EUROPEAN COMMISSION

Directorate-General for Health & Consumers

Scientific Committee on Consumer Safety

SCCS

OPINION ON

Chloroacetamide

COLIPA n° P27

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

The SCCS adopted this opinion at its 10th plenary meeting

of 22 March 2011

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

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1. BACKGROUND

2-Chloroacetamide with the CAS No 79-07-2 and the EC No 201-174-2 is currently authorized as a preservative in Annex VI entry 41 of the Cosmetics Directive (76/768/EEC) in a concentration up to 0.3%.

The first scientific opinion on 2-Chloroacetamide was delivered on July 1986 and the second scientific opinion by the scientific committee on cosmetology was delivered on the 13 October 1987 with the following conclusion:

> *"Chloroacetamide has shown considerable sensitizing potency in humans at concentrations in the range of those present in cosmetics. This property might be due to a contaminant in certain batches of the substance. The Committee agrees with provisional acceptance if information is provided on the results of ongoing surveillance."*

According to regulation EC No 1272 (2008) Annex VI, Table 3.2, 2-Chloroacetamide is currently classified as Repr. Cat 3; R62; T; R25; R43 (which corresponds to Repr.2, Acute Tox 3., Skin Sens 1 of Table 3.1. of the same regulation).

After a public call for scientific data the current submission has been compiled.

2. TERMS OF REFERENCE

1. *On the basis of the provided data the SCCS is asked to assess the risk to consumers when 2-chloroacetamide is used in cosmetic products under the current use conditions of 0.3% in cosmetic products.*

2. *Does the SCCS have any further scientific concern with regard to its use in cosmetic products?*

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Chloroacetamide (INCI name)

3.1.1.2. Chemical names

2-Chloroacetamide
Acetamide, 2-chloro
alpha-Chloroacetamide
2-Chloracetamide
2-Chloroacethanamide
2-Chloroethanamide
Acetic acid, chloro, amide

3.1.1.3. Trade names and abbreviations

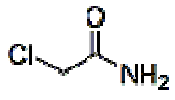
COLIPA n° P27

CA 24
Diamoll C Pulver®
KM 101
Microcide
Mergal AF®
USAF DO-29

3.1.1.4. CAS / EC number

CAS: 79-07-2
EC: 202-431-1

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: C_2H_4NOCl

3.1.2. Physical form

Colourless to pale yellow crystals, characteristic odour

3.1.3. Molecular weight

Molecular weight: 93.51 g/mol

3.1.4. Purity, composition and substance codes

The purity of technical grade 2-chloroacetamide is at least 98%.There are also specifications with a purity of 99%.

Ref.: AR 10, 60

3.1.5. Impurities / accompanying contaminants

Technical 2-chloroacetamide (purity 98%):	≤ 0.5% ammonium chloride ≤ 0.2% water
2-Chloroacetamide (purity 99%)	≤ 0.03% chloroacetic acid ≤ 0.75% ammonium chloroacetate ≤ 0.2% ammoniumchloride ≤ 0.15% water
2-Chloroacetamide (further specification)	≤ 0.2% acetamide ≤ 2.0% chloroacetic acid ≤ 0.5% water

Ref.: AR 10, 60

Comment
There is no information available on which type of chloroacetamide is used for cosmetic ingredients.

3.1.6. Solubility

Water:	90 g/L (at 20° C)
Water:	170 g/L (at 40°C)
Isopropanol:	40 g/L (at 20° C)
Butanol:	35 g/L (at 20° C)

Dissolves well in diethyl ether, dimethyl sulfoxide and N-dimethylformamide

Ref.: AR 10, 11

3.1.7. Partition coefficient (Log P_{ow})

Log Pow: -0.53 (calculated)

Comment
Log Pow was not determined by EC method A.8

BUA (2001) gives two further values (+ 0.53 and – 0.59) for log Pow, but also states, that original data was not available and that validity of the data cannot be confirmed.

3.1.8. Additional physical and chemical specifications

Melting point:	117-119 °C
Boiling point:	220 °C (decomposition)
Flash point:	170 °C
Vapour pressure:	0.07 hPa at 20 °C
Density:	730-850 kg/m³
Viscosity:	/

pKa: /
Refractive index: /
UV/Vis spectrum (200-800 nm): no absorption at λ > 290 nm (in methanol)

3.1.9. Homogeneity and Stability

Information on stability of the substance is not available.

General Comments to physico-chemical characterisation

In most instances, neither information on the methodologies used to determine physico-chemical parameters nor analytical reports are available.

The Log P_{ow} strongly depends on the pH, especially for ionisable molecules, zwitterions etc. Therefore, a single calculated value of Log P_{ow}, usually without any reference to the respective pH, cannot be correlated to physiological conditions.

3.2. Function and uses

Chloroacetamide is used as a preservative in cosmetics and in household (e.g. cleaning) products. It is used as a biocide in a variety of products such as paints and glues. It is also used as a preservative in soluble cutting oils, and in the leather-, paper-, textile- and plastic industries. To a minor extent, it is used for chemical synthesis in the pharmaceutical industry.

In cosmetics, chloroacetamide is often used as a mixture of chloroacetamide and sodium benzoate (70% / 30%) in rinse-off products at the level of 0.15% and in leave-on products at a maximum concentration of 0.3%. The 70% chloroacetamide / 30% sodium benzoate mixture is called CA 24.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

The acute oral toxicity of chloroacetamide has been determined in several old, poorly described studies. An overview on acute oral toxicity studies is given in table 1.

Table 1: Acute oral toxicity of chloroacetamide

Species	**Strain**	**Sex**	**Details on the substance**	**LD_{50} [mg/kg bw]**	**Guideline/GLP**	**Year/Reference**
Rat	No information	No information	CA 24	370	No information	SCC opinion 1 Jul 1986
Mouse	No information	No information	CA 24	150	No information	SCC opinion 1 Jul 1986
Rat	Wistar	female	Technical substance	138	No information	Hoechst, 1976 (IUCLID 25) Christian, 1991 (IUCLID 26)
Mouse	No information	No information	No information	150	No information	Hoechst, 1981 (safety data sheet chloroacetamide)
Mouse	No information	No information	No information	150 - 155	no	Hoechst, 1958 (IUCLID 27) Christian, 1991 (IUCLID 26)
Rabbit	No information	No information	No information	122	No information	Christian, 1991 (IUCLID 26)
Dog	No information	No information	No information	31	No information	Christian, 1991 (IUCLID 26)

3.3.1.2. Acute dermal toxicity

No data

3.3.1.3. Acute inhalation toxicity

No data

3.3.1.4. Acute toxicity – other routes

The acute toxicity of chloroacetamide has also been investigated by the intraperitoneal (i.p.), subcutaneous (s.c.) and intravenous (i.v.) route. An overview on the acute toxicity of chloroacetamide by these routes is given in table 2.

Table 2: acute toxicity of chloroacetamide by the intraperitoneal, subcutaneous and intravenous route.

Species / route of administration	**Strain**	**Sex**	**Details on the substance**	**LD_{50} [mg/kg bw]**	**Guideline/GLP**	**Ref.**
Rat / i.p.	No information	No information	No information	50	No information	Taken from opinion I from 1 July 1986
Rat / i.p.	No information	No information	No information	50	No information	Nielsen, 1983 IUCLID 28
Rat / i.p.	Long-Evans	No information	No information	50	No information	Thiersch, 1972 (cited from BG Chemie (2000)
Mouse / i.p.	No information	No information	No information	130	No information	Kemper 1989 IUCLID 29
Mouse /i.p.	No information	No information	No information	100 - 150	No information	Christian, 1991 (IUCLID 26)
Chinese Hamster/ i.p.	No information	No information	No information	150	No information	Kemper 1989 IUCLID 29
Mouse / i.p.	No information	No information	No information	100	No information	RTECS, 1997 (cited from BG Chemie (2000)
Rat / s.c.	CD and BD IX rats	No information	Commercial product	70	No guideline; application during embryonal development	Von Kreybig et al. (1969)
Mouse / i.v.	No information	No information	No information	180	No information	RTECS, 1997 (cited from BG Chemie (2000)

In a further study (no statement on GLP-compliance, no compliance with OECD/EU test guidelines) groups of 4 – 5 male Sprague-Dawley rats received single i.p. doses of 37.5, 75.0 and 112.5 mg/kg bw/d chloroacetamide (vehicle: 0.9% NaCl). At 112.5 mg, three of four animals died within 4-5 hours. They exhibited fatty degeneration and extensive necrosis of the centrilobular areas of the liver, accompanied by leucocytic infiltration. At 75 mg/kg bw, swelling, hydropic degeneration and single necrotic hepatocytes mainly in the peripheral and midzonal areas of the liver were observed. After administration of a single dose of 37.5 mg/kg bw no effects on liver morphology were observed.

Comment
In this study, investigation of morphology was restricted to the liver.

Ref.: AR 5

Discussion of acute toxicity

The acute toxicity of chloroacetamide has been investigated after oral, subcutaneous and intravenous application of the substance to various animal species. No data are available for acute dermal and acute inhalation toxicity. Studies are poorly reported and information on

signs of toxicity is lacking. However, from the results of the available studies chloroacetamide can be considered as toxic, because oral LD_{50} values are in the range between 31 and 370 mg/kg bw. For the other routes investigated, LD_{50} values in the range between 50 and 180 mg/kg bw have been reported. After i.p. administration of doses of 75 and 112.5 mg/kg bw chloroacetamide to rats, morphologic changes in the liver were observed.

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline: OECD TG 404; EU B.4
Species: rabbit, New Zealand White
Group: 3 animals, no information on sex
Test substance: chloroacetamide
Batch: no information
Purity: approximately 99% (IUCLID)
Dose: 500 mg
Route: dermal
Exposure time: 4 hours
Application: semi-occlusive
GLP: in compliance
Study period: Publication year: 1993

Three animals (2.5 to 2.8 kg, aged 3 – 5 months) received dermal applications of 500 mg chloroacetamide (made into a paste with 0.25 ml 0.9% saline) to the shorn skin in the dorsal region of the trunk. Treated areas were covered by a semi-occlusive dressing. After a 4 hr exposure period, the dressing was removed and the skin was washed. Assessment was performed 30 minutes, 1 hour and 1, 2, 3 and 7 days after removal of the dressing. One of the rabbits exhibited very mild erythema and oedema formation 1 hour to 3 days after removal of the dressing, a second animal showed signs of moderate erythema and oedema formation and the third animal displayed severe erythema and moderate oedema formation. All signs of irritation were reversible 7 days after removal of the dressing. The authors evaluated chloroacetamide as slightly irritating.

