SCCS/1507/13
Revision of 18 June 2013


Scientific Committee on Consumer Safety

SCCS

# OPINION ON
# DIETHYLENE GLYCOL MONOETHYL ETHER
# (DEGEE)

The SCCS adopted this opinion at its 18th plenary meeting

of 26 February 2013

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.
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In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines 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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This opinion has been subject to a commenting period of four weeks after its initial publication. Comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged. Revised opinions carry the date of revision.

Keywords: SCCS, scientific opinion, diethylene glycol monoethyl ether, directive 76/768/ECC, CAS 111-90-0, EC 203-919-7

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## TABLE OF CONTENTS

## 1. BACKGROUND

Diethylene glycol monoethyl ether (DEGEE) was evaluated by the SCCP in 2006 (SCCP/1044/06) and in 2008 (SCCP/1200/08).

The SCCS adopted the third opinion (SCCS/1316/10) on Diethylene glycol monoethyl ether (DEGEE) at its 8th plenary meeting of 21 September 2011 with the following 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 7.0% in oxidative hair dye formulations and in an on-head concentration up 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.*

In January 2012, industry requested to increase the maximum use concentration in all cosmetics products from 1.5% to 2.6%.

Moreover, the International Fragrance Association (IFRA) reported the use of DEGEE as solvent, commonly used in the Fragrance Industry to prepare fragrance compounds. Based on the available use information, an estimation of the inhalation exposure to DEGEE was submitted to support a risk assessment of its use in spray products.

## 2. TERMS OF REFERENCE

1. *On the basis of available information, the SCCS is asked to assess whether a maximum concentration of 2.6% of DEGEE can be considered safe, also taking into account the other uses previously assessed (10% in rinse-off products, 7.0% in oxidative and 5.0% in non-oxidative hair dye formulations).*
2. *Taking into account the provided exposure data, the SCCS is asked to assess the safety of DEGEE when used in spray products in a concentration up 2.6%.*
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), 16 December 2008 (SCCP/1200/08) and by the SCCS on 21 September 2010 (SCCS/1316/10) with additional data from a recent submission. New references in opinion n° SCCS/1316/10 are referred to as N Ref.: xx, and are listed separately. Additional references from the present opinion are likewise listed separately as NN Ref.: xx.

### *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

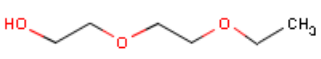
Carbitol, Carbitol solvent, Dioxitol, Dowanol 17, Dowanol DE, Ektasolve DE, Solvolsol, Transcutol CG, Transcutol P, Transcutol HP

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

Colourless liquid with a mild, pleasant odour; hydroscopic

#### 3.1.3. Molecular weight

Molecular weight: 134.2 g/mol

### 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

Miscible in water

### 3.1.7. Partition coefficient (Log Pow)

Log $P_{ow}$: 0.54 (exp.)

### 3.1.8. Additional physical and chemical specifications

| | |
|---|---|
| Melting point: | - 76 °C |
| Boiling point: | 179 – 205 °C |
| Density: | 0.988 |
| Vapour pressure: | 0.19 hPa |
| Rel. vapour pressure: | / |

Conversion
1 ppm = 5.58 mg/m³
1 mg/m³ = 0.179 ppm

### 3.1.9. Homogeneity and 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 nitrocellulose, 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, SCCS/1316/10), the SCCP positively evaluated the use of DEGEE in all cosmetic products up to 1.5% in addition to 10% in rinse-off products, and 7.0% in oxidative and 5% in non-oxidative hair dye 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 in cosmetic products including spray products up to 2.6% based on new information provided.

Cosmetic Europe point out that one third of the hydroalcoholic products would contain at least traces of DEGEE. DEGEE can be intentionally added to the fragrance compounds in order to improve diffusion properties of perfumes.

### *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 | LD50 (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

Comment
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 | LD50 (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 on acute toxicity
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 Monoethylether (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 24h 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

Human

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 12$^{th}$ 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 comment
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 hours 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 |

| | 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 DEGEE in oxidative formulation and with DEGEE in 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* (%)*** |
|---|---|---|---|

| 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 3.9 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 were applied to the filter in the first experiments while 20 mg/cm² were used in the experiments with the hair dye formulations.

The applicant applies for the use of 2.6% DEGEE in all cosmetic products. Two experiments relevant for this use with 10 cells each have been performed. In the first experiment the mean absorption was 43.2±4.3% and in the second experiment 45.6±4.8%. The study carried out with occlusion gave an absorption of 55.9. However, the experiment is not satisfactory reported with standard deviations.

Conclusions

In the MOS calculation for the use of 2.6% DEGEE in all products, SCCS will use (45.6 + 4.8) 50.4%. This value has been taken from the second experiment. In the first experiment the value was (43.2 + 4.3) 47.5%. It is noted that these experiments were performed with 2% DEGEE and the recovery was only 44 – 46%. An absorption of 55.9% was reported with occlusion (85% recovery).

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 3.9: Summary of dermal absorption studies with DEGEE

| Formulation | Incubation time | Concentration | | Dermal absorption | | Recovery % |
|---|---|---|---|---|---|---|
| | | % | µg/cm² | µg/cm² | % | |
| Shampoo (rinse-off) (20) Table 3.3 | 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 (le-ave-on) (21) Table 3.4 | 24 h | 15 | 831 | 425 ± 85 | 51.0 ± 9.1 | 52 |
| | | 15 | 859 | 385 ± 46 | 44.9 ± 4.8 | 46 |
| Repeated under occlu-sion (21) Text | 24 h | 15 | Ca 890 | 459 | 51.5 | 92 |
| Emulsified formulations (leave-on) First exp. (22) Table 3.5 | 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) Table 3.6 | 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) Text | 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) Table 3.7 | 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) Table 3.8 | 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

No inhalation study was performed by the applicant, but there exists information on study results, which were partly peer reviewed and included in the OECD SIDS documents.

