EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

Scientific Committee on Consumer Products

SCCP

OPINION ON

ETHYLENE GLYCOL MONOBUTYL ETHER (EGBE)

Butoxyethanol (INCI)

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

The SCCP adopted this opinion at its 11th plenary on 21 March 2007

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.

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Opinion to be cited as: opinion of the SCCP on ethylene glycol monobutyl ether

TABLE OF CONTENTS

1. BACKGROUND

A risk assessment of EGBE with the chemical name 2-butoxyethanol or ethylene glycol monobutyl ether was done by a member state (France). The risk assessment is based mainly on open scientific literature and on skin absorptions studies done by Industry. The risk assessment led the member state to put some restrictions on the use this substance.

According to the notification to the Commission EGBE is used in cosmetic products only as a solvent in hair dyes.

Based on a NOAEL 150 mg/kg and a skin penetration rate of 113µg/cm^2 the member state concluded, that the substance could be considered safe for the consumers, when used in a concentration up to 4% in permanent hair dyes and up to 2% in non-oxidative hair dyes.

2. TERMS OF REFERENCE

1. *Does the SCCP consider the use of EGBE as solvent in hair dyes in a concentration up to 4% in oxidative hair dyes and up to 2% in non-oxidative hair dyes safe for the consumer taken into consideration the scientific data provided?*

2. *If not, does the SCCP foresee any other restrictions to the safe use of EGBE?*

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Butoxyethanol (INCI)

3.1.1.2. Chemical names

2-butoxyethanol (UPAC name) o-butyl ethylene glycol
ethylene glycol monobutyl ether butyl glycol
2-butoxy-1-ethanol glycol butyl ether
3-oxa-1-heptanol n-butoxyethanol

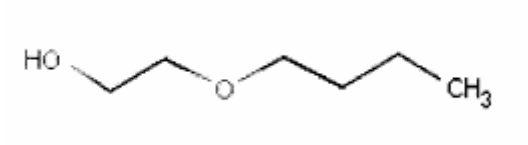
3.1.1.3. Trade names and abbreviations

Dowanol EB Butyl Oxitol
Butyl Cellosolve Eastman EB Solvent
Butyl IcinolEGBE

3.1.1.4. CAS / EINECS/ELINCS number

CAS: 111-76-2
EILINCS: 203-905-0

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_6H_{14}O_2$

3.1.2. Physical form

Liquid

3.1.3. Molecular weight

Molecular weight: 118.17

3.1.4. Purity, composition and substance codes

Purity: $\geq$ 99%

3.1.5. Impurities / accompanying contaminants

Impurities: 2-butoxyethoxyethanol (CAS 112-34-5) ≤ 0.3% w/w
1,2-ethanediol (CAS 107-21-1) ≤ 0.5% w/w
1-butanol (CAS 71-36-3) ≤ 0.2% w/w
water < 0.2% w/w

Additives: 0.008 – 0.012% w/w butylated hydroxytoluene (BHT) (CAS 128-37-0) added to prevent the formation of peroxides.

3.1.6. Solubility

In water: EGBE can be considered as highly miscible in water up to about 100 g/l

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: 0.8

3.1.8. Additional physical and chemical specifications

Appearance:	Colourless liquid
Melting point:	-74.8°C
Boiling point:	171°C
Density:	0.9 at 20°C
Rel. vap. dens.:	4.1 (air=1)
Vapour Press.:	1 hPa at 20°C

Conversion
1 ppm = 4.91 mg/m^3
1 mg/m^3 = 0.204 ppm

3.1.9. Stability

No data submitted

3.2. Function and uses

EGBE belongs to the group of glycol ethers, which are mainly used as solvents. During 2003, the production of EGBE in the European Union was approximately 161,000 tonnes. EGBE is manufactured by adding n-butyl alcohol and ethylene oxide.

EGBE has a wide range of uses as a solvent in paints and surface coatings, detergents and surface cleaners, inks or dyes. Nearly 60% of EGBE in Europe is used in paints and coatings, 11% is used in detergents and cleaners and about 0.5% is used in cosmetics and personal care products.

According to the notification to the Commission, EGBE is used in cosmetic products only as a solvent in hair dyes. It is usually used at concentrations lower than 2% (4% in oxidative hair dyes) but in certain types of dyes, especially those directly applied to the scalp without prior dilution, its concentration can be higher.

3.3. Toxicological Evaluation

Part of the toxicological evaluation is based on the EU risk assessment of 2-Butoxyethanol. The discussion of some studies has been transferred from the EU risk assessment.

Ref.: 1

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Animal experiments

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

Table 3.1: *Acute toxicity in animals after oral administration of EGBE*

Species	Sex	Validity*	LD_{50} (mg/kg bw)	Ref
Rats	M	2	2 600	2
	F	2	2 300	2
		2	1 500	3
		2	1 590	4
		2	2 420	5
		2	1 000 – 2 000	6
		2	1 746	7
Mice		2	1 230	3
		2	2 005 (fed)	7
		2	1 519 (fasted)	7
Rabbits		2	320 – 370	3
Guinea pig		2	1 200	3
		1	1 414	8

*1 valid (performed according to Guidelines and GLP)

2 valid with restrictions (not performed according to Guidelines and/or GLP, but scientific acceptable)

Human data

The acute toxicity after oral administration of EGBE has been estimated in humans. The results are summarized in Table 3.2.

Table 3.2: *Acute toxicity in humans after oral administration of EGBE*

Estimation of absorbed dose	Patient history	Patient pathology	Ref
Between 0.5 and 1 g/kg bw	50-year woman. Suicide attempt with window cleaner.	Coma, metabolic acidosis, hypokaliemia, increase in serum creatinine level and urinary excretion of oxalate crystals	9
About 1 g/kg bw	23-year woman. Suicide attempt with mixture containing EGBE.	Coma, breathing difficulties and metabolic acidosis. Haematuria and decreased Hb for 2 days.	10
About 750 mg/kg bw	53-year man. Suicide attempt with mixture containing EGBE.	Coma, tachycardia, metabolic acidosis, hypoxemia, pulmonary oedema and ARDS. Non haemolytic anaemia with thrombopenia.	11
About 1.25 g/kg bw – 2 times separated by 9 days	18-year man. Ingestion of a glass cleaner.	Metabolic acidosis and hepatic biochemical disorders. Nothing after the second ingestion.	12
About 4.5 g/kg bw.	19-year man. Ingestion of a mixture containing EGBE.	Coma, acidosis and haematuria.	13
Between 0.4 and 1.2 g/kg bw	51-year woman. Ingestion of a mixture containing EGBE.	Metabolic acidosis and mental status depression.	14

Acute human toxicity data comes from children accidental ingestion or adult suicide attempts made with mixtures containing EGBE. For oral route case reports, ingested doses are difficult to evaluate because of the lack of data concerning the body weight of all patients and the exact ingested dose, but a semi-quantitative estimation of the ingested doses was made for each case. The range of doses which lead to clinical symptoms varies between 0.5 and 4.5 g/kg bw. In all cases, patients exhibited CNS depression and metabolic acidosis. Signs of haemolysis were seen in some cases but this finding was not systematic (this showed that humans are much more resistant to haemolysis than rodents). After a first acute ingestion, a second administration some days later did not exhibit the same symptoms. This finding was also seen with animals in some studies. In these cases, EGBE was ingested together with other substances (ethanol and/or unknown substances) that could have some influence on the symptoms seen. The patients recovered totally after treatment when they had ingested between 0.5 and 1.5 g/kg bw. According to this data, a LOAEL of 400 mg/kg bw can be taken into account for acute toxicity by oral route in humans.

Comment

For the oral route, available animal studies considered reliable show LD_{50} values upwards from 1000 mg/kg bw. Humans recovered totally after ingestion of between 0.5 and 1.5 g/kg bw. A LOAEL of 400 mg/kg bw can be taken for acute toxicity by oral route in humans.

3.3.1.2. Acute dermal toxicity

The acute toxicity after dermal application of EGBE has been determined in several animal experiments. The results are summarized in Table 3.3.

Table 3.3: *Acute toxicity in animals after dermal administration of EGBE*

Species	Validity*	LD_{50} (mg/kg bw)	Ref
Rats	2	2 275	15
	1	>2 000	16
	1	>2 000	17
Guinea pig	2	6 411	2
	2	208 (intact skin)	18
	2	271 (abraded skin)	18
	2	450 – 1 800	19
	1	> 2 000	20
Rabbits	2	560	2
	2	505	3
	2	580	4
	2	100	21
	2	569	5
	2	435	22
	1	> 2 000	23
	1	841	24

*1 valid (performed according to Guidelines and GLP
2 valid with restrictions (not performed according to Guidelines and/or GLP, but scientific acceptable

Comment
For the dermal route, great differences were seen between the tested species and the mode of occlusion. The rabbit seems to be the most sensitive species with LD_{50} of about 500 mg/kg bw when administered occlusively.

3.3.1.3. Acute inhalation toxicity

The acute toxicity after inhalation of EGBE has been determined in several animal experiments. The results are summarized in Table 3.4.

Table 3.4: *Acute toxicity in animals after inhalation of EGBE*

Lethal Concentration LC_{50} (mg/kg)	Mortality and treatment related effects in function of exposure time	Validity*	Ref
Rats			
	Exposure to 800 ppm during 8 hr caused 50% mortality whereas an exposure time of 4 hr did not cause any death (6 animals in each group) Exposure to 500 ppm (2.45 mg/l) for 8 hr – did not cause any mortality (0/6) whereas a treatment period of 4 hr caused one death (out of 6) If older rats are exposed: 11/13 and 23/23 died after 7 hr of exposure	2	2,3
LC50: 486 ppm (2.38 mg/l) for males and 450 ppm for females.	4hr exposure Laboured breathing, loss of coordination, tail necrosis, renal toxicity, haemolysis. Mortality from 523 ppm (2.56 mg/l)	2	24
	Saturated vapours (617 ppm – 3 mg/l) and exposure time variable. Mortality 4/6 for 7 hr exposure, 1/6 for 3 hr exposure and no mortality for 1 hr exposure. Lethargy, necrosis of the tail and haemolysis.	2	25
Guinea Pigs			
	LT50: 7 hrs at 1300 ppm (6.37 mg/l)	2	3
> 633 ppm (3.1 mg/l) (females) > 691 ppm (3.39 mg/l) (males)	1 hr exposure. No effects	1	24

*1 valid (performed according to Guidelines and GLP)
2 valid with restrictions (not performed according to Guidelines and/or GLP, but scientific acceptable)

Comment
For the inhalation route, the 4 hour LC_{50} in rats, which are susceptible to haemolysis, was of the region of 450 ppm (2214mg/m^3). Higher values are seen in Guinea pigs.

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

Guinea pigs

Primary skin irritation was determined by application of the compound to the depilated abdomen of Guinea pigs at doses of 1, 5, 10, or 20 ml/kg. Skin responses were compared with those seen in rabbits in the same study. A dose of 0.3 g/kg in rabbit leads to a moderate irritation whereas a dose of 4.5 g/kg in guinea pigs leads to a strong irritation.

Ref.: 26

Rabbits

A skin irritation study was performed according to the method described in the US code of Federal Regulations. EGBE was administered on the abraded and intact skin of 6 rabbits during 24 hours under semi-occlusive conditions. After exposure, scoring was performed (according to the Draize method) immediately and 48 hr after the first reading. At the first reading time, 5 out of 6 animals exhibited a slight to moderate erythema and 4 out of 6 exhibited a slight oedema. At the second reading time, 4 out of 6 animals exhibited a very slight to moderate erythema and 3 out of 6 exhibited a slight oedema.

Ref.: 27

EGBE was only mildly irritating to rabbit skin when applied and left uncovered for a 4 hr period on rabbit skin. More prolonged exposure to the undiluted chemical (24 hr) can lead to moderate to severe irritation characterised by erythema, oedema and necrosis.

Ref.: 5

Guideline:	/
Species/strain:	New Zealand albino rabbits
Group size:	3 adult (EEC test method) 6 adult (Draize protocol)
Test substance:	EGBE
Batch:	Merck (purity > 99%)
Dose level:	A single topical application on a flank shaved the day before of 0.5 ml to the skin for 4 hours under occlusive patch (EEC test method) and for 24 hours in the Draize protocol. In the last method, the substance was also applied on the 2nd (scarified) flank
Route:	Topical
Exposure period:	4 hours
Observation:	72 h and 14 days
GLP:	/

EGBE and others glycol ethers were tested to assess their cutaneous irritation properties. Test substance was administrated dermally to rabbits according to 2 test methods for assessing cutaneous irritation: the EEC testing method and the Draize protocol. Three New Zealand white rabbits were used in the EEC protocol and 6 in the Draize protocol. 0.5 ml of

the test substance was applied occlusively on the flank of each animal, 4 hr for the EEC protocol and 24 hr for the Draize protocol (for the later, EGBE was also applied on the second scarified flank). Animals were observed for erythema and oedema. At 72 hr, histopathological control of the skin at the site of application completed the macroscopic observation. According to the EEC method, EGBE is classified irritant and according to the Draize method, EGBE is classified as severely irritant. These results were confirmed by histological observations. No detailed observations were available in this publication.

Ref.: 28

Comment
All the studies performed on rabbits and Guinea-pigs have shown that EGBE have caused moderate irritation (erythema and oedema) when applied occlusively on the skin for a period of 4 hours. If the substance was applied on scarified skin or for a longer period of time, signs of severe irritation sometimes leading to necrosis were reported.

3.3.2.2. Mucous membrane irritation

Eye

Rabbits

An eye irritation study was performed according to the method described in the US code of Federal Regulations. Six animals (rabbits) were instillated with 0.1 ml of undiluted EGBE and observed during 72 hours. A scoring of the effects at 24, 48 and 72 hours according to the method of Draize was performed. Effects on cornea, conjunctivae and iritis were seen in 5, 2 and 6 out of 6 animals at observation time of 24, 48 and 72 hours respectively and were not reversed after the 72 hr observation period.

Ref.: 29

Instillation of 5 µl of undiluted EGBE into rabbit eyes produced severe corneal injuries with iritis. Moderate corneal injury was observed when 0.5 ml of a 15% aqueous dilution of EGBE was instilled into the eyes of rabbits and no injuries was noted for a 5% aqueous dilution. No more details about this study are available, the value of 5 µl reported in the study is quite surprising in relation with the effects observed and the other doses tested.

Ref.: 5

EGBE (99 % purity) was instilled in the right eye of 6 New Zealand white rabbits. The test was performed in two phases. The test was performed according to OECD guideline 405 (version 1981). Erythema, chemosis, iritis and corneal opacity were scored according to the Draize scores at 4, 24, 48, 72, 96 and 168 hr after treatment. The mean erythema, chemosis, iritis and corneal opacity values (average of the 24, 48 and 72 hour observation) were: 2.59, 0.78, 1.00 and 1.33 respectively. The mean corneal upper layer damage (loss of epithelium measured by fluorescein retention on the cornea using a hand-slit lamp) was 95% after 4 hrs. Pain response was measured and described as "a few blinks only, normal within one or two minutes; animals didn't squeal or rubbing the eyes". The substance caused marked pannus in all 6 rabbits. EGBE, according to this study, should be classified as an irritant (mean erythema score >2.5, iritis =1). Pain sensation after contact with the product seems to be low, what can lead to underestimation of the irritating properties. Studies with fluorescein retention revealed a 90% loss of the upper layer of the cornea after instillation.

Ref.: 30

EGBE (99% purity) was instilled in the eye of 3 rabbits. The test was performed according to OECD guideline 405 (version 1981). Erythema, chemosis, iritis and corneal opacity were scored according to the Draize scores at 1, 2, 3, 7, 14 and 21 days after treatment. The EC scores for erythema, chemosis and corneal opacity values (average of the 24, 48 and 72 hour observation) are 2.33, 2.78 and 2.33 respectively. Opacity resolved in all 3 animals

although very slight redness (score 1), resist in 2 of 3 animals and chemosis (score 1) in one animal. According to the scores a classification is needed as irritant. It is stated that the study was performed in compliance with GLP.

Ref.: 31

Six New Zealand white rabbit were tested in a Draize irritancy test with about 100 chemicals including EGBE (purity > 99 %). The cornea, iris and conjunctivae were scored at 24, 48 and 72 hr and at 7, 10, 14 and 21 day. Ketamine was injected intramuscularly just prior to measurement at a dose of 40 mg/kg to sedate the rabbits. Corneal thickness was measured before instillation and thereafter at intervals coincident with the Draize scoring. Three measurements were made on each cornea and averaged. Based on all the substances studied, a significant linear correlation was established between Draize score and % swelling ($r = 0.86$). A great majority of chemicals showed agreement between the Draize score and the corneal swelling when compared with class irritancy rating (mild, slight, moderate, severe, extreme). For EGBE, Draize scoring was more severe than % of swelling. These differences are due to conjunctival damage, more heavily weighted by Draize scoring. But, swelling seems to detect more persistent irritation than Draize score. Resolution of ocular irritation was within 14 days.

Ref.: 32

Eye irritation potential of EGBE was assessed according to OECD guideline 405. 6 New Zealand white rabbits were instilled 0.1 ml EGBE into the conjunctival sac of the right eye. Amounts of ocular damage/irritation were noted 1, 24, 48 and 72 hr following treatment. Additional observations were made on days 7, 14 and 21 to assess the reversibility of the ocular effects. No information on test substance purity was included in the study report. The eyes were not rinsed 24 hours following treatment. Areas of corneal opacity were noted in all treated eyes on 24 and 48 hr after treatment. Reversibility was not obtained in 21 days for 1 animal. Iridial inflammation was noted in all treated animals 1, 24 and 48 hr after instillation. Reversibility was obtained in 14 days. Moderate conjunctival irritation was observed at 1, 24 and 48 hr. Effects were present until D14. Petechial haemorrhage was seen in some animals at each observation time from 1 to 72 hr. Ectropion was noted from 72 hr observation time in some animals and not reversible for 1 animal at the end of the study. One animal showing signs of discomfort was therefore killed for humane reasons immediately after the 14^{th} day. EGBE is considered to be a severe irritant to the eye in this study. It is stated that the study was performed in compliance with GLP.

Ref.: 33

The study was performed according to OECD guideline 405, 3 New Zealand white rabbits were instilled with EGBE (purity 99.6 %). Animals were observed 21 days. Eye washing was performed about 24 hours after treatment, before the 24 hour reading. One animal exhibited a slight corneal opacity reversible in 21 days. Signs of a slight iris injury were described, reversible in 7 days. A medium to severe irritation of the conjunctivae was also observed and was reversible in 21 days. According to criteria for assessment of ocular lesions, EGBE can be considered as an eye irritant. It is stated that the study was performed in compliance with GLP.

Ref.: 34

Humans

After exposure to 200 ppm of EGBE, immediate irritation of the nose and throat, followed by ocular irritation and disturbed taste was experienced by all three subjects.

Ref.: 35

Comment
Several studies have been performed to assess the eye irritation properties of EGBE. Most of them were not performed according to guidelines, but overall all studies have shown that EGBE is irritant or severely irritant to the eyes of rabbits with effects both on conjunctivae,

iris and cornea. In one well performed study EGBE produced irreversible effects on the conjunctivae and on the cornea in at least one treated rabbit. It was also demonstrated that dilution of EGBE in water decreases its irritant properties as well as rinsing of the eyes in case of exposure.