Ref.: AR 11, 59

When guinea pigs (Pirbright White) received single applications of aqueous chloroacetamide solutions to the dorsal skin for 24 hours, erythema was observed following exposure to 0.5 ml of a 25% solution. A 9% solution did not produce any effect.

Ref.: AR 11

CA 24, applied as a 5-percent aqueous solution (equivalent to 3.5% chloroacetamide and 1.5% sodium benzoate), caused no skin irritation in the Draize test (24-hour occlusive application to the shaved and scarified skin of the rabbit).

Ref.: AR 11, 46

In a non-GLP study performed in 1958, a 10% solution of chlorocetamide was not irritating to the skin of rabbits.

Ref.: AR 49

In a subacute (30 d) toxicity study, female Yellow-Silver rabbits weighing 1.9 to 3.1 kg received daily applications of 40% aqueous chloroacetamide suspensions to the depilated skin of the neck. Plastic cuffs were placed around the necks in order to prevent licking of the substance. In the lowest dose group (0.063 ml suspension/kg bw corresponding to 25 mg/kg bw), "encrustations" of the skin were observed which appeared on the first day of treatment. On treatment days 4 or 5, parts of the treated skin areas were "hardened".

Ref.: AR 11, 42

A maximisation test of chloroacteamide in the guinea pig employed acetone as solvent. It was reported that a 3% solution induced an inflammatory skin reaction in the controls.

Ref.: AR 2, 11

From the first scientific opinion on 2-chloroacetamide (SCC, 1986) the information is available that a 1% solution was not irritating to the skin of guinea pigs.

Comment
A reference for this study description could not be retraced.

Summary on skin irritation
One guideline-compliant study in rabbits is available, where chloroacetamide was slightly irritating to the skin. Three older studies not performed according to acknowledged test guideline or GLP are available. One study was performed using CA 24, the others using pure chloroacetamide. When solutions between 1 and 10% chloroacetamide were used, no irritating reactions were observed. A 25% solution on the other hand, caused erythema in the skin of guinea pigs. In other dermal studies not specifically addressing skin irritation, skin reactions were reported: in a dermal subacute study in rabbits, encrustations were observed starting with the lowest dose of a 40% solution. In a maximisation test in guinea pigs, a 3% solution of chloroacetamide induced an inflammatory skin reaction.
From the available data it can be derived, that chloroacetamide – at least when used in higher concentration – has a skin irritating potential.

3.3.2.2. Mucous membrane irritation

An eye irritation study of chloroacetamide was conducted in 3 New Zealand white rabbits (weight: 2.9 – 3.4 kg; age: 3 – 5 months) in compliance with OECD test guideline 405 and GLP. 100 mg chloroacetamide was instilled into the conjunctival sac of the left eyes. The right eyes served as controls. 24 hours after application, the eyes were rinsed with physiological saline and the eyes were assessed 1, 24, 48 and 72 hours thereafter. After 1 to 7 hours, the animals displayed severe reddening and swelling of the conjunctiva and corneal clouding could also be observed. The effects cleared up slowly but were reversible after 21 days following instillation. Therefore the authors concluded that chloroacetamide was irritating to the eye.

Ref.: AR 11, 43, 50

According to the method "Appraisal of the Safety of chemicals in Foods, Drugs and Cosmetics- Draize", 0.1 ml of a 5% aqueous dilution of CA 24 (no information on purities of the components; corresponding to 3.5% chloroacetamide) was applied into the left conjunctival sacs of the eyes of six albino rabbits. The substance was not rinsed after application, the observation period was 7 d. Under the conditions of this study, CA 24 was found to be non-irritating.

Ref.: 15 (subm. I)

From the first scientific opinion on 2-chloroacetamide (delivered on July 1986), further information on mucous membrane irritation is available, the respective study reports were not available for evaluation and study authors could not be retraced:

A single application of a 10% solution to the rabbit eye induced redness of the conjunctivae in 1/4 animals. Slight redness was observed upon daily application of 1% in an ointment for 12 days. A similar response, however, occurred in controls treated with the ointment only.

From the IUCLID data, also information on a negative rabbit study using a 10% solution is available.

Ref.: AR 48

A 5% solution of CA 24 was applied into the eyes of probands (no further information). After application, discomfort, lacrimation and blurred vision were observed which lasted for 15 – 30 min.

Ref.: AR 33

From the first scientific opinion on 2-chloroacetamide (delivered on July 1986), information on human experience is available, the respective study report is not given and study authors could not be retraced: a 0.2% preparation was well tolerated in the eye of 5 humans.

Summary on mucous membrane irritation
Mainly poorly described information on mucous membrane irritation in rabbits and humans is available, there were no original study reports or study publications for evaluation. In a guideline-compliant study in rabbits, 100 mg chloroacetamide was irritating, whereas in further (non-guideline) studies on rabbits, 10% chloroacetamide, an ointment containing 1% chloroacetamide and 5% CA 24 (corresponding to 3.5% chloroacetamide) was non-irritating to the eyes; however, 10% chloroacetamide caused redness in the eye of 1 of four animals. In humans, a 0.2% solution was judged to be well tolerated, whereas a 5% concentration of CA 24 (corresponding to 3.5% chloroacetamide) caused discomfort, lacrimation and blurred vision.

From the information available it is concluded, that pure chloroacetamide and concentrations equal or higher than 3.5% have a mucous membrane irritating potential.

3.3.3. Skin sensitisation

Guideline:	OECD TG 406 (Magnusson Kligman Maximisation test)
Species:	Guinea-pigs (Dunkin-Hartley)
Group:	5 animals/group
Test substance:	chloroacetamide
Batch:	no information
Purity:	analytical grade
Dose:	0.003, 0.01, 0.03, 0.1, 0.3%
Controls:	Freund´s adjuvans or vehicle, respectively
Route:	intradermal/dermal
GLP:	no information
Publication year:	1996

Animals received intradermal applications of Freund's adjuvant and aqueous solutions of chloroacetamide. The control group received Freund's adjuvant only. Seven days later, chloroacetamide was applied to the skin as 0.3 or 30% solution in a 1:7 polyethylene glycol/water mixture and left under occlusive cover for 48 hrs. Controls received vehicle only. After further 12 days, sensitisation was induced in all groups using 5% chloroacetamide in 1:7 polyethylene glycol/water by exposing the shorn skin for 24 hours under occlusive cover. Results were assessed 24, 48 and 72 hrs after removal of the dressing. 7 days after the first challenge, rechallenge was carried out by the same way. Irrespective of the chloroacetamide concentrations used, 60 – 100% of the chloroacetamide-treated animals showed positive response after initial challenge, while none of the control animals exhibited positive response. At rechallenge, the initial challenge proved to have induced sensitisation in the controls, with 80% of the animals showing positive reaction. From the results the authors concluded that chloroacetamide has a strong sensitising potential, as a high percentage of animals were sensitised and as the first challenge induced allergic reactions in control animals with clearly positive responses after rechallenge.

Ref.: AR 13

In a maximization test in 20 Pirbright white guinea pigs with 99% pure chloroacetamide (using for induction a 9% aqueous concentration intradermally and a concentration of 9% in vaseline topically) a challenge treatment with 3, 1 and 0.3% in distilled water given after 14 days rest did not produce sensitization. 1-chloro-2,4-dinitrobenzene (DNCB) served as positive control, distilled water served as negative control; 10 animals were used for the respective control group. In a preceding range-finding experiment undiluted, 50% diluted and 25% diluted test item caused erythema.

Ref.: 11 (subm III)

Comment
A statement on QA was provided, but the test did not comply to any guideline.

In a sensitisation test by a modified Buehler method with CA 24 twenty male Pirbright white Guinea pigs received occlusive topical induction treatments with 0.5 ml of a 0.3% CA 24 solution in water (corresponding to 0.21% chloroacetamide), once a week, for 3 weeks. 10 male untreated animals served as controls. After a 14-day rest period, a challenge treatment with 0.3% in water did not induce any sign of sensitisation.

Ref.: 1 (subm I)

Comment
No information on QA or compliance with test guideline was given.

In a further sensitisation test, a 1% aqueous solution of chloroacetamide was painted on the shaved skin of 10 male Pirbright white guinea pigs nine times at intervals of 48 hrs. The same solution was also applied to the shaved and abraded skin of 10 further male Pirbright white guinea pigs using the same exposure regimen. After a 14-day rest period the same solution applied as a challenge treatment did not induce any positive response for both experimental settings.

Ref.: 2 (subm I)

Comment
No information on QA or compliance with test guideline was given.

A further sensitisation test was conducted with a skin cleansing product containing 0.1% CA 24 (equivalent to 0.07% chloroacetamide; no information on vehicle;) by rubbing it with a glass stick into the shaved and abraded skin of the back of 8 male Albino guinea pigs in an amount of 0.1 ml, three times weekly for three weeks. Since the challenge treatment with 0.1 ml, 0.1% solution after 14 days did not produce more severe changes, the test substance was not considered a sensitizer.

Ref.: 3 (subm I)

Comment
No information on QA or compliance with test guideline was given. According to the study description (the original study was not available for evaluation) the treatment induced erythema and oedema.

A further sensitisation test was conducted in a similar way, however, with a 1.0% (instead of a 0.1) solution of CA 24. Since the challenge treatment with 0.1 ml of a 1.0% solution after 14 days did not produce more severe changes, the test substance was not considered a sensitizer.

Ref.: 4 (subm I)

Comment
No information on QA or compliance with test guideline was given. According to the study description (the original study was not available for evaluation) the treatment induced erythema and oedema.

In an open epicutaneous test without Freund's adjuvant, groups of 10 female albino guinea pigs (Pirbright White) received local exposures of the shaved skin of the flank to 3% or 1% chloroacetamide (99.0% pure) solutions (no details on skin reactions given) 5 times per week over a period of 4 weeks. A mixture of 45% methylcellosolve, 45% water and 10% Tween 80 was used as solvent. Ten days after completion of the pretreatment, a 0.2% solution was applied to the contralateral, untreated skin of the flank in order to elicit the positive response. Skin areas were evaluated 24 and 48 hrs later. No definite allergic reaction was elicited in either group of pre-treated guinea pigs when a 0.2% solution was used. In a very short expert's report the author remarks in his discussion that "it has previously been established that if Freund's adjuvant is used, a sensitising effect of chloroacetamide can be demonstrated in guinea pigs".

