Scientific Committee on Consumer Safety

SCCS

OPINION ON

Thioglycolic acid and its salts (TGA)

The SCCS adopted this opinion by written procedure on 11 November 2013

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

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

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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1. BACKGROUND

Thioglycolic acid and its salts (including the ammonium salt) are currently regulated in the Cosmetic Regulation 1223/2009 in Annex III entry 2a with the following restriction:

Thioglycolic acid and its salts may be used in:
(a) Hair products:
— general use, max concentration allowed 8% (pH 7 to 9.5)
— professional use, max concentration allowed 11% (pH 7 to 9.5)
(b) Depilatories, max concentration allowed 5% (pH 7 to 12.7)
(c) Hair rinse-off products, max concentration allowed 2% (pH up to 9.5)

The above mentioned percentages are calculated as thioglycolic acid.

The previous EU Cosmetics Directive 76/768 of 27 July 1976 did not contain any definition of "hair" or "hair product", and therefore does not contain any specific requirements for the application of hair (care) products to eyelashes, except for a number of provisions relating to some specific substances. Hair dye products containing these substances must be labelled "Do not use to dye eyelashes and eyebrows" unless intended for professional use.
The Directive was replaced as from 11 July 2013 by the Cosmetic Regulation 1223/2009. The term "hair product", introduced in the preamble to Annexes II to VI of the EU Cosmetics Regulation, is defined as "a cosmetic product which is intended to be applied on the hair of head or face, except eyelashes".

Thus, the use of thioglycolic acid and thioglycolates in hair products is in accordance with the new European cosmetics legislation, but the application of these products to eyelashes has been prohibited since 11 July 2013.
In order to ensure the legal compliance of these products, the applicant submitted a dossier for the safety assessment of thioglycolic acid and thioglycolates in cosmetic products used on eyelashes. It has been reported that eyelash-waving products based on thioglycolic acid derivatives are applied by professionals and during application a direct contact to the skin or eyes is avoided with the help of a sticking eyelash roll.

Furthermore, following concerns originally raised by Denmark regarding the local safety of "hair chemical removers" i.e. depilatory products containing thioglycolic acid and its salts (thereafter designated as TGA), Cosmetics Europe* submitted a document containing post-marketing surveillance (PMS) data on these cosmetic products.

1. TERMS OF REFERENCE

1. *Does the SCCS consider Thioglycolic acid and its salts (TGA) still safe for use as depilatories in cosmetic products in a concentration up 5.0% taking into account the data provided from Cosmetics Europe?*

2. *Taking into account the scientific data available, does SCCS consider Thioglycolic acid and its salts (TGA) safe for use on eyelashes (after mixing with oxidative hair dyes found safe for use in hair dye products) in concentrations up to 8% for general use and up to 11% for professional use applied on eyelashes?*

2. OPINION

Original studies on chemical characterisation, physico-chemical properties and toxicological endpoints of thioglycolic acid and its salts were not included in the submitted dossier. The submitted literature, as well as additional literature searched by the SCCS were used for the safety evaluation of thioglycolic acid and its salts in this Opinion.

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Thioglycolic Acid

- INCI name: Thioglycolic Acid

Thioglycolic acid salts: See Table 1.

Table 1. Thioglycolic acid and its salts used in cosmetics products in EU, according to the CosIng* database.

INCI Name	IUPAC Name	CAS No.	EC No.	Cosmetic Function and Uses
Ammonium thioglycolate	Ammonium mercaptoacetate	5421-46-5	226-540-9	Depilatory Hair waving or straightening, Reducing
Calcium thioglycolate	Calcium bis(mercaptoacetate)	814-71-1	212-402-5	Keratolytic, Depilatory, Reducing
Calcium thioglycolate hydroxide	Acetic Acid, Mercapto-, Calcium Salt (1:1), Trihydrate	29820-13-1 65208-41-5	249-881-5 212-402-5	Depilatory
Ethanolamine thioglycolate	(2-Hydroxyethyl)ammonium mercaptoacetate	126-97-6	204-815-4	Depilatory Hair waving or straightening, Reducing
Magnesium thioglycolate	Bis(mercaptoacetato-O,S) magnesium	63592-16-5	264-358-1	Depilatory Hair waving or straightening, Reducing
Potassium thioglycolate	Potassium mercaptoacetate	34452-51-2	252-038-4	Depilatory Hair waving or straightening, Reducing
Sodium thioglycolate	Sodium mercaptoacetate	367-51-1	206-898-4	Antioxidant, Depilatory Hair waving or straightening, Reducing
Strontium thioglycolate	Mercaptoacetic acid, strontium salt	38337-95-0	252-888-9	Depilatory Hair waving or straightening, Reducing

*http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=28236

3.1.1.2. Chemical names

Thioglycolic Acid:
alpha-Acetic acid, mercapto- (CAS Name), mercaptoacetic acid, 2-Mercaptoacetic acid, α-mercaptoacetic acid, 2-thioglycolic acid, 2-sulfanylacetic acid (IUPAC)

3.1.1.3. Trade names and abbreviations

/

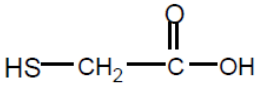
3.1.1.4. CAS / EC number

Thioglycolic Acid
CAS: 68-11-1
EC: 200-677-4

See Table 1 for thioglycolic acid salts

3.1.1.5. Structural formula

Thioglycolic Acid



3.1.1.6. Empirical formula

Thioglycolic Acid: $C_2H_4O_2S$

3.1.2. Physical form

Thioglycolic Acid: colourless liquid.

3.1.3. Molecular weight

Thioglycolic Acid: 92.12

3.1.4. Purity, composition and substance codes

Thioglycolic Acid: available as >98% pure

3.1.5. Impurities / accompanying contaminants

No information is available on impurities in TGA.

3.1.6. Solubility

Thioglycolic Acid: miscible with water, acetone, ethanol, ethyl ether, and other organic solvents; slightly soluble in chloroform.

3.1.7. Partition coefficient (Log P_{ow})

Thioglycolic Acid

- Octanol/water partition coefficient: log P_{OW} = 0.059

3.1.8. Additional physical and chemical specifications

Thioglycolic Acid
Melting point: - 16.5°C

Boiling point:	96°C, 101.5°C
Flash point:	131.5 °C at 1025 hPa
Vapour pressure:	10 mm Hg at 18°C; 0.4 mmHg at 25°C
Density:	1.325 g/cm^3 at 20°C
Viscosity:	6.55 cP at 20°C
pKa:	3.73
Refractive index:	1.5030 at 20°C
pH:	/
UV_Vis spectrum (….. nm):	/

3.1.9. Homogeneity and Stability

Stable at room temperature in closed containers under normal storage and handling conditions. It oxidizes when exposed to air.

According to information submitted by the industry (ICADA 2012) the stability of thioglycolic acid in a gel preparation, containing circa 8.8% (range 8.7% to 8.9%) thioglycolic acid, over seven months at alternating temperatures (20°C / 40°C) in different bottles was evaluated. In the polyolefin bottle, the content of the active ingredient declined to 82.8% of the initial value. In the glass bottle the thioglycolic acid was able to resist the thermal stress (the final measured content was 97.8% of the t_0-value). When the original product was stored at room temperature over one year, the content of the active ingredient declined to 94.9% of the initial value. Thus, this marketed product appeared to be stable with respect to thioglycolic acid content when stored in a glass bottle.

General Comments to physico-chemical characterisation
Information on impurities in thioglycolic acid and its salts was not available.

3.2. Function and uses

According to the Cosmetic Regulation 1223/2009 thioglycolic acid and its salts may be used in:

(a) Hair waving or straightening products:
— general use, max concentration allowed 8% (pH 7 to 9.5)
— professional use, max concentration allowed 11% (pH 7 to 9.5)
(b) Depilatories, max concentration allowed 5% (pH 7 to 12.7)
(c) Hair rinse-off products, max concentration allowed 2% (pH up to 9.5)

The above mentioned percentages are calculated as thioglycolic acid.

In the course of the normal application (i.e. application according to the labeled instructions), the eyelash-waving products marketed by ICADA members don't have any contact with the eyelid, eyeball and ocular mucosa, respectively. Therefore the product's constituents will not become systemically available. A systemic exposure would only be possible if one assumes a wrong application mode of the products. Of course this scenario is highly improbable, because the user spectrum is restricted to professionals. Nevertheless, in order to calculate a margin of safety (MoS), a skin-contact scenario must be assumed. (ICADA 2012)

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

(Taken from OECD 2009)

Thioglycolic acid and its ammonium and sodium salts are toxic by oral administration. When expressed as thioglycolate anion, whatever the salt is, the LD_{50}'s of the salts were in the range of the thioglycolic acid LD_{50} in rats.
In an acute oral toxicity study performed according to the OECD guideline # 401, 5 groups of 5 Sprague-Dawley rats per sex were dosed with 0, 40, 64, 80 and 200 mg/kg bw thioglycolic acid (purity 99%). Animals were observed for 14 days following the exposure for mortality and clinical signs. Mortality occurred at dose levels equal to and exceeding 64 mg/kg bw. Behavioural abnormalities (piloerection, lethargy, ptosis, prostration) were observed in all treated rats. The LD_{50} of thioglycolic acid was 73 mg/kg bw (Gardner, 1988).
The acute oral toxicity of ammonium and sodium thioglycolates was tested in male and female rats according to the Acute Toxic Class Method (OECD guideline # 423). The LD_{50} of the 71% aqueous solution of ammonium salt was between 50 and 200 mg/kg bw (or between 35 and 142 mg/kg bw when expressed as active ingredient) in Sprague-Dawley rats (Hönack, 1996). Sodium thioglycolate was tested pure (>98%) or as a 46% aqueous solution in Wistar rats, the LD_{50}'s were between 50 and 200 (Arcelin, 2000) or 200 and 500 mg/kg bw (Sanders, 2000), respectively.
Another study performed with ammonium thioglycolate according to the OECD guideline # 401 lead to an acute LD_{50} between 25 and 200 mg a.i./kg bw in Wistar rats (Heusener, 1998).
Former studies with sodium thioglycolate reported comparable LD_{50}'s in rats and rabbits and slightly higher in mice (von Schmidt, 1974).

