EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

Scientific Committee on Consumer Products

SCCP

OPINION ON

Diethyleneglycol monoethylether

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

The SCCP adopted this opinion at its 18th plenary of 16 December 2008

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

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

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

SCCP
Questions concerning the safety of consumer products (non-food products intended for the consumer).
In particular, the Committee addresses questions related to the safety and allergenic properties of cosmetic products and ingredients with respect to their impact on consumer health, toys, textiles, clothing, personal care products, domestic products such as detergents and consumer services such as tattooing.

Scientific Committee members
Claire Chambers, Gisela Degen, Ruta Dubakiene, Bozena Jazwiec-Kanyion, Vassilios Kapoulas, Jean Krutmann, Carola Lidén, Jean-Paul Marty, Thomas Platzek, Suresh Chandra Rastogi, Jean Revuz, Vera Rogiers, Tore Sanner, Günter Speit, Jacqueline Van Engelen, Ian R. White

Contact
European Commission
Health & Consumer Protection DG
Directorate C: Public Health and Risk Assessment
Unit C7 - Risk Assessment
Office: B232 B-1049 Brussels
Sanco-Sc6-Secretariat@ec.europa.eu

(ISSN)

http://ec.europa.eu/health/ph_risk/risk_en.htm

ACKNOWLEDGMENTS

Dr. C. Chambers
Prof. V. Kapoulas
Prof. J.-P. Marty
Prof. T. Platzek
Dr. S.C. Rastogi
Prof. V. Rogiers
Prof. T. Sanner (chairman and rapporteur)
Dr. J. van Engelen
Dr. I.R. White

Keywords: SCCP, scientific opinion, diethyleneglycol monoethylether, directive 76/768/ECC, CAS 111-90-0, EINECS 203-919-7

Opinion to be cited as: SCCP (Scientific Committee on Consumer Products), Opinion on diethyleneglycol monoethylether, 16 December 2008

TABLE OF CONTENTS

1. BACKGROUND

A risk assessment of diethylene glycol monoethyl ether (DEGEE) with the chemical name 2-(2-ethoxyethoxy)ethanol and INCI-name Ethoxydiglycol was done by a member state (France). The risk assessment was based mainly on open scientific literature and on skin absorption studies done by Industry. The risk assessment led the member state to put some restrictions on the use of this substance.

According to the notification to the Commission the substance DEGEE is used in cosmetic products in shampoos (rinse-off) in a concentration up to 5% and creams (leave-on) in a concentration up to 2%. Furthermore, the substance should not be used in products for oral hygiene and the eyes. The notifying member state concluded that the substance could be considered safe for the consumers, when used in a concentration up to 1.5% in cosmetics product except products for oral hygiene.

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

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

In addition to the overall use of DEGEE in a concentration up to 1.5%, Industry has applied for a specific use of DEGEE as a solvent in hair dyes formulations at a concentration up to 7.0%. To support the usage as solvent in hair dyeing products new percutaneous absorption data and an updated risk assessment was submitted by COLIPA[1] in December 2007.

2. TERMS OF REFERENCE

1. *Does the SCCP consider that an additional use of the substance DEGEE as solvent in an on-head concentration up 7.0% in oxidative hair dye formulations and in an on-head concentration up 5.0% in non-oxidative hair dye formulations is safe, taken into account the provided data?*

2. *And/or, does the SCCP have any further scientific concerns with regard to the overall use of DEGEE in cosmetic products in a concentration up to 1.5% and the additional use with an on-head concentration up to 7.0% in hair dyeing formulations?*

[1] COLIPA – The European Cosmetic Toiletry and Perfumery Association

3. OPINION

The present Opinion contains the information provided in the previous Opinion on DEGEE adopted by SCCP on 19 December 2006 (SCCP/1044/06) with addition of a new percutaneous absorption study received from COLIPA in December 2007 which has been added in section 3.3.4. Dermal / percutaneous absorption. Due to the new request, changes have been made in sections 3.3.13. Safety evaluation (including calculation of the MoS), 3.3.14. Discussion, 4. Conclusion, and 6. References.

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Ethoxydiglycol (INCI)

3.1.1.2. Chemical names

2-(2-Ethoxyethoxy)ethanol (IUPAC name)
Carbitol, Carbitol solvent, Diethylene glycol monoethyl ether, 3,6-Dioxa-1-octanol, Diethylene glycol ethyl ether, , Diglycol monoethyl ether, Dioxitol, Ethanol, 2,2'-oxybis-, monoethyl ether, Ethyl carbitol, Ethyl diethylene glycol, Ethyl digol,

3.1.1.3. Trade names and abbreviations

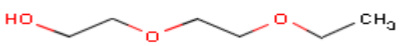
Dowanol 17, Dowanol DE, Ektasolve DE, Solvolsol, Transcutol, Transcutol P, Transcutol HP