Ref.: 3 (subm II)

Comment
No information on QA or compliance with test guideline was given.

Guinea pigs were exposed to chloroacetamide in a modified Draize test. Ten animals of approximately 350 g underwent exposure to chloroacetamide in two cycles of induction and challenge (no details on concentrations used). One of ten animals showed a positive allergic reaction. The authors discussed their method as not being very sensitive and conceded that the negative result must be interpreted with caution.

Ref.: AR 52

Comment
No information on QA or compliance with test guideline.

Summary of skin sensitisation
In animal studies, chloroacetamide was sensitising in a maximisation test performed according to OECD TG 406. An older maximisation test was negative. Modified Buehler assays performed in guinea pigs using either pure chloroacetamide (concentration: 1%) or CA 24 at concentrations between 0.1 and 3% were negative. Two further assays in guinea pigs (an open epicutaneous test using concentrations of 1% and 3% chloroacetamide and a modified Draize test) were also negative. Thus, in animals, the sensitising potential of chloroacetamide is low based on mostly poorly described tests which have mainly not been performed according to modern standards with respect to Test Guidelines and Quality assurance.
The potential of chloroacetamide to induce contact allergies in humans is described in section 3.3.11 – Human data.

3.3.4. Dermal / percutaneous absorption

An in vitro dermal absorption study is not available.
The toxicokinetic behaviour after single dermal administration of 1-^{14}C-chloroacetamide has been investigated in the rat (see section 3.3.9.- Toxicokinetics). From this study, a dermal absorption percentage of 56% is derived.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose oral / dermal / inhalation toxicity

Oral

Information on a non-published and non-GLP oral 28-day repeat-dose toxicity study is available. Male rats (no information on strain) received 20 applications of 0, 5, 10 and 20 mg/l chloroacetamide. No further information on substance was given. Behaviour and haemogram were normal. At the highest dose, protein was present in the urine. Further, dose-dependent degenerative macroscopic and histological changes in livers and kidneys could be observed.

Ref.: AR 48

Comment
The original study report was not available for evaluation. Therefore it cannot be checked whether the unit of mg/l which is given for dosage, is correct or a typing error. A NOAEL cannot be derived from this study description, the study can only be used as supporting information.

Further information on oral short-term repeat-dose studies which lack information on dosages is available. In a 30 day oral study in rabbits, spastic paralysis with or without sensory change was observed.

Ref.: AR 38

Comment
As no information on dose is available, a NOAEL cannot be derived from the study description. Therefore it can only be used as supporting information.

Dermal

Guideline:	precedes OECD test guidelines
Species:	rabbit, Yellow-Silver, weighing between 1.6 and 2.9 kg
Group:	5 female animals per dose
Test substance:	chloroacetamide, 40% aqueous suspension
Batch:	no information
Purity:	no information
Dose:	25, 50, 100, 200, 400 mg/kg bw/d (dose levels refer to chloroacetamide)
Controls:	0 mg chloroacetamide
Route:	dermal
Duration:	30 days, one application per day
GLP:	no
Study period:	1967

On 30 consecutive days, suspensions of chloroacetamide were applied to the depilated skin of the animals' necks. Plastic cuffs were used in order to prevent licking-off of the substance. Before and after the treatment period, blood was taken for investigation of haemoglobin, red blood cells, white blood cells, differential blood count and Heinz bodies. Urine was taken for investigation of protein, glucose and sediments. Body weights were determined three times a week and at the end of the study, hearts, lungs, livers, spleens, kidneys, adrenal glands, ovaries, pancreas, brains, pituitary glands and thymus glands were examined histopathologically.

Results

Local skin changes consisting of encrustations and thickening of the application site were observed in all dosed animals (all dose groups). Local skin changes were not present in the control group. One animal of the 50 mg/kg bw dose died, but no further information on findings in this animal was given. All animals survived at 100 and 200 mg/kg bw/d. Aside from local skin reactions further toxicological findings were not observed in the two lowest dose groups. Above 100 mg/kg bw/d, dose-dependent weight loss and dose-dependent histopathological changes of liver, heart and spleen could be observed. The highest dose level was lethal in 3 of 5 animals. Local changes as well as histopathological changes in liver, kidney and spleen were more pronounced in the highest dose group compared to the lower dose groups. Analysis of blood and urine revealed no pathological findings in any of the dose groups.

A dermal NOAEL of 50 mg/kg bw/d was derived from this study based on weight loss and histopathological changes of liver, heart and spleen. This dermal NOAEL of 50 mg/kg bw/d is taken for MoS calculation.

Ref.: AR 55

Comment
This study demonstrates that dermal absorption of chloroacetamide takes place. Although the study was performed prior to the implementation of OECD testguidelines and GLP principles it has been properly performed and documented and is considered as a valid study for the assessment of repeated dermal toxicity.

Subcutaneous

Guideline:	precedes OECD test guidelines
Species:	rabbit (no information on strain)
Group:	5 female animals per dose
Test substance:	chloroacetamide
Batch:	no information
Purity:	no information
Dose:	2, 10, 25, 50, 100 mg/kg bw/d
Controls:	1.0 ml 0.9% saline/kg bw/d
Route:	subcutaneous
Duration:	30 days
GLP:	no
Study period:	1967

Chloroacetamide was administered subcutaneously for 30 consecutive days to female rabbits at dose levels of 2, 10, 25, 50 and 100 mg/kg bw/d. Control animals received 1 ml 0.9% saline/kg bw. Before and after the treatment period, blood was investigated (determination of haemoglobin, leucocytes, erythrocytes and Heinz bodies) and urinalysis was performed. 3-4 days after the application period, animals were killed and selected organs (heart, lungs, liver, spleen, kidneys, adrenal glands, pancreas, ovaries, brain, thymus and pituitary gland) were taken for histological examination.

Results
In control animals and in animals treated with 2 and 10 mg/kg bw/d chloroacetamide, no adverse effects were reported. At 25 mg/kg bw, one of 5 animals died. Hindlimb paralysis, diarrhoea, thickening at the injection site and reduced haemoglobin levels were reported. At 50 mg/kg bw/d, all animals died after 7 to 13 injections. Forelimb and hindlimb paralysis, thickened skin of the neck and diarrhoea but no histopathological changes were reported. Laboratory tests have not been performed at 50 mg/kg bw/d. At 100 mg/kg bw/d, all animals died after one or two injections. No further observations on toxicity were reported, no laboratory tests and no post-mortem investigations were performed at the highest dose level.

NOAEL (sc): 10 mg/kg bw/d.

Ref.: AR 56

Intravenous

Guideline:	precedes OECD test guidelines
Species:	rabbit (no information on strain)
Group:	5 female animals per dose
Test substance:	chloroacetamide
Batch:	no information
Purity:	no information
Dose:	2, 10, 25, 50, 100 mg/kg bw/d
Controls:	0.9% saline
Route:	intravenous
Duration:	30 days
GLP:	no
Study period:	1967

Chloroacetamide was administered intravenously for 30 consecutive days to female rabbits at dose levels of 2, 10, 25, 50 and 100 mg/kg bw/d. In control animals and in animals treated with 2 and 10 mg/kg bw/d chloroacetamide, no adverse effects were reported. At 25 mg/kg bw/d, one of five animals died. Limb paralysis starting after injection 13, diarrhoea, body weight loss and a slight reduction in haemoglobin were reported. Organs were without histopathological findings. At 50 mg/kg bw/d, all animals died after 6 to 15 injections. Limb paralysis starting after injection 7 and diarrhoea were reported. Laboratory tests were not performed. Organs were without histopathological findings. At 100 mg/kg bw/d, all animals died after one or two injections, laboratory tests or post-mortem investigations were not performed.

NOAEL (iv): 10 mg/kg bw/d

Ref.: AR 57

Intraperitoneal

Repeated i.p. administrations of 37.5 mg/kg bw chloroacetamide every second day to male Sprague-Dawley rats induced slight hepatocellular swelling and hydropic degeneration in the peripheral two-thirds of the lobules of the liver within two weeks.

Ref.: AR 6

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

Oral

Guideline:	no information
Species:	rat (Sprague-Dawley)
Group:	10 animals / sex / dose
Test substance:	chloroacetamide
Batch:	no information
Purity:	no information
Dose:	12.5 and 50 mg/kg bw/d
Controls:	untreated animals (10 males, 10 females)
Route:	oral, diet
Duration:	13 weeks
GLP:	no

Study period: no information

Groups of 10 male and 10 female animals received daily doses of 0, 12.5 and 50 mg/kg bw chloroacetamide via feed. At 12.5 mg/kg bw/d body weight gain was reduced and received statistical significance at 50 mg/kg bw/d. In male animals of the lower dose soft, atrophied testes with reduced weight were observed after 13 weeks, whereas female animals of the lower dose had significantly enlarged thyroids. In the group receiving 50 mg/kg bw/d, mild sedation was seen and food consumption was reduced. Further, in male animals of the high dose group, testes weights and sizes were significantly reduced, whereas high-dose females showed enlarged thyroid glands. Behavioural observation, haematology, clinical-chemical investigations, urinalysis and hearing and visual tests did not demonstrate adverse treatment-related findings. Histopathological examinations revealed that spermatogenesis was impaired severely and in a dose-dependent manner (complete absence of mature sperm cells at the high dose). In other organs investigated (original data were not available for evaluation, in the summary of the study it is not stated, which organs were investigated), no histopathological changes were observed. However, at the high dose, indications for fatty degeneration of livers and liver atrophy were observed. A dose without adverse effects could not be identified in this study.

LOAEL: 12.5 mg/kg bw/d (based on testis atrophy in male animals and enlarged thyroids in female animals).

Ref.: AR 53

Guideline:	According to Directive 87/302/EWG, part B
Species:	rat (Wistar, Hoe: WISKf(SPF71)
Group:	10 animals / sex / dose
Test substance:	chloroacetamide
Batch:	no information
Purity:	> 98%
Dose:	2, 10 and 50 mg/kg bw/d
Controls:	0 mg chloroacetamide (diet without chloroacetamide)
Route:	oral, diet
Duration:	13 weeks, followed by a 29-day observation in half of the animals
GLP:	no
Study period:	1985

Young animals received daily doses of chloroacetamide via feed. At the end of the treatment period, 5 male and 5 female animals were observed for 29 days. At study termination (for recovery animals: at the end of the recovery phase), haematological and clinical-chemical investigations were performed. Organ weights and macroscopic changes in organs were also investigated and urinalysis was performed.