Table 2. Acute oral toxicity studies on thioglycolic acid and its salts

(Taken from OECD 2009)

Test substance	Species (strain, sex)	Results (LD_{50})		Symptoms at toxic dose levels	Reference
		As tested	Expressed as thioglycolic acid		
Thioglycolic acid (99% pure)	Rat (Sprague-Dawley, male & female)	-	73 (67-81) mg/kg bw	Piloerection, hunched posture, waddling, lethargy and pallor of extremities, ptosis, decreased respiration rate and prostration No necropsy findings	Gardner, 1988
Ammonium thioglycolate	Rat (Wistar, male & female)	>25, < 200 mg (TGA a.i)/kg bw	>21, <168 mg/kg bw	Locomotor disturbance, abdominal position & dyspnea GI tract irritation, lung emphysema & urinary bladder dilatation at necropsy	Heusener, 1998

Test substance	Species (strain, sex)	Results (LD_{50})		Symptoms at toxic dose levels	Reference
		As tested	Expressed as thioglycolic acid		
	Rat (Sprague-Dawley, male & female)	>50, <200 mg (71% TGA aq. sol.)/kg bw > 35, <142 mg (TGA a.i.)/kg bw	>30, <120 mg/kg bw	Reduced activity, hunched posture, decrease in grip, limb and body tone, piloerection and and reduced breathing rate GI tract irritation at necropsy	Hönack, 1996
Sodium thioglycolate	Rat (Wistar, male & female)	>200, <500 mg (46% NaTG aq. sol.)/kg bw >92, <230 (NaTG a.i.)/kg bw	>74, <184 mg/kg bw	Decreased respiration rate laboured respiration, hunched posture, prostration and ataxia. Haemorrhagic lung, dark liver & kidney, GI tract irritation at necropsy	Sanders, 2000
	Rat (Wistar, male & female)	>50, <200 mg/kg bw	>40, <160 mg/kg bw	Ruffled fur, respiratory distress Distended stomach with gas at necropsy in 2 females	Arcelin, 2000
	Rat (male & female)	-	♂: 114 (87-148) mg/kg bw ♀: 136 (114-161) mg/kg bw	No data	von Schmidt, 1974
	Mouse (male & female)	-	♂: 322 (298-348) mg/kg bw ♀: 275 (236-320) mg/kg bw	No data	von Schmidt, 1974
	Rabbit (male)	-	119 mg/kg bw	No data	von Schmidt, 1974

At the highest concentrations tested, the LD_{50} values from ammonium (17.5% solution) and sodium thioglycolate (5% solution) were 0.172 g/kg bw and 0.504 g/kg bw, respectively (Burnett 2009).

According to EU-CLP, thioglycolic acid is classified as Acute Tox. 3; H301 (toxic if swallowed).

3.3.1.2. Acute dermal toxicity

(Taken from OECD 2009)

Thioglycolic acid and its ammonium and sodium salts are harmful by contact with skin. When calculated as thioglycolate anion, whatever the salt is, or the concentration, the LD_{50}'s of the salts were in the range of the thioglycolic acid LD_{50}.
In a non-guideline acute dermal toxicity study, 4 groups of 2 New Zealand white rabbits per sex were dosed with 250, 500, 1000 or 2000 mg/kg bw thioglycolic acid (purity 98.2%). Animals were observed for 14 days following the exposure for mortality and clinical signs. Mortality was 0/4, 1/4, 2/4 and 4/4 on day one. No other effects than a skin irritation at the site of application was reported. The LD_{50} was 848 mg/kg bw (Rampy, 1973).
In studies performed in Sprague-Dawley rats according to the OECD guideline # 402, no mortality was observed after dermal application of 2000 mg/kg bw of 71% aqueous solution ammonium thioglycolate and clinical signs were limited to moderate skin irritation at the site of application (Klein, 2003a). With sodium thioglycolate (purity > 99%), dose levels of 1000 or 2000 mg/kg bw didn't induce any mortality and clinical signs in males, excepted dryness of the skin. At the 2000 mg/kg bw, all females were found dead or died on day 2, no clinical signs were observed prior to death in four of them and the last one was in coma prior to death. All females survived to 1000 mg/kg bw, no clinical signs were recorded, excepted a skin irritation and a reduced body weight gain. The LD_{50} was between 1000 and 2000 mg/kg bw (Klein, 2003b).
In a former study with 71% aqueous solution ammonium thioglycolate, no mortality was observed in male and female New Zealand white rabbits receiving 200 mg/kg bw on the abraded skin (Griffiths and Koschier, 1979).

Table 3. Acute dermal toxicity studies on thioglycolic acid and its salts.

(Taken from OECD 2009)

Test substance	Species (strain, sex)	Exposure duration and condition	Results (LD_{50})		Symptoms at toxic dose levels	Reference
			As tested	Expressed as thioglycolate		
Thioglycolic acid	Rabbit (New Zealand white, male & female)	24 hours, occlusive	848 (505-1430) mg/kg bw		Moderate skin erythema, slight to moderate edema and slight to moderate necrosis (no other clinical signs reported)	Rampy, 1973

Ammonium thioglycolate	Rat (Sprague-Dawley, male & female)	24 hours, semi-occlusive	> 2000 mg (71% TGA aq. sol.)/kg bw > 1430 mg (TGA a.i.[1])/kg bw	> 1200 mg/kg bw	No mortality and clinical signs No skin irritation No necropsy findings	Klein, 2003a
	Rabbit (New-Zealand white, male & female)	24 hours, occlusive on abraded skin	> 200 mg (71% TGA aq. sol.)/kg bw > 142 mg (TGA a.i.)/kg bw	> 120 mg/kg bw	No mortality Nasal discharge, diarrhoea No skin irritation reported	Griffiths and Koschier, 1979a
Sodium thioglycolate	Rat (Sprague-Dawley, male & female)	24 hours semi-occlusive	>1000, <2000 mg a.i./kg bw	>800, <1600 mg/kg bw	No clinical signs and mortality was observed in males at both dose levels, in females at 1000 mg/kg and before death in 4/5 females at 2000 mg/kg, last one was in coma prior to death Skin irritation No necropsy findings	Klein, 2003b

According to EU-CLP, thioglycolic acid is classified as Acute Tox. 3; H311 (toxic in contact with skin).

3.3.1.3. Acute inhalation toxicity

(Taken from OECD 2009)

Thioglycolic acid is harmful by inhalation exposure. There were no valid inhalation studies for ammonium thioglycolate.
In an acute inhalation toxicity performed according to the OECD guideline # 403, 5 Wistar rats per sex and per groups were exposed nose-only for 4 hours to a respirable thioglycolic acid (purity > 99%) aerosol (particle size: 2.5-3.1 µm MMAD) at analytical concentrations of 0; 0.284; 0.837; 1.441 and 3.629 mg/l Animals were observed for 14 days following the exposure for mortality and clinical signs. Mortality occurred at exposure concentrations

[1] a.i.: active ingredient

equal to and exceeding 1.441 mg/l. The clinical signs observed included respiratory distress (bradypnea, labored and irregular breathing patterns), nasal irritation (discharge and encrustations), corneal opacity and behavioral abnormalities (mobility reduced, limp, apathy, tremour, paralysis, prostration, reduced reflexes). The approximate LC_{50} for males and females was calculated to be 2.172 and 1.098 mg/l, respectively (Pauluhn, 2004).
In a non-guideline study, 2 groups of six male Sprague-Dawley rats were exposed in a 19-litre exposure chamber for 7 hours to vapours of thioglycolic acid (purity 98.2%). Air was bubbled at ambient temperature or at 125°C through thioglycolic acid and the vapours generated passed to the chamber. The nominal concentration of the atmosphere to which the animals were exposed was 620 ppm (2.4 mg/l) generated at room temperature and 8200 ppm (31.4 mg/l) generated at 125°C. The animals showed no untoward effect during the exposure or during a 2-week post-exposure observation period (Rampy, 1973).

Table 4. Acute inhalation toxicity studies on thioglycolic acid

(Taken from OECD 2009 and MAK 2013)

Test substance	Species (strain, sex)	Exposure duration	Results	Symptoms at toxic dose levels	Reference
Thioglycolic acid (Mixture of vapours and aerosols, 2.5-3.1 µm MMAD[2])	Rat (Wistar, male & female)	4 hours	Approximate LC_{50}-males: 2.172 mg/l [3] Approximate LC_{50}-females: 1.098 mg/l	Respiratory distress, nasal irritation, corneal opacity and behavioral abnormalities Discoloured lungs at necropsy	Pauluhn, 2004
Thioglycolic acid (vapours generated at room temperature or 125°C)	Rat (Sprague-Dawley, male)	7 hours	No mortality Nominal concentrations: 2.4 mg/l at room temperature and 31.4 mg/l at 125°C	No clinical signs No necropsy findings	Rampy, 1973
Thioglycolic acid (Mixture of vapours and aerosols, 76-89% <5.5 µm MMAD[4])	Rat (Wistar, male & female)	4 hours	LC_{50}: 210 ± 40 mg/m^3		Elf Atochem North America Inc 1994. (taken from MAK)

According to EU-CLP, thioglycolic acid is classified as Acute Tox. 3; H331 (toxic if inhaled).

3.3.2 Irritation and corrosivity

[2] MMAD: Mass Median Aerodynamic Diameter

[3] All concentration data represent actual concentrations of the test substance in the rats' breathing zone.