DEGEE

3.1.1.4. CAS / EINECS/ELINCS number

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

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_6H_{14}O_3$

3.1.2. Physical form

Liquid with a mild, pleasant odour; hydroscopic

3.1.3. Molecular weight

Molecular weight: 134.2

3.1.4. Purity, composition and substance codes

Gattefosse states that since May 1998, the manufacturing process of DEGEE was improved, in order to decrease the content in residual impurities:

Transcutol: inferior 99.5%
Transcutol P: > 99.7%
Transcutol HP: > 99.9%

3.1.5. Impurities / accompanying contaminants

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

3.1.6. Solubility

In water: Miscible

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: - 0.54

3.1.8. Additional physical and chemical specifications

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

Conversion:
1 ppm = 5.58 mg/m³
1 mg/m^3 = 0.179 ppm

3.1.9. Stability

/

3.2. Function and uses

DEGEE may be prepared from ethylene oxide and 2-ethoxyethanol in the presence of SO_2. It is used in the chemical and paint industries as a solvent for nitro cellulose, resins, and dyes. DEGEE is not used in food or detergent products.

Purified DEGEE (>99%) is used in cosmetics and dermatological preparations and as solvent in some medicine products. DEGEE enhance the percutaneous absorption through the skin and mucosal barriers. It is used in some drugs to enhance absorption.

According to the notification to the Commission DEGEE is used in cosmetic products in shampoos (rinse-off) in a concentration up to 5% and creams (leave-on) in a concentration up to 2%. DEGEE is used in toiletries, skin care, hair care or sun care products which may be applied on the whole body. It must not be used in products for oral hygiene and the eyes.

3.3. Toxicological Evaluation

Part of the evaluation is based on a submission "Transcutol Toxicological report summary (Last update: 31/03/2003 from Gattefosse". This submission is unsatisfactory as in contains in most cases only summaries and no details.

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

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

Table 3.1: Acute toxicity after oral administration of DEGEE

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

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

Ref.: 9

3.3.1.2. Acute dermal toxicity

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

Table 3.2: Acute toxicity after dermal administration of DEGEE

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

The Hazard Substances Data Bank (HSDB) cites:
…..cosmetic preparations containing more than 5% Carbitol should not be used even for application to small areas of body …use … for this purpose may constitute an unexpected hazard, especially if applied to broken skin or in persons with renal disorders.

Ref.: 9

3.3.1.3. Acute inhalation toxicity

LC_{50} rats = 5240 mg/m³

Ref.: 12

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

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

DEGEE (500 mg) was slightly irritant to rabbit skin after 24 hour application.

Ref.: 13

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

Transcutol was applied once at the dose level of about 0.020 ml per volunteer, on a surface of about 50 mm^2 of skin on the back of 10 volunteers. Transcutol was kept in contact with the skin under an occlusive patch test for 48 hours. This application was performed in parallel and under same condition with patch test alone as "negative" control. Cutaneous macroscopic examinations were performed about 30 min after removal of the patches. Evaluation of the erythematous and oedematous reactions was made according to a given numerical scale. No reaction of pathological irritation and significant intolerances was noted. 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

DEGEE was slightly irritant to the rabbit eye, Slight pain response, conjunctival redness, thickening of cornea were noted.

Ref.: 15

Guideline:	/
Species/strain:	New Zealand albino rabbits
Group size:	3 adult males
Test substance:	Transcutol
Batch:	/
Dose level:	0.1 ml of a 30% solution
Route:	Right eye
Exposure period:	/
Observation:	1 h and after 1, 2, 3 days
GLP:	In compliance

Transcutol was administered in a 30% solution in water for injection into the inferior conjunctival sac of the right eye of 3 male rabbits without rinsing and at the dose level of 0.1 ml per animal. The conjunctiva, iris and cornea were examined 1 h after administration and then on days 1 (24 hrs), 2, and 3. As no more abnormality was noted in the 3 rabbits on day 3, examinations were not continued. Evaluation of the lesions was performed according to the Draize scale. It was concluded that administration of Transcutol at 30% into the eye of the rabbit may be termed "Slightly irritant". The lesions observed 1 h after administration showed a total reversibility on day 1.

Ref.: 16

Guideline:	OECD 405 (1987)
Species/strain:	New Zealand albino rabbits
Group size:	3 adult males
Test substance:	Transcutol
Batch	/
Dose level:	0.1 ml
Route:	Right eye
Exposure period:	/
Observation:	1 h and after 1, 2, 3 days
GLP:	In compliance

Transcutol was administered into the inferior conjunctival sac of the right eye of 3 male rabbits without rinsing and at the dose level of 0.1 ml per animal. The conjunctiva, iris and cornea were examined 1 h after administration and than on days 1 (24 hrs), 2, and 3. It was concluded that administration of Transcutol into the eye of the rabbit may be termed "Slightly irritant". No lesions were observed at day 3.

Ref.: 17

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

General comment
DEGEE is moderately irritant to the eye and slightly irritating to the skin.