Respiratory tract

Mice

Sensory irritation was evaluated using an *in vivo* method with mice. Male Swiss-Webster mice (4/group) were exposed whole body to a series of chemicals (including EGBE). The measured response was the maximum percent decrease in respiratory rate, averaged over 4 mice, simultaneously exposed for 10 minutes. The responses obtained for various concentrations of solvents were utilized to develop a concentration-response relationship. For this, the concentration associated with a 50 % decrease in respiratory rate (RD50) was calculated. Time response relationship: after an initial decrease observed within a few seconds and characterized by a pause during the respiratory rate, a phase of recovery occurred but respiration rate was lesser than normal until the end of the exposure period. The response obtained within the concentration range tested was less than 50% decrease in respiratory rate. The RD50 calculated by extrapolation was 2825 ppm (confidence limit 1695 – 7278). These values are well in excess of the saturated vapour pressure. When compared with the criteria of evaluation, 0.01 RD50 (about 28 ppm) would cause minimal or no sensory irritation whereas 0.1 RD50 (about 280 ppm) would cause definite but tolerable sensory irritation.

Ref.: 36

Humans

Three volunteers were exposed to 100 and 200 ppm of EGBE for periods of 2 or 4 hours, separated by a 2 hour period of non-exposure. Immediate irritation of the nose and throat, followed by ocular irritation and disturbed taste was reported by all three subjects. Whether such 'irritation' was physiological or merely discomfort is not clear.

Ref.: 35

Some human studies by inhalation for toxicokinetic studies have shown that EGBE was not irritant to the eyes or respiratory tract at doses of 20 ppm during 2 hours or 25 ppm for exposure periods of 10 minutes. A study performed by Johansson in 1991 (ref. 39) in which human volunteers were exposed to 50 ppm EGBE whole body (but breathing pure air) and mouth only did not describe irritant effects, but according to the description of the study, this kind of effects were not recorded.

Ref.: 37, 38, 39

Guideline:	/
Species/strain:	Humans
Group size:	4 adult volunteers (2 males and 2 females)
Test substance:	EGBE
Batch:	/
Dose level:	50 ppm
Route:	Inhalation
Exposure period:	2 hours
Observation:	/
GLP:	/

No signs of irritation were reported after exposure to 50 ppm EGBE. Four volunteers (2 males and 2 females, aged 28-33) were exposed on 9 separate occasions, each occasion separated by at least 3 weeks. All exposures were 50 ppm EGBE for 2h. To record any physiological changes in the volunteers under the different conditions, physiological monitoring equipment was used). The published study paper does not mention if these signs were checked except the recording of the physiological changes. However, the author did indicate in a written communication that the volunteers were asked to report any adverse effects and none were reported.

Ref.: 40

Comment
Studies available did not show any signs of significant respiratory irritation. From the human data it is apparent that the NOEL is >50 ppm whilst the NOEL (based on effects of discomfort) is 100 – 200 ppm.

3.3.3. Skin sensitisation

Guinea pigs

The study was performed according to OECD guideline 406. EGBE was tested in Guinea pigs for sensitisation potential. Induction was made with a dermal injection of 0.5% EGBE in 0.9% saline and a topical application of a 25% EGBE in 0.9% saline. Challenge was made with a topical application of 10% EGBE in saline. All topical applications were made occlusively. Concentrations tested were based on results found in preliminary studies (showing a Maximal Concentration with No Irritation (CMNI) of 10%). Two challenge applications were made at one week interval. No evidence of sensitisation was seen in treated animals both at challenge and re-challenge. It is stated that the study was performed in compliance with GLP.

Ref.: 41

Maximized Magnusson Kligman test

Guideline:	/
Species/strain:	Dunkin-Hartley albino guinea pigs
Group size:	20 animals in test group and 10 in control groups (Bodyweight less than 500 g at beginning of study)
Test substance:	EGBE
Batch:	Merck (purity > 99%)
Dose level:	Intradermal injection: Test substance together with Freund's adjuvant, 10.9% aqueous NaCl-solution (1:1)
Epicutaneous induction:	Test substance
Challenge:	Test substance 1%
Route:	Intradermal and epicutaneously occlusive
Exposure period:	24 and 48 hours
Observation:	4 weeks
GLP:	/

EGBE was tested in a Maximised Magnusson & Kligman test. After induction (test substance concentrations not reported) a 1% concentration of EGBE was used for challenge. No animals exhibited signs of sensitisation.

Ref.: 28

Humans

The sensitising potential of 10% (v/v) EGBE in aqueous solution was assessed by HRIPT in human on 201 volunteers. Induction phase consisted in 9 consecutive occlusive application

of EGBE. Patches were removed 24 hr after application. Assessments of the sites of application were made at 48 hr intervals and after new patches were reapplied. Following the ninth evaluation, the subjects were dismissed for a 14-day rest period. The challenge phase was initiated during the sixth week of the study. Identical patches were applied to sites previously unexposed to EGBE. They were removed after 24 hr of exposure and the sites graded after additional 24 hr and 48 hr periods. Re-challenge if required was to be performed whenever there was evidence of possible sensitisation. The re-challenge was conducted on naïve sites on the back under both occlusive and semi-occlusive conditions approximately one or two weeks after challenge had been completed. Patches were applied for 24 hr, the patches removed and the sites evaluated 48, 72 and 96 hr after patches applications. Only 7 of the 48 hr evaluations and 12 of the 72 hr evaluations showed slight erythema. One subject at the 72 hr reading had definite erythema. More severe responses which would have been expected if delayed contact sensitisation had occurred were not observed. In conclusion, according to the authors, none of the 201 subjects completing the study showed evidence of sensitisation. Re-challenge was not required for any subject. It is stated that the study was performed in compliance with GCP.

Ref.: 42

Comment
The SCCP does not consider RIPT studies as ethical.

Two cases of dermatitis herpetiformis (DH) temporally related to the use of a cleaning solution containing EGBE. Analysis of the mixture showed the presence of EGBE, trisodium phosphate, nonylphenol polyethylene glycol ether, anthraquinone, lemon fragrance and an antifoam agent. Patch testing with the individual chemicals at the same concentration than in the mixture (except for EGBE which was diluted to avoid irritation) and at the same time precipitated a flare of DH in both patients. Causal agent remained unknown because each individual ingredient was not separately patch tested. These results are hardly reliable for EGBE risk assessment because of co-exposure.

Ref.: 43

Comment
No signs of skin sensitisation were seen in two animal studies or in a human patch test. Moreover, considering Structure Activity Relationship (SAR) in the glycol ether family, the wide dispersive use of EGBE and the fact that EGBE has never been associated with cases of skin sensitisation, it can be considered that skin sensitisation will not be expected.

3.3.4.1. Dermal / percutaneous absorption

In vivo
Rats

Guideline:	/
Species/strain:	Wistar rats
Groups:	6 male (269-290 g bw) and 6 female (172-196 g bw)
Test substance:	EGBE, 1,2-^{14}C, Spes.act 5.3 mCi/mmol
Batch:	/
Purity:	> 99%
Dose applied:	200 mg/kg
Skin preparation:	12 cm^2 shaved skin
Exposure period:	48 hours
Recovery:	20 – 23% in urine
GLP:	/

Cutaneous absorption *in vivo*: EGBE (>99% pure) was applied to 6 males and 6 females Wistar rats (12 cm^2 on shaved skin) at a dose of 200 mg/kg. To avoid oral uptake, the site of application was kept covered with a perforated glass capsule. Urine was collected for 48

hours. After termination, treated skin area was dissected for the determination of the radioactivity. Percutaneous absorption was assessed by using the measurements of urinary excretion of ^{14}C from 0 to 48 hr following administration of EGBE and expressing the results as total radioactivity (urine) following topical application / total radioactivity (urine). Within 48 hr following topical application of EGBE, approximately 20 to 23 % of the applied radioactivity was found in the urine, with no notable differences by sex (95% eliminated during the first 24 hr). At the end of the experiment only small amounts of radioactivity were found in the treated skin (4.7% in males and 8.3% in females). A percutaneous absorption of approximately 25-29 % of the applied topical dose was determined.

Ref.: 44

Male F344/N rats were exposed dermally (non occlusively) to EGBE (99.9 % purity) at dose levels of 122, 367 and 650 μmol/rat). Parent compound, CO_2, urine and faeces were collected for 72 hr. After 72 hr, rats were killed and the skin around the site of dosing was removed from the carcass. Digested tissues, urine and faeces were assayed for radioactivity. Analysis of urine from [^{14}C]-EGBE dosed animals indicated the presence of the parent compound. Some experiments demonstrated that 7% of the volatilized [^{14}C]-EGBE was trapped in the water in the urine receptacle. It demonstrates that urine dripping down the sides of the metabolic cage can absorb significant quantities of the volatilized EGBE. At the mid dose, 3 rats per time point were killed at 0.5, 1, 2, 4, 8 and 16 hr after exposure. Blood was collected and haematocrit and radioactivity measurement made. Urinary and plasma metabolites were analyzed. The total recovery of the ^{14}C was 74 to 90 %. 43 to 64 % of the dermally applied dose was trapped as volatile ^{14}C. 20 to 25 % of the dermally applied dose were absorbed and metabolized.

Ref.: 45

In vitro

Guideline:	/
Test substance:	EGBE, 1,2-^{14}C, Spes.act 5.3 mCi/mmol
Batch:	/
Purity:	>99%
Dose applied:	30 µl
Skin preparation:	Rats (fresh dorsal skin of hairless rats), pigs (dorsal skin), human skin (flexus side of the arms [obtained at autopsy])
Skin temperature:	32°C
Donor chamber:	100%, 3.5% and 10% in water alone or with 5% sodium dodecyl benzene (LAS) or 5% isopropanol (IPA)
Receptor fluid:	Physiological saline
Skin integrity:	Before and at the end checked visually
Exposure period:	Rats: 1, 6, and 16 hr – semiocclusive, 1 hr – nonocclusive; pigs: 6 hr semiocclusive (all 7 preparations). Rats and pigs: 10, 30, and 60 min – nonocclusive (3.5% in water). Rats, pigs, and humans: 1 hr semiocclusive and nonocclusive (10% in water)
Recovery:	/
GLP:	/

A modified method of Zesch and Schaefer (ref. 46) was used.
Cutaneous absorption of EGBE *in vitro* was assessed. These studies also included an evaluation of cutaneous absorption of EGBE under semi occlusive and non-occlusive conditions. Skin of rats, pigs and humans was used. Before and at the end of each study, the skin was checked visually for integrity of the stratum corneum. For application of test materials, an area of 5 cm^2 (animal skin) or 3 cm^2 (human skin) was demarcated on the skin samples. 30 µl of each of the following test solutions was applied: EGBE (100 %), EGBE (3.5 %) in water, EGBE (10 %) in water. All test solutions were evaluated for absorption in rat skin (1, 6 and 16 hr semi-occlusive, 1hr exposure non-occlusive) and pig skin (6 hr exposure semi-occlusive). Absorption of EGBE (3.5% in water) was also assessed on both

rat and pig skin under non-occlusive conditions (10, 30 and 60 min exposure). A comparative study involving samples of rat, pig and human skin was performed with EGBE (10% in water) using an exposure period of 1hr under both semi-occlusive and non-occlusive conditions. The studies with 3.5 and 10% EGBE were repeated with 5% 2-propanol (IPA) or 5% alkyl sulfonate (LAS) added to the test solution. Neither IPA nor LAS had any measurable effect on the dermal uptake of EGBE. Under semi-occlusion *in vitro*, EGBE is readily absorbed and completely absorbed after 16 hr of exposure. Penetration depends on time as well as concentration. The penetration rate of pure EGBE is slower than from aqueous solutions, but more complete after 16 hr. A comparison of EGBE penetration at 6 hr under semi-occlusive conditions showed that for all the tested formulations, the penetration through the pig skin was 2 or 3 times less rapid than through rat skin. Application of EGBE on rat skin under non-occlusive conditions results in apparently great reduction in the absorption due to the volatility of the compound. Within 10 min following application, a major proportion of the absorbed material had penetrated. In comparison with the pig skin, rat skin showed an approximate 2 fold higher absorption. Under semi-occlusive condition, the penetration rate of EGBE through human skin was comparable with that through pig skin (much slower than through rat skin). Under non-occlusive conditions, human skin exhibits the lowest percutaneous absorption (6.9 % of the applied dose).

Some of the results are summarized in the Table 3.5

Table 3.5. *Comparative in vitro percutaneous absorption of radio-labelled EGBE [a,b] in hairless rat, pig, and man*

System	Method	Percentage absorption [c]	
		600 µg/cm^2	1000 µg/cm^2
Hairless rat	Nonocclusive	14.7	11.0
	Semiocclusive	62.7	43.3
Pig	Nonocclusive	13.3	8.6
	Semiocclusive	21.1	17.7
Human	Nonocclusive		6.9
	Semiocclusive		17.3

[a] 10% EGBE in water (6 µl/cm^2 = 600 µg/cm^2; 10 µl/cm^2 = 1000 µg/cm^2, exposure time 1 hr

[b] Specific activity, 58.6 to 85.6 µC/ml

[c] Mean value of three measurements (coefficient of variation max 4%)

Ref.: 44

Comment

The percentage absorption using human skin and 1000 µg/cm^2 was 17.3%. The results in the table indicate that the percentage absorption is higher at lower concentration of EGBE. Since no data on recovery is given 17.3% is considered a minimum value. The study can, however, not be considered as valid since the recovery is unknown.

In an *in vitro* skin penetration test, the mean absorption rate of undiluted liquid EGBE (purity > 99%) through human epidermis was assessed. Disks of human abdominal skin were placed in diffusion chambers. The membrane integrity was assessed before the test by measurement of their permeability to tritiated water. The glycol ether absorption rate was measured for a period of 8 hours. After the test, the potential of the tested substance for impairing the epidermal diffusion barrier function was assessed by measuring the permeability to tritiated water again. A calculation of the damage ratio was made: Permeability constant after glycol ether contact / permeability constant before glycol ether contact. For EGBE the mean rate of penetration calculated in this study was 0.20 mg/cm^2/hr (±0.03 SEM, n=8). EGBE did not produce large alteration in permeability in this study: damage ratio = 2.07. The authors pointed out that human skin *in vitro* will always

deteriorate over time and a ratio greater than 1 would be expected. The measured ratio indicates a marginal effect with little damage to the skin following prolonged exposure.

Ref.: 47, 48

A skin penetration study *in vitro* was performed with liquid EGBE. Human abdominal skin (stratum corneum) was exposed to EGBE in Franz-type diffusion cells. The integrity of each skin sample was determined before and after the test by measuring the permeability to tritiated water. The skin absorption results for EGBE were measured twice due to high variability observed in the first study. The mean absorption rates were: 0.857 mg/cm^2/hour $\pm$ 0.282 in the first experiment and 1.52 mg/cm^2/hour $\pm$ 0.37 in the second experiment. The damage ratio was 3.25 $\pm$ 3.33 in the first experiment and 5.14 $\pm$ 4.99 in the second experiment. Due to the high variability in each experiment, mean absorption rates were calculated separately for the undamaged skin (n=8) and the damaged ones (n=4), the results from the damaged skin specimens were about 3 times higher than the undamaged ones (3.39 mg/cm^2/hour vs 1.19 mg/cm^2/hour). When the results from the four cells showing the high damage ratio are excluded from the calculation of the overall mean result, the mean damage ratio is 1.66 $\pm$ 1.31.

Ref.: 49

Guideline:	/
Test substance:	EBGE ([ethanol-2-^{14}C] specific activity 407 kBq/mg)
Batch:	/
Purity:	98%
Dose applied:	3 mg/ml or 6 mg/ml, 100 or 200 μl in the skin) or undiluted (10.5 μl) EBGE
Skin preparation:	Human breast skin (0.64 cm^2)
Skin temperature:	32 ^{0}C
Exposure period:	24 hr
Donor chamber:	EBGE
Receptor fluid:	Tissue culture medium was used as a receptor fluid with 2 % (w/v) Bovine Serum Albumin (BSA) or 2-6 % (w/v) PolyEthylene Glycol 20 (PEG 20) added for some studies
Skin integrity:	/
Recovery:	88 – 108%
GLP:	/

In vitro percutaneous absorption of liquid glycol ethers (including EGBE purity 98 %) was studied. Human breast skin, full thickness or dermatomed (stratum corneum + upper dermis) was exposed to EGBE in aqueous solution at different concentrations (3 mg/ml or 6 mg/ml, 100 or 200 μl in the skin) or undiluted (10.5 μl). Percutaneous absorption was measured for 24 hours using flow through diffusion cells. Tissue culture medium was used as a receptor fluid with 2 % (w/v) Bovine Serum Albumin (BSA) or 2-6 % (w/v) PolyEthylene Glycol 20 (PEG 20) added for some studies. In aqueous solution, a steady state flux of 544 $\pm$ 64 nmol.cm^{-2}.h^{-1} (0.064 mg/cm^2/hr) was found with dermatomed human skin. Reducing the dose to 100 μl decreased the steady state flux of EGBE by about 55 %. Using full thickness skin increased the time to steady state and reduced the steady state flux. Absorption rates of undiluted EGBE in finite dose exceeded those measured with aqueous solutions, though the apparent permeability coefficient was higher with the aqueous doses.

Ref.: 50

Oxidative hair dye formulations + developer containing hydrogen peroxide

Guideline: OECD 28

Test substance:	Oxidative hair dye formulations containing 5% and 10% EGBE ([ethanol-1,2-^{14}C] specific activity 50 mCi/mmole) mixed 50/50, w/w with developer containing hydrogen peroxide
Batch:	3442-013; New England Nuclear, Boston, USA
Purity:	>97.5%
Dose applied:	20 mg/cm²; 500 and 1000 µg/cm^2 EBGE
Skin preparation:	Human skin
Skin temperature:	32 ^{0}C
Exposure period:	30 min
Donor chamber:	EBGE in oxidative hair dye formulations + developer
Receptor fluid:	Saline in phosphate buffer
Skin integrity:	TEWL measurement
Recovery:	Mean 84% ± 4%
Number of samples:	not stated
GLP:	in compliance

Two different EGBE concentrations 5 and 10% in an oxidative hair dye formulation were mixed with an equal volume of developer containing hydrogen peroxide and applied on human skin during a period of 30 minutes. For each experiment 33 mg of the different formulations (containing 932±46 and 1874±66 µg EGBE) (corresponding to 20 mg/cm^2) were applied on the skin surface (1.76 cm^2). At the end of the 30 minutes of exposure, the surface of the skin was washed to eliminate the residual mixture. The diffusion of EGBE was monitored during the 24 hours following the applications. The receptor fluid was collected 2, 4, 6, 10, 21 and 24 hours after the beginning of exposure. At the end of the 24 hr observation period, tissues (horny layer, epidermis and dermis) were analysed for EGBE remaining. Results are expressed in µg equivalent of EGBE (µg/cm^2) and in % of the applied dose for all the compartments analysed. The numbers in the table are corrected for the skin surface (see table 3.6).