3.3.2.1. Skin irritation

Thioglycolic acid

(Taken from OECD 2009)

In vitro study
The corrosive potential of thioglycolic acid (99% pure) was assessed using a human skin model, the EpiDerm Skin Model (Directive 2000/33/EC, B.27[5]). Duplicate EpiDerm tissues were treated with 50 µl of thioglycolic acid and exposed for 3 minutes and 60 minutes. The tissues were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air for the appropriate exposure times. Toxicity was determined by the conversion of MTT[6] to formazan by viable cells in the test material treated tissues relative to the negative/solvent control-treated tissues. The study was validated by the inclusion of a positive control material, 8.0 N potassium hydroxide. The relative mean viability was 4.96% after 3 minutes exposure, and 6.60% after 60 minutes exposure. As the relative mean viability of the test material-treated tissues was <10% after 3 minutes exposure, thioglycolic acid is considered to be corrosive *in vivo* (Warren, 2002).

Studies in Animals
Thioglycolic acid was applied as a single occlusive patch test on the abdominal skin of rabbits. Skin reactions were observed and recorded at various time intervals up to 7 hours or until a chemical burn was observed. At the end of the exposure period, the skin area was washed with soap and water. Thioglycolic acid application resulted in necrosis within 5 minutes. This was accompanied by hyperemia and edema (Rampy, 1973).

Studies in Humans
Patch tests with thioglycolic acid (apparently not neutralized) at concentrations (up to 11%) described as typical of hair waving solutions were carried out with duration from 1 hour through 96 hours on the unabraded skin of 294 and the abraded skin of 63 volunteers. In the study, the data from which were not presented fully in this paper, thioglycolic acid was found to cause irritation to the skin at 2.8% or higher, being most irritating to abraded skin. Tests using 4.6% aqueous thioglycolic acid caused irritation to the skin of volunteers after 4-6 hours (McCord, 1946).

Ammonium and sodium thioglycolate

(Taken from OECD 2009)

Studies in Animals
The skin irritation potential of ammonium thioglycolate (71% solution) and sodium thioglycolate (98% pure) was evaluated in studies performed according to the OECD guideline #404 (Klein, 2003c and 2003d). Because irritant effects were anticipated, a single New Zealand albino rabbit was tested first before the other two animals were used. The test material (0.5 ml or 0.5 g) was applied to the shaved, intact skin on one flank for 3 min and for 4 h on the other flank in the first animal. The sites were semi-occluded.
The test materials were non-irritating in the first animal, so it was applied to a single flank in the other two rabbits for 4 h (the other flank served as a control). The animals were examined for signs of erythema, eschar, and edema and skin reactions were measured 1, 24, 48, and 72 h after termination of exposure. Both substances induce a slight irritation.

[5] It should be B46 according to Directive 2000/33/EC

[6] MTT = 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide

The skin irritation potential of 71% ammonium thioglycolate was also evaluated in a former study performed according to the US EPA guideline # 16 CFR 1500.41. 0.5 ml test material was applied to each of two sites, one on abraded skin, the other site on intact skin of each of 6 rabbits. The test sites were then immediately occluded for a 24-hour exposure period. At the end of 24 hours, the plastic wrappings and patches were removed. The skin sites were then individually examined and scored separately for both erythema and edema using the Draize method. After 72 hours the sites were examined and rescored.
Three of the six rabbits died during the test. The mean erythema scores over 24 and 72 hours for each surviving animal were 3.0, 3.0 and 3.0 for both the intact and abraded skin. The edema scores were 2.5, 3.0 and 2.5 for the intact skin and 2.5, 2.5 and 2.5 for the abraded skin (Griffiths and Koschier, 1979b). Under the condition of a 24-hour occlusive exposure, 71% ammonium thioglycolate was severely irritating to the skin.
The test conditions and the main results of the available studies are presented in table 5.

Table 5. Skin irritation studies with ammonium and sodium thioglycolate.

Test substance	Species (number)	Method	Conclusion	Reference
Ammonium thioglycolate (71% solution)	New Zealand albino rabbit (3)	OECD guideline # 404 Semiocclusive 4-hour exposure	Slightly irritating	Klein, 2003c
	New Zealand albino rabbit (6)	US EPA guideline # 16 CFR 1500.41 24-hour occlusive exposure on intact and abraded skin	Severely irritating	Griffiths and Koschier, 1979b
Sodium thioglycolate (98%)	New Zealand albino rabbit (3)	OECD guideline # 404 Semi-occlusive 4-hour exposure	Slightly irritating	Klein, 2003d

SCCS comment
Thioglycolic acid and ammonium thioglycolate are skin irritants; at high concentrations they can be corrosive.
According to EU - CLP thioglycolic acid is classified as Skin Corr. 1B; H314 (causes severe skin burns and eye damage).

3.3.2.2. Mucous membrane irritation

Eye Irritation

Thioglycolic acid

(Taken from OECD 2009)

The eye irritation potential of thioglycolic acid has been investigated in rabbits in 2 studies compliant with the OECD guidelines # 405. These tests were performed to evaluate the eye irritation potential of neat thioglycolic acid and a 10% aqueous solution. 0.1 ml of neat or 10% thioglycolic acid was instilled into the eyes of 1 or 6 rabbits, respectively, followed by grading at various times between 4 hours and 72 or 96 hours.
With neat thioglycolic acid, whitening of the conjunctiva due to corrosion prevented scoring for erythema, defaulting to a maximum score of 3 for erythema. The score given for corneal opacity also reached the maximum of 4; there is no indication of the evaluation of the reversibility in the publication (Jacobs,1988). Therefore, neat thioglycolic acid is considered as corrosive to the eyes.

With the 10% solution, a moderate conjunctival irritation, iritis and corneal opacity were observed. The mean scores for ocular parameters lead to consider the 10% solution as irritant to the eyes. The study was stopped at 96h, so the reversibility was not evaluated (Jacobs, 1992).

In a former study, 0.1 g of neat thioglycolic acid was instilled into the conjunctival sac of the right eye of one rabbit. After 30 seconds this eye was flushed for two minutes with a stream of flowing tap water. Thioglycolic acid was then instilled in the same way into the left eye, which was left unwashed. After instillation into the second eye, both eyes were examined for conjunctival irritation, corneal injury and internal effects (iritis) at intervals up to two weeks. Instillation of thioglycolic acid resulted in severe pain, severe conjuntival inflammation, dense corneal opacity and severe iritis. These effects were not improved at the end of 14 days after exposure. Washing immediately after exposure did not modify the response (Rampy, 1973).

Ammonium and sodium thioglycolate

The eye irritation potential of ammonium thioglycolate (71% solution) and sodium thioglycolate (98% pure) has been investigated in rabbits in studies compliant with the OECD guideline # 405 (Klein, 2003e and 2003f). A volume of 0.1 ml of ammonium thioglycolate or 100 mg of sodium thioglycolate was instilled into the conjunctival sac of one eye of the 3 test animals. The eyes were not rinsed after administration of the test item. The other eyes served as controls. The eye irritation reactions were scored approximately 1 hour, 24, 48 and 72 hours (both salts) and up to 8 days (sodium salt only) after the instillation. Both compounds induce a slight irritation.

The eye irritation potential of ammonium thioglycolate (71% solution) has also been investigated in a study compliant with the US EPA guideline # 16 CFR 1500.42 (Griffiths and Koschier, 1979c). A volume of 0.1 ml of ammonium thioglycolate was instilled into the conjunctival sac of one eye of each of the 6 test animals. The eyes were not rinsed after administration. The other eyes served as controls. The eye irritation reactions were scored approximately 1 hour, 24, 48 and 72 hours and 7 days after the instillation. Ammonium thioglycolate induce a mild irritation, mean enanthema (redness), chemosis, corneal opacity and iritis scores over 24, 48 and 72 hours were 2.6, 0.0, 0.0 and 0.0, respectively. On day 7 redness score was zero.

Table 6. Eye irritation studies with ammonium and sodium thioglycolate

(Taken from Burnett 2009 and OECD 2009)

Test substance	**Species (number)**	**Method**	**Conclusion**	**Reference**
Ammonium thioglycolate (71% solution)	New Zealand albino rabbit (3)	OECD guideline # 405	Slightly irritating	Klein, 2003
	New Zealand albino rabbit (6)	16 CFR 1500.42	Mildly irritating	Griffiths and Koschier, 1979
Sodium thioglycolate (98%)	New Zealand albino rabbit (3)	OECD guideline # 405	Slightly irritating	Klein, 2003
Cold wave (17.5%	New Zealand	Eyes of 3 animals rinsed.	Transient ocular	Consumer Product Testing

ammonium thioglycolate)	rabbits (9)	Reactions scored up to day 7 after Instillation.	reactions	Company, Inc, 1982d
Permanent waving Solution (10.98% ammonium thioglycolate)	Albino rabbits (9)	Eyes of 3 animals rinsed. Reactions scored up to day 3 after Instillation.	Nonirritant	Applied Biological Labora-tories, Inc, 1982c
Permanent waving solution (8.3% ammonium thioglycolate)	New Zealand albino rabbits (9)	Eyes of 3 animals rinsed. Reactions scored up to day 7 after Instillation.	Moderate ocular irritant	Bio-technics Laboratories, Inc, 1986a
Permanent waving Solution (7.2% ammonium thioglycolate)	New Zealand albino rabbits (9)	Eyes of 3 animals rinsed. Reactions scored up to day 7 after Instillation.	Moderate ocular irritant	Bio-technics Laboratories, Inc, 1986b
Permanent waving Solution (7.1% ammonium thioglycolate)	New Zealand albino rabbits (9)	Eyes of 3 animals rinsed. Reactions scored up to day 7 after Instillation.	Nonirritant	Bio-technics Laboratories, Inc, 1978b
Permanent waving Solution (7.0% ammonium thioglycolate)	New Zealand albino rabbits (9)	Eyes of 3 animals rinsed. Reactions scored up to day 3 after Instillation.	Borderline ocular irritant (unrinsed eyes); nonirritant (rinsed eyes)	Hill Top Testing Services, Inc, 1977a
Permanent waving Solution (7.0% ammonium thioglycolate)	Rabbits (9)	Eyes of 3 animals rinsed. Reactions scored up to day 3 after Instillation.	Minimal irritant (unrinsed eyes);non-irritant (rinsed eyes)	Micro-Bio Testing and Research Laboratories, Inc, 1982a
Permanent waving Solution (7.0% ammonium thioglycolate)	Rabbits (9)	Eyes of 3 animals rinsed. Reactions scored up to day 3 after Instillation.	Non-irritant	Micro-Bio Testing and Research Laboratories, Inc, 1982a
Permanent waving Solution (7.0% ammonium thioglycolate)	New Zealand albino rabbits (9)	Eyes of 3 animals rinsed. Reactions scored up to day 3 after Instillation.	Ocular irritant	Bio-Technics Laboratories, Inc/ 1981
Permanent waving Solution (5.8% ammonium thioglycolate)	New Zealand albino rabbits (9)	Eyes of 3 animals rinsed. Reactions scored up to day 3 after Instillation.	Moderate ocular irritant	Bio-Technics Laboratories, Inc, 1986c
Permanent waving Solution (5.8% ammonium thioglycolate)	New Zealand albino rabbits (9)	Eyes of 3 animals rinsed. Reactions scored up to day 3 after Instillation.	Non-irritant	Bio-Technics Laboratories, Inc, 1981

Conclusion
Ammonium thioglycolate
Results ranged from non-irritant to moderate ocular irritation, but the severity of the ocular irritation does not appear to be concentration related.