### 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 regimen.

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 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 was 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

In a 28-day nose only inhalation study in which Sprague Dawley rats inhaled 16, 49, or 200 ppm (90, 270, 1100 mg/m³) DEGEE for 6 hours/day, 5 days/week, no systemic effects were observed. However, mild local irritation of the larynx and nasal turbinates were found in some rats (numbers were not stated) and foci of necrosis in the small ventral cartilage of the larynx were observed in 2/5 and 3/5 males inhaling 270 or 1100 mg/m³, respectively. At 1100 mg/m³ the test atmosphere was approximately equally divided by mass into respirable droplets (aerosol) and vapour. The NOAEL from this study for systemic toxicity was determined to be 1100 mg/m³. It is stated that DEGEE was 98.6% pure. Impurities included ethylene glycol (1%) and ethyl glycol (0.24%).

Ref.: 26

Comment
A NOAEL of 90 mg/m3 was found for local effects and 1100 mg/m3 DEGEE for systemic toxicity. SCCS note that the DEGEE used contained 1% ethylene glycol.

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) toxicity (oral, dermal)

**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 |

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 (hematology [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

Comment
The SCCS notes that the concentration of ethylene glycol is higher than accepted in cosmetic preparations and that the study was not performed according to OECD guidelines and GLP.

**Dermal**

Rabbits

Rabbits received dermal treatments (not further specified) of DEGEE at dose levels of 0.1, 0.3, 1.0 and 3.0 ml/kg bw at 5times 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

In this poorly reported study in which rats were exposed for 4 months continuously to 0.2, 1 or 4 ppm (1, 5, or 25 mg/m3) DEGEE, 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”. The continuous nature of the exposure is unlikely to reflect true exposure situations.

Ref.: 32

Comment
No conclusions were drawn from this study due to the nature of exposure as well as the limited reporting of the study.

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 (Wistar) 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. 46% of the high dose (950 mg/kg bw/d) animals, 14% of the intermediate dose (200 mg/kg bw/d), and 4% of the low dose (40 mg/kg bw/d) had pathological changes (kidney and bladder damage). DEGEE with less than 0.2% ethylene glycol was less toxic. 7% of the high dose (950 mg/kg bw/d) had pathological changes (kidney and bladder damage. To effect was found among the rats receiving lower doses. The authors stated that "incidences of infections and tumours were not increased by DEGEE administration. 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

Comment
The study was performed in 1944. It is not possible to draw any conclusion from the study in relation to potential carcinogenic effects.

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*

Dermal
In a 28 day study with exposure 6 h/day (semi-occlusive), the study authors concluded that Transcutol is not toxic at dose levels up to 1000 mg/kg bw/day. No effects on growth, mortality, haematology, clinical chemistry or gross pathology were recorded in a 90 day study where rabbits were exposed to DEGEE 5 times per week. However, treatment related histopathological effect was seen in the kidneys of the animals at 1000 and 3000 mg/kg bw. The effects on the kidney are further supported by an old study from 1947 where kidney damage and treatment-related mortality were reported in rabbits following dermal application of DEGEE for 30 day (no further information available).

Oral

Table 3.10: 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<br>**1.8% in diet:** Relative kidney weight significantly increased among males.<br>**0.6% in diet:** No effects recorded.<br>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 rats died.<br>**2680 mg/kg**: 1 rat died. Relative liver, heart, and kidney weights increased.<br>**1340 mg/kg:** No effects noted | NOAEL = 1340 mg/kg bw/day | N-6, 48 |

| Study | Species | Sex | Effects | Critical doses | Ref |
|---|---|---|---|---|---|
| 90 day | Rat: Wistar | m + f | **5% in diet**: Growth significantly retarded.<br>**1% in diet:** No effect recorded.<br>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 anaemia in males. Relative kidney weigh significantly increased among both males and females.<br>**0.5% in diet:** No effects recorded.<br>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<br>(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<br>**500 mg/kg:** Hydropic degeneration of the proximal renal tubules in one of two females.<br>**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.<br>**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.<br>**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.<br>**950 mg/kg:** 7% with pathological kidney changes.<br>**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. The concentration of ethylene glycol in this study was higher than accepted in cosmetic preparations and the study was not performed according to OECD guidelines and GLP.

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.

Inhalation

In the 28 days study rats were exposed to 90, 270 and 1100 mg/m$^3$ DEGEE 6 h/day 5 days/week. Mild local irritation of the larynx and nasal turbinates were found in rats

exposed to 270 and 1100 mg/m$^3$ DEGEE. No system effects were observed. A NOAEL of 90 mg/m$^3$ was found for local effects and 1100 mg/m$^3$ DEGEE for systemic toxicity. SCCS note that the DEGEE used contained 1% ethylene glycol.

In a 4 month study where rats were exposed continuously to 1, 5, or 25 mg/m$^3$ DEGEE, the authors reported changes in the functional state of the nervous system were found during both the treatment and the recovery periods in rats. Analysis of blood samples was said to reveal indications of anemia. The authors wrote that the effects were confined mainly to rats exposed to 5 mg/m$^3$ or more.

