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 UDS: 0, 800, 2000 mg/kg bw
Exposure:	Single application
Route:	Oral (gavage)
Application volume:	10 ml/kg body weight
Vehicle:	Purified water for Transcutol® and DMN, corn oil for 2-AAF,
Sacrifice Times:	Range-finding assays: 48 hours after application UDS: 2 – 4 hours and 12 – 14 hours after application
Positive control:	2-Acetamidofluorene (2-AAF): 75 mg/kg bw (for the 12-14 h experiments); Dimethylnitrosamine (DMN): 10 mg/kg bw (for the 2-4 h experiments)
GLP:	In compliance
Date:	August 1996

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

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

In the range-finding segment, the animals did not show any mortalities or clinical findings. In the UDS study, no significant increases in the group mean or net nuclear grain (NG) count for animals treated with Transcutol were observed at any dose level or at harvest time.
The group mean NG count for the vehicle control was within historical control values, while the positive controls induced a significant increase in NG count demonstrating the sensitivity and validity of the test system.

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

N Ref.: 19

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

Ref.: 1

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

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

3.3.7. Carcinogenicity

No adequate data available

3.3.8. Reproductive toxicity

Oral route

Mice

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

Fifty mated CD1 mice were orally administered DEGEE (>99% purity) by gavage at 5500 mg/kg/day (calculated 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 (4400 mg/kg bw/day) and at 74±10 days of age paired with nonsiblings from the same dose group. A significant decrease (34%) in motile sperm from de cauda epididymis in males exposed to 2.5% DEGEE was seen. In addition the relative liver weights were increased (16% in males and 10% in females).

Ref.: 41, 42

Rats

Prenatal developmental toxicity study in rats

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

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

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

Findings in the dams:
There was no mortality or treatment-related effects on clinical condition in any of the groups. Maternal body weight gain and food consumption were statistically significantly reduced in the
2000 mg/kg bw group during the first five days of treatment. No effect on body weight/body weight gain or food consumption was observed at 300 and 1000 mg/kg bw. Necropsy examination of the adult females did not reveal any treatment-related abnormalities.

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

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

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

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

The authors concluded that the NOAEL for maternal toxicity as well as for prenatal developmental toxicity was 1000 mg/kg bw. There was no indication of teratogenicity up to the highest dose level tested and therefore the respective NOAEL was >2000 mg/kg bw.
N Ref.: 26

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

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

Fertility and general reproductive performance in rats

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

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

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

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

Conclusion
The authors concluded that the oral administration of Transcutol within the fertility and general reproductive performance study in female Sprague-Dawley rats, showed that all doses levels, up to 2000 mg/kg bw/day, were well tolerated, although minor effects on clinical condition and body weight were observed at the higher dose levels (mainly in males). There were no effects of the test article 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
Sprague-Dawley rats, a group of 20 pregnant female rats were exposed to 0, 102 ppm DEGEE for 7 h/day from day 7 to day 15 of gestation. The animals were killed for necropsy at day 20. No selective developmental toxicity was seen under the treatment conditions. It was concluded that DEGEE was not a developmental toxicant.

Ref.: 46

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

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

3.3.9. Toxicokinetics

3.3.9. 1. *In vitro* metabolism

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

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

Rat hepatocytes were isolated from 2 different rat livers, R1 and R2. Human hepatocytes were also isolated from two different human liver specimens, H1 and H2. Hepatocyte suspensions were incubated with 15, 150, and 1500 µM [14C]-DEGEE or 150 µM [^{14}C]-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 ^{14}C-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 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: 3 males and 3 females/time point (blood and plasma kinetics) 3 males and 3 females/time point (balance of excretion) 3 males and 3 females/time point (tissue distribution, oral route) BDIX Rats: 3 males and 3 females/time point (blood and plasma kinetics) 3 males and 3 females/time point (tissue distribution, oral route)
Test substance:	Non-labelled: Transcutol® HP (DEGEE) Labelled: [14C]-Diethylene glycol monoethyl ether ([14C]-DEGEE)
Batch:	Non-labelled: DEGEE: 0025005 Labelled: [14C]-DEGEE: 104-272-053 (radiochemical purity: 100%, supplied by Moravek Biochemicals Inc., USA)
Dose level:	Oral and intravenous: 20 mg/kg bw (50 µCi/kg bw)
Exposure:	Single application
Route:	Oral (gavage) and intravenous
Application volume:	Oral: 5 ml/kg bw, intravenous: 2 ml/kg bw
Vehicle:	Oral: water for injection
Intravenous:	Physiological saline solution (0.9% NaCl)
Sampling time-points:	Sprague-Dawley rats: 0.25 up to 168 hours after oral or intravenous application BDIX rats: 0.25 up to 6 hours after oral or intravenous application
GLP:	In compliance
Date:	March 2002