SCCS comment
Thioglycolic acid, ammonium, and sodium thioglycolate are ocular irritants.
According to EU - CLP thioglycolic acid is classified as Skin Corr. 1B; H314 (causes severe skin burns and eye damage).

Studies to evaluate skin and eye irritation by cosmetic products
A laboratory supplied the documentation of a human in-use test (n = 10) and another laboratory the final reports of two animal studies (n = 3), respectively, each of which tested a different safety aspect of the eyelash-waving product (EVIC Hispania 2007, Echevarne 2003a and Echevarne 2003b). When the product was applied to human eyelashes (in-use test), dermal/ocular reactions or sensations were absent, resulting in the expert judgement "very good skin and eye compatibility". Even in direct contact with rabbit eyes and skin, only slight irritancy was observed. Synoptically viewed, the generated data clearly indicated that the product was safe in the course of the normal intended use and reasonably foreseeable use.

SCCS Comment
The animal studies are not GLP-compliant, but their results can be considered reliable. Likewise the human study cannot be classified as GCP-conforming. However, substantial requirements of GCP are fulfilled by this trial, and its results are considered reliable.

3.3.3. Skin sensitisation

(Taken and slightly abbreviated from MAK 2013; animal skin sensitization studies are also summarised in Burnett 2009)

In a closed epicutaneous test four out of eight guinea pigs reacted to an application of 30% ammonium thioglycolate, not to 0.2% (CIR 1991). In a Buehler test (10% ammonium thioglycolate for induction, 5% for elicitation) 35% of the animals showed a positive reaction (Basketter 1996). Guinea pigs were not sensitized in a Buehler test with 50% ammonium thioglycolate for induction and elicitation (Burnett 2009). In seven maximization tests with permanent-wave formulations containing ammonium thioglycolate no sensitisations occurred (CIR 1991). In an open epicutaneous test 20 guinea pigs were repeatedly treated with 10% ammonium thioglycolate until severe reactions occurred. After healing, elicitation was studied with 1%, 2% and 5%; only 3 animals showed a weak reaction (Schulz, 1961).

Local Lymph Node Assay (LLNA)
In a review paper (Basketter, 1999) ammonium thioglycolate in DMSO is classified as a sensitizer. According to additional information supplied by the author, the EC3 value was 10%

(Taken from Burnett 2009)

The skin sensitization potential of ammonium thioglycolate was investigated by IKW (IKW, 2006) using CBA/Ca/Ola/Hsd female mice in a local lymph node assay. There were 5 mice in each of the 3 dose groups and the control group. The mice received 25 µL of topical solution consisting of 0%, 0.5%, 8.0%, or 20.0% (active matter) of the test material in a mixture of acetone/water/olive oil (2:2:1 by vol) on the dorsal surface of each ear lobe once

daily for 3 consecutive days. Five days after the first application of solution, all mice received radiolabeled thymidine (^{3}H-TdR) by i.v. injection in the tail vein. All mice were killed 5 hours after the ^{3}H-TdR injection. The lymph nodes of the mice were removed and studied for proliferation with ^{3}H-TdR. The values were then used to calculate the stimulation index (SI). All mice in the 20.0% dose group died after the third application. The SI values for the 0.5% and 8.0% dose groups were 2.7 and 17.0, respectively. The EC3 theoretical concentration was calculated as 0.65%. The study concluded that ammonium thioglycolate in acetone/water/ olive oil was strongly sensitizing in mice.

Human Repeat Insult Patch Test (HRIPT)

(Taken from OECD 2009)

The available Human Repeated Insult Patch Tests (HRIPT) to assess the skin sensitisation potential of ammonium thioglycolate in healthy humans are summarized in table 7. Repeated applications of ammonium thioglycolate at concentrations ranging from 14.6 to 18.0 % in aqueous solutions induced a significant level of skin irritation and a low level of allergic contact dermatitis.

Table 7. Human Repeated Insult Patch Tests with ammonium thioglycolate.

(Taken from OECD 2009)

Test item	# of subjects	Exposure conditions	Results	Reference
18.0% ammonium thioglycolate	24 males, 196 females, 18-69 years old 199 subjects completed the study	9 repeated insult (semi-occlusive) patch test	Very mild to marked irritation in approximately 27% (54/199) 1/199 subject with allergic contact dermatitis	Frentzko, 1990a
18.0% ammonium thioglycolate	25 males, 195 females, 18-66 years old 195 subjects completed the study	9 repeated insult (semi-occlusive) patch test	Very mild to moderate irritant in approximately 47% (96/205) of the population tested No evidence of induced allergic contact dermatitis	Frentzko, 1990b
14.4% ammonium thioglycolate	32 males, 208 females, 18-69 years old 220 subjects completed the study	9 repeated insult (semi-occlusive) patch test	Barely perceptible to moderate irritation in 8/220 No evidence of induced allergic contact dermatitis	Frentzko, 1990c
Cold wave product (pH 9.3-9.5) containing 9.0% ammonium thioglycolate	9 male & 43 female subjects (29-77 years old)	9 repeated insult (semi-occlusive) patch test	No evidence of induced skin irritation and allergic contact dermatitis	Townsend, 1977

Test item	# of subjects	Exposure conditions	Results	Reference
17.5% ammonium thioglycolate (pH 7.3-7.6; 14.6% thioglycolic acid)	54 subjects (18-67 years old)	10 repeated insult (semi-occlusive) patch test	3/54 with allergic contact sensitisation	Frentzko, 1982
17.5% ammonium thioglycolate (pH 7.3-7.6; 14.6% thioglycolic acid)	102 subjects (15-73 years old)	10 repeated insult (semi-occlusive) patch test	Reactions suggestive of moderate allergic contact sensitisation in 2/102 subjects	Berger, 1982
Permanent waving solution containing 12.0% ammonium thioglycolate, 5.0% urea, and 0.61 % ammonium hydroxide	139 females, 52 males	Modification of the Draize-Shelanski-Jordan patch test	No evidence of induced skin irritation and allergic contact dermatitis	Rappoport, 1983
Aqueous solution of 1.25% thioglycolic acid (adjusted to pH 9.0-9.3 with ammonia)	20 subjects	9 repeated insult (semi-occlusive) patch test	Sensitisation reactions were not observed in any of the subjects.	Voss, 1958

SCCS comment
The SCCS considers HRIPT test as unethical.

Clinical studies in humans
Exposure to ammonium thioglycolate causes allergic contact dermatitis in humans. It is occasionally seen in consumers, whilst there are several publications reporting widespread occurrence of allergic contact dermatitis from glyceryl thioglycolate in persons who are professionally exposed, such as hairdressers (Warshaw, 2012). A voluntary withdrawal from the market of glyceryl thioglycolate as a hair-waving ingredient has resulted in a sharp decrease in number of individuals with contact allergy (Uter, 2003).
Patch-tests with ammonium thioglycolate tend to show irritant reactions, while documented cases of allergic contact dermatitis allergy seem to be rare. However, due to a number of skin sensitisation in hairdressers caused by ammonium thioglycolate and due to positive test results in various dermal clinics the sensitizing effect of ammonium thioglycolate to the skin should be considered as certainty (Bundesarbeitsblatt, 1999).

Allergic skin reactions to ammonium thioglycolate were observed in number of cases as summarized in Table 8. The most comprehensive studies with patients present various cutaneous diseases, which may be due or not to professional application.

Table 8. Ammonium thioglycolate patch testing of patients with dermatitis

(Taken from OECD 2009)

Test item	**Number of subjects**	**Exposure conditions**	**Results**	**Reference**

Test item	Number of subjects	Exposure conditions	Results	Reference
Ammonium thioglycolate 1% petrolatum (pet.)	809 hairdressers	Patch test	4% positive	Frosch, 1992
Hair waving lotion containing 0.86% ammonia and 4.61 % thioglycolic acid	863 subjects initially tested, 15 subjects retested because of + reactions	Patch test	Positive reactions in 0.26% of the factory workers and 0.3% of the women in contact with cold wave permanent lotions	Behrman, 1949
0.55 N ammonium thioglycolate (~6.0% solution, pH 9.3)	1 male & 212 female subjects (18-34 years old)	Patch test with reapplication 14 days later	26/213 (12%) with immediate reaction	Downing, 1951
Hair waving lotion (pH 9.21) containing 0.86% ammonia and 4.61 % thioglycolic acid	143 male & 143 female subjects	Patch test with reapplication 20 to 40 days later	Skin irritation was not observed in any of the 286 patients tested. When patch tests were repeated (109 patients), sensitisation reactions were not observed.	Behrman, 1949
2,5 % ammonium thioglycolate in petrolatum	17 women (23-70 years old)	Patch test	2/17 with positive reaction	Morrison and Storrs, 1988
Cold permanent waving lotion 0,1, 1, 2 and 5 % ammonium thioglycolate	4 female hairdressers (19-20 years old) with eczematous dermatitis	Open patch test	Positive reactions in all 4 hair dressers Negative reaction in 18 healthy subjects and 2 hairdressers without dermatitis	Yamasaki, 1984
2.5% ammonium thioglycolate in petrolatum	8 hairdressers (age ~31) and 4 clients (age ~57) with dermatitis.	Patch test	Positive reaction in 1 hairdresser	Storrs, 1984
Eleven different cold wave solutions containing ammonium thioglycolate (0.3, 0.5, 0.7, 1, 3, 5, and 7% aqueous ammonium thioglycolate)	One hairdresser (20 years old)	Open patch test over month 8	Allergic reactions in the hairdresser were due to ATG.	Ishihara, 1983
Aqueous solution of 5% ammonium thioglycolate	7 beauticians (16-22 years old)	Open patch test	Allergic reactions observed in 3 subjects	Matsunaga, 1988

SCCS Comment

Ammonium thioglycolate is a sensitizer, but the occurrence of allergic contact dermatitis is low. No data were found on sodium thioglycolate.