Table 3.6: *Quantities of EGBE analysed in the different system compartments for the 2 tested concentrations (2.5 and 5 %) in the presence of hydrogen peroxide*

	EGBE 2.5 %		**EGBE 5 %**	
	µg/cm^2	**% of the applied dose**	**µg/cm^2**	**% of the applied dose**
Filter (F)	16 ± 2	3.13 ± 0.34	28 ± 4	2.82 ± 0.38
Washing (W) (30 min)	343 ± 38	68.6 ± 7.5	652 ± 95	65.2 ± 9.5
Skin excess (F+W)	359 ± 39	71.7 ± 7.8	680 ± 98	68.1 ± 9.8
Epidermis (E) Including stratum corneum (SC)	0.060 ± 0.035	0.012 ± 0.007	0.150 ± 0.060	0.015 ± 0.006
Dermis (D)	0.010 ± 0.01	0.002 ± 0.001	0.05 ± 0.03	0.005 ± 0.003
Receptor fluid (RF)	61 ± 29	12.1 ± 5.9	125 ± 73	12.5 ± 7.3
Total skin +RF (SC+E+D+RF)	61 ± 29 highest value measured 98 ± 7	12.1 ± 5.9	125 ± 73 highest value measured 231 ± 14	12.5 ± 7.3
Total recovery (%)	84 ± 4		81 ± 4	

Ref.: 51

Comment
Under semi-occlusive conditions, dermal uptake of pure EGBE in rats was between 20 and 30% of the administered dose. Dermal uptake of aqueous dilutions of 5, 10 and 20% EGBE was similar to that of the pure substance. If EGBE was administered under non-occlusive conditions, the dermal uptake decreased dramatically (uptake < 10%), mainly because of the volatility of EGBE.

Some *in vitro* studies demonstrated that dermal uptake for pig skin was 2 or 3 times slower than rat skin. The results found with human skin were more or less equivalent to those seen with pig skin. Oxidative hair dye formulations containing 5 and 10% EGBE + developer containing hydrogen peroxide was studied in a well-conducted experiment. With 2.5% EGBE in the presence of hydrogen peroxide the dermal absorbance was 61 ± 29 μg/cm^2 (12.1 ± 5.9%).

In calculation of MOS, (61 ± 2x29) 119 μg/cm^2 reduced from 2.5% to 2.0% (119 x 2.0/2.5) ***95 μg/cm^2*** is used.

3.3.4.2. Oral absorption

Absorption of EGBE orally administered is rapid and essentially complete (assumed to be 100%)

Ref.: 1

3.3.4.3. Inhalation

Measurements performed on human volunteers showed a real absorption of 55 to 60%. A 60% of absorption for EGBE inhalation should be used in risk characterisation.

Ref.: 1

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated dose oral toxicity

Rat

Groups of 10 Albino rats (CR, COBS, CD-BR) were treated with various glycol ethers (including EGBE - purity > 99.5 %) via oral gavage, 5 days per week for ***6 weeks***. Doses administered were: 0, 222, 443 and 885 mg/kg bw. Body weights were recorded twice a week during the first week of exposure and every week after until termination of the study. Animals were observed daily for clinical signs. Blood samples were taken before euthanasia for the determination of haematology indices and biochemistry. After termination, histological examinations were performed on the following organs: lung, heart, thymus, kidneys, liver, spleen, brain, salivary glands, stomach, intestinal tract, pancreas, oesophagus, adrenal glands, pituitary, thyroid, parathyroid, trachea, mesenteric lymph node, testes and annexes, bone marrow, tongue, nasal cavities and eyes. Before fixation, liver, kidneys, heart, testes, brain and spleen were weighed.

2 out of 10 animals were found dead in the high dose. No mortality was seen in any other groups. A significant decrease in body weight gain was seen from day 13 in animals treated with the high dose of EGBE. In other groups there was a trend to a decrease in body weight gain comparable to controls and a lower food consumption in the middle dose group (not significant). Clinical symptoms were limited to bloody urine in the mid and high dose groups. Only one of the ten rats given the low dose of EGBE had bloody urine. This persisted through the third week of treatment. Other clinical signs at the mid and high dose were lethargy, unkempt hair coats, piloerection, slight weakness and inactivity. All doses produced significant effects on RBC parameters, showing a characteristic pattern of haemolytic anaemia. No effects were seen on WBC. For biochemistry: a significant increase of alkaline phosphatase (PAL) was seen from the middle dose. Moreover, in the high dose, a significant increase of SGPT and a decrease of glucose (GLU) were noted. These changes were slightly different compared to controls so their toxicological significance is uncertain. Absolute and relative spleen weights were increased for the middle and high dose of EGBE. A small increase of the liver weight (only significant for relative weight) was seen for the high dose group. No effects were seen on testes weight (relative or absolute) at any dose

group. For gross pathology, only enlarged, dark spleens were seen for high and intermediate dose. Histopathological lesions were hyperkeratosis and acanthosis in the stomach epithelium in all treated animals. Hepatomegaly was also seen in 4 out of 10 animals of the high dose group. Haemosiderin deposition was seen in some animals (7/10 and 6/10 in the high and mid dose group respectively). Thymus atrophy was seen in one animal of the high dose group. Congestion of the spleen was seen in all treated animals. Extramedullary haematopoiesis was reported for one animal in the high dose group and haemosiderin deposit in the majority of animals of the high and mid dose group. For this study, the NOAEL can be considered to be lower than 222 mg/kg (which is the Low Observable Adverse Effect Level: LOAEL) based on effects seen on spleens and RBC parameters.

Ref.: 52

Five groups of Sherman rats (5/sex/group) were fed with diet containing 0, 0.03, 0.125, 0.5 and 2% Butyl cellosolve (EGBE purity unknown) corresponding to doses of 0, 18, 76, 310 and 1540 mg/kg/d for ***90 days***. No deaths were seen attributable to the direct action of EGBE. Appetite was not affected and no pertinent micropathology was discovered. Tests for blood in pooled urine sample from the 2%, 0.5% and controls groups after 3 and 6 days were negative. The mean weight gain was lower than controls for the 2% group. Relative kidney and liver weight were increased at 2% dose and relative liver weight was increased at 0.5% dose. The NOAEL can be considered to be 0.125% (76 mg/kg bw/d) but considering that the test conditions were poorly reported (no control of the administered substance, evaporation?) this NOAEL will not be taken into account for the risk characterisation. The LOAEL was 310 mg/kg bw/d.

Ref.: 53

Groups of young DW albino rats (10 males and 10 females/group) were given food with EGBE (purity unknown) at doses of 0, 0.01, 0.05, 0.25, and 1.25% (corresponding to 0, 7, 38, 188, and 919 mg/kg bw in males and 0, 9, 41, 222, and 976 mg/kg bw in females respectively) during 3 months. Animals were weighted 4 times during the first week and once a week thereafter. After euthanasia liver and kidneys were weighed. Organs were examined for signs of pathology. The urinary bladder was examined for concretions. Histology was performed on representative tissues. One animal died at day 73 after the beginning of the study in the high dose group. Food consumption was decreased in both males and females at 1.25%. In males a significant decrease of food consumption was also observed at 0.25% dose. A significant decrease of body weight gain was seen in animals dosed with 1.25% EGBE. It was also seen in males at doses of 0.25%. The mean liver and kidney weight of males and females were definitely increased at the 1.25% dose. At both 1.25% and 0.25% testes were atrophied. The NOAEL for this study can be considered to be 0.05%. The purity of the EGBE used in this study is not given, nor does there appear to be analytical verification of EGBE doses, homogeneity and stability.

Ref.: 54

Comment
The effects on testis is difficult to interpret given for example that testicular atrophy was not reported in other oral, repeated-dose studies even at higher doses of EGBE. Given the availability of numerous other more recent and robust studies, this one cannot be considered reliable to derive the critical NOAEL.

Guideline:	/
Species/strain:	F344/N rats
Group size:	10 males and 10 females
Test substance:	EGBE
Batch:	Lot BT00504LP Aldrich Chemical Co., USA
Purity:	≈ 99%
Dose levels:	0, 750, 1500, 3000, 4500, and 6000 ppm

Route: Oral, drinking water
Exposures: 13 weeks
GLP: In compliance

EGBE was administered in drinking water at concentrations of 0, 750, 1500, 3000, 4500 or 6000 ppm to groups of 10 male and 10 female F344/N rats for 13 weeks. Estimates of compound consumption based on water consumption by rats were 69, 129, 281, 367 and 452 mg/kg bw/day for males and 82, 151, 304, 363 and 470 mg/kg bw/day for females. Supplemental groups of 10 rats/sex/group/time point were included for haematology and clinical chemistry observations at weeks 1 and 3. Rats were held in the testing laboratory for 11 - 12 days and were 5 to 7 weeks old when the study began. All rats were observed twice daily throughout the study. Body weights were recorded at the beginning of the study and then weekly until the end of the experiment. Clinical observations also were recorded weekly throughout the experiment. Complete autopsies were made on all study rats. Microscopic examinations were made on all control group and treated rats.

No rats treated with EGBE died or were killed before the scheduled end of the study and there were no clinical signs of toxicity. Body weight gains were reduced in both male and female rats of the 4500 and 6000 ppm treatment groups. In males and females there were reductions in drinking water consumption in the higher dose groups, this being clearly concentration related in females, from a mean of 18.8 ml/day in the control group to 10.7 ml/day in the 6000 ppm exposure group. A markedly macrocytic and mildly hypochromic anaemia was observed at each time point and reticulocyte counts were moderately increased in weeks 1 and 13. For males there was a decrease in erythrocyte counts at all time points in the 3000 ppm and greater groups, while in females the decrease occurred in the 1500 ppm and greater groups. A consistent thrombocytopenia was observed at all time points in males and females of the 4500 and 6000 ppm groups; it also occurred in females of the 3000 ppm group at week 13. The most consistent blood chemistry observation was an increase in alkaline phosphatase, particularly in the high dose groups. This observation is consistent with mild cholestasis. Thymus weights were significantly reduced in males of the 4500 ppm group and males and females of the 6000 ppm group. Other organ weight changes were found, but these appeared to be secondary to body weight gain reduction (spleen was not weighed). Histopathological lesions occurred in the liver, spleen and bone marrow and male and female rats. Hepatic changes included primarily centrilobular hepatocellular degeneration and primarily centrilobular Kupffer cell pigmentation. These changes were present in the majority of dosed rats, but they were more prevalent in the 3000 ppm and greater dose groups and were slightly more severe in females. In addition, the cytoplasm of hepatocytes of rats at all dose levels was more eosinophilic and lacked the ampholytic-to-basophilic granularity typical of the controls. In the spleen there was an increase in haematopoiesis and deposition of haemosiderin. In bone marrow there was a hyperplasia characterised by an increase of haematopoietic cells and decrease in marrow fat cells. All of these lesions were present in the majority of dosed rats, but they were more prominent in the 3000 ppm and greater groups. There was no treatment effect on testis weights but there was a reduction in the size of the uterus in females at 4500 and 6000 ppm. Changes in uterine weight were considered by the authors to be secondary to the reduction in body weight gain rather than a direct effect of EGBE. The only spermatozoal measurement that showed significant change relative to the control group was sperm concentration which was slightly decreased in all groups of treated males; however, this effect was not dose-related. There were no significant differences from the control group in oestrous cycle length for treated females, although females treated at 4500 and 6000 ppm spent more time in dioestrous than the other groups. This correlates with the smaller uterine size, which was attributed to a secondary consequence of reduced body weight gain. A LOAEL was identified in this study, based on cytoplasmic alterations in hepatocytes of both male and female rats at 750 ppm, equal to 69 mg/kg bw/d and 82 mg/kg bw/d in males and females respectively. Alternatively, based on mild anaemia indicated by a decrease in RBC counts in male rats and liver damage in the three highest dose groups a

NOAEL of 129 mg/kg bw/d may be used. The French Authorities (The Conseil Supérieur d'Hygiéne Publique de France (CSHPF), 7 November 2002) used 150 mg/kg bw/d representing the NOAEL for female rats since the effects in the female rats were more severe than in the male rats for their MOS calculation.

Ref.: 55

Mice

Guideline: /
Species/strain: $B6C3F_1$ mice
Group size: 10 males and 10 females
Test substance: EGBE
Batch: Lot BT00504LP Aldrich Chemical Co., USA
Purity: ≈ 99%
Dose levels: 0, 750, 1500, 3000, 4500, and 6000 ppm
Route: Oral, drinking water
Exposures: 13 weeks
GLP: In compliance

EGBE was administered in drinking water at concentrations of 0, 750, 1500, 3000, 4500 or 6000 ppm to groups of 10 male and 10 female $B6C3F_1$ mice for ***13 weeks***. Estimates of compound consumption based on water consumption by mice were 118, 223, 553, 676 and 694 mg/kg bw/d for males and 185, 370, 676, 861 and 1306 mg/kg bw/d for females. Mice were held in the testing laboratory for 11 - 12 days and were 5 to 7 weeks old when the study began. All mice were observed twice daily throughout the study. Body weights were recorded at the beginning of the study and then weekly until the end of the experiment. Clinical observations were recorded weekly throughout the experiment. Complete autopsies were made on all study mice. Microscopic examinations were made on all control group and treated mice.

No mice treated with EGBE died or were killed before the scheduled end of the study and there were no significant clinical signs of toxicity. Body weight gains were slightly reduced in both male and female mice of the 3000 and 6000 ppm treatment groups. No particular pattern was evident in drinking water consumption. Organ weight changes that were found were secondary to body weight gain reduction. No treatment related gross or microscopic lesions in male or female mice were found. The NOAEL of 223/370 mg/kg bw is not very reliable because no haematological analysis were performed on animals during or after the study.

Ref.: 55

The subchronic toxicity after oral administration of EGBE has been determined in several animal experiments. The results are summarized in Table 3.7.

Table 3.7. *Summary of studies performed by oral route*

Study	NOAEL (mg/kg bw/d)	Effects	Ref.
Rats			
6 weeks gavage	LOAEL = 222	Haematological effects at all doses and irritant effects on the stomach	52
90 days in food	NOAEL = 76 LOAEL = 310	Poorly reported	53
90 days in food	NOAEL = 0.05 % (38 and 41 mg/kg in male and females respectively)	Decrease in food consumption and body weight gain in males. Testes atrophy.	54
13 weeks in drinking water	LOAEL of 69 and 82 mg/kg bw/day for males and females respectively. (Alternatively, based on mild	Slight cytoplasmic alterations in hepatocytes of both male and female rats	55

Study	NOAEL (mg/kg bw/d)	Effects	Ref.
	anaemia indicated by a decrease in RBC counts in male rats in the three highest dose groups a NOAEL of 129 mg/kg bw/d (males) or 150 mg/kg bw (females) may be used)		
Mice			
13 weeks in drinking water	NOAEL = 223 and 370 mg/kg bw/d for males and females respectively LOAEL = 553 and 676 mg/kg bw/d for males and females respectively	Slight decrease in body weight gain	55

General comments
Effects seen by oral route were body weight reduction, haemolysis, hepatic effects and local irritation effects. Irritation to the forestomach was seen after gavage dosing. A LOAEL of 69 and 82 mg/kg bw/d (in males and females respectively) were observed in rats based on slight cytoplasmic alterations in hepatocytes of both male and female rats. Alternatively, based on mild anaemia indicated by a decrease in RBC counts and liver damage in male rats in the three highest dose groups in ref 55 a NOAEL of 129 mg/kg bw/d may be used. The French Authorities (The Conseil Supérieur d'Hygiéne Publique de France (CSHPF), 7 November 2002) used 150 mg/kg bw/d representing the NOAEL for female rats since the effects in the female rats were more severe than in the male rats for their MOS calculation. It should be noted (see 3.3.1.1) that humans are much more resistant to haemolysis than rodents.

3.3.5.2. Repeated dose dermal toxicity

Mice

Guideline: /
Species/strain: BALB/c female mice
Group size: /
Test substance: EGBE
Batch: /
Purity: /
Dose levels: 0, 100, 500, 1000 and 1500 mg/kg bw/day
Route: Dermal
Exposures: 4 days
GLP: /

EGBE was administered to BALB/c mice (5-6 weeks old) to assess the effects on the immune system. Doses of 0, 100, 500, 1000 and 1500 mg/kg bw/day (about 25µl in a 4:1 mixture of acetone and olive oil vehicle) were applied during ***4 days*** to the backs of the mice. The numbers of animals per dose level is not indicated in the study report. Except for one group of mice tested for IgM plaque-forming cell (PFC) response, animals were killed 24 hours after the last dose. Body weights were recorded. Thymus and spleen were removed and weighed prior to preparation of cell suspensions. For evaluation of the PFC response, mice were immunised by intraperitoneal injection with sheep erythrocytes on the third day of dosing and killed 72 hours after the final dose. Cell suspensions of lymphoid organ tissue were prepared for spleen and thymus, cellularity and viability were assessed. Lymphoproliferative assays (Non-specific mitogenic stimulation of either B or T lymphocytes), mixed lymphocyte response (MLR) assay, activity of natural killers (NK) cells, cytotoxic T lymphocyte (CTL) activity and plaque-forming cell response to sheep red blood cells were assessed using standard procedures.

No effects on body weight gain or thymus weight were seen at any dose tested. At the 1500 mg/kg bw dose, an increase in the relative spleen weight and an increase of splenic cellularity of 29 % were seen. There was no changes in the lymphoproliferation assay in the B cells population whereas the T lymphocyte sub-population exhibited a decrease of the proliferative responses significant only at doses of 500 and 1000 mg/kg (a decrease was also seen for the 1500 mg/kg bw dose but not statistically significant, this weakens the relevance of this finding). The same results were seen in the MLR assay (decreased responses at the high doses, significant only at 500 and 1000 mg/kg bw). NK, CTL activity and T-dependent PFC response was unchanged after treatment. The lack of a dose-related response in these observations throws doubt on their significance for EGBE toxicology. The NOAEL for immunological effects was 1000 mg/kg bw, based on spleen weight reduction at 1500 mg/kg bw/d.

Ref.: 56

Rabbits

EGBE was administered occlusively via dermal route to 4 groups of New Zealand (NZ) white rabbits (5 animals/sex) at concentrations of 5, 25, 50, and 100% (1 ml/kg of mixture corresponding to 45, 225, 450, and 900 mg/kg bw) during 6 hr/day for ***9 days*** (dosed 5 days, no dosed 2 days and dosed 4 days). Control group was treated with vehicle only. Dermal irritation score were determined after each test exposure. Body weight of animals was measured regularly: all males were submitted every day (and also after exposure period) to an ophthalmologic examination (discoloration, discharge, gross abnormality, reddening). Urine samples were collected prior to exposure on the 2, 5 and 9 day of exposure and on the day of sacrifice. Blood samples were collected before dosing the last day of dosing and the day of sacrifice and assessed of RBC count, haematocrit, MCV, MCH, MCHC, WBC count. Fourteen days after the last dosing, animals were euthanized. Tissues were collected and fixed. Only kidneys were examined histopathologically. Liver and kidneys of all animals were weighed. In males testes were also weighed. In the preliminary studies: LD50 was calculated for males: 0.707 ml/kg and for females: 0.630 ml/kg.

