dose toxicity

In five rat studies the NOAELs have varied from 180 to 1340 mg/kg bw/day while in a mice study a NOAEL of about 850 – 1000 mg/kg bw/day was obtained. In a pig study a NOAEL of 167 mg/kg bw/day based on the finding of hydropic degeneration of the proximal renal tubules in one of two female pigs (no effects in three male pigs) at the next higher dose (500 mg/kg bw/day) was found. None of these studies were performed according to guidelines or GLP. A NOAEL of 400 mg/kg bw/day for oral (gavage) administration of DEGEE (purity >99.9%) based on liver effects was found in a new well conducted guideline and GLP compliant 13 week study with dogs. In the previous opinions on DEGEE (SCCP/1044/06, SCCP/1200/08) a NOAEL of 200 mg/kg bw/day from an albino 2 year oral study from 1964 was used in the calculation of MOS. For the present opinion, the SCCS

decided to use the 13 week dog study from 2007 with a NOAEL of 400 mg/kg bw/day in the calculation of MOS.

Mutagenicity/Genotoxicity

DEGEE was tested for mutagenicity/genotoxicity in a range of validated and/or scientifically reasonable studies *in vitro* and *in vivo*. No genotoxic/mutagenic potential was noted in reliable bacterial gene mutation assays *in vitro* with *Salmonella typhimurium* in the presence or absence of metabolic activation. *In vivo*, DEGEE did not possess a mutagenic/genotoxic potential covering two independent endpoints. No indication of clastogenicity was observed in a 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. In conclusion, DEGEE can be considered to be of no genotoxic/mutagenic risk to humans. There is no need for any further studies.

Carcinogenicity

No adequate carcinogenicity study is available.

Reproduction toxicity

Two reliable studies on reproductive toxicity exist with regards to fertility and reproductive performance and developmental toxicity in the rats, performed by the applicant following internationally accepted guidelines under GLP conditions with characterized and analysed test material. In addition, there are also published studies available, which, however, can only be considered as additional information due to methodological limitations. The oral administration of DEGEE within the fertility and general reproductive performance study in female Sprague-Dawley rats, showed that all doses levels, up to 2000 mg/kg bw/day, were well tolerated, although minor effects on clinical condition and body weight were observed at the higher dose levels (mainly in males). There were no effects of the test article on gonadal function, fertility and reproductive performance in any group. The NOAEL for fertility and general reproductive performance was 2000 mg/kg bw, while the NOAEL for systemic toxicity was 1000 mg/kg bw.

In the prenatal developmental toxicity study, the oral administration of DEGEE to pregnant Sprague-Dawley rats from implantation to day 17 of gestation resulted in maternal toxicity at 2000 mg/kg bw in form of retarded body weight gain and reduced food consumption. Gestation was not affected at any dose level. Evidence of embryo-foetal toxicity was restricted to minor skeletal findings which principally included an increase in the incidence of reduced ossification of cranial bones. These minor skeleton findings were not considered to be indicative of a teratogenic potential. The SCCS concluded in the previous opinions on DEGEE (SCCP/1044/06, SCCP/1200/08) a NOAEL of 300 mg/kg bw/day for embryo-foetal toxicity. However, in the recent submission, additional argumentation for the NOAEL of 1000 mg/kg bw/day was provided and the SCCS considers 1000 mg/kg bw/day being the NOAEL for maternal and embryo-foetal toxicity. It was concluded that there was no indication of teratogenicity at any dose level used in the study

Toxicokinetics and metabolism

An *in vitro* metabolism study to determine its metabolism profile, DEGEE was slowly metabolized by rat hepatocytes to several different unidentified metabolite peaks that accounted for approximately 1-17% of the total radioactivity. Human hepatocytes did not metabolize DEGEE significantly.

In vivo, the absorption, distribution and excretion of DEGEE was investigated comparably in two strains of rats after a single oral or intravenous dose of 20 mg¹⁴C-DEGEE /kg bw each. It was demonstrated that the radioactivity was rapidly excreted in urine, irrespectively of

sex and route of administration. After intravenous injection, the maximum plasma concentration of the radioactivity was observed 0.25 hours post dose, while after oral administration it was observed at 0.25 - 0.50 hours post dose. The plasma half-life corresponded to 37 to 84 hours and measurable concentrations were observed in almost of the tissues 168 hours post dose. The absolute bioavailability of the radioactivity is very high (79 – 95 %). The tissue distribution of the radioactivity was characterized by high concentrations observed in pituitary, thyroid, adrenals and bone marrow with regards to the concentrations observed in blood / plasma (100 to 1000 times less) at the same sampling time. The radioactivity measured in tissues was significantly decreased at 48 hours. No biologically relevant differences were observed within both strains of rats. In studies on the metabolic fate and excretion of DEGEE it could be shown that after a single oral administration, 90% of the administered radioactivity was excreted in the urine within the first 24 hours and ¹⁴C-DEGEE was intensively metabolised, as only 3% of the urinary excreted radioactivity correspond to unchanged compound. The two major urinary metabolites were identified as ethoxyethoxyacetic acid and diethylene glycol, which represented 83% and 5.4% of the excreted urinary radioactivity, respectively. In plasma, only ethoxyethoxyacetic acid and unchanged ¹⁴C-DEGEE were detected, which was consistent with urinary results.

4. CONCLUSION

The SCCP previously concluded in its opinion of 16.12.08 (SCCP/1200/08) that the use of diethylene glycol monoethyl ether (DEGEE) as a solvent in an on-head concentration of up to 7.0% in oxidative hair dye formulations and in an on-head concentration of up to 5.0% in non-oxidative hair dye formulations in addition to the use of DEGEE at concentrations up to 1.5% in all cosmetic products, except products for oral hygiene and eye products, does not pose a risk to the health of the consumer, provided that the level of ethylene glycol in DEGEE used is < 0.2%.

Based on the new information submitted, SCCS is of the opinion that:

1. the use of DEGEE as a solvent in cosmetic products in a concentration up to 10% in rinse-off products does not pose a risk to the health of the consumer.
2. the use of DEGEE as a solvent in cosmetic products in a concentration up to 5.5% in leave-on products does pose a risk to the health of the consumer.
3. an additional use of the substance DEGEE as solvent in an on-head concentration up to 7.0% in oxidative hair dye formulations and in an on-head concentration up to 5.0% in non-oxidative hair dye formulations does not pose a risk to the health of the consumer.
4. the additional use of DEGEE at concentrations up to 1.5% in all cosmetic products does not pose a risk to the health of the consumer.
5. DEGEE must not be used in products for oral hygiene and the eyes. The level of ethylene glycol in DEGEE used should be less than 0.2%

The opinion relates to the dermal application of cosmetic products only and does not include any other cosmetic exposure, such as exposure from possible aerosol/spray products. Aggregate exposure to diethylene glycol monoethyl ether (DEGEE) from non-cosmetic sources has not been considered.

5. MINORITY OPINION

Not applicable

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