3.3.3. Skin sensitisation

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

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 of them (a man) abandoned the study on the 12th day.

The protocol of the irritation and sensitisation study was allocated into 3 distinct periods.
Induction period. 9 consecutive applications, to the same area, of 0.02 ml, per volunteer, of Transcutol by the occlusive epicutaneous route to the skin of the arm during a 2 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

Human volunteers showed neither irritation nor signs of sensitisation when the material was tested at a 20 percent level in petroleum for a 48 hour closed patch test.

Ref.: 2

General comments
DEGEE has not been demonstrated to cause sensitisation.
The SCCP considers human induction studies as unethical

3.3.4. Dermal / percutaneous absorption

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
Recovery:	91%
GLP:	in compliance

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 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 and dermis) and analysed for DEGEE remaining. Results are expressed in µg equivalent of DEGEE (µg/cm²) and in % of the applied dose for all the compartment 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
Recovery:	About 50%
GLP:	in compliance

A 15% DEGEE "leave on" hydro-alcoholic gel formulation was tested in two experiments. The formulation was applied on human skin during a period of 24 hours. The receptor fluid

was completely collected after 3, 6, 9, 12 hours and replaced by fresh fluid, the last sampling point was 24 hours. At the end of the 24 hr observation period, the different skin layers were separated (horny layer, epidermis and dermis) and analysed for DEGEE remaining. Results are expressed in μg equivalent of DEGEE (μg/cm²) and in % of the applied dose for all the compartment 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 full test was also performed "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
Recovery:	44 – 52%
GLP:	in compliance

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 % of the applied dose for all the compartment analysed (see table 3.5 and 3.6).

First experiment

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

	DEGEE 2%		DEGEE 5%		DEGEE 10%	
	µg/cm²	% of the applied dose	µg/cm²	% of the applied dose	µg/cm²	% of the applied dose
Washing (W)	0.87 ± 0.36	0.87 ± 0.36	1.56 ± 0.67	0.63 ± 0.29	1.82 ± 0.89	0.35 ± 0.18
Total absorbed (E+D+RF)	43.7 ± 7.0	43.2 ± 4.3	140 ± 28	56.1 ± 12.5	267 ± 43	50.4 ± 7.3
Total recovery (%)	44 ± 4		57 ± 12		51 ± 7	

Second experiment

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

	DEGEE 2%		DEGEE 5%		DEGEE 10%	
	µg/cm²	% of the applied dose	µg/cm²	% of the applied dose	µg/cm²	% of the applied dose
Washing (W)	0.98 ± 1.25	0.85 ± 1.08	1.05 ± 0.37	0.36 ± 0.13	1.32 ± 0.37	0.24 ± 0.11
Total absorbed (E+D+RF)	52.7 ± 7.0	45.6 ± 4.8	128 ± 22	44.4 ± 5.1	294 ± 32	51.6 ± 3.3
Total recovery (%)	46 ± 4		45 ± 5		52 ± 3	

The percutaneous absorption study was conducted "without occlusion". The "mass balance" of the experiment was low. The low recovery at the end of the 24 hours of diffusion was related to the evaporation of DEGEE from the skin surface. Therefore, the full test was also performed "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 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%.

In the MOS calculation, the results of the 2% emulsified formulation will be used since the maximum concentration in the term of reference was 1.5%. Thus, a dermal absorption of 45.6 + 2 x 4.8 = 55.2% will be used in the MOS calculations.

Percutaneous absorption data on hair colorant product usage (Submission from December 2007)

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 were prepared by mixing an oxidative hair colorant with placebo developer (without hydrogen peroxide). Final concentrations of [^{14}C]-DEGEE, 7%, 3.5% and 2%. 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 non-oxidative in a typical semi-permanent hair dye formulation were used. Final concentrations of [^{14}C]-DEGEE, 5%, 3% and 1%. 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 the 7% DEGEE in oxidative formulation and with 5% DEGEE in non-oxidative formulation are summarized in Table. 3.7 and Table 3.8, respectively.

Table 3.7: Distribution of Radioactivity in µg equiv./cm^2 (%) at 24 h Following Topical Application of [14C]-DEGEE in Test Preparation 1 (7%, w/w) to Human Split-Thickness Skin