The absorption, distribution and excretion of Transcutol® HP was investigated comparably in male and female Sprague-Dawley or BDIX rats after a single oral (gavage) administration or intravenous injection at a dose level of 20 mg [^{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) Labelled: [14C]-Diethylene glycol monoethyl ether ([14C]-DEGEE)
Batch:	Non-labelled: DEGEE: 0025005 Labelled: [14C]-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 Urine: pre-dose, 0 – 8 h, 8 – 24 h Feces: pre-dose, 0 – 24 h
Analysis:	Liquid scintillation counting
GLP:	/
Data:	September 2003

The metabolic fate and excretion of Transcutol HP was investigated in 4 male Sprague-Dawley rats after a single oral administration of 1000 mg [^{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 [^{14}C]-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 on toxicokinetics and metabolism by the applicant

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

In vivo, the absorption, distribution and excretion of Transcutol® was investigated comparably in two strains of rats after a single oral or intravenous dose of 20 mg [^{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, while after oral administration it was observed at 0.25 - 0.50 hours post dose. The plasma half-life corresponded to 37 to 84 hours and measurable concentrations were observed in almost of the tissues 168 hours post dose. The absolute bioavailability of the radioactivity is very high (79 — 95%). The tissue distribution of the radioactivity was characterised by high concentrations observed in pituitary, thyroid, adrenals and bone marrow with regards to the concentrations observed in blood / plasma (100 to 1000 times less) at the same sampling time. The radioactivity measured in tissues was significantly decreased at 48 hours. No biologically relevant differences were observed within both strains of rats. In studies on the metabolic fate and excretion of Transcutol it could be shown that after a single oral administration, 90% of the administered radioactivity was excreted in the urine within the first 24 hours and [^{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.

3.3.10. Photo-induced toxicity

No data submitted

3.3.11. Human data

See sections 3.3.2 and 3.3.3

3.3.12. Special investigations

Local tolerance study in the rabbit

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

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

Result and conclusion

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

N Ref.: 11, 12

3.3.13. Safety evaluation (including calculation of the MoS)

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

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

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

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

[3]The retention factor was introduced by the SCCNFP to take into account rinsing off and dilution of finished products by application on wet skin or hair (e.g. shower gels, shampoos, …) [SCCNFP/0321/00]
[4] Not applicable since these products do not contain DEGEE according to the applicant
[5] MOS calculations performed separately

CALCULATION OF THE MARGIN OF SAFETY

Diethylene glycol monoethyl ether (DEGEE)

The safety calculation is only considering dermal exposure.

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

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

Leave-on products

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

Exposure 13 g/day, 5.5% DEGEE		=	715 mg/day
Maximum absorption through the skin	715 x 68.6/100	=	490.5 mg/day
Typical body weight of human		=	60 kg
Systemic exposure dose (SED)	490.5 /60	=	8.2 mg/kg bw
Margin of Safety	**NOAEL / SED**	**=**	**49**

Rinse-off products

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

Exposure 0.22 g/day, 10% DEGEE		=	22 mg/day
Maximum absorption through the skin	22 x 21.4/100	=	4.7 mg/day
Typical body weight of human		=	60 kg
Systemic exposure dose (SED)	4.7 /60	=	0.08 mg/kg bw
Margin of Safety	**NOAEL / SED**	**=**	**5000**

Total exposure	490.5 +4.7	=	495.2 mg/day
Systemic exposure dose (SED)	495.2/60	=	8.25 mg/kg bw
Margin of Safety	**NOAEL / SED**	**=**	**48**

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

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

A dermal absorption of (34.2 + 2x15.0) 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	=	0.62 mg/kg bw
No observed adverse effect level	NOAEL	=	400 mg/kg bw
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.