Results
In top dose animals, food consumption was reduced during the first 30 days of treatment and body weight gain was impaired. At the end of the feeding period, males of the top dose exhibited a significant reduction in testicular weight which was still present after the 29 day observation (recovery) period. Sizes of testes and epididymes were reduced. Histopathological examination revealed depression and/or cessation of spermiogenesis as well as moderate proliferation of Leydig cells immediately at the end of the feeding period. The epididymis contained neither mature nor immature sperm cells. At the end of the 29 day observation period there were signs of partial regeneration of the testicular tubules. No other histopathological changes were seen in the males. The females of all dose groups were without histopathological findings at both time points of examination. In top dose females, liver weights were statistically significantly reduced. Haematological investigations, clinical chemistry and urinalysis were conducted at the end of the study. The only treatment-related finding was an increase in leucocytes in all top dose animals of both

sexes. In groups receiving 2 and 10 mg/kg bw/d, no substance-related organ changes were observed.

A NOAEL of 10 mg/kg bw/day was derived from this study.

Ref.: AR 54

Comment
This study was aimed at identifying a dose without effect, which could not be established in a previous oral 90 d study (the study resulting in a LOAEL of 12.5.mg/kg bw/d). Thus, for calculation of the MoS based on oral exposure, the NOAEL of 10 mg/kg bw/d is taken.

In a further, non-GLP-compliant study (the original study report was not available for evaluation) using groups of 20 Wistar rats (no further information on substance and whether both sexes of animals were used), dosages 0, 12.5 and 50.0 mg/kg bw/d were administered via diet for a period of 13 weeks. At 12.5 mg/kg bw/d, reduction of testes sizes and impaired spermiogenesis was reported. At the high dose (50 mg/kg bw/d), body weight gain and scrubby fur were observed in addition to reduced testis size and impairment of spermiogenesis.

LOAEL: 12.5 mg/kg bw/d.

Ref.: AR 50

Dermal

In a 90 day dermal application study, the commercially available preservative CA 24 (no details with respect to the purity of the two components), was applied to the shorn skin of male Wistar rats weighing approximately 210 g (no details of the number of applications per week). Groups of 10 rats were treated with 12.5 mg/kg bw CA 24 (as 1% solution in Lanette (*Lanette*® is a mixture of 50% Cetylalcohol (Hexadecan-1-ol) and 50% Stearylalcohol (Octadecan-1-ol)) or with 50 mg/kg bw CA 24 (either as 2% solution in Lanette or as 2% solution in distilled water). A control group was treated with Lanette. The dose level of 50 mg/kg body weight of CA 24 corresponds to 35 mg chloroacetamide/kg body weight and the dose level of 12.5 mg/kg body weight of CA 24 corresponds to 8.75 mg chloroacetamide/kg body weight. No reasons were given for the choice of dosages. The study parameters, which included body weight, feed consumption and histopathological examination of the testis and epididymis, gave no indications of pathological changes attributable to the substance. There are no data on the chemical's dermatological actions during the treatment.

NOAEL: 35 mg/kg/bw (highest dose applied).

Ref.: AR 11

Comment
The original study report was not available for evaluation. As substantial information (e.g. on the number of applications and on parameters investigated) is lacking, the study will not be used for risk assessment.

Summary on repeat-dose toxicity

Short-term (14d), subacute (28d/30d) and subchronic (90 d) repeat-dose studies on chloroacetamide have been performed but none of them met modern standards with respect to test guideline compliance and quality assurance. Most of the original study reports were not available for evaluation and most of the studies were poorly described. Subacute studies have been performed by the oral, dermal, subcutaneous and intravenous

route in rats and rabbits. In these studies, apart from local and acute effects, systemic effects on liver (e.g. fatty infiltration of the liver, degenerative macroscopic changes), kidneys, spleen and heart were observed. Three subchronic studies in rats using the dietary administration pathway are available. In male animals, the reproductive tissues were adversely affected: testes and epididymes were identified as target tissues. Size reductions were observed for both tissues and for testes, significant weight reduction was observed. Spermatogenesis was impaired dose-dependently and Leydig cell proliferation was observed. In female animals, reductions in liver sizes and enlargement of thyroid glands was observed. At high doses (50 mg/kg bw/d), increases of leucocytes were observed in both sexes. In a teratological study (described in section 3.3.8.2 Teratogenicity), where Wistar rats received chloroacetamide by gavage from GD 7 to GD 17, reduced thymus weight and shrinking of the thymus was observed in female animals at a dose level of 48 mg/kg bw at termination of the study. A poorly described subchronic dermal study with CA 24 in rats gave no indications of pathological changes attributable to the substance.
Effects on the male reproductive system were the most sensitive effects observed in subchronic studies. Based on testis effects, an oral NOAEL of 10 mg /kg bw/d and an oral LOAEL of 12.5 mg/kg bw/d were derived from 90-d studies. An oral NOAEL of 10 mg/kg bw/d and a dermal NOAEL of 50 mg/kg bw/d are used for two possibilities of MOS calculation.

3.3.5.3. Chronic (> 12 months) toxicity

No data available.

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

The in vitro genotoxicity of chloroacetamide has been investigated in bacterial assays and in a SHE assay. An overview is given in table 3.

Table 3: *In vitro* genotoxicity tests performed with chloroacetamide

Species /Strain	Guideline/ GLP-compliance	Substance, Batch, Purity	Concentrations	Remarks (metabolic activation, positive control)	Findings	Year/Reference
Ames test Salmonella typhimurium TA 98/100/1535/1537/ 1538; no information on strains	No information	CA 24	0.5 - 1000 µg/plate	With and without metabolic activation (liver enzymes from Aroclor-induced rats); positive controls: sodium azide, 2-Aminoacridine, 2-Nitrofluorene, 2-Anthramine	Negative; bacteriotoxic effects at higher dose levels	1979 Ref. 9, Subm. I 1986;BG Chemie IBR 1979a IUCLID26 (J.Am Coll.Toxicol)
Ames test Salmonella typhimurium TA 98/100/1535/1537	No information	Chloroacetamide, purity ≥ 98 %	4 – 2500 µg/plate	With and without metabolic activation (S9 mix from Aroclor 1254-induced rat liver) no information on controls or toxic ranges	negative	IUCLID 40; Hoechst AG Pharma Forschung Toxikologie Ames-Test – Substanz 128/79 – Chloracetamid, unpublished report No. 351/79 A, 1979: reported in BG Chemie
Ames test Salmonella typhimurium TA 98/100/1537	No information	Chloroacetamide, no information on purity	0.5 – 10 mg/plate	With and without metabolic activation (S9 mix from Aroclor 1254-induced rat liver) no information on controls or toxic ranges	Negative, but bacteriotoxic at 20 mg/plate	Voogd et al., 1989 (reported in BG Chemie)
Klebsiella Pneumoniae	No information	No information	0.1 – 2 g/l	20 hr incubation in beef bouillon; Subsequent incubation on agar plates which had partly been prepared with streptomycin was used to determine the number of bacteria to have acquired streptomycin resistance by mutation.	At concentrations of and above 0.5 g/L the mutation rate increased up to tenfold in a concentration-dependent manner. At 0.5 g/l, the substance did not exhibit any marked bacteriotoxicity. At 2 g/L, however, 80 to 85% of the bacteria were destroyed.	Voogd et al., 1989 (reported in BG Chemie)
Syrian hamster embryo cells (Syrian hamster embryo cell transformation assay)	No information	No information	Up to 50 µg/ml	No details	The highest concentration tested, 50 µg/mL, did not reduce the cell survival rate below 40% of the solvent control. No morphological transformations were observed. The testing program included both positive and negative controls	IUCLID 41 CCR (1987): Project No. 108505 (HOE 87.1436); reported in Am Chem Toxicol.10 (1991), 21-30

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

In vivo micronucleus test

A Micronucleus test reported to be in compliance with GLP and OECD TG 474 was performed in NMRI mice using technical grade chloroacteamide (purity ≥ 98%). Groups of 5 male and 5 female mice were dosed twice by gavage using dose levels of 0 (control), 1, 10 or 100 mg/kg bw (dosing interval: 24 hr). Positive controls were treated with cyclophosphamide at a dose level of 100 mg/kg bw. 6 hr after the second administration, animals were sacrificed; smears of the femoral bone marrow were prepared and stained. 2000 polychromatic erythrocytes were examined for micronuclei and the ratios of juvenile forms and normocytes as well as numbers of normocytes containing micronuclei were determined. Compared to negative controls, chloroacetamide exposure did not cause significant changes in any of the measured parameters whereas cyclophosphamid caused a marked increase in the number of micronuclei.

Ref.: AR 58

Chromosomal aberration (and _in vivo_ micronucleus test)

Chinese hamsters (no information an the number of animals) received two i.p. injections of CA 24 at dose levels of 12.5, 25.0 and 50 mg/kg bw (corresponding to 35, 17.5 and 8.75 mg chloroacetamide/kg bw/d, respectively) at an interval of 24 hours. No structural or numerical chromosome aberrations were observed in blood smears from the bone marrow and in spermatogonia. No increase was observed in the number of micronuclei in the blood smears of the bone marrow. (Study report not available for evaluation).

Ref.: 10 (subm I)

Dominant lethal test

In a dominant lethal test, groups of 10 male NMRI mice received single intraperitoneal injections of CA 24, dissolved in 0.5% methylcellulose, at dose levels of 114 and 123 mg/kg bw (corresponding to 79.8 and 86.0 mg chloroacetamide/kg bw). Control animals received 0.5% methylcellulose. Treated male animals were each mated with 3 virgin females per week over a period of 10 weeks. Implantations were reduced at the end of weeks 1, 2 and 3 (reaching statistical significance at weeks 2 and 3), and a reduction in fertility index was seen after weeks 1 and 2. The number of foetuses after weeks 1, 2 and 3 were clearly lower compared to controls (reaching statistical significance at 3 weeks). After week 4, the effects were no longer observed. The authors concluded that the observed effects were due to a toxic effect of the preservative on male animals during the first 3 weeks. The number of resorptions, the mutagenicity index and the number of dead foetuses remained unchanged throughout the entire study. Thus, the authors concluded that a mutagenic effect seemed unlikely, however, there were clear effects on fertility.