	Cell Number and Donor Number													
	Cell 88 0127	Cell 89 0127	Cell 91 0154	Cell 92 0154	Cell 93 0154	Cell 95 0162	Cell 96 0162	Cell 97 0162	Cell 99 0164	Cell 100 0164	Cell 101 0164	Cell 102 0164	Mean	SD
Total Dislodgeable Dose	1355.5	1372.1	1410.9	1388.6	1382.8	1380.5	1377.0	1331.4	1358.7	1369.0	1349.4	1323.4	1366.6	24.6
Stratum Corneum	0.7	0.3	0.5	1.1	0.3	0.7	0.1	0.6	0.4	0.2	0.4	0.3	0.5	0.3
Unexposed Skin	0.5	0.5	0.6	0.4	0.2	1.2	0.5	0.7	0.3	0.3	0.3	0.3	0.5	0.3
Total Unabsorbed	1356.7	1372.9	1411.9	1390.1	1383.3	1382.4	1377.6	1332.7	1359.4	1369.5	1350.1	1324.0	1367.6	24.7
Exposed Skin	0.8	0.7	0.4	0.6	0.5	1.2	0.6	2.2	0.7	0.4	0.5	0.5	0.8	0.5
Receptor Fluid	51.5	*33.0	*11.2	*16.3	*11.7	*38.6	*28.2	*54.1	*37.7	*28.5	*45.1	*44.9	°33.4	°14.7
Receptor Rinse	*0.0	*0.0	*0.0	*0.0	*0.0	*0.0	*0.0	*0.1	*0.1	*0.0	*0.0	*0.0	°0.0	°0.0
Total Absorbed	51.5	33.0	11.2	16.3	11.7	38.6	28.2	54.2	37.7	28.5	45.1	44.9	33.4	14.7
Dermal Delivery	52.4 (3.7)	33.7 (2.4)	11.6 (0.8)	17.0 (1.2)	12.2 (0.9)	39.8 (2.8)	28.9 (2.1)	56.4 (4.0)	38.4 2.8)	28.9 (2.1)	45.5 (3.3)	45.4 (3.3)	34.2 (2.4)	15.0 (1.1)
Mass Balance	1409.1 (100.7)	1406.6 (100.5)	1423.6 (101.8)	1407.1 (100.6)	1395.5 (99.7)	1422.1 (101.7)	1406.5 (100.5)	1389.1 (99.3)	1397.8 (99.9)	1398.4 (100.0)	1395.5 (99.8)	1369.4 (97.9)	1401.7 (100.2)	14.6 (1.0)

Table 3.8: Distribution of Radioactivity in µg equiv./cm^2 (%) at 24 h Following Topical Application of [14C]-DEGEE in Test Preparation 4 (5%, w/w) to Human Split-Thickness Skin

	Cell Number and Donor Number													
	Cell 42 0154	Cell 43 0154	Cell 44 0154	Cell 45 0151	Cell 46 0151	Cell 47 0151	Cell 48 0162	Cell 49 0162	Cell 50 0162	Cell 51 0164	Cell 52 0164	Cell 53 0164	Mean	SD
Total Dislodgeable Dose	1006.4	996.91	1009.8	1014.8	1027.2	1021.6	1007.9	1008.0	1015.0	1038.6	1018.9	1011.4	1014.7	10.9
Unexposed Skin	0.3	0.32	0.4	0.4	0.3	0.4	0.4	0.8	0.4	0.3	0.2	0.3	0.4	0.2
Total Unabsorbed	1007.3	997.38	1010.7	1015.5	1027.7	1022.5	1009.0	1009.0	1015.6	1039.2	1019.2	1011.8	1015.4	10.8
Exposed Skin														
Receptor Fluid	0.6	0.2	0.5	0.2	0.2	0.2	0.2	0.5	0.4	0.1	0.1	0.2	0.3	0.2
Receptor Rinse	*8.8 *0.0	*6.4 *0.0	*10.7 *0.0	*5.1 *0.0	*4.5 *0.0	*7.7 *0.0	*6.7 *0.0	*12.6 *0.0	*17.1 *0.0	*11.5 *0.0	*12.2 *0.0	*11.6 *0.3	°9.6 °0.0	°3.7 °0.0
Total Absorbed	8.8	6.4	10.8	5.2	4.5	7.7	6.7	12.6	17.1	11.5	12.2	11.6	9.6	3.7
Dermal Delivery	9.4 (0.92)	6.7 (0.65)	11.3 (1.10)	5.4 (0.52)	4.7 (0.46)	8.0 (0.78)	7.0 (0.68)	13.1 (1.29)	17.6 (1.72)	11.6 (1.14)	12.3 (1.21)	11.8 (1.16)	9.9 (0.97)	3.8 (0.37)
Mass Balance	1016.7 (99.7)	1004.0 (98.5)	1021.9 (100.2)	1020.9 (100.1)	1032.4 (101.3)	1030.5 (101.1)	1016.0 (99.6)	1022.1 (100.2)	1033.1 (101.3)	1050.9 (103.1)	1031.5 (101.2)	1023.6 (100.4)	1025.3 (100.6)	11.6 (1.14)

The results with the oxidative formulations and with the non-oxidative formulation of DEGEE are summarized in Table 3.9 and Table 3.10, respectively.

Table 3.9: Dermal absorption obtained with 3 different concentration of DEGEE in oxidative formulations

DEGEE concentration in unmixed formulation %	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* (%)
14	7	33.42 ± 14.70	0.77 ± 0.5	**34.18 ± 14.99 (2.4 ± 1.1)**
7	3.5	13.51 ± 5.88	0.25 ± 0.09	**13.76 ± 5.92 (2.0 ± 0.8)**
4	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 3.10: 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. 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.