### 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

Comment
The study is from 1986 and is poorly reported. Its reliability is considered as limited.

| | | |
|---|---|---|
| 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 bacterio-toxicity 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 *in vivo*

**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<br>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<br>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 $^{3}$H-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, there were no significant increases observed at any dose level or at

harvest time in the group mean or net nuclear grain (NG) count for animals treated with Transcutol. 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 vitro*
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.
*In vivo*
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 (premating 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

Comment
SCCS consider NOAEL for parental systemic toxicity 2200 mg/kg bw/d, NOAEL for F1 reproduction 2200 mg/kg bw/d, There was no effect on fertility despite decrease in sperm motility in F1 males at 4400 mg/kg bw/d. Increased liver weight was also seen at this dose.

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: 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 fetuses were weighed, sexed and examined for external abnormalities. Half of the fetuses were examined internally prior to processing for skeletal examination. The remaining fetuses 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 embryofetal 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: | 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<br>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 or 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 route**

Rats

| | |
|---|---|
| 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.

**Inhalation**

Rats

| | |
|---|---|
| Guideline: | / |
| Species/strain: | Rat/Sprague-Dawley |
| Group size: | Control 16, exposed 21 females |
| Test substance: | Technical grade DEGEE; purity 98 – 99.5% |
| Batch: | / |
| Dose level: | 102 ppm (570 mg/m$^3$), 7 hr/day |
| Route: | Inhalation |
| Exposure period: | Day 7 – 15 of gestation |
| GLP: | / |
| Date: | 1984 |

Males (>300g) were individually placed in cages with three females (200-300 g). Vaginal smears were taken daily, and the presence of sperm was considered day 0 of gestation. Pregnant females were caged alone. Feed and water intake were recorded on days 7, 14 and 21. Signs of maternal toxicity were noted daily.

Animals were placed individually in wire mesh cages within the exposure chambers. 20 pregnant animals were exposed to test material. Exposures were conducted 7 hr/day on gestation days 7 – 15. Females were weighed and euthanized on day 20. The uterus was removed and the numbers of resorption sites and live foetuses were counted. Foetuses were removed, weighed, sexed, and examined for external malformation. Two-thirds of the foetuses were fixed and examined for visceral abnormalities with a dissection microscope. The rest of the foetuses were examined for skeletal defects.

Results
Test material did not cause toxicity in dams or in foetuses. There was no effect of treatment on the number of pregnancies (15/16 in control, 20/21 in treated) implants/dam (13.1 in control and 13.4 in treated), resorptions per litter (1.3 in control and 0.9 in treated) live foetuses (174 in control and 250 in treated), live foetuses/litter (11.6 in control and 12,5 in treated), live foetal weight, or the number of litters with abnormal foetuses (5[33%] in control and 6[30%] in treated). There was no statistical significant effect of treatment on the number of foetuses with visceral or skeletal malformations or on the specific types of malformations or variations noted.

Ref.: 46

Comment
The SCCS considers, on the basis of the above experiment, the NOAEL of maternal toxicity and teratogenicity to be 102 ppm (570 mg/m$^3$; 11.4 mg/kg bw/d) based on no effects at the only dose tested.

*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 foetuses. In a rat study the dose of 1000 mg/kg bw/day was considered a NOAEL for maternal and embryofetal toxicity. In an inhalation study no effects were found at the single dose studied (102 ppm; 11.4 mg/kg bw/d).

## 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)<br>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<br>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<br>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 [$^{14}$C]-DEGEE or 150 µM [14C]-EGEE. Incubation medium aliquots were removed at 0, 1, and 4 h after the addition of [$^{14}$C]-DEGEE or [$^{14}$C]-EGEE. Control incubations without hepatocytes were included with the first experiment using 1500 µM [$^{14}$C]-DEGEE or 150 µM [$^{14}$C]-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 [$^{14}$C]-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 pmol 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 [$^{14}$C]-DEGEE and 70% of the radioactivity was [$^{14}$C]-DEGEE in cells incubated with the lowest concentration (15 µM [$^{14}$C]-DEGEE). The rest of the radioactivity was associated with several peaks that were not identified. Rat hepatocyte incubations with [$^{14}$C]-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 [$^{14}$C]-DEGEE in the H1 and H2 hepatocyte preparations. When H1 hepatocytes were incubated for 4 h with 150 µM [$^{14}$C]-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:<br>3 males and 3 females/time point (blood and plasma kinetics)<br>3 males and 3 females/time point (balance of excretion)<br>3 males and 3 females/time point (tissue distribution, oral route)<br>BDIX Rats:<br>3 males and 3 females/time point (blood and plasma kinetics)<br>3 males and 3 females/time point (tissue distribution, oral route) |
| Test substance: | Non-labelled: Transcutol® HP (DEGEE)<br>Labelled: [$^{14}$C]-Diethylene glycol monoethyl ether ([$^{14}$C]-DEGEE) |
| Batch: | Non-labelled: DEGEE: 0025005<br>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<br>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 [$^{14}$C]-DEGEE /kg bw each.

Results and conclusion
After administration of 20 mg/kg of [$^{14}$C]-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)<br>Labelled: [$^{14}$C]-Diethylene glycol monoethyl ether ([$^{14}$C]-DEGEE) |
| Batch: | Non-labelled: DEGEE: 0025005<br>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 [14C]-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<br>Urine: pre-dose, 0 – 8 h, 8 – 24 h<br>Faeces: 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 [$^{14}$C]-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. [$^{14}$C]-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 [$^{14}$C]-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 by the applicant on toxicokinetics and metabolism
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 [$^{14}$C]-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 (first sampling time), 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 administrated radioactivity was excreted in the urine within the first 24 hours and [$^{14}$C]-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 [$^{14}$C]-DEGEE were detected.