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

A dermal absorption of (9.9 + 2x3.8) 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.

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

3.3.14. Discussion

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

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

Acute toxicity
The acute toxicity of DEGEE after oral or dermal application as well as inhalation can be regarded as very low in all species investigated. The LD50 values for acute oral and acute dermal toxicity were generally much higher than 2000 mg/kg bw and the available LC50 value for acute inhalation was >5000 mg/m^3 (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% (mean + SD; 113.7 µg/cm^2) was used.

Use in concentrations up to 5.5% in leave-on cosmetic products
In a hydro-alcoholic formulation (leave-on) containing 15% DEGEE 51 ± 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%). With emulsified formulations (leave-on) containing 2, 5, and 10% DEGEE, two experiments were performed at all three concentrations. No occlusion was used and the recovery was only between 44 and 57%. The mean absorption in the six experiments varied from 43.2% to 56.1%.

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

Use as solvent in an on-head concentration up 7.0% in oxidative hair dye formulations and in an on-head concentration up 5.0% in non-oxidative hair dye formulations
Two *in vitro* studies on percutaneous absorption through human skin are available. In both studies a contact time of 30 min were used. The final DEGEE concentrations were 2, 3.5, and 7% in the case of the oxidative formulations and 1, 3 and 5% in the case of the non-oxidative formulations. The systemically available levels for the relevant concentrations used were 34.2 ± 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.

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

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

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

Mutagenicity/Genotoxicity
DEGEE was tested for mutagenicity/genotoxicity in a range of validated and/or scientifically reasonable studies *in vitro* and *in vivo*. No genotoxic/mutagenic potential was noted in reliable bacterial gene mutation assays *in vitro* with *Salmonella typhimurium* in the presence or absence of metabolic activation. *In vivo,* DEGEE did not possess a mutagenic/genotoxic potential covering two independent endpoints. No indication of clastogenicity was observed in a limited micronucleus tests in mice after intraperitoneal injection and no increased repair synthesis as measure of DNA damage in the hepatocytes of the treated rats was observed. In conclusion, DEGEE can be considered to be of no genotoxic/mutagenic risk to humans. There is no need for any further studies.

Carcinogenicity
No adequate carcinogenicity study is available.

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

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

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

In vivo, the absorption, distribution and excretion of DEGEE was investigated comparably in two strains of rats after a single oral or intravenous dose of 20 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, while after oral administration it was observed at 0.25 - 0.50 hours post dose. The plasma half-life corresponded to 37 to 84 hours and measurable concentrations were observed in almost of the tissues 168 hours post dose. The absolute bioavailability of the radioactivity is very high (79 — 95 %). The tissue distribution of the radioactivity was characterized by high concentrations observed in pituitary, thyroid, adrenals and bone marrow with regards to the concentrations observed in blood / plasma (100 to 1000 times less) at the same sampling time. The radioactivity measured in tissues was significantly decreased at 48 hours. No biologically relevant differences were observed within both strains of rats. In studies on the metabolic fate and excretion of DEGEE it could be shown that after a single oral administration, 90% of the administered radioactivity was excreted in the urine within the first 24 hours and 14C-DEGEE was intensively metabolised, as only 3% of the urinary excreted radioactivity correspond to unchanged compound. The two major urinary metabolites were identified as ethoxyethoxyacetic acid and diethylene glycol, which represented 83% and 5.4% of the excreted urinary radioactivity, respectively. In plasma, only ethoxyethoxyacetic acid and unchanged ^{14}C-DEGEE were detected, which was consistent with urinary results.

4. CONCLUSION

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

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

1. the use of DEGEE as a solvent in cosmetic products in a concentration up to 10% in rinse-off products does not pose a risk to the health of the consumer.

2. the use of DEGEE as a solvent in cosmetic products in a concentration up to 5.5% in leave-on products does pose a risk to the health of the consumer.

3. an additional use of the substance DEGEE as solvent in an on-head concentration up 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.

5. MINORITY OPINION

Not applicable

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