In the MOS calculation, the mean + 2 SD may be used, which is 64.2 µg/cm² for the oxidative hair colorant formulation and 17.5 µg/cm² for the non-oxidative formulation.

General comment on dermal absorption studies
Table 3.11 summarize 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. In one experiment with 30 min contact time the dermal absorption was about 20% with shampoo formulations. This latter study may be compared with the new experiments with hair dye formulations, where a dermal absorption of 1 – 2% was reported. The SCCP finds it difficult to explain the large difference between these results since the concentrations of DEGEE in the formulations used were in the same range in both studies. One factor that may contribute to the difference is that 5 mg/cm² of the formulations was applied to the filter in the first experiments while 20 mg/cm² was used in the experiments with the hair dye formulations.

Table 3.11: Summary of dermal absorption studies with DEGEE

Formulation	Incubation time	Concentration		Dermal absorption		Recovery %
		%	µg/cm²	µg/cm²	%	
Shampoo (rinse off)	30min	5	279	60.5 ± 22.3	21.6 ± 10.6	91
		10	530	92.9 ± 20.8	17.5 ± 3.9	91
Hydro-alcoholic gel (leave on)	24 h	15	831	425 ± 85	51.0 ± 9.1	52
		15	859	385 ± 46	44.9 ± 4.8	46
Emulsified formulations (leave on) First exp.	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.	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	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	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	30 min	1	200	3.7 ± 2.2	1.8 ± 1.1	100
		3	600	8.3 ± 2.3	1.4 ± 0.4	100
		5	1000	9.9 ± 3.8	0.9 ± 0.4	100

3.3.5. Repeated dose toxicity

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

Oral

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/d) 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/d.

Ref.: 24

Dermal

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

Ref.: 10

Guideline: /
Species/strain: Young adult New Zeeland albino rabbits
Group size: 5 males and 5 females
Test substance: Transcutol
Batch: Lot No. 96933
Purity: /
Dose levels: 0, 100, 300, and 1000 mg/kg bw/day
Route: Dermal
Exposures: 28 days for a period of 6 h each day

GLP: In compliance

New Zeeland rabbits, groups of 5 males and 5 females, received 0, 100, 300, and 1000 mg/kg bw/d for 28 days. Transcutol were applied dermally and allowed to remain in contact with the skin for a period of 6 h each day. The test site was covered with one 4 x 6 inch 6-ply gauze pad. The animals were observed for signs of toxicity and mortality each day. Blood was collected from all animals on day 1 and at termination for haematology and blood chemistry evaluation. Complete necropsies were performed on all rabbits.

All treated animals survived and gained weight. Apart from several instances of transient soft faeces during the study, all animals appeared active and healthy. There were no signs of gross toxicity, adverse pharmacologic effects or abnormal behaviour. Animals from the exposed groups exhibited barely perceptible erythema and/or oedema and desquamation. The incidence of irritation increased with increasing dose level. Gross necropsy revealed small black masses on the ovaries of 3 females from group 1 (control), 3 (300 mg/kg bw/d), and 4 (1000 mg/kg bw/d). 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. It was 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/d.

Ref.: 25

Inhalation

Rats
SD rats were exposed to 0, 16, 50, and 200 ppm DEGEE (nose-only) for 28 days. There were no signs of systemic intoxication, but there were histopathological changes indicative of mild non-specific irritation in the upper respiratory tract at the mid- and high-exposure levels.

Ref.: 26

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

Ref.: 27

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

Oral
Rats

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/d.

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% 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/d.

Ref.: 29

Comment
In the documentation received, it is in addition to the two studies above (ref.: 28, 29) referred to a 90 day rat subchronic gavage study (ref.: 30). This study has not been submitted. It is 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/d.

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/d after 3 weeks
Route:	Oral in diet
Exposures:	90 days
GLP:	/

White pigs, groups of 3 males and 3 females, received 0, 167, 500, and 1500 mg/kg bw/day DEGEE (top dose decreased to 1000 mg/kg bw/d 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/d. It is concluded that NOAEL was 167 mg/kg bw/d.

Ref.: 29

Dermal

Rabbits

Rabbits receiving dermal treatments (not further specified) of DEGEE for 90 days showed no effects on growth, mortality, haematology, clinical chemistry or gross pathology at dose level up to 300 mg/kg bw/d. A treatment related histopatological effect was seen in the kidneys of animals at 1000 and 3000 mg/kg bw/d.

Ref.: 31

Inhalation

Rats
Continuous DEGEE inhalation exposure of rats at 0.27 and 4.5 ppm for 4 months followed by a recovery period resulted in changes in blood cell (anaemia) and chemistry profiles as well as CNS effects.