Comment

SCCS notes that there is no evidence as to the extent of hydrolysis of the ether among species and hence it is not clear what animal would be the best model for man.

### 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

*Exposure from spray application*
When using spray application it is expected that the dermal dose is the same as when applying directly to the skin, but in addition there will be some exposure by inhalation.

<u>Calculations by the applicant</u>
The inhalation exposure was calculated by the applicant for fine fragrances, hair sprays, antiperspirants and deodorants with the use of a computer program developed by RIVM (ConsExpo 4.1) assuming a DEGEE concentration in the spray of 2.6%. The main results are presented in Table 3.11.

*Table 3.11. Calculation of daily exposure by the applicant*

| Substance | Peak concentration Zone 1 (mg/l) | Peak concentration Zone 2 (mg/l) | Total exposure (mg) | Daily exposure (mg/kg bw/d) |
|---|---|---|---|---|
| Fine fragrance | 0.192 | 0.0228 | 2.34 | 0.0390 |
| Hair spray | 0.0270 | 0.0108 | 1.13 | 0.0188 |
| Ap/Deo | 0.0462 | 0.0065 | 0.672 | 0.0111 |
| Total | | | | 0.0689 |

Total inhalation exposures (per body weight per day) to DEGEE by the applicant were estimated to 0.0390 mg/kg for fine fragrance, 0.0188 mg/kg for hair spray, and 0.0111 mg/kg for and 0.0111 mg/kg. Total 0.0689 mg/kg bw/d.

NNref.: 1

Comment
The SCCS notes that the applicant considers 2 zones (as scenarios) in relation to the inhalation exposure. Zone 1 (Cloud) is 1 $m^3$ and time in zone 1 is 1 min. Zone 2 (Bathroom) is 10 $m^3$ and time in zone 2 is 20 min. Moreover, the inhalation rate was considered to be only 9 l/min.

Calculation by SCCS (calculation based on ppm).
A conservative estimation for DEGEE was made using extrapolation from the DCM opinion. Based on the DCM Opinion (SCCS/1408/11) using the ConsExpo exposure calculation tool exposure for a consumer using a hair spray was calculated (evaporation module). This model assumes use of a hair spray in a small room (bathroom, 10 $m^3$) with a low ventilation rate (2$m^3$/h). This scenario is described in the fact sheet 'Cosmetic Products'[1] . Other assumptions are: the sprayed amount is 6.8 g, the hairspray contained 35% dichloromethane, the consumer is in the bathroom for 5 minutes. Using this model, the exposure is calculated to be in average 62 ppm (219 mg/$m^3$). It is assumed that consumers use the hairspray twice a day, so they will be exposed to 62 ppm for 5 minutes twice each day. Assuming 2.6% DEGEE, this will represent (62 x 0.026 / 0.35) 4.6 ppm or 25.7 mg/$m^3$ or 0.0257 mg/l. This "Peak concentration has then been used for Fine fragrance and Antiperspirant/Deodorant by scaling according to the amount sprayed according to the ConsExpo fact sheet. Total daily exposure is based on two times 5 minutes and an inhalation rate of 23.1 l/min. See Table 3.12.

Table 3.12: Calculation of daily exposure by SCCS

| Substance | Spraying time (s) | Amount sprayed (g) | Peak concentration (mg/l) | Total exposure (mg) | Daily exposure (mg/kg bw/d) |
|---|---|---|---|---|---|
| Fine fragrance | 5.0 | 0.70 | 0.0026 | 0.61 | 0.0101 |
| Hair spray | 14.4 | 6.8 | 0.0257 | 5.94 | 0.0989 |
| Ap/Deo | 10 | 4.0 | 00151 | 3.49 | 0.0582 |
| Total | | | | | 0.167 |

The highest peak exposure is calculated to 0.0257 mg/l or 25.7 mg/$m^3$. Since a NOAEL of 90 mg/$m^3$ was found for local effects in rats in a 28 day study with 6 hours exposure per day, it is unlikely that 2 times 5 min exposure per day at 25.7 mg/$m^3$ will produce any adverse effects in humans.

### 3.3.13. Safety evaluation (including calculation of the MoS)

[1] www.consexpo.nl

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 2.6% DEGEE in all cosmetic products and 10% DEGEE in rinse-off products**

Table 3.13: Calculation of aggregate exposure through cosmetic use for preservatives

| Type of exposure | Product | g/day |
|---|---|---|
| Rinse-off skin & hair cleansing products | Shower gel | 0.19 |
| | Hand wash soap | 0.20 |
| | Shampoo | 0.11 |
| | Hair conditioner | 0.04 |
| *Total rinse-off products* | | *0.54* |
| Leave-on skin & hair care products | Body lotion | 7.82 |
| | Face cream | 1.54 |
| | Hand cream | 2.16 |
| | Deo non-spray | 1.50 |
| | Hair styling | 0.40 |
| Make-up products | Liquid foundation | 0.51 |
| | Make-up remover | 0.50 |
| | Eye make-up | 0.02 |
| | Mascara | 0.025 |
| | Lipstick | 0.06 |
| | Eyeliner | 0.005 |
| *Total leave-on products* (products used for the eye are excluded) | | *14.45* |
| Oral care cosmetics | Toothpaste | 0.14 |
| | Mouthwash | 2.16 |
| **TOTAL** | | **17.4** |

CALCULATIONS OF THE MARGIN OF SAFETY

Diethylene glycol monoethyl ether (DEGEE)

The safety calculation is considering dermal and inhalation exposure.

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

The applicant wants to use DEGEE in a concentration up to 2.6% in all cosmetic products and in a concentration up to 10% in rinse-off products.