No male rabbits died during the study. One female rabbit was found moribund on day 5, the condition was apparently unrelated to treatment. No body changes were seen in male whereas female rabbits treated with undiluted EGBE exhibited a decrease in body weight gain throughout the study. All ophthalmologic examinations were negative for the male rabbits. The females were not examined. Severe necrosis and moderate oedema were observed in all animals dosed with undiluted EGBE by the 6 day of treatment. The 50% dilution produced necrosis of the skin of 1 out of 5 males as compared to 4 out of 5 females. Erythema preceded necrosis in every instance. Slight oedema was also observed in those rabbits exhibiting necrosis. Dosage related erythema was observed at the lower dosage levels. Very slight reddening was seen in the male rabbits in the water control group. No statistical comparisons were performed on the skin irritation readings. On day 2 of treatment there was haemoglobin in the urine of two of four male rabbits dosed with undiluted EGBE. There also was a slight increase in urinary protein in four males dosed with undiluted material on day 2 of treatment. Both of these conditions returned to normal by treatment day 5. On day 2 of treatment there was blood in the urine of three of five female rabbits dosed with undiluted EGBE. There was a corresponding increase of urinary protein in two of these animals. On day 5 of treatment, four of the five females dosed with undiluted material had blood in their urine, as well as one dosed with the 50% dilution. By day 9 of treatment only one dosed with undiluted sample still had blood in the urine; however, at this time all five females dosed with the 50 % dilution had blood in their urine. There was no evidence of haemoglubinuria at any dosage level at the time of sacrifice (14 days post exposure). No abnormalities were seen in any of the other measures of urinary effect assessed and there was no occult found in the urine of rabbits dosed with the 25% or the 5% dilutions. Statistically significant depressions of mean erythrocyte count, haemoglobin

and mean corpuscular haemoglobin concentration were present on treatment day 9 in females dosed with undiluted substance and the mean corpuscular haemoglobin was significantly increased. Although not statistically significant, the contemporaneous decreased haematocrit and increased mean corpuscular volume are consistent changes. The presence of larger red blood cells with more haemoglobin is indicative of response to red cell loss by early release of less mature cells into the circulation. These effects were no longer seen after the 14-day observation period. Females in the three lower dosage groups had no indication of biologically significant haematological effects. The increased mean red blood cell counts on day 9 of treatment in the 5% dilution group and on day 14 after exposure in the 25% dilution group are considered to be statistical artefacts. No changes were noted in any organ weight measured in all animals. Gross findings included some thickening of the skin in male rabbits given undiluted substance and possibly dose related patchy colour change of the kidneys of the three females. For this study, the NOAEL of 450 mg/kg can be taken into account mainly based on gross findings observed in animals dosed with pure substance. Haematological effects seen during the study were transient and recovery was complete after the 14-day observation period.

Ref.: 57

Four groups of 10 males and 10 females NZ white rabbits were treated dermally (occlusively) with EGBE (high purity) at concentration of 0% - 2.8% - 14.3% and 42.8% in distilled water (corresponding to dose levels of 0, 10, 50, and 150 mg/kg) for a 6 hr period/day 5 d/w for ***13 weeks.*** Animals were observed for clinical symptoms twice daily. Prior to treatment, the site of application of each animal was graded using the Draize skin reaction scoring system. Scoring was performed daily for the first 3 weeks of the study, the once weekly thereafter. Bw and food consumption were measured weekly. Blood was collected before the study, during week 4 and just before termination. The following parameters were determined: Haematology: white blood cell count, mean corpuscular haemoglobin concentration, erythrocyte Count, mean corpuscular haemoglobin, haemoglobin, differential white blood cell count, haematocrit, erythrocyte fragility (termination only), mean corpuscular volume. Clinical chemistry: glucose, direct bilirubin, total bilirubin, albumin, blood urea nitrogen, total bilirubin, alkaline phosphatase. All rabbits were subjected to a gross necropsy examination. The following organs were weighed at sacrifice for at least 6 animals/sex/level: heart, thymus, testes, spleen, liver, ovaries and kidneys. The following tissues were also examined briefly and conserved: adrenals, aorta, bone (sternebrae), brain (3 transverse sections), epididymis (both, tail), oesophagus, eyes, gall bladder, heart, intestines (caecum, duodenum, jejunum, ileum and colon), kidneys, liver (left & median lateral lobes), lung (left), lymph node (mesenteric & thoracic), mammary gland, ovaries, pancreas, parathyroids, pituitary, prostate, sciatic nerve, seminal vesicles, skeletal muscle, skin (test site & adjacent normal skin), spleen, stomach (pylorus and fundus), submandibular salivary gland, testes, thyroids, thymus, tongue, trachea, urinary bladder, uterus (both horns), and vagina.

Red coloured faeces and red liquid material on cage paper (probably blood) were seen in some treated animals (each group). Some irritant effects were sometimes seen in animals of both control and treated groups. There was no increase in the severity of these effects in treated animals versus controls. There were no treatment related deaths. No significant effects were seen on body weight gain or food consumption in any group treated compared to control animals. Sporadic changes in haematology parameters and RBC fragility values were noted but values were within normal ranges for this laboratory and probably not related to the test material. Sporadic changes were also noted in WBC counts but were not related to test material administration. No changes in biochemistry were related to treatment. No test material related effects were noted on absolute or relative organ weights or final body weights. The NOAEL for this study was equal to 150 mg/kg bw, the highest tested dose. The actual NOAEL may be of a higher value. It is stated that the study was performed in compliance with GLP.

Ref.: 58

The subchronic toxicity after dermal application of EGBE has been determined in several animal experiments. The results are summarized in Table 3.8.

Table 3.8. *Summary of the studies performed by dermal route*

Study	NOAEL (mg/kg bw/d)	Effects	Ref.
Mice			
4 days. Doses: 0, 100, 500, 1000, 1500 mg/kg bw/day	NOAEL=1000	Effects on splenic cellularity at 1500.	56
Rabbits			
9 days. Doses: 45, 225, 450, 900 mg/kg bw/d	NOAEL = 450	Transient haematological effects	57
13 weeks. Doses: 10, 50, 150 mg/kg bw/d	NOAEL > 150	No effects	58

Comment
Two studies are available on rabbits to assess the toxicity of repeated doses of EGBE administered dermally. In only one study, signs of toxicity were recorded and were limited to transient signs of haemolysis. This study led to a NOAEL of 450 mg/kg bw/d due to haematological effects seen at 900 mg/kg bw/d. Given that this study was performed only during 9 days, the NOAEL of the second study, which was performed during 13 weeks, could be more reliable for the risk characterisation. This NOAEL was 150 mg/kg bw/d. The mouse study designed for the assessment of EGBE effects on the immune system, gives a NOAEL of 1000 mg/kg.

3.3.5.3. Repeated dose inhalation toxicity

Rats

Groups of Alderly Park rats (4 males and 4 females/group) were exposed (whole body) to various concentrations of EGBE and for different periods of time. Rats were maintained in the exposure chamber for periods of up to 6 hours, and between repeated daily exposures they were returned to their cages where food and water were freely available. In the initial experiments the concentrations were selected to produce, if possible, acute effects after short exposures. Thereafter the exposure period was extended and the concentration lowered until the animals could survive 6-hour exposures, five days a week, for up to four weeks. With liquids, the concentrations which could be tested in these acute and subacute experiments. The rats were weighed and examined each morning, and their conditions and behaviour were recorded throughout the exposure period. Urine was collected overnight after the last exposure day for biochemical tests. Blood was collected during euthanasia for haematological tests. After a gross examination of the organs, the lung were collected and examined. The following organs were also taken for microscopic examination: lungs, liver, kidneys, spleen, adrenals and occasionally heart, jejunum, ileum and thymus. The results are summarized in Table 3.9.

Table 3.9. *Experimental pattern of exposure*

Dose	Number of exposures	Symptoms
250 ppm (1208 mg/m^3)	4 x 6-hr exposures	initial haemaglobinuria and lethargy, low HB and MCHC, weight loss
100 ppm (483 mg/m^3)	15 x 6-hr exposures	no toxic signs, urine and blood tests normal apart from increased red cell osmotic fragility: autopsy, organs normal
50 ppm (242 mg/m^3)	15 x 6-hr exposures	as 100 ppm experiment
20 ppm (97 mg/m^3)	15 x 6-hr exposures	no toxic signs, blood normal: autopsy, organs normal

In this study, the Lowest Observable Adverse Effect Concentration: LOAEC based on haematological signs was 50 ppm (15 exposures) leading to a NOAEC of 20 ppm. Other effects than those seen on haematological parameters were seen only at 250 ppm (4 exposures).

Ref.: 59

Groups of Fisher 344 rats (8 animals per sex in each group with an addition of 7 females to the high exposure and control groups, 7 males to the control group and 8 males to the high exposure group - the additional animals were used to determine, if necessary, the reversibility of any effect - these animals were observed during 14 days after the last exposure) were exposed to 0, 20, 86 and 245 ppm (0, 97, 415 and 1183 mg/m^3) of EGBE 6 hr/day for 5 consecutive days –followed by 2 days without exposure and then 4 days of additional exposure. All animals were observed before, during and following exposure for any signs indicative of toxic effects. The modified Irwin Screen test was performed on five rats per sex from the 245 ppm exposure group and five rats per sex from the control group for signs indicative of behavioural and/or neuromuscular abnormalities. Animals were observed on exposure days 1, 2, 5, 6 and 7 prior to following exposure and preceding sacrifice. During the post-exposure period, reversibility groups were screened on days 4, 7 and 14. Evaluations were made on the following: corneal response, pupil response, tail pinch, toe pinch, righting reflex, locomotor activity, impaired gait, respiration, tremors, convulsions, salivation, piloerection, diarrhoea, tail elevation, lacrimation, stereotypy. All rats had a ophthalmologic examination prior to group assignments. Except for reversibility groups, an eye examination was also done on all rats at sacrifice. The body weight for each rat was determined on the morning preceding the 1, 2, 5, 6 and 7 exposure days and again prior to sacrifice. During the post-exposure period the body weights of the reversibility group were recorded on post-exposure days 4 and 7 and prior to sacrifice, day 14. Haematological tests were performed on blood samples collected from all rats on the day prior to sacrifice. The analysis included: haemoglobin, haematocrit, erythrocyte count, white blood cell count, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. Reticulocyte and white blood cell smears were made for each animal, but were only evaluated for high exposure and control animals. All rats were killed for necropsy on the day following the final exposure (except the rats of the reversibility groups which were sacrificed on the 14 day following the final exposure. Liver, lungs, kidneys and testes were weighed.

No deaths occurred during the study. After the first and second exposure, only red staining of urine was seen in animals of the high dose group. A slight increase over the control values for audible respiration and nasal discharge were observed in male rats of the 245 ppm group. Urogenital area wetness was observed in a few females of the 245 ppm exposure group. Male rats in the 245 ppm or control groups appeared normal during the modified Irwin Screen testing sessions. One female rat of the 245 ppm exposure group exhibited an abnormal righting reflex following exposure. A decrease in body weight gain was observed for animals treated with 245 ppm and for females treated with 86 ppm. During post exposure observation, animals recovered and no differences were seen with controls at post exposure day 14. Erythrocyte count, haemoglobin and MCHC values were significantly lower in males and females of the 245 ppm group compared to controls. These animals also exhibited a significant increase in MCV, nucleated red blood cells, reticulocytes and in males only, lymphocytes. Haematological values in both males and female rats exposed to 86 ppm were significant for haemoglobin (decrease) and MCV (increase). The females of the 86 ppm group also had significant decreases in MCH and MCHC and an increase in the haematocrit values. All effects seem to be dose-related. No haematological effects were seen in the 20 ppm group. In the reversibility group, animals exhibited a substantial recovery following 14 day post exposure period. However a significant decrease in erythrocyte count (male only) and a significant increase in MCV (all animals) and in MCH (male only) were still present in treated animals. The leukocyte count returned to control value during this recovery period. The mean liver weight values (relative and absolute) for the female of the 245 ppm group were higher than the controls. In addition, the relative

liver weight for the females of the 86 ppm group and for the males of the 245 ppm group were higher than the controls. The mean relative testes weight for the males of the 86 ppm group was lower than controls. This can be considered to be an isolated finding not related to the treatment. After 14 day recovery period, no significant differences from controls in male or in female organ weight were observed. At necropsy, no finding could be specifically related to EGBE exposure. In this study, a NOAEC of 20 ppm can be identified.

Ref.: 60

EGBE (purity 99.4 %) vapour was administered by whole body exposure to Fischer 344 rats at concentrations of 0, 5, 25, and 77 ppm 0, (24, 121, 372 mg/m^3), 6 hr/day, 5 day/week for approximately 13 weeks (16 males and 16 females/group). Animals were observed for signs of toxicity before, during and after exposures. Body weight determinations were performed regularly. Food consumption was determined weekly. All rats had an ophthalmologic examination prior to group assignments, male rats of the 77, 25 ppm and controls were re-examined prior to terminal sacrifice. Only the females of the 77 ppm group were examined. Haematological tests were performed on blood samples collected from all rats on the day prior to sacrifice. Clinical examinations included urinalysis, haematology and serum clinical chemistry. Urine from rats of the high exposure group was analysed for haemoglobin and red blood cells. Urine from the same 5 rats per sex was analysed prior to exposure for the first 5 exposure days and prior to exposure each Wednesday thereafter. Urine was also collected and a more comprehensive analysis was performed one week prior to the terminal sacrifice from all rats. An interim sacrifice was performed on 6 rats per sex per exposure level. All remaining rats were necropsied at the final sacrifice on the day following the last exposure. A gross dissection and an evaluation were performed on each animal. The following organs were weighed at terminal sacrifice: liver, kidneys, lungs, and testes. No organ weight was obtained for the rats at the interim sacrifice. At the time of the necropsy, histopathological examinations were performed on the usual tissues selected for such studies.

No death occurred related to treatment. No effects were seen concerning ophthalmologic examination and Irwin Screen test. For rats that were killed at the interim sacrifice time, after 9 exposures, the females of the 77 ppm group had lower body weight gain values comparing to the controls. No differences were found among the females of the lower exposure groups or males of any exposure group. For the main group, similar results were obtained for the same periods of time. However, following this time period the depression in body weight gain noted in the females was reversible and returned to values similar to the values of the control group. The decreases of food consumption are consistent with the changes noted in body weight gain. Slight signs of haemolytic anaemia were seen in females exposed to high doses of EGBE. Subtle haematological effects noted in females were characterised by minimal depression of red blood cell counts, haemoglobin and haematocrit with an attendant slight increase in MCH. These effects were noted at the interim sacrifice time and persisted throughout the study without increasing in severity. There was no indication of red blood cells or haemoglobin in the urine collected daily during the first exposure week or weekly thereafter from the male and female rats of the high exposure level. Moreover, there was no treatment related alteration in erythrocyte fragility at either the interim or terminal sacrifice times. No other haematological, urinary or clinical chemistry findings of toxicological significance were observed among the rats. Several incidental lesions were present in various organs, however no treatment-related, gross or microscopic lesions were found in either the male or female rats sacrificed at the end of the study. On organ weights, the only significant finding was lower relative kidney weight among the males of all treated groups. This finding is not believed to be related to the treatment. The NOAEC was 25 ppm (about 9 mg/kg bw/d[1]), based on the non-progressive haematological effects in females and a transient reduced body weight gain at two weeks seen in females of the 77 ppm dose group.

[1] The systemic doses have been calculated for rats assuming an inhalation volume of 6 l/h and a weight of 500g for male rats and 350 g for female rats.

Ref.: 61

Guideline:	/
Species/strain:	F344/N rats
Group size:	10 animals per sex and dose
Test substance:	EGBE
Batch:	Dow Chemical USA Lot QP-911021-26D1 and QP-921215-26D2 (purity > 99%)
Dose level:	0, 31.2, 62.5, 125, 250, and 500 ppm by inhalation 6 h per day, 5 days per week
Route:	Inhalation
Exposure period:	14 weeks
GLP:	In compliance

EGBE was administered to groups of 10 male and 10 female F344/N rats by whole-body exposure to 0, 31, 62.5, 125, 250 or 500 ppm (0, 150, 302, 604, 1208, and 2416 mg/m^3) EGBE vapour (lot no. QP-921215-26D2, Dow Chemical, Plaquemine, LA, USA, purity > 99 %) for 6 h per day, 5 days per week for 14 weeks. Rats were held in the testing laboratory for 11 - 12 days and were 6 weeks old when the study began. All rats were observed twice daily throughout the study. Body weights were recorded at the beginning of the study and then weekly until the end of the experiment. Clinical observations also were recorded weekly throughout the experiment. Haematological examinations were performed at the end of the experiment. Differential leukocyte and nucleated erythrocyte counts were made by microscopic examination of blood films. Autopsies were made on all study rats. Organ weights measured were for the heart, right kidney, liver, lungs, right testis and thymus. A complete histological examination was performed on the chamber control and 500 ppm males and females and on the 250 ppm female rats.

Six female rats were found moribund and killed during the study: five in the 500 ppm group (four in week 1, one in week 5) and one in the 250 ppm group (in week 8). By the end of the study, body weight gains were significantly reduced in females of the 500 ppm group, but were unaffected in all other groups. Clinical findings were most prevalent in rats of both sexes exposed to 125, 250 or 500 ppm and included abnormal breathing, pallor, red urine stains, nasal and eye discharge, lethargy and either increased salivation or lacrimation. All females of the 500 ppm group, particularly during the first two weeks, developed alternating blue and white bands on their tails that caused them to self-mutilate and loose the distal portion of their tails. Haematological examination showed that inhalation of EGBE resulted in the development of a persistent and exposure-related macrocytic, normochromic, responsive anaemia, as indicated by decreased haematocrit values, haemoglobin concentrations and erythrocyte counts in the 125 ppm or greater group males and in all groups of exposed females. The effects were dose related. This evidence of a sex difference in the severity of the anaemia was also seen in the 500 ppm group, in which the indicators were slightly more severe in the females than in the males. Evidence of an erythropoietic response was shown by increases in reticulocyte and nucleated erythrocyte counts in males of the 125 ppm or greater groups and females of the 62.5 ppm or greater groups. Other haematological changes were decreases in lymphocyte and monocyte counts in males of the 125 ppm or greater groups and increased platelet counts in females of the 125 or 500 ppm groups. Some organ weight changes were observed. These were: increases of the kidney of males in the 500 ppm group and females in the 125 ppm or greater groups; increases of the liver of males in the 250 or 500 ppm and females in the 125 ppm or greater groups; and decreases of the thymus of females in the 500 ppm group. Thrombosis was observed in the tail vertebrae, femur, incisors, nasal cavity, lung, heart and liver of most females in the 500 ppm group, but not at all in the 250 ppm group. Additionally, in the 500 ppm group females, there was degeneration of the hepatic centrilobular areas and renal tubules (4/5 in each case) and atrophy of the thymus (4/5) and spleen (1/5). Haematopoietic cell proliferation was observed in the spleen of 2/5 group same group. Bone

marrow hyperplasia was recorded in males of the 250 and 500 ppm groups and females in all groups from 62.5 ppm. The severity of this response was dose related In the forestomach of females in the 500 ppm group, but not in lower dose groups, observations made were of inflammation (3/5), necrosis (2/5), ulcers (2/5) and hyperplasia (1/5). Effects also observed in the 250 ppm group females were hepatic necrosis, pigmentation of Kupffer cells and renal tubule cells and bone marrow hyperplasia. No NOAEC was found for female rats. LOAEC was 31 ppm based on haematological effects seen at all doses tested. A NOAEC of 62.5 ppm was found for male rats, based on haematotoxic effects seen at 125 ppm.