Ref.: 32

3.3.5.3. Chronic (> 12 months) toxicity

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

Ref.: 33

Albino rats receiving two grades of DEGEE through three generations (Fo, F1 and F2) during a 2-year period. One grade contained 0.2% ethylene glycol and the other 29.5% ethylene glycol. The drinking water levels were 0, 0.01, 0.04, 0.2, and 1% (10, 40, 200 and 950 mg/kg bw/d). F I and F 2 generations received the same dosage levels as the parents, and all survivors were killed off 718 days from the start of the test. The sample that contained 29.5% ethylene glycol was considerably more toxic than the purer grade. The "toxic" group constituted 16 rats showing severe injury, notably kidney damage or bladder concretions; the animals comprised 39% of animals receiving 950 mg/kg bw and 11% of animals receiving 200 mg/kg bw DEGEE with 29.5% ethylene glycol and 7% of animal receiving 950 mg/kg bw DEGEE with 0.2% ethylene glycol. It was concluded that the maximum safe dose of the impure material was 10 mg/kg bw/d whereas it was about 200 mg/kg bw/d for the purer sample.

Ref.: 34

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

Ref.: 1

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

Ref.: 37

General comment
Three oral subchronic oral studies in rats are discussed. DEGEE was added to the diet in two of the studies. In one study with Wistar rats a NOAEL of 800 mg/kg bw/d was reported and in a study with CFE rats a NOAEL of 250 mg/kg bw/d (0.5% DEGEE in the diet) was concluded. In the third study the rats received DEGEE by gavage. No significant effects were observed at the only dose tested. The French authorities used this study for their NOAEL of 180 mg/kg bw/d.

In an oral mice study with DEGEE added to the diet a NOAEL of 850 – 1000 mg/kg bw/d was concluded. In a similar small study with white pigs and the following doses; 0, 167, 500, and 1500 mg/kg bw/d, NOAEL of 167 mg/kg bw/d was concluded. Histological

examination showed hydropic degeneration of the proximal renal tubules at the highest level of treatment and at 500 mg/kg bw/d.

From a two year chronic drinking water study with albino rats, a NOAEL of 200 mg/kg bw/d was obtained with a preparation containing 0.2% ethylene glycol. This value will be used in calculation of MOS.

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

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

Ref.: 1

Guideline:	/
Species/strains:	*Salmonella typhimurium* TA98, TA100, TA102, TA1535, and TA1537
Test substance:	Transcutol P
Batch:	/
Purity:	/
Replicates:	Two independent experiments in triplicate
Concentrations:	Experiment 1: 0 (control), 52, 164, 512, 1600, and 5000 µg/plate Experiment 2: 0 (control), 492, 878, 1568, 2800, and 5000 µg/plate
Controls:	Negative and positive (substances not given)
Preincubation test:	/
Test conditions:	Standard plate test and preincubation test both with and without metabolic activation (Type not stated)
GLP:	In compliance

Transcutol P was tested on 5 strains of *Salmonella typhimurium* (TA98, TA100, TA102, TA1535, and TA1537) in the presence and absence of metabolic activation. Concentrations of Transcutol P used are stated above. No signs of cytotoxicity (such as a reduction of the bacterial lawn and/or a decrease in number of revertants with evidence of a dose relationship) and no precipitate were noted. It was concluded that under the experimental conditions that Trancutol P did not induce any mutagenic effect in the test either with or without metabolic activation.

Ref.: 37

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

DEGEE did not induce micronuclei in CD-1 mouse bone marrow following 2 daily i.p. injections at 1980 mg/kg bw.

Ref.: 1

Unscheduled DNA synthesis (UDS) test in primary rat hepatocytes *in vivo*

Guideline:	/
Species/strain:	Wistar rats
Group size:	5 males
Test substance:	Transcutol
Concentrations:	800 and 2000 mg/kg bw
GLP:	In compliance

Groups of 5 male rats were treated once with Transcutol at 800 and 2000 mg/kg bw by gavage at a dose volume of 10 ml/kg bw. 75 mg/kg bw of 2-acetamidofluorene (2-AAF) was used as positive control animals for the 12 –14 h experiment and 10 mg/kg bw of dimethylnitrosamine (DMN) was used as positive control for the 2 – 4 h experiment.

Approximately 12 – 14 h (experiment 1) and 2 – 4 h (experiment 2) after dosing, the animals were killed and their livers perfused with collagenase to provide a primary culture of hepatocytes. Cultures were made from 3 animals in each dose group and were treated with [^{3}H] thymidine. Six slides from each animal were prepared with fixed hepatocytes and of these 3 were dipped in photographic emulsion to prepare autoradiograms. Slides were examined microscopically and net grain count (NNG) was determined for each of two of the three slides, each animal and dose group. Negative control gave a group mean NNG value of less than zero with only 0.7 – 1% cells in repair. Group mean NNG values were increased by 2-AAF and DMN treatment to more than 5 and more than 50% cells found to be in repair. Treatment with 800 and 2000 mg/kg Transcutol dit not produce NNG value greater than –1.7 nor were more than 0.7% cells found in repair at either dose. It was concluded than Trancutol did not induce UDS detectable under the experimental conditions employed.