Ref.: 11 (subm I)

Summary on mutagenicity/Genotoxicity

In vitro, chloroacetamide was non-mutagenic in three Salmonella assays using *S. typhimurium* strains TA98, 100, 1535, 1537 and 1538 (with and without metabolic activation, respectively). One bacterial *in vitro* fluctuation test using Klebsiella pneumoniae yielded positive results. In an *in vitro* cell transformation assay using Syrian Hamster Embryo cells, chloroacetamide did not produce morphological transformations (with and without metabolic activation). *In vivo*, negative results were obtained in a Chinese hamster chromosome aberration test examining bone marrow and spermatogonia, in a micronucleus test in Chinese hamster, a micronucleus test in mice and in a dominant-lethal test

performed in mice. From the results of the *in vitro* and *in vivo* assays performed it can be deduced that chloroacetamide is not mutagenic and genotoxic.

3.3.7. Carcinogenicity

No data available

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data available

3.3.8.2. Teratogenicity

Long-Evans rats (no information on number) received oral applications (no further information on the way of application) of 20 mg/kg bw chloroacetamide either as single dose on day 7 or as two applications on the 11th and the 12th day of gestation. On day 21 of gestation, caesarean sections were performed in both groups and placental weights, foetal weights, numbers of implantations and foetal malformations were assessed. Signs of embryotoxicity or teratogenicity could not be observed. Also in the dams there were no signs of toxicity.

Ref.: 12 (subm I)

Comment
It is unusual to investigate teratogenic effects after administration of test substance on single days of gestation only. Maybe the critical window of pregnancy was not met by this type of administration.

CD and BDIX rats (3 pregnant animals per strain) received single subcutaneous doses of 50 mg/kg bw chloroacetamide on day 13 and 14 of gestation. The dose was toxic and resulted in postnatal death of 50% of the pups. There were no cases of malformations. In surviving animals, development was normal. No details were given on the criteria used to assess pups (pups dying postnatally as well as surviving pups) and dams.

Ref.: 13, 14 (subm I)

Comment
It is unusual to investigate teratogenic effects after administration of test substance on single days of gestation only. Maybe the critical window of pregnancy was not met by this type of administration.

Guideline:	not stated
Species/strain:	Rat, Wistar, JcL
Group size:	(a) 20 pregnant females (b) 7 pregnant animals
Test substance:	chloroacetamide
Batch:	no information
Purity:	> 97.0%
Dose levels:	(a) 3, 12 and 48 mg/kg bw/d (b) 24 mg/kg bw/d
Route:	oral (gavage)
Vehicle:	distilled water
Dosing schedule:	(a) daily from day 7 to 17 of gestation (b) daily from day 14 of gestation until postnatal (PND) day 2

Dosage volume: 2 ml/kg
Controls: vehicle (distilled water) in groups of 20 (a) and 5 (b) animals
GLP: no information
Study period: the study was published in 2003

Dose selection was based on a preliminary experiment in which oral doses of 0, 1, 3, 10, 30 and 60 mg/kg bw/d had been administered to pregnant animals on gestation days 7- 17. In the highest dose group of the preliminary experiment, reductions in body weight gain and reduction of thymus weights were observed in dams, body weights of viable foetuses were reduced. External examination of the pups did not reveal any abnormalities.

The main study consisted of two parts. In one part (designated part a), the teratological effects of chloroacetamide were assessed, in the other part (designated as part b), effects on the reproductive systems of the offspring were assessed. Subsequently, the two parts are described separately. For both parts, pregnant female animals were used.

Part a:

Weight and food intake of animals were recorded daily. Animals were killed on day 20 of gestation. Selected tissues (thymus, stomach, adrenal glands, kidneys and gravid uteri) were assessed. Corpora lutea, number of implantations, viable fetuses, fetuses dying early and at later stage (reabsorbed or dead foetuses) were examined. Viable fetuses were weighed, checked for external malformation, sexed and fixed in order to check for visceral and skeletal abnormalities.
Results part a: there were no treatment-related effects in any dose group. In dams, body weight gain was significantly depressed at 12 and 48 mg/kg bw/d; in high-dose dams, weights of gravid uteri and thymus weights were significantly reduced and shrinking of the thymus gland was observed after termination on gestation day (GD) 20. Incidences of absorbed or dead fetuses, the number of viable foetuses and sex ratio were not affected by chloroacteamide treatment when compared to controls. However, the weight of viable fetuses was significantly reduced at 48 mg/kg bw/d. In pups, no effects on reproductive organs were observed after chloroacetamide treatment when compared to controls. In the 12 and 48 mg/kg/d groups the number of ossified sternebrae and the number of ossified forelimb phalanges were reduced.

Part b:

Weight and food intake of parental animals were recorded daily. On PND 4, pups were culled into groups of 4 males and 4 females. Weights were determined on the day of birth, PNDs 4 and 7 and in weeks 3, 6, 9 and 13. Ano-genital distance (AGD) was investigated on postnatal days 4 and 10. In female pups, vaginal opening was determined on PND 27, in male animals, preputial separation was determined on PND 37. Pups were killed in week 13 selected organs (in male animals: testes, epididymes, seminal vesicles, thymus, adrenal gland and pituitary gland, in female animals: ovaries, uteri, oviducts, adrenal glands and pituitary glands) were taken and weighed.
Results part b: in treated dams, body weight gain and food consumption were significantly reduced towards the end of gestation. In pups of both sex, there was a tendency for lower body weights between PNDs 0 and 4. No difference in the number of pups born and number of pups surviving until PND 4 was found between treated and control animals. Also with respect to AGD, vaginal opening and preputial separation, there were no differences between treated and control animals. At 13 weeks of age, no differences in the weights of adrenal glands, pituitary glands and male and female reproductive organs were observed between treated and control animals.

Results
From the teratological part of the study, study, a NOAEL of 3 mg/kg bw/d was derived for dams (based on body weight reductions) and pups (based of ossified sternebrae and the

number of ossified forelimb phalanges). Repeated administration of 24 mg/kg bw/d from GD 14 to PND 2 did not adversely affect the reproductive tissues of the offspring.

Ref.: AR 73

Comment

The study was available as translation from a Japanese original version. The quality of the translation could not be shown, however, some issues in the translated version raise some doubts on the study and/or translation (e.g. preputial separation is mentioned in the context of female animals). Thus, the NOAEL derived from this study is not taken into account for MOS calculation.

Summary on Reproductive toxicity
No guideline-compliant studies on reproductive toxicity of chloroacetamide are available. In two poorly described teratological studies, which used the oral and the subcutaneous application pathway, no malformations in foetuses were observed after treatment of maternal animals during certain days of gestation. However, as in both studies substance was administrated in single days during gestation, the critical window of development might not have been targeted. In a further study, the effects chloroacetamide on development and reproductive organs of the offspring were investigated after oral gavage administration to maternal animals during gestation. No adverse effects on reproductive tissues of the offspring were observed after repeated administration of 24 mg/kg bw/d from GD 14 to PND 2. In a dominant-lethal test in NMRI mice (described in section 3.3.6.2 – Genotoxicity/Mutagenicity *in vivo*), fertility was clearly impaired at single i.p. dose levels of 79.8 and 86 mg/kg bw chloroacetamide. Based on body weight reduction in maternal animals and based on skeletal findings in the offspring, a NOAEL of 3 mg/kg bw/d was derived for dams and pups from a combined teratology/reproductive toxicity study, whose quality could not be ascertained.

3.3.9. Toxicokinetics

Guideline:	not stated
Species/strain:	Rat, Wistar
Group size:	10 male animals per application pathway (5 animals for determination of blood levels, 5 animals for determination of excretion and tissue distribution, respectively)
Test substance:	[1-^{14}C]-chloroacetamide
Batch:	12o59 I (121 mCi/g) and 12o59 II (46.6 mCi/g)
Radiochemical purity:	> 98.0%
Dose level:	approximately 2 mg/kg bw
Route:	oral (gavage), intravenous and dermal
Vehicle:	distilled water (for oral and i.v. administration) distilled water containing 10 µl/ml Genapol® (for dermal administration)
Dosing schedule:	single application
Controls:	no controls
GLP:	no information
Study period:	1985

Groups of animals received single doses of (1-^{14}C)-chloroacteamide by the oral, i.v. or dermal pathway. Dermal application was onto shaved skin of the back, oral intake from treated skin was prevented by fixing a vessel onto the treated skin area. Treated skin areas were washed off 6 hrs after substance administration. After application, animals were housed individually (for determination of excretion and tissue distribution) or as two animals (for blood analysis and exhalation) in metabolic cages which allowed separation of urine and faeces. After oral application, exhaled radioactivity was also investigated. Termination was after 24 hrs for dermal application and after 7 days after oral and i.v. administration. Radioactivity was determined by liquid scintillation and whole-body autoradiography.
Results: after i.v. administration, rapid distribution of radioactivity occurred, because blood levels were only 4% of theoretical value 5 min after injection. At later time points, radioactivity in blood increased again. Elimination of radioactivity from blood occurred biphasic with two half-lives of about 5 and 500 hrs. 87% of radioactivity was excreted via urine within 7 days after administration; radioactivity determined in cage wash and faeces was 1.5%. At study termination after 7 d, radioactivity could still be detected in plasma. After oral administration, maximal plasma levels were observed 1-3 hrs after administration. Two elimination half-lives of 5.6 and 520 hrs were determined for blood. Within 7 days after administration, 90% of radioactivity was excreted via urine, whereas radioactivity in faeces and cage wash amounted to 3.6%. At study termination after 7 d,

radioactivity could still be detected in plasma. Remaining total body radioactivity after 7 days was 9% of the administered radioactivity. Highest amounts were found in blood, heart, lungs, liver and spleen.
After dermal administration, maximal plasma levels were observed between 2 and 6 hrs after application. Two elimination half-lives of approximately 6 and 180 hrs were determined for blood. Within 24 hrs, 43% of the administered radioactivity was excreted via urine, whereas 0.7% of the administered radioactivity was excreted via faeces. At study termination after 24 hrs radioactivity (33% of the applied amount) was still present in organs, tissues, site of application and remaining body. Autoradiography revealed that radioactivity was present in blood, liver, kidneys and also in skin areas that had not been treated. At study termination, 43.4% and 0.68% of the applied radioactivity had been excreted via urine and faeces, respectively. 11.8% of the applied radioactivity was determined in tissues and body compartments excluding treated skin areas, 21.1% of the applied radioactivity was detected in skin of the application site. In total, 77.5% of the applied radioactivity was recovered. Thus, from the percentages of radioactivity excreted and radioactivity present in body compartments different from the application site, 56% of the applied radioactivity is taken as amount absorbed.