3.3.4. Dermal / percutaneous absorption

(Taken from OECD 2009)

The dermal absorption/percutaneous penetration of [^{14}C]-radiolabelled ammonium thioglycolate out of a representative permanent hair waving formulation (13% in the formulation, pH 9.5) was studied on the clipped excised skin of four Landrace large white cross pigs. The pig skin, dermatomed to a mean thickness of 0.80 mm, was used because it shares essential penetration characteristics with human skin. The dermal absorption/percutaneous penetration of the test substance was investigated for the open application of about 20 mg formulation per cm² pig skin. Therefore the resulting dose of ammonium thioglycolate was approximately 2.63 mg/cm² skin (equivalent to 2.1 mg thioglycolic acid/cm²). Skin discs of about 3.14 cm² were exposed to the formulations for 30 min., terminated by gently rinsing with a commercial shampoo solution diluted with water. The amount of ammonium thioglycolate systemically available (epidermis/dermis plus receptor fluid) was found to be 19.83 µg/cm² (0.77%), corresponding to 16.74 µg/cm² when calculated for thioglycolic acid. OECD Guideline 428 (Skin Absorption: *In Vitro* Method).
The mean cutaneous absorption for 11% ammonium ^{14}C-thioglycolate at pH 6, pH 7, and pH 8 was 1.09%, 0.81%, and 0.86%, respectively.

(Taken from Burnett 2009)

The absorption of thioglycolic acid as ammonium thioglycolate was studied in groups of Sprague-Dawley rats (5/sex; ~ 200 g) (IKW,2006). A solution of [^{14}C] thioglycolic acid was neutralized with a 25% solution of ammonia resulting in 3 solutions of 11% ammonium [^{14}C] thioglycolate with pH 6, pH 7, and pH 8, respectively. Each exposure group of rats received approximately 300 mg of test material on the clipped dorsal skin for 30 minutes followed by a washing of the site. The test site was then neutralized with 0.3 mL of a "natural styling solution" containing 2.1% hydrogen peroxide for 10 minutes followed by a washing of the site. These applications were to mimic human exposure to hair waving products. After the second wash, the test sites were covered with 4 layers of gauze and the rats were placed into metabolism cages for 72 hours. Following the observation period, the animals were killed. The test sites and surrounding skin were excised and dissolved in Soluene-350 for radioactivity analysis.
Most of the radiolabel was removed from the rat skin during washing of the test material and neutralization solution (mean 96.1%-96.8%). The mean ^{14}C recovered in urine and feces in the pH 6, pH 7, and pH 8 exposure groups was 0.139%, 0.119%, and 0.137%, respectively. The mean ^{14}C content of the skin at the application site for the pH 6, pH 7, and pH 8 exposure groups was 0.82%, 0.57%, and 0.60%, respectively. The mean cutaneous absorption for 11% ammonium [^{14}C] thioglycolate for the pH 6, pH 7, and pH 8 exposure groups was 0.27%, 0.24%, and 0.26%, respectively.

The absorption of [^{35}S] sodium thioglycolate was investigated using male rabbits (2-3 kg, strain not stated). Five animals were fed during a period of approximately 24 hours and then fasted for 24 hours (Freeman, 1956). A 25.0% solution of [^{35}S] thioglycolic acid
(330 mg/kg) was then applied to clipped skin of the back via inunction. At the end of 1 hour, 5% to 8% of the dose of [^{35}S] thioglycolic acid applied had been excreted in the urine. The amount excreted at 5 hours varied from 30% to 40%. The increased excretion of 35S per unit time may not have been attributable directly to percutaneous thioglycolate absorption because sodium thioglycolate may have altered the metabolism of other sources of sulfur in the body. No further increase in the absorption and excretion of thioglycolate per unit time was observed when a larger dose of the test solution (660 mg/kg) was applied to 3 additional rabbits (same procedure). The authors concluded that the total amount absorbed over an extended period of time probably was related to the amount applied.

SCCS Comment
Using a pKa of 3.73 for thioglycolic acid, the portion of thioglycolate at pH 6 is calculated to be ≥99.5% compared to a portion of ≤0.5% free acid in the Burnett 2009 study. At pH 7 or 8, the portion of the free acid further decreases by one and two orders of magnitude, respectively. The data is consistent with the absorption of thioglycolate in the pH range 6 – 8 as the absorption is not dependent on the varying concentrations of the free acid in the pH range studied. The dissociation rates at different pH values studied seem not to influence the dermal absorption dramatically.
Dermal absorption rate of 16.74 µg/cm^2 has been determined from a study using 13% concentration in hair waving formulation. However in depilatories, the maximum concentration allowed is 5%.

3.3.5. Repeated dose toxicity

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

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

(Taken from OECD 2009)

The repeated dose toxicity of sodium thioglycolate was evaluated by dermal and oral administrations.
In a repeated dose dermal toxicity study completed by National Toxicology Program (NTP) and using a method comparable to the OECD guideline # 411, sodium thioglycolate was administered via dermal route, 5 days per week, for 13 weeks at dose levels of 0, 11.25, 22.5, 45, 90, or 180 mg/kg bw /d to Fischer 344 rats and 0, 22.5, 45, 90, 180, or 360 mg/kg bw/d to B6C3F1 mice. All animals survived the 13 weeks administration. The only treatment related effect was skin irritation at the site of application. The Lowest-Observed-Effect-Levels (LOELs) at the application site were 11.25 and 45 mg/kg bw/d in rats and mice, respectively, based on skin irritation, there was no No-Observed-Effect-Level (NOEL) at the application site. The NOAELs for systemic toxicity can be estimated to be higher than 180 and 360 mg/kg bw/d in rats and mice, respectively.
In an oral repeated dose toxicity study compliant with the OECD guideline # 408, sodium thioglycolate was administered by gavage, 7 days per week, for 13 weeks, to Sprague-Dawley rats at dose levels of 0, 7, 20 and 60 mg/kg bw/d. The main effects, fully reversible, seem to be related to the inhibition of the β-oxidation of fatty acids. The NOAEL was established at 20 mg/kg bw/d and the NOEL at 7 mg/kg bw/d.

(Taken from Burnett 2009)

The dermal toxicity of cold wave solutions (pH 9.0-9.5) containing 7.0% ammonium thioglycolate was evaluated using albino rabbits. Four cold wave solutions were applied to the skin via injunction at doses of 0.5,1.0, 2.0, and 4.0 mL/kg, respectively, for 90 days. Eleven of 18 rabbits given 4.0 mL/kg doses and 2 of 17 rabbits given 2.0 mL/kg doses died. No deaths occurred in groups doses with 1.0 mL/kg (15 rabbits). A mild dermatitis was observed at microscopic examinations of sections of skin from approximately 50 animals.

SCCS comment
In the NTP repeated dose dermal toxicity study in rats, the systemic NOAEL was 180 mg/kg bw/d and the local LOAEL was 11.25 mg/kg bw/d. The systemic NOAEL (corrected for 5 days treatment per week: 180 x 5 / 7 = 129 mg/kg bw/d) was used to calculate the MoS.

3.3.5.3. Chronic (> 12 months) toxicity

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

(Taken from OECD 2009)

Gene mutation tests in bacteria

The most recent bacterial gene mutations assay was conducted with ammonium thioglycolate following a protocol compliant with the OECD guideline 471. The direct plate incorporation procedure was performed with *Salmonella typhimurium* TA98, TA100, TA102, TA1535 and TA1537 at concentrations up to 5000 µg/plate. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Cytotoxic effects were observed in the absence and in the presence of a metabolic activation at the concentration of 5000 µg/plate. Ammonium thioglycolate did not induce a biological relevant increase in revertants in this bacterial gene mutation test in either the absence or presence of metabolic activation in any strain tested compared to the untreated controls. The positive and negative controls included in the experiment showed the expected results. Under the experimental conditions used, ammonium thioglycolate was not mutagenic in this gene mutation test in bacteria (Thompson, 2003)

In a study performed according to the US NTP protocol, four *S. typhimurium* strains TA97, TA98, TA100 and TA1535 were exposed to thioglycolic acid in a pre-incubation assay. Test concentrations were based on the results of a preliminary toxicity assay. Liver S9 fraction from Aroclor 1254-induced rats or uninduced hamsters was used as exogenous metabolic activation system. Based on a preliminary toxicity assay, concentrations up to 3333 µg/plate were used with or without metabolic activation in all four strains. Cytotoxicity was observed at 2000 µl/plate and above with S9-mix and at 1000 µl/plate and above without S9-mix. Thioglycolic acid did not induce a biological relevant increase in revertants compared to the untreated controls in these studies. Positive and solvent controls gave the expected results. Under the experimental conditions used, thioglycolic acid was not mutagenic in this gene mutation test in bacteria (Zeiger, 1987).