Ref.: 38

General comment
In one purely reported study 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. 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 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/d
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 LD_{10} 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 F_1 generation from the final litters was reared and continuously treated with 0 or 2.5% DEGEE 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. In addition the relative liver weights were increased (16% males and 10% females).

Ref.: 41, 42

Rats
Rats, groups of 24 males and 24 females, received 0, 300, 1000, and 2000 mg/kg bw/d with Transcutol HP with gavage daily during premating (63 days for males, 14 days for females) and during mating. Dosing continued until gestation day 7 for females and until the day prior to necropsy for males (20 days after mating). All dose levels of Transcutol HP up to 2000 mg/kg bw/d were well tolerated in rat, although minor effects on clinical condition and body weight was observed at the higher dose levels (mainly in males). There were no effects of DEGEE on gonadal function, fertility and reproductive performance in any group. It was concluded that NOAEL for fertility was 2000 mg/kg bw/d and that NOAEL for general toxicity was 1000 mg/kg bw/d.

Ref.: 43

Pregnant female rats received 0, 300, 1000, and 2000 mg/kg bw/d with Transcutol by gavage from day 6 to day 17 of gestation. The animals were killed at day 20 for maternal, foetal soft tissue and skeletal evaluations. The highest dose level of 2000 mg/kg bw/d of Transcutol HP resulted in slight maternal toxicity characterised by reduction in body weight gain and food consumption. There was no evidence of maternal toxicity in lower dose groups. Evidence of embryo-foetal toxicity was restricted to minor skeletal findings which principally included a dose-related increase in the incidence of reduced ossification of cranial bones in the 1000 and 2000 mg/kg bw/d groups. These minor skeleton findings were considered not to be indicative of a teratogenic potential of Transcutol HP, but suggested a selective effect on the developing foetuses in view of the limited maternal toxicity in the high dose group and the absence of an effect on foetal weight. The dose of 1000 mg/kg bw/d was considered a NOAEL for maternal toxicity. The dose of 300 mg/kg bw/d was considered NOAEL for embryo-foetal toxicity. It was concluded that there was no indication of teratogenicity at any dose level used in the study.

Ref.: 44

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
Sprague-Dawley rats, a group of 15 pregnant female rats were exposed to 0, 100 ppm DEGEE for 7 h/d from day 7 to day 15 of gestation. The animals were killed for necropsy at day 20. No selective developmental toxicity was seen under the treatment conditions. It was concluded that DEGEE was not a developmental toxicant.

Ref.: 46

Comment
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, but suggested a selective effect on the developing foetuses. In a rat study the dose of 1000 mg/kg bw/d was considered a NOAEL for maternal toxicity. The dose of 300 mg/kg bw/d was considered to be the NOAEL for embryo-foetal toxicity.

3.3.9. Toxicokinetics

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

3.3.10. Photo-induced toxicity

No data submitted

3.3.11. Human data

No data submitted

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Diethylene glycol monoethyl ether (DEGEE)

The safety calculation is only considering dermal exposure.

NOAEL based on kidney damage or bladder concretions in albino rats was 200 mg/kg bw/d

DEGEE in all cosmetic products, except products for oral hygiene and eye products at a concentrations up to 1.5 %

Dermal absorption 45.6±4.8. Uses mean + 2 SD
Exposure 17.79 (total) – 3.52 (oral product) = 14.27 g/d, 1.5% DEGEE = 214 mg/d.

Maximum absorption through the skin	214 x 55.2/100	=	118 mg/d
Typical body weight of human		=	60 kg
Systemic exposure dose (SED)	118/60	=	1.97mg/kg bw
No observed adverse effect level (albino rats 2 year oral study)	NOAEL	=	200 mg/kg bw
Margin of Safety	**NOAEL / SED**	**=**	**102**

A MOS of 102 is considered to give sufficient protection in relation to the use of DEGEE as a solvent in cosmetics (with exception of oral products).

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

Dermal absorption 34.2±15.0. Uses mean + 2 SD

Maximum absorption through the skin	A (µg/cm²)	=	64.2 µg/cm²
Skin Area surface	SAS (cm²)	=	700 cm²
Dermal absorption per treatment	SAS x A x 0.001	=	44.9 mg
Typical body weight of human		=	60 kg
Systemic exposure dose (SED)	SAS x A x 0.001/60	=	0.75 mg/kg bw
No observed adverse effect level (albino rats 2 year oral study)	NOAEL	=	200 mg/kg bw

Margin of Safety	**NOAEL / SED**	**=**	**267**

A MOS of 267 gives sufficient protection in relation to the use of DEGEE as a solvent in oxidative hair dye formulations.