Ref.: 62, 63

Guideline:	/
Species/strain:	F344/N rats
Group size:	50 animals per sex and dose
Test substance:	EGBE
Batch:	Dow Chemical USA Lot QP-911021-26D1 and QP-921215-26D2 (purity > 99%)
Dose level:	0, 31.2, 62.5, and 125 ppm by inhalation 6 h per day, 5 days per week
Route:	Inhalation
Exposure period:	105 weeks
GLP:	In compliance

EGBE was administered to groups of 50 male and 50 female F344/N rats by whole-body exposure to 0, 31.2, 62.5 or 125 ppm (0, 150, 302, and 604 mg/m^3) EGBE vapour (lot no. QP-921215-26D2, Dow Chemical, Plaquemine, LA, USA, purity > 99 %) for 6 hr per day, 5 days per week for **105 weeks**. For haematological and bone marrow analyses, additional groups of 27 male and 27 female F344/N rats were exposed to 0, 62.5 or 125 ppm for evaluation at 3, 6 and 12 months and 9 male and 9 female rats were exposed to 31.2 ppm for 3 months (for haematological examination only) and 6 months. Rats were held in the testing laboratory for 18 days and were 7 – 8 weeks old when the study began. All rats were observed twice daily throughout the study. Body weights were recorded at the beginning of the study, after which they were recorded monthly from week 5 until week 89 and then every two weeks from week 92 until the end of the experiment. Clinical observations also were recorded monthly from week 5 until week 89 and then every two weeks until the end of the experiment. Haematological examinations were performed after 3, 6, and 12 months. Differential leukocyte and nucleated erythrocyte counts were made by microscopic examination of blood films. Autopsies and microscopic examinations were made on all of the main study animals that had not been used for blood and bone marrow sampling. Organ weights were not measured. At autopsy, all organs and tissues were examined for grossly visible lesions and all major tissues were fixed and processed for histological examination. A quality assessment pathologist evaluated the slides from all tumours and potential target organs, which included bone marrow, forestomach, kidney, liver, lung, nose and spleen of males and females and the adrenal and clitoral glands of females.

Survival of exposed male and female rats was similar to the chamber control groups, the mean numbers of survivors at the end of the experiment in each of the 0, 31.2, 62.5 and 125 ppm groups were 19, 11, 21 and 24 males and 29, 27, 23 and 21 females, respectively. No clinical signs were attributed to EGBE exposure. Body weights of male and female rats in the 31.2 and 62.5 ppm groups and male rats of the 125 ppm group were generally similar to the controls throughout the experiment. Body weights of female rats of the 125 ppm group were generally lower than those of the chamber control from week 17 until the end of the experiment. Haematological examination showed that inhalation of EGBE resulted in the development of a persistent and exposure-related macrocytic, normochromic, responsive anaemia, as indicated by decreased haematocrit values, haemoglobin concentrations and erythrocyte counts. These changes occurred at 3, 6 and 12 months in 62.5 ppm group females and 125 ppm group males. Some anaemia also occurred

at 3 and 6 months in the 31.2 ppm group females and at 12 months in the 62.5 ppm group males. Increases in circulating reticulocyte and nucleated erythrocyte counts are consistent with an erythropoietic response to the anaemia. In bone marrow there were approximately 15% - 35% decreases in the myeloid/erythroid ratio in the 125 ppm rats of both sexes, but particularly in females. Bone marrow hyperplasia was recorded in males of the 250 and 500 ppm groups and females in all groups from 62.5 ppm. The severity of this response was dose related. Non-neoplastic effects of exposure were observed in the nose, liver and spleen. In the nose there were significant increases in the incidences of hyaline degeneration of the olfactory epithelium in all groups of males (chamber control, 13/48; 31.2 ppm, 21/49; 62.5 ppm, 23/49; 125 ppm, 40/50) and in females exposed to the two higher concentrations of EGBE (chamber control, 13/50; 31.2 ppm, 18/48; 62.5 ppm, 28/50; 125 ppm, 40/49). The severity of the lesion was minimal and did not change with increasing exposure concentration. Incidences of Kupffer cell pigmentation of the liver increased significantly in all exposed groups of male rats (chamber control, 23/50; 31.2 ppm, 30/50; 62.5 ppm, 34/50; 125 ppm, 42/50) and in the two higher exposure groups of female rats (chamber control, 15/50; 31.2 ppm, 19/50; 62.5 ppm, 36/50; 125 ppm, 47/50). The severity of the lesion increased in the 125 ppm group of both sexes. In the spleen, incidences of fibrosis were significantly increased in the two higher exposure groups of male rats (chamber control, 11/50; 31.2 ppm, 14/50; 62.5 ppm, 19/50; 125 ppm, 20/50), but not in females. No NOAEC could be derived for non-neoplastic effects in this study. The LOAEC for both male and female rats was 31 ppm (about 8 mg/kg bw/d [males], about 11 mg/kg bw/d [females]), based on an increased incidence of minimal Kupffer cell pigmentation and on significant haematological effects in females.

Ref.: 62

Mice

Groups of 70 male C3H mice were exposed to 0, 100, 200 or 400 ppm (0, 483, 966, and 1932 mg/m^3) EGBE 7 hr/day for up to **90 days**. Body weights were recorded weekly. Serial sacrifices were performed on 15 mice from each group after 30 and 60 exposures, on 10 mice after 90 exposures, and on 11 to 18 mice after 42 days rest following 90 days of exposure. Ten mice from each group were used for serial determination of erythrocyte fragility after various exposure periods. The blood samples were obtained by section of the cervical cord. Liver and kidney weight were measured. The kidneys were cut for histological examination.

No mortality or gross pathology or renal tissue changes were observed. Statistically significant alterations of liver weights occurred in the mice exposed to 400 ppm for 30, 60 and 90 days. A similar occurrence was noted in the mice exposed for 60 days to 200 ppm. The number of mice which had bloody urine immediately after exposure was proportional to the exposure concentration. No case of bloody urine was noted after the third exposure. Increased erythrocyte fragility was found in the mice exposed to all three concentrations. The increase in erythrocyte fragility appeared to be as great after the first exposure as it was after the 89 exposure. In all cases the erythrocyte fragility value fell to essentially normal levels when blood samples were taken from mice after 17 hr rest.

Ref.: 35

Guideline:	/
Species/strain:	B6C3F1 mice
Group size:	50 animals per sex and dose
Test substance:	EGBE
Batch:	Dow Chemical USA Lot QP-911021-26D1 and QP-921215-26D2 (purity > 99%)
Dose level:	0, 62.5, 125, 250, and 500 ppm by inhalation 6 h per day, 5 days per week

Route: Inhalation
Exposure period: 14 weeks
GLP: In compliance

EGBE was administered to groups of 10 male and 10 female $B6C3F_1$ mice by whole-body exposure to 0, 31.2, 62.5, 125, 250 or 500 ppm (0, 150, 302, 604, 1208, and 2416 mg/m^3) EGBE vapour (lot no. QP-921215-26D2, Dow Chemical, Plaquemine, LA, USA, purity > 99%) for 6 hr per day, 5 days per week for **14 weeks**. Mice were held in the testing laboratory for 11 - 12 days and were 6 weeks old when the study began. All mice were observed twice daily throughout the study. Body weights were recorded at the beginning of the study and then weekly until the end of the experiment. Clinical observations also were recorded weekly throughout the experiment. Haematological examinations were performed at the end of the experiment. Differential leukocyte and nucleated erythrocyte counts were made by microscopic examination of blood films. Autopsies were made on all study mice. Organ weights measured were for the heart, right kidney, liver, lungs, right testis and thymus. A complete histological examination was performed on the chamber control and 500 ppm male and female mice.

In the 500 ppm group, in week 2 of the experiment, two male and two female mice died and two male and two female mice were found moribund and killed. All other mice survived. By the end of the study, body weight gains were significantly reduced in males of the 125, 250 and 500 ppm groups, but were unaffected in all other groups. Clinical findings were found only in males and females of the 500 ppm group that died or were killed and included abnormal breathing, red urine stains and lethargy. Haematological examination showed that inhalation of EGBE resulted in the development of a persistent and exposure-related normocytic (unlike rats), normochromic, responsive anaemia, as indicated by decreased haematocrit values, haemoglobin concentrations and erythrocyte counts in the 125 ppm or greater group males and in all groups of exposed females. Normocytic and normochromic erythrocytes were demonstrated by the lack of change in the mean cell volumes and mean cell haemoglobin concentrations, respectively. Other haematological changes were increased numbers of polychromatophilic erythrocytes in the 500 ppm group and increased platelet counts in males of the 500 ppm group and females of the 250 and 500 ppm groups. Increases were found in absolute and relative liver weights of males of the 500 ppm group and in relative liver weights of males of the 250 ppm group and females of the 500 ppm group. Haemosiderin deposition in Kupffer cells was the only change observed in livers of the 500 ppm group males and the 250 and 500 ppm group females. Haemosiderin pigmentation was also increased in renal tubule cells of both males and females of the 500 ppm group. Extramedullary haematopoietic cell proliferation (primarily erythroid) and haemosiderin pigmentation were present in males exposed to 125 ppm or greater and females exposed to 250 or 500 ppm. In the forestomach, there were significant increases in females only in the incidence of inflammation in the 250 and 500 ppm groups and epithelial hyperplasia in the 125 ppm and greater groups. Effects observed in the males and females of the 500 ppm group that were either killed or died included forestomach inflammation, necrosis and ulceration, suppurative inflammation of the peritoneum and mediastinum, atrophy of the spleen, thymus and lymph nodes, renal tubule degeneration and, in males, testicular degeneration and epididymidal necrosis. No NOAEC for female mice could be derived for non-neoplastic effects in this study, the LOAEC for female mice being 31 ppm (about 46 mg/kg bw/d[2]), the lowest dose tested, based on significant haematological effects. The NOAEC for male mice was 62.5 ppm, based on haematological effects at 125 ppm.

Ref.: 62

[2] The systemic doses have been calculated for mice assuming an inhalation volume of 1.8 l/h and a weight of 30 g for male mice and 25 g for female mice.

Guideline:	/
Species/strain:	B6C3F1 mice
Group size:	50 animals per sex and dose
Test substance:	EGBE
Batch:	Dow Chemical USA Lot QP-911021-26D1 and QP-921215-26D2 (purity > 99%)
Dose level:	0, 62.5, 125, and 250 ppm by inhalation 6 h per day, 5 days per week
Route:	Inhalation
Exposure period:	105 weeks
GLP:	In compliance

EGBE was administered to groups of 50 male and 50 female $B6C3F_1$ mice by whole-body exposure to 0, 62.5, 125 or 250 ppm (0, 150, 302, 604 mg/m^3) EGBE vapour (lot no. QP-921215-26D2, Dow Chemical, Plaquemine, LA, USA, purity >99 %) for 6 hr per day, 5 days per week for **105 weeks**. For haematological and bone marrow analyses, additional groups of 30 male and 30 female $B6C3F_1$ mice were exposed to 0, 62.5, 125 or 250 ppm for evaluation at 3, 6 and 12 months. Mice were held in the testing laboratory for 18 days and were 7 weeks old when the study began. All mice were observed twice daily throughout the study. Body weights were recorded at the beginning of the study, after which they were recorded monthly from week 5 until week 93 and then every two weeks from week 94 until the end of the experiment. Clinical observations also were recorded monthly from week 5 until week 93 and then every two weeks from week 94 until the end of the experiment. Haematological examinations were performed after 3, 6, and 12 months. Differential leukocyte and nucleated erythrocyte counts were made by microscopic examination of blood films. Bone marrow cellularity was measured. Cytological evaluations of bone marrow cell morphology and myeloid/erythroid ratios were made microscopically. Autopsies and microscopic examinations were made on all of the main study mice that had not been used for blood and bone marrow sampling. Organ weights were not measured. At autopsy, all organs and tissues were examined for grossly visible lesions and all major tissues were fixed and processed for histological examination. A quality assessment pathologist evaluated the slides from all tumours and potential target organs, which included bone marrow, forestomach, kidney, liver, lung, nose and spleen of males and females and the preputial gland, prostate, skin (prepuce), testis and urinary bladder of males.

Survival of male mice exposed to 125 or 250 ppm was significantly less than that of the chamber control group, whereas survival in the lower dose group was similar to the chamber control group. The mean numbers of survivors at the end of the experiment in each of the 0, 62.5, 125 and 250 ppm groups were 39, 39, 27 and 26 males and 29, 31, 33 and 36 females, respectively. No clinical signs were attributed to exposure to EGBE. Body weights of exposed male mice were generally less than those of the chamber control during the last 25 weeks of the experiment. Body weights of female rats of the 250 ppm group were generally lower than those of the chamber control from week 30 until the end of the experiment, the difference being about 20 % for much of that time. Body weights of the 62.5 and 125 ppm group females were generally lower than the chamber controls from about week 60 until the end of the experiment. Haematological examination showed that inhalation of EGBE resulted in the development of a persistent and exposure-related normocytic and normochromic, responsive anaemia, as indicated by decreased haematocrit values, haemoglobin concentrations and erythrocyte counts; it lacked, in general, changes in mean cell volumes and mean cell haemoglobin concentrations. These changes occurred at 3, 6 and 12 months in 125 and 250 ppm group male and female mice. Some anaemia also occurred at 6 months in the 62.5 ppm group females and there was a minimal increase in cell volume, suggesting a macrocytosis, in females of the 250 ppm group at 12 months. Increases in circulating reticulocyte counts that were observed in 125 and 250 ppm male and female mice at 3 and 6 months and 250 ppm female mice at 12 months, are consistent with an erythropoietic response to the anaemia. In bone marrow there was no change in cell counts and no decrease in the myeloid/erythroid ratio, unlike the observations reported for rats (above). Additionally, thrombocytosis developed, as shown by increased platelet counts

in the 250, 125 and 62.5 ppm exposed groups of males and females at 12 months, in the 250 ppm group males at 6 months and females at 6 and 3 months and in the 125 ppm group females at 6 months. Incidences of haemosiderin pigmentation in Kupffer cells of the liver was significantly increased in males of the 125 and 250 ppm groups and females of all groups exposed to EGBE in a dose-dependent manner. Haematopoietic cell proliferation in the spleen was increased in males exposed to 125 and 250 ppm and females exposed to 250 ppm, but it was not accompanied by any change in myeloid/erythroid cell ratio. Incidences of haemosiderin pigmentation in the spleen were significantly increased in all exposed groups of males and in the 125 and 250 ppm groups of females. These observations were attributed to the primary haemolytic effect of EGBE that was followed by regenerative hyperplasia of the haematopoietic tissue. Increases in the incidence of hyperplasia were also observed in the bone marrow of males exposed to 125 and 250 ppm. In the nose there were increases in the incidences of hyaline degeneration in all groups of female mice of the olfactory epithelium (chamber control, 6/50; 62.5 ppm, 14/50; 125 ppm, 11/49; 250 ppm, 12/50) and in the respiratory epithelium (chamber control, 17/50; 62.5 ppm, 35/50; 125 ppm, 26/49; 250 ppm, 23/50). The severity of the lesion was minimal and did not change with increasing exposure concentration. There was no clear dose response relationship. In males, the incidence was similar to the chamber controls. Non-neoplastic lesions of the forestomach consisted of ulcer (full thickness defect of the forestomach epithelium), particularly in females, epithelial hyperplasia (an increased thickness of the stratified squamous epithelium sometimes accompanied by an increased thickness of the keratinised layer) that was usually focal and, particularly in females, frequently associated with ulceration. There were a number of inflammatory changes in the urogenital system. Effects were only seen in male mice. In the kidney, incidences of glomerulosclerosis and hydronephrosis were significantly increased at 125 ppm (but not at 250 ppm) whilst the incidence of chronic inflammation was increased significantly at 250 ppm. The incidences of inflammation of the preputial and prostate glands in males exposed to 250 ppm were significantly increased as were incidences of inflammation of the bladder. The incidence of ulcer of the transitional epithelium of the urinary bladder was significantly increased at 125 ppm but not at 250 ppm). It is likely that the condition was exacerbated by the irritative effects of EGBE or its metabolites but is not primarily mediated by the substance. Incidence of non-neoplastic lesions in the urogenital system of male mice following 2-years exposure to EGBE. No NOAEC could be derived for non-neoplastic effects in female mice in this study, the LOAEC being 62.5 ppm, the lowest dose tested, based on marginal, but significant haematological effects. The NOAEC for male mice was 62.5 ppm, based on body weight gain decrement, marginal, significant haematotoxic effects and inflammation of the kidney, urinary bladder and prepuce at 125 ppm.

Ref.: 62

The subchronic and chronic toxicity after inhalation of EGBE has been determined in several animal experiments. The results are summarized in Table 3.10.

Table 3.10. *Summary of subchronic and chronic studies on animals performed by inhalation route*

Study	NOAEC (ppm)	Effects	Ref.
Rats			
15 exposures to 0, 20, 50 and 100 ppm. 4 exposures to 250 ppm	20 ppm (haematological effects)	Haematological effects	59
9 days, 6 hours a day. Doses: 0, 20, 86, 245 ppm	20 ppm (haemotoxicity)	Haematological effects and effects on liver and body weight gain.	60
13 weeks. Doses: 0, 5, 25, 75 ppm	25 ppm (haematological effects only)	Haematological effects only	61
14 weeks. Doses: 0, 31, 62.5, 125, 250, 500 ppm	LOAEC of 31 ppm	Study performed to describe the vascular and bone lesion observed in moribund female.	62, 63
105 weeks. Doses: 0,	None identified for	Haematological effects. Effects on liver	62

Study	NOAEC (ppm)	Effects	Ref.
31, 62.5, 125ppm	haematological effects; 31.2 ppm may be considered as an LOAEL.	(Kupffer cell pigmentation).	
Mice			
14 weeks. Doses: 0, 31, 62.5, 125, 250, 500 ppm	None identified for haematological effects; LOAEC of 31 ppm	Haematological effects. Effect on body weight gain. Irritant effects on the forestomach.	62
105 weeks. Doses 0, 62.5, 125, 250ppm	None identified LOAEC of 62.5 ppm	Haematological effects. Effects on liver (Kupffer cell pigmentation).	62

Comments
The main effect in rodent after inhalation of EGBE was haemolysis, which was consistently observed and sometimes associated with secondary hepatic effects (Kupffer cells pigmentation and absolute and relative liver weight increases). Other effects were decreases of body weight gain, effects on the forestomach and effects on the WBC sub-populations (T lymphocyte). In these studies, a NOAEC of 25 ppm in female rats (about 9 mg/kg bw/d) and a LOAEC of 31 ppm in female mice (about 46 mg/kg bw/d) and rats (about 8 mg/kg bw/d [males], about 11 mg/kg bw/d [females]) can be established (based on haemolysis, as the only significant primary effect).