Ref.: AR 85

Most probably, chloroacetamide conjugates with glutathione (GSH).

Ref./ AR 5, 6, 10, 31

Summary on toxicokinetics

Chloroacetamide is rapidly absorbed after oral and dermal administration. After administration of radiolabelled chloroacetamide, radioactivity is rapidly distributed. Two half-lives of elimination can be calculated after oral, dermal and i.v. administration. Whereas the first elimination half-lives range between 5- 6 hrs for all application routes investigated, considerably longer second elimination half-lives of 500 hrs, 520 hrs and 180 hrs were calculated for the iv, oral and dermal route.
Elimination occurs mainly via urine, only minor amounts are excreted via faeces. After oral administration, most radioactivity is distributed into blood, heart, lungs and spleen. After dermal administration, most amounts of radioactivity are distributed into blood, liver and kidneys. Data indicate that approximately 96% and 56% of radioactivity from radiolabelled chloroacteamide can be systemically bioavailable after oral and dermal administration, respectively. Concerning metabolism, there is information available, that chloroacetamide binds to glutathione.

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

A patch test was performed with 209 eczema patients. Single applications of a 0.5% solution of CA 24 (chloroacetamide: 99% purity, sodium benzoate: DAB-7) were used. CA24 was well tolerated and non-irritating. No irritation occurred when there was exposure to sun.

Ref.: 1 (subm II)

From the limited information available, chloroacetamide can be regarded as being non photo-irritating.

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data available

3.3.11. Human data

Skin irritation

The skin damaging potential of the preservative CA 24 was investigated in 10 healthy subjects without dermatological problems and in 15 persons with allergy for benzoic acid, p-hydroxybenzoic acid ester, or Peruvian balsam. The subjects were treated daily for 14 days using a 0.1% aqueous solution of CA 24 (corresponding to 0.07% chloroacetamide) which was applied to skin and then rubbed into the skin. Neither of the subjects developed any signs of skin irritation.

Ref.: 2 (subm II)

Comment
The concentration used for this investigation might have been too low to elicit skin irritation.

Mucous membrane irritation (see also section 3.3.2.2-Mucous Membrane Irritation)

A 5% solution of CA 24 (corresponding to 3.5% chloroacetamide) was applied into the eyes of probands (no further information). After application, discomfort, lacrimation and blurred vision were observed which lasted for 15 – 30 min.

Ref.: AR 33

From the first scientific opinion on 2-chloroacetamide (SCC, 1986), information on human experience is available, the respective study report is not given and study authors could not be retraced: a 0.2% preparation was well tolerated in the eye of 5 humans.

Skin sensitization

A large number of case reports and of reports of positive patch tests in different patient groups and healthy volunteers is available. A non exhaustive list of the frequency of chloroacetamide induced contact allergy is given in table 4; a non-exhaustive list of published case reports is given in table 5. Among the reports given in table 4 that of Prins and Smeenk (1972) clearly demonstrated that allergy in 17 of the patients was attributable to chloroacetamide contained in the ointments. From table 4 it can also be seen, that positive allergic reactions to chloroacetamide can be induced at concentrations of 0.1 or 0.2%, i.e. below those concentrations which are intended to be used for cosmetic products (0.3%). Table 5 shows, that individuals with contact allergies to chloroacetamide are frequently observed among patients at dermatological institutes. It can also be seen, that sources for chloroacetamide exposure not only result from the use of cosmetics but also from other applications of the substance (e.g. from paints, wallpaper glues, substances used at certain workplaces) and that even airborne allergic contact dermatitis is possible (Finkbeiner and Kleinhans, 1994). As chloroacetamide is also frequently used as CA 24, a mixture of choroacetamide and sodium benzoate, one might claim that allergic reaction can also be due to sodium benzoate. Nater (1971) demonstrated in one of his patients, that allergic reactions were clearly due to chloroacetamide and not to sodium benzoate. In chloroacetamide-sensitive patients, a dose-response relationship in skin reactions was observed after challenge with different doses of chloroacetamide (Marzulli and Maibach, 1976).
From the most recent retrospective analysis (Brasch and Uter, 2011) comprising information from 34631 patients, positive reactions to chloroacetamide were reported in

0.83%. This is in line with an older report (Schnuch and Geier, 1997), where the percentages of chloroacetamide allergies were 1% among 11630 women and < 1% among 6389 men.

Table 4: Chloroacetamide-induced contact allergy in different patient groups

Patient information	**Number of patients**	**Substance**	**Concentration**	**Information on frequency of application an reading**	**Results**	**Ref.**
Retrospective analysis of patch test data in Germany from 1995 - 2008	34631	Chloroacetamide not further specified	0.2% pet.	Single, lasting for 2 days	Positive in 0.83%	Brasch and Uter, 2011
Patients with signs of chronic venous insufficiency	36	Chloroacetamide not further specified	0.2% pet	Single, lasting for 2 days	Positive in 5.6%	Gallenkemper et al., 1998
Patients who had attended patch testing	2000	Chloroacetamide not further specified	No information	No information	Positive in 1 patient (0.05%)	Gawkrodger and Paul, 2008
Patients allergic to chloroacetamide	1832	Chloroacetamide not further specified	No information	No information	30 positive reactions (1.6%)	(subm V) Note to CEC on 2-chloroacetamide – COLIPA P27
Eczema patients	209	70% chloroacetamide (purity 99%) and 30% sodium benzoate (DAB-7)	0.5%	single	Non-irritating, well tolerated, no irritation when exposed to sun	Ref.: 1 (subm II) Prof. Dr. Schmidt-La Baume in Zusammenarbeit. Prof. Dr. R. Pfister. Stadt, Hautklinik, Karlsruhe Baden-Baden, 25.5.1966 (Report not available for evaluation)
Eczema patients and patients without dermatological problems	200 humans for single application, 200 humans for daily application during 2 weeks 15 eczema patients for daily application	70% chloroacetamide (purity 99%) and 30% sodium benzoate (DAB-7)	0.1%	Single and daily during 2 weeks	No toxic or allergic skin reactions	Ref.: 2 (subm II) 2. Prof. Dr. med. H. Röckl. Universitäts-Klinik und Poliklinik für Hautkrankheiten,

Patient information	Number of patients	Substance	Concentration	Information on frequency of application an reading	Results	Ref.
	during 2 weeks					Würzburg 16.11.1970
Eczema patients	296	chloroacetamide (purity 99%)	0.2%	single	4 positive reactions of 162 women (2.5%); 3 positive reactions of 134 men (2.3%)	Ref.: 3 (subm II) Prof. Dr. med. K.H. Schulz Universitäts-Hautklinik Abteilung für Allergologie Hamburg 18.12.1984
Male and female patients, mostly Caucasians	150; 117 males and 33 females	Chloroacetamide, manufacturer and purity unknown	0.5%	3 times per week during 3 weeks, repeated application after 14 days	19 positive reactions of 33 women (57.5%): 28 positive reactions of 117 men (24.6%); high degree of positive reactions is discussed to be probably due to impurities	Ref.: 5 (subm I) W.P. Jordan, Jr. and S.E. King. "Delayed hypersensitivity in females" Contact Dermatitis 3, 19-26 (1977)
18 Females in one test; 200 humans in the follow-up	18 and 200	Chloroacetamide, manufacturer and purity unknown	0.18% (in a cream)	No information	2 positive reactions of 18 (11%) 1 positive reaction of 200 (0.5%)	Ref. 6 (subm II) Nater 1971. Allergic reactions due to chloracetamide Dermatologica 142. 191-192
Eczema patients	465 (230 femalesand 235 males)	Chloroacetamide, manufacturer and purity unknown	0.1%	No information (full paper not available)	7 positive reactions of 465 patients (1.5%)	Ref. 7 from submission I: Meynadier (1982): Allergie aux conservateurs, Ann. Dermatol. Venerol. 109, 1017-1023 (Report not

Patient information	Number of patients	Substance	Concentration	Information on frequency of application an reading	Results	Ref.
						available for evaluation)
4 different Patch tests (setting a-d); setting (d) involved 84 eczema patients	14 (a), 10 (b), 102 (c) and 25 (d) humans	70% chloroacetamide (purity 99%) and 30% sodium benzoate (DAB-7)	(a) 1% (b) 0.5% (c) 1% (d) 0.1 and 0.2%	(a) single application (b) single application and irritation by tape stripping (c) no information (d) daily for 2 weeks	No irritation, no positive reaction	Ref.: 10 (subm III) Prof. Dr. med Franz Klaschka, Berlin 26.07.1985 (Report not available for evaluation)
Patients with stasis dermatitis of the lower limbs	104	Chloroacetamide (Merck)	1% in pet.	Performed according to Fregert et al. 1969 (not available for evaluation)	Positive patch tests in 3%	Angelini et al., 1975
Patients not further specified	3254	Chloroacetamide (no further information)	0.1% in pet.	No information	Positive patch tests in 0.3%	Agren et al., 1980
Patients allergic to wool wax	51	Chloroacetamide not further specified	2% in water	No information	Positive reactions in 53.3% of the patients	Auth et al., 1984 (Report not available for evaluation)
Patients not further specified	51	Chloroacetamide not further specified	2% in pet.	No information	Positive reaction in 1 patient (2%)	Lama et al., 1986
						Andersen & Rycroft, 1991
Contact dermatitis patients	501	Chloroacetamide not further specified	0.2% in pet.	Patch test performed according to ICDRG recommendations	Positive reaction in 3 patients (0.6%)	De Groot et al., 1986
Male house painters with current and/or previous skin disease	180	Chloroacetamide not further specified	0.1% in pet.	Patch test not further specified	Positive reaction in 5 patients (2.8%)	Högberg & Wahlberg, 1980
Patients with and without allergy to Hirudoid ointment	100 (a) 125 (b) 25 (c) (patient without allergy to Hirudoid ointment) 27 (d)	CA 24	0.2%in water	Patch test not further specified	Positive reaction in (a) 12 patients (12%) (b) 1 patient(0.8%) (c) 0 patients (0%) 17 patients (63%)	Smeenk & Prins, 1972
Patients with occupational contact dermatitis	736	Chloroacetamide not further specified	No information	Patch test performed according to ICDRG	Positive reaction in 1 patient (0.1%)	Aalto-Korte et al., 1996

