EUROPEAN COMMISSION

Directorate-General for Health & Consumers

Scientific Committee on Consumer Safety

SCCS

OPINION ON

DIETHYLENE GLYCOL MONOETHYL ETHER

(DEGEE)

Scientific Committees
on consumer safety
on emerging and newly identified health risks
on health and environmental risks

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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TABLE OF CONTENTS

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 COLIPA[1]. 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 EFfCI[2], 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, Solvolsol, 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: $C_6H_{14}O_3$

3.1.2. Physical form

Liquid with a mild, pleasant odour; hydroscopic

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/m^3 = 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 SO_2. 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

LC_{50} 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)

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 mm^2 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/cm² of formulation, 279.3 and 529.6 µg/cm² 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%	
	µg/cm²	% of the applied dose	µg/cm²	% of the applied dose
Washing (W)	194 ± 4	69 ± 1	389 ± 28	73 ± 5
Receptor fluid (RF)	53.8 ± 22.3	19.37 ± 8	89.7 ± 19.6	16.9 ± 3.0
Total absorbed (E+D+RF)	60.5 ± 29.8	21.6 ± 10.6	92.9 ± 20.8	17.5 ± 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 µCi/g of formulation)
Batch: 104-272-053 from ADME BIOANALYSES (30 310 Vergeze, France)
Purity: 98.2%
Dose applied: 5 mg/cm² of formulation, about 831.4 and 859.1 µg/cm²
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 (µg/cm²) 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	
	µg/cm²	% of the applied dose	µg/cm²	% of the applied dose
Washing (W)	6.34 ± 1.84	0.77 ± 0.23	7.80 ± 1.64	0.91 ± 0.20
Total absorbed (E+D+S+RF)	425 ± 85	51.0 ± 9.1	385 ± 46	44.9 ± 4.8
Total recovery (%)	52 ± 9		46 ± 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 µg/cm² (51.5%) with a recovery of 92 ± 6%.

Ref.: 21

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}C] DEGEE 53 mCi/mmole, Specific activity at time of application to the skin 112 – 130 µCi/g of formulation)
Batch:	104-272-053 from ADME BIOANALYSES (30 310 Vergeze, France)
Purity:	98.2%
Dose applied:	5 mg/cm² of formulation, 100 – 571 µg/cm^2
Skin preparation:	Human skin
Skin temperature:	37 ^{0}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 (µg/cm²) 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%	
	µg/cm²	% of the applied dose	µg/cm²	% of the applied dose	µg/cm²	% 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%	
	µg/cm²	% of the applied dose	µg/cm²	% of the applied dose	µg/cm²	% 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 μg/cm² (55.9%) with a recovery of 85±14%, 5%: 167 μg/cm² (63.8%) with a recovery of 92±3%, 10%: 319 μg/cm² (56.4%) with a recovery of 93±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 ± 10.6% was absorbed using a shampoo with 5% DEGEE (total recovery 91%). With 10% DEGEE 17.5 ± 3.9% was absorbed (total recovery 91%). In the second study with a hydro-alcoholic formulation (leave-on) containing 15% DEGEE 51± 9.1% was absorbed. The total recovery was however, only 52 ± 9%. The low recovery was due to evaporation as the recovery increased to 92 ± 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 ± 4.3, 56 ± 12.5, and 50.4 ± 7.3%, respectively in the first experiment and 45.6 ± 4.8, 44 ± 5.1, and 51.6 ± 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 μCi/mg)
Purity:	97.6%
Dose applied:	20 mg/cm² of formulation, 1400, 700, and 400 μg/cm² 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 [^{14}C]-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/cm² of formulation, 1000, 600, and 200 µg/cm² 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 [^{14}C]-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/cm²****	***Amount left in epidermis/dermis after 24 hours µg/cm²****	***Systemically Available Level µg/cm²* (%)***
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 µg/cm²****	***Amount left in epidermis/dermis after 24 hours µg/cm²****	***Systemically Available Level µg/cm²* (%)***
5	9.59 ± 3.69	0.29 ± 0.17	**9.89 ± 3.75 (0.9 ± 0.4)**
3	8.22 ± 2.26	0.12 ± 0.04	**8.33 ± 2.28 (1.4 ± 0.4)**
1	3.56 ± 2.11	0.11 ± 0.06	**3.67 ± 2.15 (1.8 ± 1.1)**

* values provided as mean ± 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 ± 14.99 µg/cm² (2.4 ± 1.1%) was absorbed in the study with the oxidative hair colorant formulation using 7% DEGEE (total recovery 100%) and 9.89 ± 3.75 µg/cm² (0.9 ± 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±12.5% and in the second experiment 44.4±5.1%. If the two studies are considered together (altogether 20 cells), the mean absorption will be 50.0±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 x 15.0) 64.2 µg/cm² and for the non-oxidative hair colorant formulation (9.9 + 2 x 3.8) 17.5 µg/cm². 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/cm²	µg/cm²	%	
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

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

Dermal

Rabbits

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 Zeeland 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 Zeeland 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/m^3) 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 Cmax) 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 Cmax 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 Cmax, 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 AUC0-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 spleenic 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, urinanalysis, 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:	/

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

Dermal

Rabbits

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

Inhalation

Rats

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/m^3 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 phosphatise 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 cervisiae* (D7)

Ref.: 1

Guideline:	OECD 471
Species/strains:	*Salmonella typhimurium* 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