**All cosmetic products (except products for oral hygiene and the eyes)**

*Leave on products*

Total leave-on products (except products for oral hygiene and the eyes) 14.45 g (see Table 3.13)

A dermal absorption of 50.4% is used in the MOS calculations.

| | | | |
|---|---|---|---|
| Exposure 14.45 g/day, 2.6% DEGEE | | = | 376 mg/d |
| Maximum absorption through the skin | 376x50.4/100 | = | 189 mg/d |
| Typical body weight of human | | = | 60 kg |
| Systemic exposure dose (SED) | 189/60 | = | 3.16mg/kg bw/d |

| | | |
|---|---|---|
| ***Margin of Safety*** *(leave on products)* ***NOAEL / SED*** | **=** | ***127*** |

*Rinse-off products*

Total rinse-of products 0.54 g (see Table 3.13)

A dermal absorption of 21.4% is used in the MOS calculations.

| | | | |
|---|---|---|---|
| Exposure 0.54 g/day, 10% DEGEE | | = | 54 mg/d |
| Maximum absorption through the skin | 54 x 21.4/100 | = | 11.6 mg/d |
| Typical body weight of human | | = | 60 kg |
| Systemic exposure dose (SED) | 11.6 /60 | = | 0.19mg/kg bw/d |

| | | | |
|---|---|---|---|
| ***Margin of Safety*** *(rinse-off products)* | ***NOAEL / SED*** | **=** | ***2077*** |

**All cosmetic products (dermal exposure, except products for the eyes))**

Total dermal systemic exposure dose (3.16 + 0.19) **=** 3.35 mg/kg bw/d

| | | | |
|---|---|---|---|
| **Margin of Safety** | **NOAEL / SED** | **=** | **119** |

***Inhalation from spray products***

Systemic exposure dose (2.6% in spray products (see Table 3.12)): 0.167 mg/kg bw/d

| | | | |
|---|---|---|---|
| **Margin of Safety** | **NOAEL / SED** | **=** | **2395** |

***Total dermal and inhalation exposure***

Total Systemic exposure dose (3.35 + 0.17) 3.52 mg/kg bw/d

| | | | |
|---|---|---|---|
| **Margin of Safety** | **NOAEL / SED** | **=** | **114** |

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

A dermal absorption of 64.2 µg/cm² is used in the MOS calculations.

| | | | |
|---|---|---|---|
| Maximum absorption through the skin | A (µg/cm²) | = | 64.2 µg/cm² |
| Skin Area surface | SAS (cm²) | = | 580 cm² |
| 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 | = | 0.62mg/kg bw/d |
| No observed adverse effect level | NOAEL | = | 400 mg/kg bw/d |

| | | | |
|---|---|---|---|
| **Margin of Safety** | **NOAEL / SED** | **=** | **645** |

A MOS of 645 gives sufficient protection in relation to the use of DEGEE as a solvent in oxidative hair dye formulations.

If we consider that the exposure from dermal, spray and oxidative hair dye 3.52 + 0.62 = 4.14 mg/kg bw/d, resulting in a MOS = 97

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

A dermal absorption of 17.5 µg/cm² is used in the MOS calculations.

| | | | |
|---|---|---|---|
| Maximum absorption through the skin | A (µg/cm²) | = | 17.5 µg/cm² |
| Skin Area surface | SAS (cm²) | = | 580 cm² |
| 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** |

A MOS of 2353 gives sufficient protection in relation to the use of DEGEE as a solvent in oxidative hair dye formulations.

(If we consider that the exposure from dermal, spray and non-oxidative hair dye 3.52 + 0.17 = 3.69 mg/kg bw/d, resulting in a MOS = 108

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

In this Opinion, the safety has been evaluated for both dermal and inhalation exposures.

*Physico-chemical specifications*
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. It is stated that Transcutol CG which is used in cosmetics contains < 0.062% 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 mg/m³ (i.e. >5 mg/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 ± 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 MOS calculation of rinse-off products 17.5 + 3.9% = 21.4% was used.

Use in concentrations up to 2.6% in leave-on cosmetic products

Two experiments with 10 cells each have been performed with 2% DEGEE. In the first experiment the mean absorption was (43.2 + 4.3) 47.5%. In the second experiment it was (45.6 + 4.8) 50.4%. In the MOS calculation for use of 2.6% DEGEE in leave-on cosmetic products SCCS used 50.4%.

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 ± 15.0 µg/cm² (2.4 ± 1.1%) for the oxidative formulation and 9.9 ± 3.8 µg/cm² (0.9 ± 0.4%) for the non-oxidative formulation. The total recovery was 100%. In the MOS calculation 64.2 µg/cm² (mean + 2SD) is used for the oxidative hair dye formulation and 17.5 µg/cm² (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 determined.

*Repeated dose toxicity*

In five oral 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 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 with a NOAEL of 400 mg/kg bw/day in the calculation of MOS.

In a 28-day nose only inhalation study which rats inhaled 16, 49, or 200 ppm (90, 270, 1100 mg/m³) DEGEE for 6 hours/day, 5 days/week, no systemic effects were observed. However, mild local irritation of the larynx and nasal turbinates were found in some rats exposed to 270 or 1100 mg/m³. Thus, a NOAEL of 90 mg/m³ was found for local effects and 1100 mg/m³ DEGEE for systemic toxicity. The SCCS notes that the DEGEE used contained 1% ethylene glycol.