Patient information	Number of patients	Substance	Concentration	Information on frequency of application an reading	Results	Ref.
				recommendations		
Evaluation of IVDK data from 1992 - 1995	(a) 11630 females (b) 6389 males	Chloroacetamide not further specified	No information	No information	(a) positive reaction in 1% of the patients (b) positive reaction in < 1% of the patients	Schnuch & Geier, 1997
Metal workers (58 male, 6 female) with contact dermatitis	64	Chloroacetamide not further specified	No information	2 d application, reading at 2 and 4 days	Positive in 5 (male) patients (7.8%)	Shah et al., 1996
Metalworkers with suspected metalworker fluid dermatitis	171	Chloroacetamide not further specified	No information	Single, exposure time 1 or 2 days, reading on day 3	Probably positive in 2 patients (1.2%)	Geier et al., 2004
Patients of a contact allergy clinic	8521	Chloroacetamide not further specified	No information	According to IDCRG Guidelines, reading on day 2, 3 or 4	Positive in 20 patients (0.23%)	Goossens et al., 1998
Patients patch tested to an extended facial/cosmetics series	553	Chloroacetamide not further specified	0.2%	Standard protocol, reading on day 2 and 4	Positive in 5 patients (0.9%)	Hughes and Stone 2007
Female hairdressers with suspected occupational skin disease	36	Chloroacetamide not further specified	No information	No information	Positive in 2 patients (5.6%)	Leino et al., 1998
Chinese eczema patients	506	Chloroacetamide not further specified	No information	Single application, duration: 2 days, readings on days 2 and 3	Positive in 4 patients (0.8%)	Li et al., 2007
Shoefactory workers with and without occupational allergic contact dermatitis (OACDD)	246 (amongst them 16 OACD)	Chloroacetamide not further specified	0.2% (pet.)	Single application, duration: 2 days, readings on days 3 and 4	Positive in 1 patient (0.4%); (positive in 6.24% OACD patients)	Mancuso et al., 1996
Outpatients with suspected contact dermatitis	2295	Chloroacetamide not further specified	0.2% (pet.)	Single application, duration: 2 days, readings on days 2 and 3	Positive in 1.5%	Perrenoud et al., 1994
75 male inmates of a penitentiary, 75 free males and females	150	Chloroacetamide not further specified	0.5% (aq.)	Modified Draize test /Repeat insult patch test (application on 3 days of 3 consecutive weeks, 2 week rest, two consecutive 48 hr challenges)	Positive in 47 probands (31%)	Jordan and King, 1977
Healthy male paid subjects aged 21- 50	205	No information	1.25%	Repeated Insult Patch Test	Positive in 35 test persons (17%)	Marzulli and Maibach,

Patient information	Number of patients	Substance	Concentration	Information on frequency of application an reading	Results	Ref.
years						1973

Comment
The SCCS does not consider HRIPT studies for determining sensitisation potential to be ethical.

Table 5: Chloroacetamide-induced allergic skin reactions – case reports

Patient (age)	Observed lesion	Type of contact with chloroacetamide	Concentration eliciting a positive patch test response [%]	Ref.
Female (25)	Itchy dermatitis on the sides of the fingers of both hands	Baby body lotion	0.18%	Nater, 1971
Male (45)	Hand dermatitis 15 years ago, lasting one year	Wallpaper glue	0.1%	Bang Pedersen and Fregert, 1976
Male (52)	Hand dermatitis	Wallpaper glue	0.1%	Bang Pedersen and Fregert, 1976
Male (30)	Dermatitis of the back of the hands	Cosmetic hand lotion (certain batch)	0.2% (in pet. And in water)	Suhonen, 1983
Female (40)	Dermatitis (face, cheeks, periorbital area)	Facial aerosol spray astringent	0.2% (in pet.)	Koch et al., 1985
Male car mechanic (34)	Chronic (5 year) dermatitis of the hands	Cutting oil used at the workplace	2.0% (pet.)	Lama et al., 1986
Female (46)	Itchy burning swelling of eyelids	Anti-wrinkle serum	0.2% (pet.)	De Groot and Weyland, 1986
Female (25)	Itching erythema with vesicles in the perioral area and on both ears	Ointments for labial herpes	0.07% (aq.)	Detmar and Agathos, 1988
Male fork lift truck driver (48)	15 month history of hand eczema	paint	0.2% (in pet.)	Jones and Kennedy, 1988
Male woodcutter (27)	Acute dermatitis of the dorsa, bilateral pompholyx dermatitis of the soles of the feet	Substances used to treat leather	0.2% (pet.)	Jelen et al., 1989
Male (71)	Redness, swelling and scaling of the vermilion border of	toothpaste	0.2% (pet.)	Macháčková and Smid, 1991

Patient (age)	Observed lesion	Type of contact with chloroacetamide	Concentration eliciting a positive patch test response [%]	Ref.
	the lips			
Male (51)	Contact dermatitis of the forearm	Ointment against mosquito bites	0.001%	Wantke et al., 1993
Female (33)	Acute dermatitis in the face, erythema and desquamation of face and neck, less severe erythema and paules on the forehead and back of the neck, i.e. airborne contact dermatitis	Environment room paint	0.1% (pet.)	Finkbeiner and Kleinhans, 1994
Female (55)	dermatitis of the face, hands, and bilateral axillary dermatitis	Roll-on deodorant containing CA 24	0.2% (pet.)	Taran and Delaney, 1997
Female (55)	Contact dermatitis, erythema, pruritus and scaling of the neck	Hair dye	No information	Assier-Bonnet and Revuz, 1999
Male carpenter (36)	Dermatitis on palms and fingertips	glue	0.2% (pet.)	Pereira et al., 1999
Male pensioner (57)	Dermatitis on face, neck and chest	Facial cleanser	No information	Klaschka, 1975
Female worker (43) in cosmetics factory	Dermatitis on fingers and right hand	disinfectant	0.5%	Klaschka, 1975
Male worker in a factory producing modelling plasticine (50)	Acute dermatitis of the face, forearms and lower arms	Plasticine and preventol ® D5	0.2% (in pet.)	Farli et al., 1987
No information	Eczema patient having contact sensitivities	Ingredients of vehicles (of ointments)	0.5% (in pet.)	Hannuksela et al., 1976
Male enginefitters apprentice (19)	hand eczema of 1-year duration	Preservative in coolant oil	5.0 % (in pet.)	Hjorth, 1979
Female secretary (27)	history of allergic reactions to iodine derivatives	body massage cream	0.1% in yellow petrolatum; 0.015% in a body massage cream	Dooms-Goossens et al., 1981
Male (59)	Itchy, symmetrical, red, scaly oedema of the eyelids	Wall paint	0.3% (in aq.)	Bogenrieder, 2001
Female student (22)	Burning eczema on face, neck, scalp and retro-auricular region	Cosmetics or material used for chemical engineering	0.1% (pet.)	Dooms-Goossens et al., 1990

Patient (age)	Observed lesion	Type of contact with chloroacetamide	Concentration eliciting a positive patch test response [%]	Ref.
Female machine operator (59)	Suspected occupational contact dermatitis	Cooling fluid	No information	Slodownik et al., 2009

Summary on human data

Repeated application of a 0.1% solution of CA 24 to the skin of human volunteers for 14 days did not induce skin irritation. However, the concentration of chloroacetamide (0.07%) might have been too low to elicit irritating effects.
Chloroacetamide was well tolerated in the eyes of 5 humans when applied as 0.2% solution; when applied as a 5% solution of CA 24 (3.5% chloroacetamide), discomfort, lacrimation and blurred vision were observed. Thus, at least at higher concentrations, chloroacetamide has a mucous membrane irritating potential in humans.
Numerous reports are available demonstrating that chlorocetamide can elicit contact allergy in humans. It could be verified for many products that chloroacteamide is the causative agent for contact allergies and that allergic reactions can be caused at concentrations lower than those intended to be used in cosmetic products. Retrospective surveys reveal, that the percentage of chloroacetamide allergy is around 1%.

3.3.12. Special investigations

In isolated hepatocytes from male Sprague-Dawley rats, the effects of chloroacetamide on glutathione (GSH) depletion, lipid peroxidation and cell lysis were investigated. Primary hepatocytes (obtained by the collagenase/perfusion method) were incubated with chloroacetamide (concentration of chloroacetamide: 0.2 mM) for 4 to 5 hours. During the first hour of incubation, a sharp drop in intracellular glutathione content could be observed with further drop until the end of the incubation period. Cell membrane permeability (measured by latency of NADH oxidation) increased sharply after two hours of incubation with further increase up to 5 hrs. Lipid peroxidation (measured as accumulation of malondialdehyde in the incubated cells) also increased sharply after 2 hrs of incubation with further increase thereafter. By adding methionine which stimulates hepatocellular glutathione synthesis, it was possible to prevent these affects.

Ref.: AR 5

Male Sprague-Dawley rats received single intraperitoneal doses of 37.5, 75 and 112.5 mg chloroacetamide/kg bw. Animals were killed at different time points after administration and livers were removed. Glutathione (GSH) and thiobarbituric acid (TBA)-reactive material was determined in liver homogenates. For some animals, small pieces of liver tissue were fixed and stained for histological examination. In animals receiving 75 mg/kg bw, hepatic glutathione content fell by approximately 90% during the first hour, but recovered within 48 to 72 hours. Three to six hours after chloroacetamide administration, lesions developed in the peripheral midzonal parts of the hepatic lobules (swelling and hydropic degeneration). Hydropic degeneration of hepatic lobules showed remission by 1/3 one week after treatment. During this period of time, an enhancement of lipid peroxidation was measured by means of the thiobarbituric acid test. A single i.p. dose of 37.5 mg/kg bw had no effect on rat liver cell morphology, whereas the dose of 112.5 mg/kg bw caused death of three of four animals after 4-5 hours (see also section 3.3.1 Acute toxicity). Histopathological examination of the livers of these animals revealed fatty degeneration, necrosis and leucocytic infiltration. Repeated i.p. injections of 37.5 mg/kg bw every second day resulted in hepatocellular swelling and hydropic degeneration within 2 weeks, which were comparable to the effects observed after single administration of 75 mg/kg bw. The results

demonstrate, that mechanisms previously observed in vitro (Anundi et al., 1979) also take place in vivo in certain areas of the liver.