In a second study performed according to the US NTP protocol, four *S. typhimurium* TA98, TA100, TA1535 and TA1537 were exposed to sodium thioglycolate in a pre-incubation assay. Test concentrations were based on the results of a preliminary toxicity assay. Liver S9 fraction from Aroclor 1254-induced rats or uninduced hamsters was used as exogenous metabolic activation system. Based on a preliminary toxicity assay, concentrations up to 1000 µg/plate were used with or without metabolic activation in all four strains. Sodium thioglycolate did not induce a biological relevant increase in revertants compared to the untreated controls in these studies. Positive and solvent controls gave the expected results. Under the experimental conditions used, sodium thioglycolate was not mutagenic in this gene mutation test in bacteria (Zeiger, 1987).

In a fourth gene mutation test in bacteria, with very little detail on the performance of the test, described by Gocke *et al.*, (1981) the mutation frequency in the *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 was determined after exposure to sodium thioglycolate with concentrations up to 3600 µg/plate. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system.
Sodium thioglycolate did not induce a biological relevant increase in revertants compared to the untreated controls in this study (Gocke, 1981).

Gene mutation assay in mammalian cells

Ammonium thioglycolate was tested in a mouse lymphoma forward mutation assay performed according to the OECD guideline 476. Cells were exposed for 4 and 24 h in the presence of S9-mix or for 24 h in absence of S9-mix. After 4 h treatment both without and with S9-mix ammonium thioglycolate induced cytotoxicity at all concentrations up to 1600

µg/ml (= 10 mM). At 24 h, cytotoxicity was observed at and above 800 µg/ml. Ammonium thioglycolate did not induce a biological relevant increase in the mutant frequency in the mouse lymphoma L5178Y heterozygous $TK^{+/-}$ cells, with or without metabolic activation compared to the untreated controls. The spontaneous mutant frequencies and the levels of activity of the positive controls confirmed the sensitivity of the test system. Under the experimental conditions used, ammonium thioglycolate was not mutagenic in this mouse lymphoma assay using the *tk* locus as reporter gene (Wollny, 2004).

Chromosome aberration test in human lymphocytes
Thioglycolic acid was tested at concentrations of 0 up to 300 µg/ml without metabolic activation and of 0 up to 1000 µg/ml with metabolic activation in an *in vitro* chromosome aberration test in human lymphocytes. Exposures were for 24 and 48 hours in absence of S9-mix and 2 hours in presence of S9-mix. Liver S9-fraction from rats was used as exogenous metabolic activation system. Cytotoxicity was observed at a concentration of 300 µg/plate without S9-mix and at and above 1000 µg/ml with S9-mix. Thioglycolic acid did not induce a biological relevant increase in the number of cells with structural chromosome aberrations compared to the untreated controls in this test. Under the experimental conditions used, thioglycolic acid was not genotoxic (clastogenic) in this chromosome aberration test in human lymphocytes (Molinier, 1994).

SCCS Comment on the *in vitro* studies
Very little details on the performance of the tests were reported, sometimes because the data are from a publication in the open literature. Information on the number of replicates, batch number, purity and solvent are often lacking. The tests were generally not conducted in accordance with OECD test guidelines and not in compliance with GLP. The performance of the tests does not comply with the present standard requirements. Although the tests have only limited value, they give no indication for a genotoxic potential.

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Mouse bone marrow micronucleus test

(Taken from OECD 2009)

Thioglycolic acid, adjusted to pH4, was administered dermally over a 2-day period to three groups of five male and five female Swiss mice at dose-levels of 0, 250, 500 and 1000 mg/kg bw/day for males or 0, 125, 250 and 500 mg/kg bw/day for females. Treatments were 24 h apart and bone marrow cells were collected 24 h after the last treatment. Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic to normochromatic erythrocytes (PCE/NCE ratio). Bone marrow preparations were stained and examined microscopically for the PCE/NCE ratio and micronuclei. Negative and positive controls were included.

Results
The polychromatic erythrocytes/normochromatic erythrocytes ratios (PCE/NCE) in the treated groups were equivalent to those of the control groups. However, systemic exposure was confirmed by the mortality observed in males given 1000 mg/kg bw/day as well as by the clinical signs observed in males treated with 500 and 1000 mg/kg bw/day.
A biologically relevant increase in the number of micronucleated polychromatic erythrocytes was not observed in the bone marrow harvested 24 hours after the treatment(s) compared to the untreated controls. Positive and vehicle controls gave the expected results.

Conclusion
Under the experimental conditions used thioglycolic acid did not induce an increase in the number of bone marrow cells with micronuclei in treated mice and, consequently, thioglycolic acid was not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice.

SCCS Comment
The test was not conducted in compliance with GLP.

Mouse bone marrow micronucleus test
Sodium thioglycolate was administered by single gavage to three groups of five male and five female NMRI mice at dose-levels of 0, 62.5, 125 and 250 mg/kg bw. The mice were treated once and bone marrow cells were collected 24 and 48 h after treatment. Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic to normochromatic erythrocytes (PCE/NCE ratio). Bone marrow preparations were stained and examined microscopically for the PCE/NCE ratio and micronuclei. Negative and positive controls were included.

Results
The polychromatic erythrocytes/normochromatic erythrocytes ratios (PCE/NCE) in the treated groups were equivalent to those of the control groups. However, in a preliminary study death was found at 500 mg/kg bw. whereas clinical signs were observed in males and females receiving 250 mg/kg bw of sodium thioglycolate.
A biological relevant increase in the number of micronucleated polychromatic erythrocytes was not observed in the bone marrow harvested 24 and 48 hours after the treatment compared to the untreated controls. Positive and vehicle controls gave the expected results.

Conclusion
Under the experimental conditions used sodium thioglycolate did not induce an increase in the number of bone marrow cells with micronuclei in treated mice and, consequently, sodium thioglycolate was not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice (Honarvar, 2005).

SCCS Comment
The test was not conducted in compliance with GLP.

Micronucleus test on mouse peripheral blood
Sodium thioglycolate was administered by daily dermal applications for 13 weeks to B6C3F1 mice at dose-levels of 0, 22.5, 45, 90, 180 and 360 mg/kg bw/day. Peripheral blood cells of the mice were collected 24 h after the last treatment. A positive control was not included.
A statistically significant increase in the number of blood cells with micronuclei was observed in female mice treated with 360 mg/kg bw/day only. A biological relevant increase in the number of micronucleated blood cells was not observed in males compared to the untreated controls. Under the experimental conditions used sodium thioglycolate did induce an increase in the number of blood cells with micronuclei in treated female mice and, consequently, sodium thioglycolate was genotoxic (clastogenic and/or aneugenic) in blood cells of mice. The authors of the OECD report of 2009 considered this result of doubtful significance because thioglycolic acid did not induce structural chromosomal aberrations *in vitro*, and thioglycolic acid and its sodium salt failed to show any evidence of clastogenic potential when administered acutely by the dermal and oral routes up to the maximum tolerated dose in the two mouse bone marrow micronucleus assays performed following the OECD guideline 474.
The SCCS agrees with this explanation.

Sodium thioglycolate was administered by two daily intraperitoneal applications to NMRI mice at dose-levels of 0, 114 and 285 mg/kg bw/day. Bone marrow cells of the mice were collected 6 h after the last treatment. All mice survived the treatment with sodium thioglycolate. A statistically significant increase in the number of bone marrow cells with micronuclei was not observed as compared to the untreated control mice. Under the experimental conditions used sodium thioglycolate did not induce an increase in the number of bone marrow cells with micronuclei in treated female mice and, consequently, sodium thioglycolate is not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice.

Sex linked recessive lethal mutation test in Drosophila
Sodium thioglycolate was tested for sex linked recessive lethal mutation test in *Drosophila*. Only one concentration of 25 mM which is close to the LD, was applied by the adult feeding method in 5% saccharose. About 1200 X-chromosomes were tested per experiment in each of 3 successive broods (3-3-4 days). In repeat experiments, sometimes only single broods were tested. F2 progeny cultures with 2 or fewer wild-type males were routinely retested in the F3 generation to confirm X-linked recessive lethal mutations (RLs). Mosaics were not counted. "Clusters" of 2 were included because their occurrence was compatible with statistical expectation of independent origin. A biologically relevant increase in sex linked lethal mutations in *Drosophila's* treated with sodium thioglycolate as compared to the untreated controls was not found. Under the experimental conditions used sodium thioglycolate was not mutagenic in this gene mutation test in *Drosophila*.

SCCS Comment
The tests were partly performed before the implementation of the OECD guidelines. The data are partly from a publication in the open literature. Very little details on the performance of the tests were reported. Information on batch number, purity and vehicle is lacking. The tests were not conducted in compliance with GLP. The performance of the tests does not comply with the present standard requirements. Although the tests have only limited value, they give no indication for a genotoxic potential.

3.3.7. Carcinogenicity

Topical application

Mice

Guideline:	/
Species/strain:	Female Swiss mice from the Eppley colony
Group size:	50 animals in the exposed groups, 40 animals in the positive control and 100 animals in the unexposed control
Test substance:	Sodium thioglycolate (Fisher Scientific Co., Fair Lawn, N.J.)
Purity:	/
Batch No.:	/
Positive control:	7,12-Dimethylbenz[a]anthracene (DMBA) (Aldrich Chemical Co.)
Dose level:	0.02 ml of a solution containing 1 and 2% solutions of sodium thioglycolate in acetone
Route:	Topical, 2 weekly to a 1-cm diameter regularly-shaved area of interscapular skin
Exposure period:	Until the mice died spontaneously or were killed when moribund
GLP:	/
Study period:	Before 1977

The experiment involved in addition to sodium thioglycolate the following substances: p-aminonitrophenol and p-phenylenediamine. DMBA was used as positive control. The test compounds were dissolved in acetone, in two concentrations selected on the basis of solubility and preliminary determinations of the maximum tolerated dose in mice. The concentrations used were 1 and 2% for sodium thioglycolate, 5 and 10% for p-aminonitrophenol and p-phenylenediamine, and 0.1% for DMBA.