Humans

Guideline: /
Humans: Workers in a beverage package production plant
Group size: 31 male workers, control group 21 not exposed male workers
Test substance: EGBE
Batch: /
Purity: /
Dose levels: 2.91 $\pm$ 1.30 mg/m^3 (0.59 $\pm$ 0.27 ppm)
Route: Inhalation
Exposures: /
GLP: /

31 male workers exposed to EGBE in a beverage package production plant were examined according to their external EGBE and internal butoxy acetic acid (BAA) (see section 3.3.9. Toxicokinetics). The effect of this exposure on several clinical parameters was examined. Average airborne concentration of EGBE was 2.91 $\pm$ 1.30 mg/m^3 (0.59 $\pm$ 0.27 ppm). There was co-exposure to methyl ethyl ketone (MEK). The subjects were between 22 and 45 years old and had been employed for 1 to 6 years in the company. 21 persons not exposed to EGBE were chosen to constitute a control group. There was a relatively good correlation between external and internal exposure estimated by measuring BAA post-shift urine samples (average 10.4 mg/g creatine; r = 0.55; P = 0.0012). Compared to the control group the exposed workers had a significant decrease (3.3%; p=0.03) in haemocrit, while mean corpuscular haemoglobin concentration (MCHC) was increased (2.1%; p=0.02). No significant difference was observed either in other erythroid parameters or hepatic and renal biomarkers.

Ref.: 64

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

The tables are reproduced from the EU Risk assessment report on 2-butoxyethanol.

Ref.: 1

The mutagenic activity in bacteria of EGBE and its major metabolites BAL and BAA (see section 3.3.9) has been summarized in Table 3.11.

Table 3.11. *Test for gene mutation induction in bacteria by EGBE and its metabolites*

Test system	**Source & purity of chemical**	**Result[a]**		**Dose[b] (LED/HID)**	**Reference**
		Without exogenous metabolic system	With exogenous metabolic system		
EGBE					
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	Dupont de Nemours, USA (purity not stated)	-	-	20,000 μg/plate	Sippel & Krahn, 1977
Salmonella typhimurium TA100, TA1535, TA1537, TA97, TA98, reverse mutation	Fisher Scientific, USA > 99 %	-	-	10,000 μg/plate	Zeiger et al., 1992
Salmonella typhimurium TA100, TA98, TA102 reverse mutation	Merck, Germany > 99 %	-	-	14,000 μg/plate	Hoflack et al., 1995
Salmonella typhimurium TA97a, reverse mutation	Merck, Germany > 99 %	+	+	2,200 μg/plate	Hoflack et al., 1995
Salmonella typhimurium TA97a, TA100 & *Escherichia coli* WP2*uvrA* reverse mutation	Dow Chemical Co., USA 99.04 %	-	-	10,000 μg/plate	Gollapudi et al., 1996
BAL					
Salmonella typhimurium TA100, TA98, TA97a, TA102 reverse mutation	Chemical synthesis > 91 %	-	-	7,000 μg/plate	Hoflack et al., 1995
BAA					
Salmonella typhimurium TA100, TA98, TA97a, TA102 reverse mutation	Janssen Chimica, Belgium > 99 %	-	-	1,000 μg/plate	Hoflack et al., 1995

[a] +, positive; –, negative; NT, not tested;
[b] LED, lowest effective dose; HID, highest ineffective dose

The genetic and related effect in cultured mammalian cells by EGBE and its major metabolites has been summarized in Table 3.12.

Table 3.12. *Tests for genetic and related effects in cultured mammalian cells by EGBE and its metabolites*

Test system	**Source & purity of chemical**	**Result [a]**		**Dose [b] (LED/HID)**	**Reference**
		Without exogenous metabolic system	**With exogenous metabolic system**		
EGBE					
Unscheduled DNA synthesis, rat primary hepatocytes (scintillation counting)*	Union Carbide, USA 99.4 %	?		0.0001%	Slesinski & Weil, 1980

Table 3.12. *Tests for genetic and related effects in cultured mammalian cells by EGBE and its metabolites*

Test system	Source & purity of chemical	Result [a]		Dose [b] (LED/HID)	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
Gene mutation, Chinese hamster ovary CHO cells, *hprt* locus	Union Carbide, USA 99.4 %	-	-	1% (v/v)	Slesinski & Weil, 1980
Gene mutation, Chinese hamster ovary CHO-AS52 cells, g*rt* locus	Aldrich, USA (purity not stated)	-		7.6 mM	Chiewchanwit & Au, 1995
Gene mutation, Chinese hamster lung V79 cells, *hprt* locus	Merck, Germany 99 %	+		20 mM	Elias et al., 1996
Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	Union Carbide, USA 99.4 %	-	-	0.25 %	Slesinski & Weil, 1980
Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	Dow Chemical, USA > 99 %	-	-	3,500 μg/ml	NTP, 2000
Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	Merck, Germany 99 %	±		15 mM	Elias et al., 1996
Sister chromatid exchange, human lymphocytes *in vitro*	Not stated	+		500 ppm	Villalobos-Pietrini et al., 1989
Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	Dow Chemical, USA > 99 %	-	-	5,000 μg/ml	NTP, 2000
Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	Merck, Germany 99 %	-		0.3 mM	Elias et al., 1996
Chromosomal aberrations, human lymphocytes *in vitro*	Merck, Germany 99 %	-	NT	0.3 mM	Elias et al., 1996
Chromosomal aberrations, human lymphocytes *in vitro*	Not stated	-	NT	3,000 ppm	Villalobos-Pietrini et al., 1989
Micronuclei, Chinese hamster lung V79 cells *in vitro*	Merck, Germany 99 %	±	NT	8 mM	Elias et al., 1996
Aneuploidy, Chinese hamster lung V79 cells *in vitro*	Merck, Germany 99 %	±	NT	16.8 mM	Elias et al., 1996
Cell transformation, Syrian hamster embryo cells, focus assay	Merck, Germany 99 %	-	NT	200 mM	Elias et al., 1996
Cell transformation, Syrian hamster embryo cells, focus assay	Not stated	+	NT	8 mM	Kerckaert et al., 1996
Cell transformation, Syrian hamster embryo cells, focus assay	Sigma Chemical Co., USA >99 %	-	NT	20 mM (7 days)	Park et al., 2002
Inhibition of gap-junctional intercellular communication, Chinese hamster lung V79 cells *in vitro*	Merck, Germany 99 %	+	NT	8 mM	Elias et al., 1996
BAL					
Gene mutation, Chinese hamster ovary CHO-AS52 cells, g*rt* locus	Aldrich, USA (purity not stated)	-	NT	7.6 mM	Chiewchanwit & Au, 1995
Gene mutation, Chinese hamster lung V79 cells, *hprt* locus	Chemical synthesis 91.4 %	+	NT	20 mM	Elias et al., 1996

Table 3.12. *Tests for genetic and related effects in cultured mammalian cells by EGBE and its metabolites*

Test system	Source & purity of chemical	Result [a]		Dose [b] (LED/HID)	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	Chemical synthesis 91.4 %	±	NT	15 mM	Elias et al., 1996
Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	Chemical synthesis 91.4 %	+	NT	0.2 mM	Elias et al., 1996
Chromosomal aberrations, human lymphocytes *in vitro*	Chemical synthesis 91.4 %	+	NT	0.2 mM	Elias et al., 1996
Micronuclei, Chinese hamster lung V79 cells *in vitro*	Chemical synthesis 91.4 %	±	NT	8 mM	Elias et al., 1996
Aneuploidy, Chinese hamster lung V79 cells *in vitro*	Chemical synthesis 91.4 %	±	NT	0.09 mM	Elias et al., 1996
Cell transformation, Syrian hamster embryo cells, focus assay	Chemical synthesis 91.4 %	-	NT	NG	Elias et al., 1996
Inhibition of gap-junctional intercellular communication, Chinese hamster lung V79 cells *in vitro*	Chemical synthesis 91.4 %	-	NT	8 mM	Elias et al., 1996
BAA					
Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	Janssen Chimica, Belgium >99 %	-	NT	0.9 mM	Elias et al., 1996
Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	Janssen Chimica, Belgium >99 %	-	NT	NG	Elias et al., 1996
Micronuclei, Chinese hamster lung V79 cells *in vitro*	Janssen Chimica, Belgium >99 %	+	NT	2.5 mM	Elias et al., 1996
Aneuploidy, Chinese hamster lung V79 cells *in vitro*	Janssen Chimica, Belgium >99 %	-	NT	0.36 mM	Elias et al., 1996
Cell transformation, Syrian hamster embryo cells, focus assay	Spectrum Chemicals, USA >99%	-	NT	20 mM (7 days)	Park et al., 2002
Inhibition of gap-junctional intercellular communication, Chinese hamster lung V79 cells *in vitro*	Janssen Chimica, Belgium >99 %	-	NT	8 mM	Elias et al., 1996

[a] +, positive; ±, weak positive –, negative; ? questionable result; NT, not tested;

[b] LED, lowest effective dose; HID, highest ineffective dose

* Scintillation counting is no longer considered a reliable method for measuring unscheduled DNA synthesis.

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

The *in vivo* genotoxicity of EGBE and its major metabolites are summarized in Table 3.13.

Table 3.13. *In vivo tests in mammals for the genotoxicity of EGBE and its metabolites*

Test system	Source & purity of chemical	Result[a]	Dose[b] (LED/HID)	Reference
EGBE				
DNA adducts, Sprague-Dawley rat brain, kidney, liver, spleen & testis, *in vivo* (^{32}P-post-labelling)	Merck, Germany 99 %	- (at 24 h)	120 mg/kg bw orally x 1	Keith et al., 1996
Methylation level of DNA, Sprague-Dawley rat brain, kidney, liver, spleen & testis *in vivo*	Merck, Germany 99 %	-		Keith et al., 1996
Methylation level of DNA, FVB/N transgenic mouse brain, kidney, liver, spleen & testis *in vivo*	Merck, Germany 99 %	-		Keith et al., 1996
Micronucleus test, CD-1 mouse bone-marrow cells *in vivo*	Merck, Germany 99 %	-	800 mg/kg bw i.p. x 1	Elias et al., 1996
Micronucleus test, B6C3F$_1$ mouse bone-marrow cells *in vivo*	Dow Chemical, USA >99 %	-	550 mg/kg bw i.p. x 3	NTP, 2000
Micronucleus test, male F344/N rat bone-marrow cells *in vivo*	Dow Chemical, USA >99 %	-	450 mg/kg bw i.p. x 3	NTP, 2000
BAL	No data available			
BAA				
Micronucleus test, CD-1 mouse bone-marrow cells *in vivo*	Janssen Chimica, Belgium >99 %	-	200 mg/kg bw i.p. x 1	Elias et al., 1996

[a] +, positive; –, negative; NT, not tested;
[b] LED, lowest effective dose; HID, highest ineffective dose

Reference:
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Kerckaert GA, Isfort RJ, Carr GJ, Aardema MJ, LeBoeuf RA. 1996. A comprehensive protocol for conducting the Syrian hamster embryo cell transformation assay at pH 6.70. *Mutat.Res.*, **356**, 65-84.
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Summary of genotoxicity

EGBE is not mutagenic in bacteria. In one study it was reported that EGBE gave a significant response in *S. typhimurium* TA97a. This was, however, not substantiated by another study specifically designed to investigate this finding. Neither BAL nor BAA was mutagenic in bacteria. Two of three mammalian cell gene mutation assays did not indicate any mutagenic activity for EGBE while a positive result was obtained in a poorly reported study with V79 hamster cells using a very high concentration (20 mM). The same publication reported a positive result at 20 mM with BAL, whereas another study found no effect with CHO hamster cells at concentrations up to 7.6 mM. There have been no mammalian cell gene mutation studies with BAA.

No evidence for chromosomal aberration induction has been found in a number of mammalian cell culture studies with EGBE or in one experiment with BAA. On the other hand, BAL induced chromosome aberrations. Weak aneugenic effects were obtained in the only available study with EGBE and BAL, but not with BAA. Induction of micronuclei was found in *in vitro* studies with BAA and, to a much lesser extent with EGBE and BAL.

There have been reports of significant activity of EGBE in the Syrian hamster embryo cell transformation assay, but again, the results have been inconsistent. There is also some indication of inhibition of gap-junctional intercellular communication in a single study with EGBE while no inhibition was found with BAL and BAA. A single assay for UDS induction used a technique that is now considered to be invalid.

In vivo, there is no evidence for micronucleus induction in bone marrow cells of mice or rats with EGBE or in mice with BAA. No interaction with DNA in several organs of rats was detected in experiments with EGBE. The balance of the evidence suggests that EGBE does not have relevant mutagenic potential *in vivo*.

3.3.7. Carcinogenicity

Inhalation studies

Rats

Guideline:	/
Species/strain:	F344/N rats
Group size:	50 animals per sex and dose
Test substance:	EGBE
Batch:	Dow Chemical USA Lot QP-911021-26D1 and QP-921215-26D2 (purity > 99%)
Dose level:	0, 31.2, 62.5, and 125 ppm by inhalation 6 h per day, 5 days per week
Route:	Inhalation
Exposure period:	105 weeks
GLP:	In compliance

US National Toxicology Program carried out the study.

F344/N rats, groups of 50 males and 50 females (7 weeks old), were exposed to 31.2, 62.5, and 125 ppm EGBE by inhalation 6 h per day, 5 days per week for 105 weeks. After the 105 week period of compound administration the remaining animals were sacrificed. Fifty animals of each sex were used as controls.

Survival of exposed male and female rats was similar to the chamber control groups. Animal surviving to study termination were: Males 19 (38%) control, 11 (22%) low dose, 21 (42%) middle dose, and 24 (48%) high dose. Females 29 (58%) control, 27 (54%) low dose, 23 (46%) middle dose, and 21 (42%) high dose. The mean body weights of females exposed to 125 ppm were generally less than the chamber control group. The most consistent exposure-related effect on the haematopoietic system was an exposure concentration related mild macrocytic, normochromic, regenerative anaemia present at 3, 6, and 12 months, with females more affected than males. Significant increases in bone marrow cellularity and decreases in the myeloid/-erythroid ratio relative to the chamber controls were observed at all time points in females exposed to 125 ppm, and a decrease in the myeloid/erythroid ratio was observed in males exposed to 125 ppm at 12 months.
The incidence of benign or malignant pheochromocytoma (combined) of the adrenal medulla in females exposed to 125 ppm was not significantly increased compared to the chamber controls but exceeded the historical control range. Exposure related increases in the incidences of hyaline degeneration of the olfactory epithelium and Kupffer cell pigmentation of the liver were observed in male and female rats.

It was concluded that under the conditions of this 2-year inhalation study, there was *no evidence of carcinogenic activity* of EGBE in male F344/N rats exposed to 31.2, 62.5, or 125 ppm. There was *equivocal evidence of carcinogenic activity* of EGBE in female F344/N rats based on the increased combined incidences of benign or malignant pheochromocytoma (mainly benign) of the adrenal medulla.

Ref.: 62

Mice

Guideline: /

Species/strain:	B6C3F1 mice
Group size:	50 animals per sex and dose
Test substance:	EGBE
Batch:	Dow Chemical USA Lot QP-911021-26D1 and QP-921215-26D2 (purity > 99%)
Dose level:	0, 62.5, 125, and 250 ppm by inhalation 6 h per day, 5 days per week
Route:	Inhalation
Exposure period:	105 weeks
GLP:	In compliance

US National Toxicology Program carried out the study.

B6C3F1 mice, groups of 50 males and 50 females (7 weeks old), were exposed to 62.5, 125, and 250 ppm EGBE by inhalation 6 h per day, 5 days per week for 105 weeks. After the 105 week period of compound administration the remaining animals were sacrificed. Fifty animals of each sex were used as controls.

Survival of male mice exposed to 125 or 250 ppm was significantly less than that of the chamber control group. Animal surviving to study termination were: Males 39 (78%) control, 39 (78%) low dose, 27 (54%) middle dose, and 26 (52%) high dose. Females 29 (58%) control, 31 (62%) low dose, 33 (66%) middle dose, and 36 (72%) high dose. The mean body weights of exposed males were generally less than those of the chamber control group during the last 6 months of the study. The mean body weights of exposed female mice were less than those of the chamber control group; the reductions were greater and occurred earlier than those observed in males. The most consistent exposure-related effect on the haematopoietic system was an exposure concentration related minimal normocytic, normochromic, regenerative anaemia present at 3, 6, and 12 months, with females affected slightly more than males.

In females exposed to 250 ppm, incidences of forestomach squamous cell papilloma and squamous cell papilloma or carcinoma (combined) were significantly increased relative to the chamber controls, and these incidences exceeded the ranges in historical chamber controls. In EGBE exposed males, there were possible exposure-related increases in the incidences of squamous cell papilloma of the forestomach, although the increases were not significant and the incidences were within the historical control range for chamber controls. Accompanying these neoplasms in females and, to a lesser extent, in males were exposure-related increases in the incidences of ulcer and epithelial hyperplasia of the forestomach. In male mice exposed to 250 ppm, the incidence of haemangiosarcoma of the liver was significantly increased relative to chamber controls and exceeded the range in historical controls; in addition, there were possible exposure-related increases in the incidence of hepatocellular carcinoma. Incidences of haemosiderin pigmentation in the Kupffer cells were significantly increased in 125 and 250 ppm males and all exposed groups of females. The incidences of splenic haematopoietic cell proliferation and haemosiderin pigmentation were generally increased in males and females, and the incidences of bone marrow hyperplasia were increased in males. The incidences of hyaline degeneration of the olfactory and respiratory epithelia of the nose were increased in female mice.

It was concluded that under the conditions of this 2-year inhalation study, there was *some evidence of carcinogenic activity* of EGBE in male B6C3F1 mice based on increased incidences of haemangiosarcoma of the liver. A marginal increase in the incidences of forestomach squamous cell papilloma and an increase in the incidences of hepatocellular carcinoma may have been exposure related. There was *some evidence of carcinogenic activity* of EGBE in female B6C3F1 mice based on increased incidences of forestomach squamous cell papilloma or carcinoma (mainly papilloma).

Ref.: 62

Comment

Forestomach tumours in rodents induced by non-genotoxic chemicals are in general not considered relevant for humans (Predictive Value of Rodent Forestomach and Gastric Neuroendocrine Tumours in Evaluating Carcinogenic Risks to Humans. IARC Technical Publications No 39, 2003). US EPA has recently evaluated the NTP carcinogenicity study and state that: "Information available to the Agency at this time indicate that nonlinear modes of action are likely responsible for the increased incidence of tumours observed by NTP (2000 [Ref.: 62]) in mice following chronic EGBE exposure. Application of nonlinear quantitative assessment methods indicate that the noncancer RfD (0.5 mg/kg bw/day) and RfC (13 mg/m^3) values developed for EGBE are adequately protective of these carcinogenic effects (An Evaluation of the Human Carcinogenic Potential of Ethylene Glycol Butyl Ether, EPA 600/R-04/123, February, 2005).

According to the criteria for classification of carcinogens in EU, EGBE should not be classified as a carcinogen on the basis of the studies available.