*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 embryofoetal toxicity. It was concluded that there was no indication of teratogenicity at any dose level used in the study

Males rats (>300g) were individually placed in cages with three females (200-300 g). The presence of sperm in vaginal smears was considered day 0 of gestation. Pregnant females were caged alone. 20 pregnant animals were exposed to 570 mg/m3 DEGEE 7 hr/day on gestation days 7 – 15. Females were weighed and euthanized on day 20. The uterus was removed and the numbers of resorption sites and live foetuses were counted. Foetuses were removed, weighed, sexed, and examined for external malformation. Two-thirds of the foetuses were fixed and examined for visceral abnormalities with a dissection microscope. The rest of the foetuses were examined for skeletal defects. Test material did not cause toxicity in dams or in foetuses. SCCS considers on the basis of the above experiment the NOAEL of maternal toxicity and teratogenicity 102 ppm (570 mg/m3; 11.4 mg/kg bw/d) based on no effects at the only dose tested.

*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 mg $^{14}$C-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 (earliest sampling point), 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 $^{14}$C-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 $^{14}$C-DEGEE were detected, which was consistent with urinary results.

There is no evidence as to the extent of hydrolysis of the ether among species and hence it is not clear what animal would be the best model for man.

## 4. CONCLUSION

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

1. The use of DEGEE at a maximum concentration of 2.6% in cosmetic products taking into account the other uses previously assessed (10% in rinse-off products, 7.0% in oxidative and 5% in non-oxidative hair dye formulation) does not pose a risk to the health of the consumer.

2. the use of DEGEE in the following spray products, fine fragrances, hair sprays, and antiperspirants and deodorants in a concentration up to 2.6% does not pose a risk to the health of the consumer.

3a. Since the systemic daily dose with the current levels and uses of DEGEE considered to be safe for the consumers, is higher than that permitted in previous Opinions, SCCS is of the opinion that the level of ethylene glycol impurity in DEGEE should be decreased from <0.2% to ≤0.1% (DEGEE conforming to this specification are commercially available) in order to avoid consumer exposure to higher dose of this toxic impurity.

3b. The use of DEGEE in products for oral hygiene and the eyes has not been evaluated.

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

## 5. MINORITY OPINION

Not applicable

## 6. REFERENCES

Received with previous submissions

1. Berte F, Bianchi A, Gregotti C, Bianchi L, Tateo F. In vivo and in vitro toxicity of carbitol. Boll Chim Farm 125: 401-403, 1986
2. Clayton, G. D. and F. E. Clayton (eds.). Patty's Industrial Hygiene and Toxicology: Volume 2A, 2B, 2C: Toxicology. 3rd ed. New York: John Wiley Sons, 1981-1982., p. 3959-3961, 1982
3. Laug EP, Calvery HO, Morris HJ, Woodard G. The toxicology of some glycols and derivatives. J Ind Hyg Toxicol 21: 173-201, 1939
4. Hanzlik PJ, Luduene FP, Lawrence WS, Hanzlik H. Acute toxicity and general systemic actions of diethylene monoethyl ether (carbitol). J Ind Hyg Toxicol 29: 190-195, 1947
5. Weil CS. Statistics vs safety factors and scientific judgement in the evaluation of safety for man. Toxicol Appl Phamacol 21: 454-463, 1972
6. Budavari, S. (ed.). The Merck Index - Encyclopedia of Chemicals, Drugs and Biologicals. Rahway, NJ: Merck and Co., Inc., p. 272, 1989
7. Gattefosse, Acute oral toxicity TOX94205, 1994
8. Smyth HF, Seaton J, Fischer L. The single dose toxicity of some glycols and derivatives. J Ind Hyg Toxicol 23: 259-268, 1941
9. Browning E. Toxicity of metabolism of industrial solvents. Elsevier, Amsterdam, Netherlands. Pp 632-634, 1965
10. Hanzlik PJ, Lawrence WS, Fellows JK, Luduene FP, Laqueur GL. Epidermal applications of diethylene monoethyl ether (carbitol) and som other glycols. . J Ind Hyg Toxicol 29: 325-341, 1947
11. National Technical Information Service. Vol. OTS0516797
12. BG Chemie: Toxicological Evaluations, Five Potential Health Hazards of Existing Chemicals. Vol. 5, Pg. 91, 1993
13. Draize JH, Woodward G, Calvery HO. Methods for the study of irritation and the toxicity of substances applied to the skin and mucous membranes. J Pharmacol Exp Therap. 82: 377-390, 1944
14. Gattefosse, Acute cutaneous irritation test (Single patch test) TOX9269, 1992
15. Jacobs GA, Matines MA. An objective methods for the evaluation of eye irritation in vivo Food Chem Toxicol 27: 255-258, 1989
16. Gattefosse, Acute ocular irritation test (JORF) TOX96334, 1996
17. Gattefosse, Acute ocular irritation and corrosion test TOX96335, 1996
18. Grant, W.M. Toxicology of the Eye. 3rd ed. Springfield, IL: Charles C. Thomas Publisher, p. 335, 1986
19. Gattefosse, Repeated dose cutaneous irritation and sensitization test (Marzulli and Maibach's method) TOX9370, 1993
20. PMIC. Percutaeous absorption of diethylene glycol mono ethyl ether through human skin in vitro. Shampo Formulations. Antony, France 24 March, 2004
21. PMIC. Percutaeous absorption of diethylene glycol mono ethyl ether through human skin in vitro. Hydro-Alcoholic Gel Formulation. Antony, France 15 April, 2004
22. PMIC. Percutaeous absorption of diethylene glycol mono ethyl ether through human skin in vitro. Emulsified Formulations. Antony, France 15 April, 2004
23. Walther R. The toxicology of glycols. Arch Gewerbehyg 11: 327-344, 1942
24. Smyth HF, Carpenter CP, Further experience with the range-finding test in the industrial toxicology laboratory. J Ind Hyg 30: 63-68, 1948
25. Gattefosse, Repeat dose dermal toxicity (28 days) TOX95241, 1994
26. Hardy CJ, Coombs DW, Lewis DJ, Klimisch HJ. Twenty-eight-day repeated-dose inhalation exposure of rats to diethylene glycol ether. Fundam Appl Toxicol. 38: 143-147, 1997
27. Lehmann KB, Flury F. Toxiclogy and hygiene of industrial solvents. Williams and Wilkins, Baltimore, Maryland, USA, 1943