Swiss mice, 7 weeks old, groups of 50 females received the test substances applied in 0.02 ml acetone twice weekly to a 1-cm diameter regularly-shaved area of interscapular skin. 100 untreated females served as controls and 40 mice received DMBA (0.1%) and were kept as positive controls. The mice were allowed to die spontaneously or were killed when moribund. Complete autopsies were performed on all animals.

There was no significant decrease in the lifespan of the treated mice. About 10% of the animals were alive after 90 – 100 weeks. The mice suffered no marked weight loss. No treatment-related epidermal hyperplasia, ulceration or dermatitis was observed. No epidermal tumours occurred, although mice from all treatment groups showed a few dermatofibromas - benign circumscribed tumours composed of collagen and fibroblasts in the dermis.

In the low-dosed sodium thioglycolate treated mice 19 of 49 affected animals (39%) had altogether 22 tumours and 22 of 45 high-dosed mice (49%) had altogether 24 tumours. 39 of the negative controls (42%) had altogether 46 tumours while 38 of the positive controls (95%) had altogether 67 tumours. The incidence of tumours in the different organs of the treated mice was not statistically different from that of untreated controls.

It is noted that also in the case of p-aminonitrophenol and p-phenylenediamine the incidence of tumours in the different organs of the treated mice was not statistically different from that of untreated controls (Stenbäck 1977).

Rabbits

Guideline:	/
Species/strain:	Female rabbits (not further specified)
Group size:	5 animals in all in all groups
Test substance:	Sodium thioglycolate (Fisher Scientific Co., Fair Lawn, N.J.)
Purity:	/
Batch No.:	/
Positive control:	7,12-Dimethylbenz[a]anthracene (DMBA: Aldrich Chemical Co.)
Dose level:	0.02 ml of a solution containing 1 and 2% solutions of sodium thioglycolate in acetone
Route:	Topical, 0.02 ml twice weekly to the inside of the left ear of the rabbits
Exposure period:	The experiment was terminated at wk 85
GLP:	/
Study period:	Before 1977

The experiment involved, in addition to sodium thioglycolate the following substances: p-aminonitrophenol and p-phenylenediamine. DMBA was used as positive control. The test compounds were dissolved in acetone, in two concentrations selected on the basis of solubility and preliminary determinations of the maximum tolerated dose in mice in relation to the experiment described above. The concentrations used were 1 and 2% for sodium thioglycolate, 5 and 10% for p-aminonitrophenol and p-phenylenediamine, and 0.1% for DMBA.

Rabbits (strain not stated), 8 weeks old, groups of 5 females received the test substances applied in 0.02 ml acetone twice weekly to the inside of the left ear. 5 untreated females served as controls and 5 rabbits treated with DMBA (0.1%) were kept as positive controls. The experiment was terminated at week 85. Complete autopsies were performed on all animals.

No differences in food intake, weight, behavior or overall appearance were observed between the groups. The survival rate varied from group to group in a manner unrelated to treatment and dependent on non-specific infectious diseases, such as pneumonia and diarrhea. No abnormalities were found in the blood or urine of the rabbits. No treatment-related local changes were observed in the ears. Tumours were not found in other organs in any of the groups exposed to sodium thioglycolate or the two other substances studied in

the present experiment. The positive controls showed 15 proliferating papillomas in the 5 rabbits (Stenbäck, 1977).

Comment by SCCS
Due to the low sensitivity of animal long-term studies to detect possible carcinogenic effects, animal studies on carcinogenicity are normally performed at doses much higher than those humans may be exposed to. In the case of sodium thioglycolate the concentrations used in the two studies above was lower than those which may be used in cosmetic products (see Terms of references).

SCCS is of the opinion that based on the present experiments it is not possible to draw any conclusions in relation to possible carcinogenic effects of thioglycolic acid and its salts.

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

(*Taken from ECHA website*)

A two-generation reproduction toxicity study is available in the ECHA registration dossier database, however this information is subject to copyright laws. Only a detailed summary of this study is published on the ECHA website and the complete report was not made available to the SCCS.
The results of this study support the conclusion on the NOAEL identified in the teratogenicity study.

3.3.8.2. Teratogenicity

(Taken from OECD 2009)

In a reproduction/developmental toxicity screening test performed following the OECD guideline # 421, the NOAEL for parental toxicity was 20 mg/kg bw/d (based on deaths at 40 and 80 mg/kg bw/d), the NOEL for reproductive performance (mating, fertility and delivery) was 20 mg/kg bw/d (based on deaths at 40 and 80 mg/kg bw/d) and the NOEL for toxic effects on progeny was 40 mg/kg bw/d (based on the dead litter at 80 mg/kg bw/d which cannot definitively be attributed to maternal condition).

In the 13-week dermal subchronic toxicity study in rats and mice with sodium thioglycolate, no treatment-related effects on sperm density and motility, caudal epididymal sperm, spermatid head counts in the testes and testis weights, as well as oestrous cycles, were observed up to dose levels of 180 and 360 mg/kg bw/d, respectively. (This study is also described above in paragraph 3.3.5).

The developmental toxicity of ammonium and sodium thioglycolates has been investigated in standard oral and dermal studies in rats and/or rabbits compliant or comparable to OECD guideline # 414, respectively.

Sodium thioglycolate was topically applied to pregnant rats from gestational days 6-19 and to pregnant rabbits from gestational days 6-29. In rats, there was one reported maternal death at 200 mg/kg bw/day. Feed consumption, water consumption, and body weights of the dams all significantly increased. The body weights of the foetuses were significantly lower than the controls; however there was no other evidence of embryo/foetal toxicity. In rabbits, moderate to severe erythema occurred at the dosing site in all groups; however no maternal systemic toxicity, embryo/foetal toxicity, or treatment-related teratogenicity were observed in any group. The LOAEL for maternal toxicity was 50 mg/kg bw/day in rats and

the NOAEL was 65 mg/kg bw/day (the highest dose tested) in rabbits. The developmental toxicity NOAEL was 100 mg/kg bw/day for rats and 65 mg/kg bw/day for rabbits.

When ammonium thioglycolate was administered by gavage, the NOAELs for maternal and embryo-foetal toxicity were 15 and 75 mg/kg bw/d, respectively. No teratogenic effects were observed in all studies (study reports from 1998, summary available on the ECHA website.

Overall, thioglycolic acid and its salts are not considered to be developmental or reproductive toxicants, except at dose levels associated with maternal lethality.

3.3.9. Toxicokinetics

(Taken from OECD 2009)

Toxicokinetics *in vivo*:

Absorption and excretion

The urinary excretion of sodium thioglycolate was evaluated using rabbits (weights and strain not stated) after i.v. injection of sodium ^{35}S-thioglycolate at doses of 70, 80, 80, and 123 mg/kg (one animal per dose). Two animals served as controls. Urine was then collected over a period of 24 h. A few drops of liquid petrolatum were placed in each container to prevent air oxidation of possible sulfhydryl compounds. Quantities of organic sulphate, inorganic sulphate, and neutral sulphur in each urine sample were expressed as the percentage of administered radioactivity.

Sodium thioglycolate caused a considerable increase in excretion of iodine reducing material, more than enough to account for the compound administered indicating the breakdown of body constituent and was excreted mostly as inorganic sulphate and neutral sulphur. The radioactivity in the urine indicated that 63-83% of the compound was excreted in the first 24 h after its administration.

The urinary excretion of sodium thioglycolate was also evaluated in rats (weight and strain not stated) injected i.p. with 12.5 to 75.0 mg/kg of a 2.5% solution of sodium ^{35}S-thioglycolate. Urine was collected over a period of 24 h. Quantities of inorganic sulphate excreted, expressed as % of administered radioactivity, ranged from 23 to 72%. The total labelled sulphur excreted during the first 24 hours was 59-96% of the dose. Two of the rats excreted 9% or 11% on the second day and 2% or 6% on the third day respectively (Freeman, 1956).

^{35}S-thioglycolic acid (100 mg/kg adjusted to pH 7.2-7.4 with NaOH) was administered to Holtzman rats (weight = 200-250 g), by i.v. (n= 12) and i.p (n=10). Also, 2 rats were each given 75 mg/kg via i.p. injection. Urine samples were collected 24 h after injection and excretion percentages were determined. The mean urine sulphate contents rats were 82.3 ± 1.6% and 90.6 ± 1.8% for i.v. and i.p. dosed rats, respectively. Most of the radioactivity was excreted in the form of neutral sulphate (Bakshy and Gershbein, 1972).

Two male New Zealand rabbits (weights not stated) were injected i.p. with ^{35}S-thioglycolic acid (100 mg/kg adjusted to pH 7.2-7.4 with NaOH) and one rabbit was injected i.p. with 200 mg/kg. Urine samples were collected 24 h after injection. The mean urine sulphur content of the 3 rabbits was 88% of the administered dose. Most of the radioactivity was excreted in the form of neutral sulphate.

Distribution

The distribution of radioactivity was determined two hour after i.v. injection of 50 mg/kg ^{35}S-thioglycolic acid (adjusted to pH 7.2-7.4 with NaOH) to one Holtzman rat. The small intestine, kidney, liver and stomach exhibited the greatest activity, respectively 0.07, 0.03, 0.02 and 0.02 % of the dose. It is possibly consistent with the generally rapid elimination of thioglycolate in the urine and bile. The greatest content of ^{35}S, 0.66% of the total administered, was detected in the faeces. This observation may have been due to

contamination of the faeces with urine missed during the rinsing of urine residue from the cage after collection (Bakshy and Gershbein, 1972).
The distribution of ^{35}S thioglycolic acid (adjusted to pH 7.2-7.4 with NaOH) in whole blood was evaluated in five Holtzman rats injected i.v. with 100 mg/kg of the test substance and bled during periods of up to 7 h. Four of the 5 had less than 3% residual activity at 1 hour, while one had 5.3% residual activity. At 4-7 hours after the injection, only 0.1% activity or less remained.
The distribution of ^{35}S-thioglycolic acid in the blood was further investigated in the New Zealand rabbit after i.v. injection of ^{35}S-thioglycolic acid (adjusted to pH 7.2-7.4 with NaOH), with emphasis on binding to the following serum protein fractions: α1, α2, β, and γ-globulins and albumin. The test substance (75 mg/kg) was injected i.v. Most of the radioactivity was bound to albumin. The extent of this uptake amounted to 0.14% at 20 min post-injection and had diminished to 0.016% at 3 h. The small amount of radioactivity detected in albumin might have been due to isotopic exchange (Bakshy and Gershbein, 1972).
A female monkey given 300 mg ^{35}S-labelled sodium thioglycolate/kg body weight by i.v. injection, excreted labelled sulphur in the urine (for up to 10 hours) entirely as neutral "sulphur". Tissue samples from 10 organs showed the largest amounts of label in the kidney, lungs and spleen (Freeman, 1956).