Subcutaneous administration

Transgenic mice

A study for increased tumour incidence or decreased tumour latency was conducted in FVB/N trangenic mice (Oncomice Neo 01™), which carry the viral Harvey *ras* (v-Ha-*ras*) oncogene controlled by the mouse mammary tumour virus (MMTV) promoter. These are mice that present a stable, heritable mutation in the Ha-*ras* gene. The endpoints of the assay can be achieved, in principle, by both genotoxic and non-genotoxic agents, but the limitations of the assay have not been defined. Two groups of seven-week old mice (25/sex/group) were dorsally implanted with subcutaneous minipumps (2002 Alzet™, Alza Corp.) delivering either saline or EGBE continuously at a rate of approximately 120 mg/kg bw per day for two weeks. Sub-groups of mice were killed for histological examination on days 5, 10, 15, 60 and 120 after implantation of the minipumps. Special attention was given to the development of mammary gland masses. There was no adverse effect upon either food or water consumption. At the 120-day sampling time, the mammary glands of six control female mice and five treated mice showed cellular hyperplasia. In addition, a control female mouse had a papillary cystic mammary adenoma and a treated mouse had an anaplastic carcinoma of the bladder. The authors conclude that EGBE does not promote initiated cells carrying the v-Ha-*ras* oncogene. There is some uncertainty about the numbers of mice used in the experiment and each sub-group.

Ref.: 65

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

Mice

Guideline:	/
Species/strain:	Swiss CD-1 mice
Group size:	See below
Test substance:	EGBE
Batch:	/
Purity:	>99%
Dose levels:	0, 0.5, 1.0, and 2.0% in drinking water
Route:	Oral, drinking
Exposures:	7 days prior to and during a 98-day cohabitation period
GLP:	In compliance

These studies were conducted using the US National Toxicology Program's Continuous Breeding Protocol. The protocol is divided into four tasks. Task 1 is a 14-days dose setting study which uses five dose groups and a control group (n = 8/sex/group. Task 2 is the continuous breeding phase, consisting of a control group (40 breeding pairs) and three dose groups (20 pairs/group). The animals are housed as breeding pairs for 98 days following 7 days of premating dosing with the test chemical. At the end of the 98 days, the pairs are separated and housed one animal/cage with continued dosing. Any litters born after the continuous breeding phase are reared by the dam until weaning, after which test chemical is provided at the same concentration as during Task 2. These animals are then used for fertility assessment of the second generation. When an effect on fertility is detected during Task 2, a 1-week crossover mating trial (Task 3) is performed with the parental animals after the last Task 2 litter is weaned to determine the affected sex. It consists of three groups of 20 pair each. Chemical treatment is discontinued for all animals during the 7 days breeding period and then reinstated until necropsy. Task 4; offspring assessment. In this phase the last litter from Task 2 is exposed to the test chemical.

Male and female Swiss CD-1 mice received 0, 0.5, 1.0, and 2.0% EGBE (purity > 99%), equivalent to daily intakes of 0, 720, 1340 and 2050 mg/kg bw in their drinking water during a continuous breeding phase (CBP) with a 7-day pre-mating period and a 98-day cohabitation period). During the cohabitation period, deaths occurred in the female mice: 13/20 (65%) in the 2% group, 6/20 (30%) in the 1% dose group, 1/20 (5%) in the 0.5 % dose group and 1/40 (2.5%) in the control group. The average body weights in the female 2% dose group were consistently lower than the controls. In the male mice, no deaths occurred but weight loss (1-2% of initial body weight) in the two highest doses and reduced weight gain were noted. Reduced fluid consumption was observed at all dose levels in both sexes (22%, 18% and 36% reduction relative to controls at 0.5%, 1.0%, and 2.0%, respectively after 14 weeks of dosing). The numbers of fertile pairs from the surviving pairs were 38/39, 19/19, 13/14 and 5/7 at 0, 0.5, 1.0 and 2.0% dose levels, respectively. Significant reduction in reproductive performance occurred at 1 and 2% dose levels as indicated by dose-related decrease in number of litters per fertile pair, litter sizes, pup viability and live pup weight. A small but significant reduction (by 5%) of live pup weight was also observed in the 0.5% dose group without other significant reproductive effects.

At the completion of the continuous breeding phase, the F_0 breeding pairs were separated and housed individually and exposure to EGBE continued. When the last litter was weaned F_0 males and females from the 1% dose group were mated with male and female control animals in a one-week crossover mating study to determine any sex-related reproductive effects of EGBE. Exposure to EGBE was discontinued during the one-week mating period and then reintroduced at 1% dose level (estimated daily intake 1830 mg/kg bw). Control males and females were also mated for comparative purposes. The proportion of successful copulations from the breeding pairs was similar in all groups. However, the number of fertile females was significantly reduced in the group where treated females were mated with control males. When evaluated over the 7 day period prior to necropsy, proportionally more females (7/13) in this 1% treated group than controls (9/38) had oestrus cycles longer than 7 days. At necropsy, male and female mice from this 1% dose group had significantly lower body weights and increased relative kidney weights. Female also had significant increases in relative liver weight. No significant differences were observed between the control and treated animals for the weights of reproductive organs, sperm motility, morphology or average oestrous cycle length and frequency. In the only histopathological examination carried out on the treated females, no treatment related kidneys lesions were observed. The cross-breeding results suggest that the fertility effects were only due to effects on the female mice. Furthermore, these effects may have been an indirect consequence of the severe general systemic toxicity rather than a direct effect of EGBE on the reproductive organs.

A final phase was conducted to assess the fertility and reproductive effects of EGBE in second generation (F_1) pups. The pups selected were those born after the CBP and when

the maternal animals were individually housed. As there were insufficient pups in the 1 and 2% dose groups, only the pups from the 0.5 % dose group were used. The F_1 generation pups were nursed, weaned and reared to sexual maturity. After weaning, the mice received 0.5% EGBE in their drinking water (estimated daily intake 950 mg/kg bw). At 74 ± 10 days of age, the F_1 animals from different litters were mated. The animals were necropsied after delivery. No significant fertility and reproductive effects were observed in the F_1 animals as indicated by the proportions of successful copulation and fertile females, litter size, pup viability and live pup weights. Similarly, no treatment-related changes in the weights of reproductive organs, sperm motility, morphology and the oestrous cycle length and frequency were noted. However, a significant increase in relative kidney weight in the females and a significant increase in relative liver weight in both the males and females were observed.

Ref.: 66, 67, 68

Comment
Significant adverse reproductive effects were observed in the females at very high dose levels (1340 mg/kg bw/day and above) which also caused severe toxicity, including death. Under the conditions of the study, the NOAEL for reproductive toxicity of EGBE (fertility) can be set as 720 mg/kg bw/day. For developmental toxicity, no NOAEL can be derived. A conservative LOAEL of 720 mg/kg bw/day can be taken as only a slight decrease in pup weight ($p<0.05$) was observed at this dose.

3.3.8.2. Teratogenicity

Oral route
Rats

Guideline:	/
Species/strain:	Fisher 344 rats
Group size:	See below
Test substance:	EGBE
Batch:	/
Purity:	>99%
Dose levels:	0 (control), 30, 100, and 200 mg/kg bw/day (GD 9-11); 0 (control), 30, 100, and 300 mg/kg bw/day (GD 11-13)
Route:	Oral, gavage
Exposures:	Gestation days 9 through 11 or 11 through 13
GLP:	In compliance

The teratogenicity of EGBE (> 99.0 % purity) was evaluated using Fisher 344 rats. The purpose of the study was to determine whether the exposure of pregnant rats to EGBE during critical periods of cardiovascular development adversely affected the structure of the foetal heart and great vessels. Other developmental toxicity was also recorded. EGBE (in distilled water) was administered via gavage to a total of 298 rats; 104 rats served as controls. The distribution of doses for the 184 animals dosed on gestation days (GD) 9 through 11 was as follows: 46 rats (0 mg/kg bw/day), 46 rats (30 mg/kg bw/day), 47 rats (100 mg/kg bw/day), and 45 rats (200 mg/kg bw/day). A total of 93 rats (all dose groups included) were killed on GD 12 of gestation, and the remaining 91 rats, on GD 20. For the 220 rats dosed on GD 11 through 13, the distribution of doses was as follows: 58 rats (0 mg/kg bw/day), 52 rats (30 mg/kg bw/day), 59 rats (100 mg/kg bw/day), and 51 rats (300 mg/kg bw/day). A total of 104 rats (all dose groups included) were killed on GD 14, and the remaining 116 rats, on GD 20. Also, on GD 20, foetuses were killed and examined.

The maternal effects of EGBE given from GD 9 - 11 or from GD11 - 13 at doses of and greater than 100 mg/kg bw/day included marked reductions in body weight and/or weight gain, increases in organ weights (kidney and spleen) and severe haematotoxicity. In

particular, dramatic reductions in circulating red blood cells, haematocrit and haemoglobin resulted by 24 hours after treatment. By GD20 the haematotoxic effects were nearly reversed. The changes observed in haematological parameters and organ weights in this study are typical of haemolytic anaemia and the compensatory haematopoietic response associated with recovery. The maternal NOAEL is 30 mg/kg bw/day based on the effects describes before.

Embryo/foetal effects were: increased resorptions, nonlive implants, and adversely affected implants per litter in the 200 mg/kg bw/day group dosed on GD 9 - 11, and decreased platelet count but no embryolethality in the 300 mg/kg bw/day group dosed on GD 11 - 13.

EGBE exposure during gestation did not increase the incidence of foetal malformations; particularly, no cardiovascular malformations were observed. When 200 mg/kg were given from day 9 to 11, an increased foetal lethality without malformations was noted. When 300 mg/kg were given from day 11 to 13, a decrease platelet count was seen in foetuses. It was noted that for developmental toxicity the NOAEL is 100 mg/kg when EGBE is administered from GD 9 - 11 and the conservative NOAEL is 100 mg/kg when EGBE is administered GD11 – 13 (although the NTP conclusion was for a NOAEL of equal to or greater than 300 mg/kg bw/day).

Ref.: 69, 70, 71

Mice

In a gavage probe study in pregnant CD-mice, EGBE (purity 97 %)was administered in distilled water at 0, 350, 650, 1000, 1500, and 2000 mg/kg bw/day (6 animals per group) during gestational days (GD) 8-14 (Note GD0 = vaginal sperm plug and sacrificed GD18). Haemolytic effects in the dams were observed from 650 mg/kg bw/day. At 1500 mg/kg bw/day the maternal mortality rate was 3/6 and at 2000 mg/kg bw/day 6/6. Increased resorption rates and numerically reduced number of viable foetuses were observed at 1000 and 1500 mg/kg bw/day. 4/43 foetus (in one litter) at 1000 mg/kg bw/day and 1/25 at 1500 mg/kg bw/day had cleft palates. For this study, the NOAEL for maternal toxicity is 350 mg/kg bw/day and the NOAEL for developmental toxicity is 650 mg/kg bw/day.

Ref.: 72, 73

Guideline:	/
Species/strain:	Swiss CD-1 mice
Group size:	See below
Test substance:	EGBE
Batch:	/
Purity:	99%
Dose levels:	See below
Route:	Oral, gavage
Exposures:	Pregnant mice, days 7 through 14 of gestation
GLP:	In compliance

In a subsequent reproduction study (following a Chernoff modification study design with treatment of the pregnant mice (20 per group) at 650 or 1000 mg/kg bw/day between GD 7-14, the animals were allowed to give birth and the offspring were observed till Post Natal Day (PND)22). No significant effects on pup growth or survival resulted. No adverse developmental effects were reported. Signs of haemolytic effects and bw gain reduction were seen at 1000 mg/kg in dams. The NOAEL for maternal toxicity is 650 mg/kg bw/day in this study, and as no effects were seen in pups, the NOAEL for developmental toxicity can be considered to be greater than 1000 mg/kg bw/day.

In another Chernoff assay, fifty mated CD1 mice were orally administered EGBE (99 % purity) by gavage at 1180 mg/kg bw/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. 20 % of the dams died, maternal weight gain was reduced and, of 31 surviving pregnant females, there were only 24 viable litters (77 %) compared with 97 % control litter viability. No external malformations were seen, pup survival to PND was unaffected and no other indication of specific developmental toxicity was found. A maternal NOAEL could not be calculated and, although pup development was unaffected, the observation of reduced numbers of live litters precludes a calculation of the developmental NOAEL.

Ref.: 74

Comment
The lowest foetal NOAEL is 100 mg/kg bw/day in the rat study. It is based on effects seen at 200 mg/kg bw/day: increase foetal lethality without malformations. These effects were seen with maternal toxicity (haemolytic anaemia) and retarded body weight gain evidenced from 100 mg/kg bw/day. The maternal NOAEL is 30 mg/kg bw/day in this study. This NOAEL based on 3 days exposure to 100 mg/kg bw/day is considerably lower than NOAEL based on up to 90 days repeated toxicity studies. On the other hand a similar value was found in a reprotox inhalation study.

Inhalation route

Rats

The teratogenicity of EGBE (98 to 99.5% purity) was evaluated using female SD rats. The animals were exposed whole body to EGBE vapour on GD 7 – 15 (GD0 = sperm positive vaginal smear) for 7hr/day (GLP status unknown). Sixteen and fifteen rats were exposed to 150 ppm and 200 ppm, respectively. The untreated control group consisted of 34 dams. Higher dose levels (250 – 500 ppm) in a preliminary study were associated with dose-related maternal deaths, haematuria and tail necrosis.

On the first day of exposure, haematuria was observed in the dams in 150 and 200 ppm groups. This was the only effect observed in dams. There were no significant differences in the number of resorptions foetal weights and the incidence of malformations or variations between the group exposed to 200 ppm EGBE and the control group. A statistically significant decrease in foetal weights was observed in the 150 ppm exposure group. Since differences in foetal weights between experimental and control groups were slight, and, also, that significant differences were not observed in the 200 ppm exposure group, these differences were not thought to have been of biological significance. For this study a very conservative LOAEL of 150 ppm can be determined for dams for haematuria seen in the first day of treatment. For developmental toxicity, the NOAEL is 200 ppm.

Ref.: 75

Mated female Fischer 344 rats (36 per group) were exposed whole body to 0, 25, 50, 100, and 200 ppm (0, 121, 242, 483, and 966 mg/m^3) EGBE vapour (purity 99.6 %) for 6hr/day on GD 6-15 (GD0 = sperm plug positive). No adverse reproductive or developmental effects were observed in animals exposed to 25 ppm or 50 ppm. Maternal toxicity was observed in a dose-related incidence during the exposure period, included evidence of haematuria from 100 ppm and pale, cold extremities with necrosis of the tail tip at 200 ppm. Dose-dependent maternal weight loss was observed at 100 ppm and 200 ppm, associated with significant reductions of food consumption (for both groups) and water consumption (for the 200 ppm group). Haematological findings from blood samples taken at necropsy on GD21 (six days after the last exposure) evidenced signs of haemolytic anaemia for treatment with 100 ppm EGBE and higher.

At sacrifice, maternal gravide uterine weight was significantly reduced and the absolute and relative spleen and relative kidney weight were elevated compared to controls at 200 ppm.

Exposure to 200 ppm was associated with a statistically significant increase in the number of totally resorbed litters and a reduction in the number of viable implants and in percentage of live foetuses per litter. However, there were no statistical significant increases in incidences of external, visceral, skeletal, or total malformations associated with exposure to EGBE. Exposure to 200 ppm was also associated with a significant increase in the number of litters with one or more foetuses with unossified skeletal elements and poorly ossified skeletal elements (anterior arch of atlas, cervical centra 5 and 6 and forelimb proximal phalanges) and poorly ossified skeletal elements (cervical arches and sternebrae 1, 3, 4 and 6). Primarily because the skeletal elements were poorly ossified there was a decreased incidence of bilobed cervical centra 5 and 6 and bilobed thoracic centra 9 and 13. There was also a decreased incidence of poorly ossified hindlimb proximal phalanges. At 100 ppm, the number of litters with one or more foetuses with unossified cervical centrum 6 was significantly increased and, because fewer also showed ossification of cervical centrum 5, the number bilobed was significantly decreased. No significant differences were observed for other aspects of skeletal ossification. The occurrence of unossified skeletal elements was considered by the authors to be an indication of delayed development in animals exposed to EGBE under maternally toxic concentrations. For this study the NOAEC is 50 ppm for maternal toxicity based on haemotoxicity and increase in bodyweight (the increase in bodyweight gain was reduced by about 30%; $P<0.05$) and food consumption retardation at 100 ppm (reduction about 13%; $P<0.01$). A LOAEC of 100 ppm for developmental toxicity is due to the small increase in skeletal ossification retardation, but without any other foetal effects, variant, observed at this dose level, this leads to a NOAEC of 50 ppm for developmental toxicity. It is stated that the study was performed in compliance with GLP.

Ref.: 76, 77

Rabbits

Mated New Zealand white rabbits (24 per group) were exposed whole body to 0, 25, 50, 100 or 200 ppm (0, 121, 242, 483 or 966 mg/m^3) EGBE vapour (purity 99.6 %) for 6hr/day on GD 6-18 (GD0 = day of copulation). No adverse reproductive or developmental effects were observed in animals exposed to 25 ppm or 50 ppm. At 200 ppm four females died or were sacrificed by the third day after the onset of dosing and four aborted. The blood samples taken at necropsy on GD29 (eleven days after the last exposure) showed increased haemoglobin concentration and haematocrit at 100 and 200 ppm, but statistical significance was only evident at 100 ppm. Maternal body weight loss was observed in all groups including controls during exposure, but the difference was greatest at 200 ppm and, by GD15, the actual body weight at 200 ppm was significantly lower. Exposure to 200 ppm produced a significant reduction in maternal body weight, gravide uterine weight and number of total implants and viable implants. There were no significant increases in the number of foetuses or litters with one or more affected foetuses with pooled external, visceral, skeletal (total) malformations in any treatment group. For individual malformations, there was a significant increase relative to controls in the number of litters at 100 ppm with one or more foetuses exhibiting fusion of papillary muscles in the left ventricle. Five foetuses from 4/19 litters were affected. However, since this malformation was not observed at the higher or other concentrations it was considered by the authors, the reviewers and the rapporteur as coincidental and not treatment-related. The incidences of skeletal variations did not indicate an adverse treatment effect on ossification. There were significant decreases in the incidences of litters at 200 ppm with unossified sternebra 6 and with rudimentary ribs on lumbar vertebra 1. However the former observation was indicative of better ossification and the latter observation was because more ribs at 200 ppm were full (extra) rather than rudimentary and there were actually no intergroup differences in supernumerary rib numbers. For this study, the NOAEC for maternal toxicity is 50 ppm and the NOAEC for developmental toxicity is 100 ppm based on fewer viable implants seen at 200 ppm. It is stated that the study was performed in compliance with GLP.

Ref.: 76, 77

Comment
Based on inhalation studies the developmental NOAEC of 50 ppm can be taken into account for the risk assessment (rats). This value is based on variations seen at 100 ppm. These effects were seen in presence of maternal toxicity (rats) (haemolytic anaemia, reduced food consumption and bodyweight gain), which was seen at 100 ppm and higher. Effects on relative organ weight were observed at 200 ppm. The maternal NOAEC for this effect was 50 ppm [6 hour exposure to 50 ppm in female rats corresponds to about 25 mg/kg/d].