28. Hall DE, Lee FS, Austin P, Fairweather FA. Short-term feeding study with diethylene glycol monoethyl ether in rats. Fd Cosmet Toxicol 4: 263-268, 1966
29. Gaunt IF, Colley J, Grasso P, Lansdown ABG, Gangolli SD. Short-term toxicity of diethylene glycol monoethyl ether in the rat, mouse and pig. Fd Cosmet Tox 6: 689-705, 1968
30. Krasavage WJ, Vlaovic MS. Comparative toxicity of nine glycol ethers: Six weeks repeated dose study. Unpublished data. Corporate health and Enviroment Laboratories, report No TX-82-06 March 15, 1982
31. Gingell R, Boatman RJ, Bus JS, Cawley TJ, Knaak JB, Krasavage WJ, Skoulis NP, Stack CR, Tyler TR. In Clayton, GD, FE. Clayton (eds.). Patty's Industrial Hygiene and Toxicology 4th revised edition. Volume II, part D 2761-2966, Wiley, New York, USA, 1994
32. Krotov YA, Lykova AS, Skachkov MA, Mitrofanova AI. Sanitary-toxicological characteristics of diethylene glycol ethers (carbitols) used in air pollution control. Gig Sanit 2: 14-17, 1981
33. Morris HJ, Nelson AA, Calvery HO. Observations of the chronic toxicities propylene glycol, ethylene glycol, diethylene glycol, ethylene glycol mono-ethyl ether, and diethylene glycol mono-ethyl-ether. J Pharmacol Exp Therap. 74: 266-273, 1942
34. Smyth HF, Carpenter CP, Boyd SC. Summary of toxicological data – A 2 year study of diethylene glycol monoethyl ether in rats. Food Cosmet Toxicol 2: 641-642, 1964
35. Hanzlik PJ, Lawrence WS, Laqueur GL. Comparative chronic toxicity of diethylene monoethyl ether (Carbitol) and related glycols. J Ind Hyg Toxicol 29: 233-241, 1947
36. Butterworth KR, Gaunt IF, Grasso P. A nine-month toxicity study of diethylene glycol monoethyl ether in the ferret. Research report no 15/1975. Br Ind Bio Res Assolc, UK. 1975
37. Gattefosse, Bacteria reverse mutation test (Ames) TOX99483, 1999
38. Gattefosse, Genetic toxicity: DNA damage and repair (Unscheduled DNA synthesis in mammali) TOX96340, 1996
39. Schuler RL, Hardin BD, Niemeier RW, Booth G, Hazelden K, Piccirillo V, Smith K. Results of testing 15 glycol ethers in a short term in vivo reprotoxicity assay. Env Health Perspectives, 57: 141-146, 1984
40. Hardin BD, Schuler RL, Burg JR, Booth GM, Hazelden KP, MacKenzie KM, Piccirillo VJ, Smith KN. Evaluation of 60 chemicals in a preliminary developmental toxicity test. Ter Carc Mut 7: 29-48, 1987
41. Williams J, Reel JR, George JD, Lamb JC. Reproductive effects of diethylene glycol and diethylene glycol monoethyl ether in Swiss CD-1 mice assessed by a continous breeding protocol. Fund Appl Toxicol 14: 622-635, 1990
42. Chapin RE, Sloane RA. Reproductive assessment by continous breeding: evolving study design and summaries of ninety studies. Environ Health Perspect 105 suppl 1: 199-395, 1997
43. Gattefosse. Reproduction/developmental toxicity test: Fertility study – Segment I, 2003
44. Gattefosse. Reproduction/developmental toxicity test: Embryofetal development study – Segment II, 2003
45. Hardin BD, Goad PT, Burg JR. Developmental toxicity of 4 glycol ethers applied cutaneously to rats. Env Health Perspectives, 57: 69-74, 1984
46. Nelson BK, Setzer JV, Brightwell WS, Mathinos PR, Kuczuk MH, Weaver TE, Goad PT. Comarative inhalation teratogenicity of four glycol ether solvents and an amino derivative in rats. Environ Health Perspect 57: 261-271, 1984
47. Fellows JK, Luduene FP, Hanzlik PJ. Glucuronic acid excreation after diethylene glycol monoethyl ether (Carbitol) and some other glycols. J Pharmacol Exptl Therap 89: 210-213, 1947
48. Kamerling JP, Duran M, Bruinvis L, Ketting D, Wadman SK, de Groot CJ, Hommes FA. (2-Ethoxyethoxy)acetic acid. An unusual compound found in gas chromatographic analysis of urinary organic acids. Clin Chim Acta 77: 397-405, 1977

49. The In Vitro Percutaneous Absorption of Radiolabelled Diethylene Glycol Monoethyl Ether at Six Different Concentrations in Hair Dye Formulations Through Human Skin, P&G Study # 10534-61756, December 2007

New references received in Submission II. The references are referred to as N Ref.: xx in the text.