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

Dermal absorption 9.9±3.8. Uses mean + 2 SD

Maximum absorption through the skin	A (µg/cm²)	=	17.5 µg/cm²
Skin Area surface	SAS (cm²)	=	700 cm²
Dermal absorption per treatment	SAS x A x 0.001	=	12.3 mg
Typical body weight of human		=	60 kg
Systemic exposure dose (SED)	SAS x A x 0.001/60	=	0.20 mg/kg bw
No observed adverse effect level (albino rats 2 year oral study)	NOAEL	=	200 mg/kg bw

Margin of Safety	**NOAEL / SED**	**=**	**1000**

A MOS of 1000 gives sufficient protection in relation to the use of DEGEE as a solvent in oxidative hair dye formulations.

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

3.3.14. Discussion

The safety has only been considered for dermal exposure.

The influence of possible evaporation in the various experiments has not been considered.

Physico-chemical specification
The stability of diethylene glycol monoethyl ether (DEGEE) is not reported. The physico-chemical characterisation and purity of the substance is not reported in several studies. Commercial products of DEGEE may contain an appreciable amount of ethylene glycol. Part of the evaluation is based on a submission "Transcutol Toxicological report summary (Last

update: 31/03/2003 from Gattefosse". This submission is unsatisfactory as in contains in most cases only summaries and no details.

General toxicity
The toxicity data on DEGEE indicates that this glycol ether produces little repro- or haemato-toxicity, which are of concern with several other glycol ethers. However, some systemic toxicity has been reported. DEGEE may contain ethylene glycol as impurity. It is recommended that the level of ethylene glycol in DEGEE preparations used in cosmetics should be <0.2%.

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

Three oral subchronic oral studies in rats are discussed. DEGEE was added to the diet in two of the studies. In one study with Wistar rats a NOAEL of 800 mg/kg bw/d was reported and in a study with CFE rats a NOAEL of 250 mg/kg bw/d (0.5% DEGEE in the diet) was concluded. In the third study the rats received DEGEE by gavage. No significant effects were observed at the only dose tested. The French authorities used this study for their NOAEL of 180 mg/kg bw/d.

In a oral mice study with DEGEE added to the diet a NOAEL of 850 – 1000 mg/kg bw/d was concluded. In a similar study with white pigs and the following doses; 0, 167, 500, and 1500 mg/kg bw/d, NOAEL of 167 mg/kg bw/d was concluded. Histological examination showed hydropic degeneration of the proximal renal tubules at the highest level of treatment and at 500 mg/kg bw/d.

A NOAEL can be calculated from several oral studies. In studies of rats the NOAEL varied from 180 to 800 mg/kg bw/day, in mice it was about 850 – 1000 kg/kg bw/d, while in pigs a NOAEL of 167 mg/kg bw/d was reported after gavage. In the MOS calculation a NOAEL of 200 mg/kg bw/d was found based on kidney damage or bladder concretions in a two year drinking water study with albino rats.

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.

Irritation /sensitisation
DEGEE is moderately irritant to the eye and slightly irritating to the skin. It has not been demonstrated to cause sensitisation.

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, but suggested a selective effect on the developing foetuses. In a rat study the dose of 1000 mg/kg bw/d was considered a NOAEL for maternal toxicity. The dose of 300 mg/kg bw/d was considered NOAEL for embryo-foetal toxicity.

Dermal absorption
(for use in concentrations up to 1.5% in cosmetic products)
Three well-conducted *in vitro* studies on percutaneous absorption through human skin are available. 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%.

In the MOS calculation the results of the 2% emulsified formulation will be used since the maximum concentration in the term of reference was 1.5%. Thus, a dermal absorption of 45.6 + 2x4.8 = 55.2% will be used in the MOS calculations.

(for 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 well-conducted *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 were for the highest concentrations used 34.2 ± 15.0 µg/cm² (2.4 ± 1.1%) for the oxidative formulation and 9.9 ± 3.8 µg/cm^2 (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.

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

Mutagenicity
In one poorly reported study DEGEE displayed a weak mutagenic activity at high concentrations in some tested *Salmonella typhimurium* strains (TA1535, TA1537, TA1538) and in *Saccharomyces cervisiae* (D7) while no mutagenic activity were reported in another Salmonella test. 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 DEGEE did not induce micronuclei in CD-1 mouse bone marrow following 2 daily i.p. injections at 1980 mg/kg bw. It is noted that no *in vitro* mammalian genotoxicity studies are available. However, two negative *in vivo* studies are available and based on the structure of the substance it is not expected that DEGEE will have relevant mutagenic potential *in vivo.* On this basis SCCP will not ask for an *in vitro* mammalian genotoxicity study.

Carcinogenicity
No adequate carcinogenicity study is available.

4. CONCLUSION

The SCCP has previously concluded that, based on the information provided, the use of diethylene glycol monoethyl ether (DEGEE) in all cosmetic products, excluding products for oral hygiene and eye products at a concentrations up to 1.5% does not pose a risk to the health of the consumer, provided that the level of ethylene glycol in DEGEE used is < 0.2% (SCCP/1044/06, 19 December 2006).

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

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

5. MINORITY OPINION

Not applicable

6. REFERENCES

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
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