Dermal administration

Rats

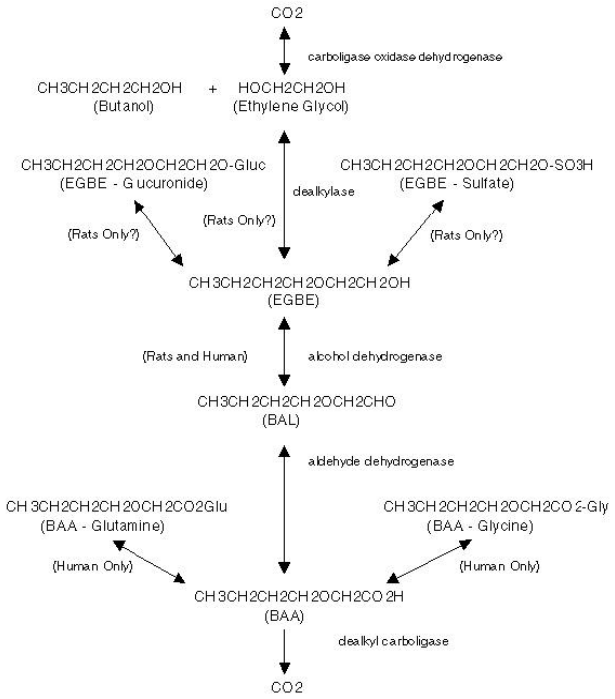
Guideline:	/
Species/strain:	Sprague-Dawley rats
Group size:	9 rats, control 17 rats
Test substance:	EGBE
Batch:	Lot 703549
Purity:	/
Dose levels:	0.12 ml x 4 per day from GD 7 – 16
Route:	Skin
Exposures:	10 days
GLP:	/

EGBE was applied to the skin (unoccluded) of 9 pregnant SD rats to investigate its potential for developmental toxicity. Four doses each 2.5 hours apart of 106 mg EGBE (total daily dose of 424 mg, 1760 mg/kg bw/day) were applied daily to shaved interscapular skin of rats on GD 7 – 16 (GD0 = sperm positive). A previous replicate of females dosed at a concentration three times higher had been terminated because of marked toxic effects (haematuria, tail necrosis, death after 3-7 days treatment). No maternal, embryotoxic, foetotoxic, or teratogenic effects were detected with EGBE treatment at the lower concentration of approximately 1760 mg/kg bw/day.

Ref.: 78

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

3.3.9. Toxicokinetics



Proposed pathways for the metabolism of EGBE in rats and humans are presented in Figure 1.

Ref.: 79, 80

The two main oxidative pathways of EGBE metabolism observed in rats are alcohol dehydrogenase (ADH) and O-dealkylation by a cytochrome P450 dealkylase (CYP 2E1). EGBE may also form conjugates with glucuronide and sulfate to some extent. Primarily because BAA is excreted in the urine of both rats and humans following EGBE exposure, it has been suggested that the former pathway, which involves production of BAA through formation of butoxyacetaldehyde (BAL) by ADH, would be applicable to both rats and humans. However, the other three proposed metabolic pathways of EGBE may be applicable only to rats, as the metabolites of these pathways—ethylene glycol (EG), EGBE glucuronide, and EGBE sulfate—have been observed only in the urine of rats and not in the urine of humans. In addition, approximately two-thirds of the BAA formed by humans is conjugated with glutamine and, to a lesser extent, glycine. These BAA glutamine and BAA glycine conjugation pathways have not been detected in the rat.

The metabolic basis for the haematotoxicity of EGBE was studied in male F344 rats using pyrazole and cyanamide as metabolic inhibitors of alcohol and aldehyde dehydrogenases, respectively. Male F344 rats (9-13 weeks) were pretreated with pyrazole or cyanamide followed by administration of 500 mg/kg EGBE by gavage. Pyrazole protected rats from EGBE-induced haematotoxicity and resulted in a 10-fold lower ratio of BAA to conjugated EGBE excreted in urine. Cyanamide treatment significantly reduced the haematotoxic response in a manner similar to that of pyrazole, but it also resulted in a high mortality rate in rats given cyanamide and EGBE, an effect not observed in animals treated with cyanamide or EGBE alone. Pyrazole completely blocked the increase in spleen weight/body weight ratios seen in EGBE-treated animals. Gavage administration of either BAL or BAA at doses molar equivalent to 125 mg/kg EGBE produced identical increased spleen weight/body weight ratios and identical increases in free haemoglobin (Hgb) levels in plasma. Pretreatment of rats with cyanamide prior to administration of BAL provided

significant protection against BAL-induced haematotoxicity. These studies confirm the central role of BAA in the haematotoxic response elicited in rats.

The elimination kinetics of EGBE and BAA appear to be independent of the route of exposure. The half-lives for the elimination of EGBE and BAA averaged 0.66 hour and 3.27 hours, respectively. For whole-body exposures under exercise conditions, the elimination half-lives for EGBE and BAA were 0.66 hour and 4 hours, respectively. For dermal exposure to neat liquids, the half-lives for elimination of EGBE and BAA were 1.3 hours and 3.1 hours, respectively. For dermal exposure to vapours, the elimination half-life for EGBE was 0.53-0.6 hour.

Ref.: 81

Mechanistic studies have shown that EGBE causes haematotoxicity *in vivo* in rats and that BAA causes the same effects *in vitro* at very low concentration. When metabolic pathways leading to the formation of BAA were blocked, no effects were seen on RBC. It can be concluded that BAA is responsible of haematotoxicity *in vivo*. Some species were very sensitive to EGBE- or BAA-induced haemolysis: rat, mouse, hamster, baboon whereas other species were resistant to these effects: dog, guinea pig, pig, cat, rabbit and humans (30 x less sensitive than rats). In one study, dogs were very sensitive to EGBE but not to BAA.

Ref.: 1

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

No data submitted

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

In human, all epidemiological studies, except one, studying glycol ethers, showed an increased risk of malformation (cleft lip, neural tube defect). For EGBE, these studies did not allow to draw any conclusion about its potential effects on humans because no studies are able to define clearly an unique source of glycol ether; Usually studies described co-exposure to various glycol ethers, including known developmental toxins such as EGME and other chemicals as well.

Ref.: 1

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

Since all key effects are induced by haemolysis in rodents, a NOAEL based on haemotoxicity will be used in the risk characterisation. The selection of an appropriate interspecies chemical safety assessment factor (CSAF) must take into account the lower sensitivity of humans than rats (or mice) to the metabolite BAA. WHO (1994) has proposed splitting the CSAF in two components representing the toxicokinetic and toxicodynamic adjustment factors (Inter-species kinetics (4.0) and Inter-species dynamics (2.5) (see also The SCCP Notes of guidance for the testing of cosmetic ingredients and their safety evaluation, 6th revision). The toxicokinetic factor is taken account of by use of the PBPK model. The

toxicodynamics factor needs to be set to an appropriate value to reflect the lower sensitivity of humans to haemolysis. The data available on the most sensitive measure (pre-haemolytic changes) suggests that a value of 0.01 would be realistic. However, a more cautious and conservative initial approach would be to propose a value of 0.1. This implies that a factor of 0.4 (4.0 x 0.1) is justified for species differences and gives a minimal MOS of 4 taking intra species variation into consideration.

Ref.: 1

CALCULATION OF THE MARGIN OF SAFETY

Ethylene glycol monobutyl ether
EGBE
(Oxidative/non-oxidative hair dyes)

The safety calculation is only considering dermal exposure.

Maximum dermal absorption of test substance reported was 95 µg/cm²

Maternal NOAEL based on marked reductions in body weight and/or weight gain, increases in organ weights (kidney and spleen) and severe haematotoxicity was 30 mg/kg bw/d.

Maximum absorption through the skin	**DA_a (µg/cm²)**	**=**	**95 µg/cm²**
Skin Area Surface (scalp)	**SAS**	**=**	**700 cm²**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**66.5 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/ 60**	**=**	**1.11mg/kg bw**
No observed adverse effect level (rat, maternal toxicity)	**NOAEL**	**=**	**30 mg/kg bw**

Margin of Safety	**NOAEL / SED**	**=**	**27**

Based on a minimal MOS of 4, the calculated MOS equal to 27 is considered to give sufficient protection in relation to the use of EGBE as solvent in hair dye preparations.

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 ethylene glycol monobutyl ether (EGBE) is not reported. The physico-chemical characterisation and purity of the substance is not reported in several studies.

General toxicity
Early studies identified the red blood cells as the primary target tissue of EGBE toxicity in animals. Haemolysis that leads to haemoglobinuria is a commonly observed clinical sign of toxicity in rodents and rabbits. It has been adequately demonstrated that the acid metabolite, 2-butoxyacetic acid (BAA) is responsible for the haemolytic effects of EGBE. The two main oxidative pathways of EGBE metabolism observed in rats are formation of butoxyacetaldehyde (BAL) catalysed by alcohol dehydrogenase (ADH) and formation of

ethylene glycol by O-dealkylation by a cytochrome P450 dealkylase. BAA is formed by oxidation of BAL.

Only rare cases of intravascular haemolysis appear to have been reported in humans. It has shown that human red blood cells have low sensitivity to glycol ethers and that the maximal plasma concentration of BAA reached in humans after cutaneous administration or inhalation of EGBE are not sufficient to cause haemolytic effects. The formation of ethylene glycol seems to play a minor role in humans. The elimination kinetics of EGBE and BAA appear to be independent of the route of exposure. The half-lives for the elimination of EGBE and BAA averaged 0.66 hour and 3.27 hours, respectively.

EGBE has low acute toxicity. For the oral route, available animal studies show LD_{50} values for EGBE upwards from 1000 mg/kg bw. Humans have recovered totally after ingestion of between 0.5 and 1.5 g/kg bw. A LOAEL of 400 mg/kg bw can be taken for acute toxicity by oral route in humans. For the dermal route of EGBE, great differences were seen between the tested species and the mode of occlusion. The rabbit seems to be the most sensitive species with LD_{50} of about 500 mg/kg bw when administered occlusively. For the inhalation route, the 4 hour LC_{50} in rats, which are susceptible to haemolysis, was of the region of 450 ppm (2214mg/m^3).

A large number of animal subchronic and chronic studies have been performed. Effects seen by oral route were body weight reduction, haemolysis, hepatic effects and local irritation effects. Irritation to the forestomach was seen after gavage dosing. LOAELs of 69 and 82 mg/kg bw/d (in males and females respectively) were observed in rats based on slight cytoplasmic alterations in hepatocytes of both male and female rats. Alternatively, based on mild anaemia indicated by a decrease in RBC counts and liver damage in male rats a NOAEL of 129 mg/kg bw/d may be used. The French Authorities (The Conseil Supérieur d'Hygiéne Publique de France (CSHPF), 7 November 2002) used 150 mg/kg bw/d representing the NOAEL for female rats since the effects in the female rats were more severe than in the male rats for their MOS calculation. This value is also in agreement with results from the skin painting experiment below.

Two studies are available on rabbits to assess the toxicity of repeated doses of EGBE administered dermally. In only one study, signs of toxicity were recorded and were limited to transient signs of haemolysis. This study led to a NOAEL of 450 mg/kg bw/d due to haematological effects at higher doses. Given that this study was performed only during 9 days, the NOAEL of the second study, which was performed during 13 weeks, could be more reliable for the risk characterisation. This NOAEL was 150 mg/kg bw/d. A mouse study designed for the assessment of EGBE effects on the immune system, had a NOAEL of 1000 mg/kg.

The main effect in rodent after inhalation of EGBE was haemolysis, which was consistently observed and sometimes associated with secondary hepatic effects (Kupffer cells pigmentation and absolute and relative liver weight increases). Other effects were decreases of body weight gain, effects on the forestomach and effects on the WBC sub-populations (T lymphocyte). In these studies, a NOAEC of 25 ppm in rats (about 9 mg/ kg bw/d) and a LOAEC of 31 ppm in mice and rats was established (based on haemolysis, as the only significant primary effect).

Compared to a control group, workers exposed to EGBE (0.59 ± 0.27 ppm) had a significant decrease (3.3%; p=0.03) in haematocrit, while mean corpuscular haemoglobin concentration (MCHC) was increased (2.1%; p=0.02). No significant difference was observed either in other erythroid parameters or hepatic and renal biomarkers.

Reproductive toxicity
The lowest foetal NOAEL is 100 mg/kg bw/day in an oral rat study. It is based on effects seen at 200 mg/kg bw/day: increase in foetal lethality without malformations. These effects

were seen with maternal toxicity (haemolytic anaemia) and retarded body weight gain evidenced from 100 mg/kg bw/day. The maternal NOAEL is 30 mg/kg bw/day in this study. This value is based on retarded bodyweight gain and severe haematotoxicity during 3 days of dosing.

Based on inhalation studies the developmental NOAEC of 50 ppm can be taken into account for the risk assessment (rats). This value is based on variations seen at 100 ppm. These effects were seen in presence of maternal toxicity (haemolytic anaemia), which was seen at 100 ppm and higher. The maternal NOAEC for this effect was 50 ppm.

Calculation of minimal MOS
Since all key effects are induced by haemolysis in rodents, a NOAEL based on haemotoxicity will be used in the risk characterisation. The selection of an appropriate interspecies chemical safety assessment factor (CSAF) must take into account the lower sensitivity of humans than rats (or mice) to BAA. WHO (1994) has proposed splitting the CSAF two components representing the toxicokinetic and toxicodynamic adjustment factors (Inter-species kinetics (4.0) and Inter-species dynamics (2.5) (see also The SCCP Notes of guidance for the testing of cosmetic ingredients and their safety evaluation, 6th revision). The toxicokinetic factor is taken account of by use of the PBPK model. The toxicodynamics factor needs to be set to an appropriate value to reflect the lower sensitivity of humans to haemolysis. The data available on the most sensitive measure (pre-haemolytic changes) suggests that a value of 0.01 would be realistic. However, a more cautious and conservative initial approach would be to propose a value of 0.1. This imply that a factor of (4.0 x 0.1) 0.4 is just for species differences and gives a minimal MOS of 4 taking intra species variation into consideration.

The lowest NOAEL was 30 mg/kg bw/d obtained a reproductive toxicity study and represent maternal toxicity after 3 day exposure. The effect was due to haematological effects and probably secondary reaction to this at a dose of 100 mg/kg bw/d.

Irritation / sensitisation
Studies performed on rabbits and guinea pigs have shown that EGBE have caused moderate irritation (erythema and oedema) when applied occlusively on the skin for a period of 4 hours. If the substance was applied on scarified skin or for a longer period of time, signs of severe irritation sometimes leading to necrosis were reported.

Several studies have been performed to assess the eye irritation properties of EGBE. Most of them were not performed according to guidelines. But overall, all studies have shown that EGBE is irritant or severely irritant to the eyes of rabbits with effects both on conjunctivae, iris and cornea. In one well performed study EGBE produced irreversible effects on the conjunctivae and on the cornea in at least one treated rabbit. Dilution of EGBE in water or rinsing of the eyes decreased the irritant effects.

Studies available did not show any signs of significant respiratory irritation. From the human data, it is apparent that the NOEL is >50 ppm but <100 ppm based on discomfort.

No signs of skin sensitisation were seen in two animal studies or in a human patch test. Moreover, considering Structure Activity Relationship (SAR) in the glycol ether family, the wide dispersive use of EGBE and that EGBE has never been associated with cases of skin sensitisation it can be considered that skin sensitisation will not be expected.

Dermal absorption
Under semi-occlusive conditions, dermal uptake of pure EGBE in rats was between 20 and 30% of the administered dose. Dermal uptake of aqueous dilutions of 5, 10 and 20% EGBE

was similar to that of the pure substance. If EGBE was administered under non-occlusive conditions, the dermal uptake decreased dramatically (uptake < 10%), mainly because of the volatility of EGBE.

Some *in vitro* studies demonstrated that dermal uptake for pig skin was 2 or 3 times slower than rat skin. The results found with human skin were more or less equivalent to those seen with pig skin. Oxidative hair dye formulations containing 5 and 10% EGBE + developer containing hydrogen peroxide was studied in a well-conducted experiment. With 2.5% EGBE in the presence of hydrogen peroxide the dermal absorbance was 61 ± 29 μg/cm^2 (12.1 ± 5.9%). In calculation of MOS, (61 ± 2x29) 119 μg/cm^2 reduced from 2.5% to 2.0% (119 x 2.0/2.5) ***95 μg/cm^2*** is used.

Absorption of orally administered EGBE is rapid and essentially complete (assumed to be 100%)
Measurements performed on human volunteers showed a real absorption of 55 to 60%. A 60% of absorption for EGBE inhalation should be used in risk characterisation.

Mutagenicity
EGBE is not mutagenic in bacteria. Neither BAL nor BAA was mutagenic in bacteria. Two of three mammalian cell mutation assays did not indicate any mutagenic activity for EGBE while a significant result was obtained in a poorly reported study with V79 hamster cells using a very high concentration (20 mM). The same publication reported also a significant result with BAL.

No evidence for chromosomal aberration induction has been found in a number of mammalian cell culture studies with EGBE or in one experiment with BAA. On the other hand, BAL induced chromosome aberrations. Weak aneugenic effects were obtained in the only available study with EGBE and BAL, but not with BAA. Micronuclei found in *in vitro* studies with BAA and, to a much lesser extent with EGBE and BAL.

There have been reports of significant activity of EGBE in the Syrian hamster embryo cell transformation assay, but again, the results have been inconsistent. There is also some indication of inhibition of gap-junctional intercellular communication in a single study with EGBE while no inhibition was found with BAL and BAA. A single assay for UDS induction used a technique that is now considered to be invalid.

In vivo, there is no evidence for micronucleus induction in bone marrow cells of mice or rats with EGBE or in mice with BAA. No interaction with DNA in several organs of rats was detected in experiments with EGBE. The balance of the evidence suggests that EGBE does not have relevant mutagenic potential *in vivo*.

Carcinogenicity
A 2-year carcinogenicity study by inhalation exposure has been carried out under the US National Toxicology Program. It was concluded that under the conditions of the study, there was *no evidence of carcinogenic activity* of EGBE in male F344/N rats. There was *equivocal evidence of carcinogenic activity* of EGBE in female F344/N rats based on the increased combined incidences of benign or malignant pheochromocytoma (mainly benign) of the adrenal medulla. There was *some evidence of carcinogenic activity* of EGBE in male B6C3F1 mice based on increased incidences of haemangiosarcoma of the liver. A marginal increase in the incidences of forestomach squamous cell papilloma and an increase in the incidences of hepatocellular carcinoma may have been exposure related. There was *some evidence of carcinogenic activity* of EGBE in female B6C3F1 mice based on increased incidences of forestomach squamous cell papilloma or carcinoma (mainly papilloma).

Forestomach tumours in rodents induced by non-genotoxic chemicals are in general not considered relevant for humans (Predictive Value of Rodent Forestomach and Gastric Neuroendocrine Tumours in Evaluating Carcinogenic Risks to Humans. IARC Technical Publications No 39, 2003).

According to the criteria for classification of carcinogens in EU, EGBE should not be classified as a carcinogen on the basis of the studies available.

4. CONCLUSION

Based on the information provided, the SCCP is of the opinion that the use of ethylene glycol monobutyl ether (EGBE) as a solvent at a concentration up to 4.0% in oxidative hair dye formulations and up to 2.0% in non-oxidative hair dye formulations, does not pose a risk to the health of the consumer.

The opinion relates to the direct application to the hair/scalp. It does not include any other cosmetic exposure, such as exposure from other type of cosmetics or possible aerosol/spray products.

5. MINORITY OPINION

Not applicable

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