6. ECB (2000) IUCLID Dataset, 2-(2-ethyoxyethoxy) ethanol, CAS 111-90-0, European Commission – European Chemicals Bureau, 18 february 2000 (http://ecb.jrc.ec.europa.eu/IUCLID-DataSheets/111900.pdf)
10. Gattefosse (1974) Transcutol® - Evaluation de la tolerance primaire cutanee chez le lapin (Translation: Skin tolerance test in th rabbit, 22 december 2003), CDREB-R No. 740607, Parexel International Corporation of Quality Assurance Documents, Gattefosse No. GAT-7472
11. Gattefosse (1979a) Transcutol® P (Ref 75033) – Tolerance locale après injection intramusculaire chez le lapin (Translation: Local tolerance after im injection in the rabbit), Product 75033, 50% in water, Report No. 901210, Institut Francais de Recherches et Essais Biologique (IFREB), Les Oncin, L'Arbresle, France, Gattefosse No. GAT-7972, 17 January 1979
12. Gattefosse (1979b) Transcutol® P (Ref 75033) – Tolerance locale après injection intramusculaire chez le lapin (Translation: Local tolerance after im injection in the rabbit), Product 75033, 30% in olive oil, Report No. 901211, Institut Francais de Recherches et Essais Biologique (IFREB), Les Oncin, L'Arbresle, France, Gattefosse No. GAT-7972, 17 January 1979
17. Gattefosse (1996a) Transcutol - Ocular irritation and reversibility test in the rabbit (O.I.R.), Report No. 8596, Pharmakon Europe, Les Oncin, L'Arbresle, France, Gattefosse No. GAT-96335, 14 June 1996
18. Gattefosse (1996b) Transcutol - Ocular irritation test in the rabbit (O.I.), Report No. 8696, Pharmakon Europe, Les Oncin, L'Arbresle, France, Gattefosse No. GAT-96334, 14 June 1996
19. Gattefosse (1996c). Transcutol: Measurement of unscheduled DNA synthesis in rat liver using an in-vitro/in-vivo procedure, Report No. 1449/2-1052, Corning Hazleton, Harrogate, North Yorskshire, England, Gattefosse No. GAT-96340, 09 August 1996
21. Gattefosse (2001a) In-vitro metabolism of Diethylene Glycol Monoethyl Ether (DEGEE) and Ethylene Glycol Monoethyl Ether (EGEE) by rat and human hepatocytes, Report No. B014-98, SRI International, Menlo Park, CA, USA, Gattefosse No. GAT-SRI B014-98, 07 June 2001
22. Gattefosse (2001b) Transcutol HP – Dose range finding fertility and embryotoxicity study by the oral route (gavage) in the rats, Report No. 935/107, MDS Pharma Service, Les Oncin, L'Arbresle, France, Gattefosse No. GAT-MDS 935/107, 29 May 2001
23. Gattefosse (2001c) Transcutol HP – Fertility study by the oral route (gavage) in the rat(Segment I), Study No. 935/105, MDS Pharma Service, Les Oncin, L'Arbresle, France, Gattefosse No. GAT-MDS 935/105, 06 November 2001
24. Gattefosse (2002a) A critical review of the literature and available data regarding the toxicology of Transcutol® (Diethylene Glycol Monoethyl Ether – DEGEE) – A modern Perspective (GLP), Gad SC, Gad Consulting Services, USA, 09 July 2002
25. Gattefosse (2002b) Plasma kinetics, absorption and tissue distribution of 14C-Diethylene glycol monoethyl ether administered by intravenous and oral route to the male and female Sprague-Dawley rats and BDIX rats, Report No. 2/175, ADME Bioanalyses, Vergeze, France, Gattefosse No. GAT/DIE/01001, March 2002
26. Gattefosse (2002c) Transcutol HP – Embryo toxicity study by the oral route (gavage) in the rat (Segment II), Study No. 935/122, MDS Pharma Service, Les Oncin, L'Arbresle, France, Gattefosse No. GAT-MDS 935/122, 14 June 2002

27. Gattefosse (2003a) Metabolite determination of 14C-DEGEE administered by oral route to the male sprague-dawley rat, ADME Bioanalyses, Vergeze, France, Gattefosse No. GAT- ADMEGAT/DIE/02001, 20 Septemer 2003
28. Gattefosse (2003b) Metabolism study of Diethyl Glycol Monoethylether administered by oral route to male Sprague-Dawley rats, ADME Bioanalyses, Vergeze, France, Gattefosse No. GATADMEGAT
34. Gattefosse (2007a) A 13-week oral (gavage) toxicity study oof Transcutol® HP in beagle dogs, Report No. WIL-261017, WIL Research Laboratories, Ashland, OH, USA, Gattefosse No. GAT-WIL 261017, 11 October 2007
35. Gattefosse (2007b) An escalating and repeated dose oral (gavage) toxicity range-finding study of Transcutol® HP in female beagle dogs, Report No. WIL-261016, WIL Research Laboratories, Ashland, OH, USA, Gattefosse No. GAT-WIL 261016, 07 June 2007/DIE/02002, 23 December 2003
48. OECD (2005) SIDS Initial Assessment Report for SIAM 21 (Draft), Diethylene Glycol Ethers, Washington, DC, USA, 18 – 21 October 2005 (http://www.oecd.org/document/63/0,3343,en_2649_34379_1897983_1_1_1_1,00.html)
51. SCCP (2006) Scientific Committee on Consumer Products, Opinion on Diethylene Glycol Monoethylether (DEGEE), adopted 19 December 2006

New references received in Submission III. The references are referred to as NN Ref.: xx in the text.

1. Cosmetics Europe (2012). Estimation of the inhalation exposure to diethylene glycol monoethyl ether (DEGEE) from cosmetic products.