Metabolism
Unlabelled thioglycolic acid (100 or 150 mg/kg) was administered to a group of seven rats via i.p. injection. Significant concentrations of dithioglycolate (average concentration 28%) were detected in the urine at 24 h post-injection. Only negligible concentrations of thioglycolate were detected (Bakshy and Gershbein, 1972).

Studies in Humans

No data available.

Conclusion
No data is available on the absorption of thioglycolic acid and/or its salts by inhalation or oral exposure. However, the physico-chemical properties of the thioglycolates, small ionisable water-soluble molecules with a very low $\log K_{ow}$ (ECHA, 2008) as well as the acute oral and inhalation toxicity data suggest that thioglycolic acid and/or its salts are significantly absorbed by the inhalation and oral routes.

SCCS comments
The data of these studies do not indicate any bioaccumulation of the thioglycolic acid.

3.3.10. Photo-induced toxicity

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3.3.11. Human data

(Taken from Burnett 2009)

Fourteen asthmatic patients (13-60 years old) inhaled mists of the following dilutions of ammonium thioglycolate: 1:10, 1:100, 1:10,000, and 1:100,000. After exposure, 13 patients had the following signs and symptoms: asthmatic breathing, an uncontrollable paroxysmal cough, pharyngeal irritation, and blocked nasal passages or nasal drip (UCLA, 1985).
Pharyngeal irritation lasted 0.5 to 2 hours, depending on the degree of sensitivity of the patient. Eight control patients (nonasthmatic and nonatopic) did not have positive reactions to the test substance.

Nasal provocation tests were performed on hairdressers suffering from occupational rhinitis (Prowis, 1976). One patient of 31 responded positive to a 0.6% ammonium thioglycolate solution pH 7.00 (Hytonen, 1997).

A postmarketing surveillance report was submitted by industry (Cosmetics Europe, formerly Colipa) on two marketed products containing a maximum concentration of 4.5% and 4.95% respectively (Colipa 2008). Skin irritation likely or very likely related to the use over a 5 year and a 18 month period was about one (1.2 and 0.94 respectively) event per one million units sold.

3.3.12. Special investigations

3.3.13.1 Safety evaluation (including calculation of the MoS)

Thioglycolic acid

CALCULATION OF THE MARGIN OF SAFETY – depilatories 5%

Product applied to the skin area surface 2000 cm^2 *		**= 2000 mg**
Amount of thioglycolic acid applied (5% of 1960 mg)		**= 100 mg**
Typical body weight of human		**= 60 kg**
External exposure dose	**100/60**	**= 1.66 mg/kg bw**
No observed adverse effect level (dermal 13 weeks toxicity study, rat)	**NOAEL**	**= 180 mg/kg bw/d**
Adjusted NOAEL for 5 days treatment/week		**= 129 mg/kg bw/d**

* Lower legs, axillae and bikini lines (US EPA 1997, Cowan 2008)

Margin of Safety	**Adjusted NOAEL/SED**	**= 78**

CALCULATION OF THE MARGIN OF SAFETY – eyelashes up to 11%

Product applied to the skin area surface 24 cm^2 (*)		**= 24 mg**
Amount of thioglycolic acid applied (11% of 24 mg)		**= 2.64 mg**
Typical body weight of human		**= 60 kg**
External exposure dose	**2.64/60**	**= 0.044 mg/kg bw**
No observed adverse effect level (dermal 13 weeks toxicity study, rat)	**NOAEL**	**= 180 mg/kg bw/d**
Adjusted NOAEL for 5 days treatment /week		**= 129 mg/kg bw/d**

(*) Approximative surface area of the eyelids and therefore indicative of the worst case exposure scenario

Margin of Safety	**Adjusted NOAEL/SED**	**= 2932**

SCCS comment

In the absence of reliable data on dermal absorption of thioglycolic acid in the use concentration of depilatory preparations, MoS calculation was based on a dermal toxicity study.

Since the depilatories are not intended for daily use, the SCCS considers a MoS of 78 as acceptable under the conditions of reasonably foreseeable use.

3.3.14. Discussion

The use of thioglycolic acid and its salts as depilatories or hair-waving products is not intended to be used daily. An application frequency of 17 times per year is assumed to be realistic (RIVM 2006).

Physico-chemical properties
Thioglycolic acid and its salts are used in hair waving or straightening products, depilators and hair care products which are removed after application. Physico-chemical properties of thioglycolic acid and ammonium thioglycolate described in this Opinion are extracted from the available scientific literature.
Esters of thioglycolic acid including glyceryl thioglycolate may also be used in products for permanent waves. However, current safety evaluation does not deal with the esters of glycolic acid.

General toxicity
The acute toxicity has been evaluated in several studies by oral, dermal and inhalation routes (see 3.3.1).
The repeated dose toxicity of sodium thioglycolate was evaluated by dermal and oral administrations.
In the repeated dose dermal toxicity study of NTP in rats, the systemic NOAEL was 180 mg/kg bw/d and the local LOAEL was 11.25 mg/kg bw/d. The systemic NOAEL (corrected for 5 days treatment per week: 180 x 5 / 7 = 129 mg/kg bw/d) was used to calculate the MoS.
Thioglycolic acid and its salts are not considered to be developmental or reproductive toxicants, except at dose levels associated with significant maternal toxicity.

Irritation / sensitisation
Thioglycolic acid and ammonium thioglycolate are skin irritants; at high concentrations they can be corrosive. Thioglycolic acid, ammonium, and sodium thioglycolate are ocular irritants.
However under professional use there should be no contact with the eyes. Application as a depilator is intended to be occasional only. Thioglycolates are sensitizers .

This opinion does not deal with the esters of thioglycolic acid. Contact allergy to glyceryl thioglycolate was seen in consumers. There are several publications reporting widespread occurrence of allergic contact dermatitis to glyceryl thioglycolate in persons who are professionally exposed, such as hairdressers (Warshaw 2012). A withdrawal from some markets of glyceryl thioglycolate as hair-waving ingredient has resulted in a sharp decrease in number of individuals with contact allergy (Uter 2003) to glyceryl thioglycolate.

Dermal absorption
A dermal absorption rate of 16.74 µg/cm^2 has been determined from a study using 13% concentration in hair waving formulation. However in depilatories, the maximum concentration allowed is 5%. Therefore this absorption rate cannot be used for the safety evaluation.

Toxicokinetics
Only studies with i.v. or i.p. application were conducted. No data is available on the absorption of thioglycolic acid and/or its salts by inhalation or oral exposure. However, the physico-chemical properties of the thioglycolates, small ionisable water-soluble molecules with a very low logK_{ow} (ECHA, 2008) as well as the acute oral and inhalation toxicity data suggest that thioglycolic acid and/or its salts are significantly absorbed by the inhalation and oral routes. The data of these studies do not indicate any bioaccumulation of the thioglycolic acid.

Mutagenicity / genotoxicity
Overall, the genotoxicity of thioglycolic acid and its ammonium and sodium salts was investigated in genotoxicity tests for the 3 endpoints of genotoxicity: gene mutations,

chromosome aberrations and aneuploidy. Aneuploidy was only investigated *in vivo*. The quality of the tests was, however, often poor with very little detail of the performance of the tests. Vital information like batch number, purity and vehicle is mostly lacking.
In a gene mutation tests with bacteria or mammalian cells thioglycolic acid and its ammonium and sodium salts were not mutagenic in the presence and absence of metabolic activation. Thioglycolic acid was not clastogenic, with or without metabolic activation, in an *in vitro* chromosomal aberration assay in human lymphocytes. The negative results found in the *in vitro* chromosome aberration test were confirmed in 3 *in vivo* micronucleus tests. However, a micronucleus assay in peripheral blood of mice treated dermally for 13 weeks with sodium thioglycolate showed a slight but statistically significant increase in the frequency of the micronucleated normochromatic erythrocytes in female mice at the top dose level of 360 mg/kg bw/day. The SCCS agrees with the authors of the OECD report of 2009 who considered this result of doubtful significance.
Based on the results of the present test, thioglycolic acid and its ammonium and sodium salts itself can be considered to have no *in vivo* genotoxic potential. The strength of this conclusion may suffer from by the poor quality of the tests performed.

Carcinogenicity
Due to the low sensitivity of animal long-term studies to detect possible carcinogenic effects, animal studies on carcinogenicity are normally performed at doses much higher than those humans may be exposed to. In the case of sodium thioglycolate the concentrations used in the two studies was lower than those which may be used in cosmetic products (see 3.3.7).

SCCS is of the opinion that based on the two topical application experiments (one with mice and one with rabbits) available, it is not possible to draw any conclusions in relation to possible carcinogenic effects of thioglycolic acid and its salts.

3. CONCLUSION

The SCCS is of the opinion that the use of thioglycolic acid (up to 5%) and its salts is safe for use as a depilatory when used as intended (i.e. use not daily and not on large surfaces, see MoS calculation in 3.3.13).

The SCCS is of the opinion that the general use (personal use by consumers at home) on eyelashes is not recommended because of eye irritation potential. However, it is safe up to 11% when applied by a professional for use on eyelashes.

The safety of these types of cosmetic products highly depends on responsible risk management including warnings and extensive guidance for use.

4. MINORITY OPINION

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