Ref.: AR 6

Male Sprague-Dawley rats received i.p. doses of 70, 80 and 150 mg/kg bw chloroacetamide. Immediately after substance administration, animals were placed in closed breathing chambers where air circulated. The effect of chloroacetamide on lipid peroxidation was investigated by an ethane/pentane breath test, i.e. exhaled ethane and pentane exhaled over periods of 2 and 4 hours were measured. At the lowest dose level, amounts of ethane and pentane at 4 hrs were small but increased with increasing dose, thus indicating the induction of dose-dependent lipid peroxidation in vivo. Remarkable increase of ethane and pentane exhalation was observed at the highest dose after 2 hrs. Further, two animals of the top dose died after 2 hours. The findings are interpreted by the authors as chloroacetamide-induced lipid peroxidation in vivo.

Ref.: AR 16

Summary on special investigations

In one *in vitro* and two *in vivo* studies it was demonstrated that chloroacetamide caused glutathione depletion and lipid peroxidation. These events are key events in the formation of cell damage and of morphological changes in the liver of animals treated i.p. with chloroacetamide.

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Chloroacetamide

(I) MoS calculation based on an oral 13 week study in rats.
NOAEL: 10 mg/kg bw/d based on testis effects in male animals and enlarged thyroids in female animals

An aggregate value of 17.4 g/d for the amount of cosmetics applied daily is taken for MoS calculation according to the SCCS's Notes of Guidance (2011).

Absorption through the skin	**DAp (%)**	**=**	**56%**
Amount of cosmetic product applied daily	**A (g/d)**	**=**	**17.4 g/d**
Concentration of ingredient in finished product	**C (%)**	**=**	**0.3%**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose	**(SED)**	**=**	**0.4872**
A (g/d) x 1000 mg/g x C (%)/100 x Da_p (%)/100 /60			
No adverse observed effect level (13 week oral rat study)	**NOAEL**	**=**	**10 mg/kg bw/d**

MOS	**NOAEL/SED**	**=**	**21**

(II) MoS calculation based on a dermal 30 day study in rats
NOAEL: 50 mg/kg bw/ d based on systemic effects (dose-dependent histopathological changes in liver, heart and spleen) after dermal administration

An aggregate value of 17.4 g/d for the amount of cosmetics applied daily is taken for MoS calculation according to the notes of guidance.

Dermal dose **A (g/d) x 1000 mg/g x C (%)/100 /60**		**=**	**0.87**
No adverse observed effect level (30 day dermal rat study)	**NOAEL**	**=**	**50 mg/kg bw/d**
No adverse observed effect level (corrected for study duration from subacute to subchronic by a factor of 3)	**NOAEL**	**=**	**16.6 mg/kg bw/d**

MOS corrected	**NOAEL/SED**	**=**	**19**

MoS calculations based on the NOAEL from oral and dermal studies lead to an almost equal outcome.

3.3.14. Discussion

Physico-chemical properties
Chloroacetamide is white crystalline powder which is readily soluble in water and in some organic solvents. Information on impurities and physicochemical data is available from IUCLID data. The purity of technical chloroacetamide is at least 98%. For most studies utilized for this report, the exact specification of the substance cannot be traced back. Also, only limited information on the methods applied for determination of physico-chemical properties is available.
For many applications, a mixture of 70% chloroacetamide and 30% sodium benzoate, called CA 24 is used. Toxicological studies have been performed either with chloroacetamide itself or with CA 24.

Acute toxicity
The acute toxicity of chloroacetamide has been investigated after oral, subcutaneous and intravenous application of the substance to various animal species. No data are available for acute dermal and acute inhalation toxicity. Studies are poorly reported and information on signs of toxicity is lacking. However, from the results of the available studies chloroacetamide can be considered as toxic, because oral LD_{50} values are in the range between 31 and 370 mg/kg bw. After i.p. administration of doses of 75 and 112.5 mg/kg bw chloroacetamide to rats, morphologic changes in the liver were observed.

Skin and mucous membrane irritation
Skin irritation: One guideline-compliant study in rabbits is available, where chloroacetamide was slightly irritating to the skin. Three older studies not performed according to acknowledged test guideline or GLP are available. One study was performed using CA 24, the others using pure chloroacetamide. When solutions between 1 and 10% chloroacetamide were used, no irritating reactions were observed. A 25% solution on the other hand, caused erythema in the skin of guinea pigs. In other dermal studies not specifically addressing skin irritation, skin reactions were reported: in a dermal subacute study in rabbits, encrustations were observed starting with the lowest dose of a 40%

solution. In a maximisation test in guinea pigs, a 3% solution of chloroacetamide induced an inflammatory skin reaction.
From the available data it can be derived, that chloroacetamide – at least when used in higher concentration – has a skin irritating potential.

Mucous membrane irritation: Mainly poorly described information on mucous membrane irritation in rabbits and humans is available, there were no original study reports or study publications for evaluation. In a guideline-compliant study in rabbits, 100 mg chloroacetamide was irritating, whereas in two other (non-guideline) studies on rabbits, 10% chloroacetamide, an ointment containing 1% chloroacetamide and 5% CA 24 (corresponding to 3.5% chloroacetamide) were non-irritating to the eyes; however, 10% chloroacetamide caused redness in the eye of 1 of four animals. In humans, a 0.2% solution was judged to be well tolerated, whereas a 5% concentration of CA 24 (corresponding to 3.5% chloroacetamide) caused discomfort, lacrimation and blurred vision.
From the information available it is concluded, that pure chloroacetamide and concentrations equal or higher than 3.5% have a mucous membrane irritating potential.

Skin Sensitisation
In animal studies, chloroacetamide was sensitising in a maximisation test performed according to OECD TG 406. An older maximisation tests was negative. Modified Buehler assays performed in guinea pigs using either pure chloroacetamide (concentration: 1%) or CA 24 at concentrations between 0.1 and 3% were negative. Two further assays in guinea pigs (an open epicutaneous test using concentrations of 1% and 3% chloroacetamide and a modified Draize test) were also negative. Thus, in animals, the sensitising potential of chloroacetamide is low based on mostly poorly described tests which have mainly not been performed according to modern standards with respect to Test Guidelines and Quality assurance.

Dermal absorption
Based on an in vivo toxicokinetic study in the rat, a 56% dermal absorption value is derived.

Repeat Dose toxicity
Short-term (14d), subacute (28d/30d) and subchronic (90 d) repeat-dose studies on chloroacetamide have been performed but none of them met modern standards with respect to test guideline compliance and quality assurance. Most of the original study reports were not available for evaluation and most of the studies were poorly described. Subacute studies have been performed by the oral, dermal, subcutaneous and intravenous route in rats and rabbits. In these studies, apart from local and acute effects, systemic effects on liver (e.g. fatty infiltration of the liver, degenerative macroscopic changes), kidneys, spleen and heart were observed. Three subchronic studies in rats using the dietary administration pathway are available. In male animals, the reproductive tissues were adversely affected: testes and epididymes were identified as target tissues. Size reductions were observed for both tissues and for testes, significant weight reduction was observed. Spermatogenesis was impaired dose-dependently and Leydig cell proliferation was observed. In female animals, reductions in liver sizes and enlargement of thyroid glands was observed. At high doses (50 mg/kg bw/d), increases of leucocytes were observed in both sexes. In a teratological study where Wistar rats received chloroacetamide by gavage from GD 7 to GD 17, reduced thymus weight and shrinking of the thymus was observed in female animals at a dose level of 48 mg/kg bw at termination of the study. A poorly described subchronic dermal study with CA 24 in rats gave no indications of pathological changes attributable to the substance.
Effects on the male reproductive system were the most sensitive effects observed in subchronic studies. Based on testis effects, an oral NOAEL of 10 mg /kg bw/d and an oral

LOAEL of 12.5 mg/kg bw/d were derived from 90-d studies. From repeat-dose studies available, an oral NOAEL of 10 mg/kg bw/d and a dermal NOAEL of 50 mg/kg bw/d were taken for two possibilities of MOS calculation.

Mutagenicity
In vitro, chloroacetamide was non-mutagenic in three Salmonella assays using S. typhimurium strains TA98, 100, 1535, 1537 and 1538 and in a gene mutation test using Syrian hamster embryo cells (with and without metabolic activation, respectively). One bacterial in vitro fluctuation test using Klebsiella pneumoniae yielded positive results. In vivo, negative results were obtained in a Chinese hamster chromosome aberration test examining bone marrow and spermatogonia, in a micronucleus test in Chinese hamster, a micronucleus test in mice and in a dominant-lethal test performed in mice. From the results of the in vitro and in vivo assays performed it can be deduced, that chloroacetamide is not mutagenic and genotoxic.

Chronic Toxicity and Carcinogenicity
No data available

Reproductive toxicity
No guideline-compliant studies on reproductive toxicity of chloroacetamide are available. In two poorly described teratological studies, which used the oral and the subcutaneous application pathway, no malformations in foetuses were observed after treatment of maternal animals during certain days of gestation. However, as in both studies the substance was administered in single days during gestation, the critical window of development might not have been targeted. In a dominant-lethal test in NMRI mice, fertility was clearly impaired by chloroacetamide. In a further study, the effects chloroacetamide on development and reproductive organs of the offspring were investigated after oral gavage administration to maternal animals during gestation. No adverse effects on reproductive tissues of the offspring were observed after repeated administration of 24 mg/kg bw/d from GD 14 to PND 2. Based on body weight reduction in maternal animals and based on skeletal findings in the offspring, a NOAEL of 3 mg/kg bw/d was derived for dams and pups.

Toxicokinetics
Chloroacetamide is rapidly absorbed after oral and dermal administration. After administration of radiolabelled chloroacetamide, radioactivity is rapidly distributed. Two half-lives of elimination can be determined after oral, dermal and i.v. administration. Whereas the first elimination half-lives range between 5- 6 hrs for all application routes investigated, considerably longer second elimination half-lives of 500 hrs, 520 hrs and 180 hrs were calculated for the iv, oral and dermal route.
Elimination occurs mainly via urine, only minor amounts are excreted via faeces. After oral administration, most amounts of radioactivity are distributed into blood, heart, lungs and spleen. After dermal application, most amounts of radioactivity are distributed into blood, liver and kidneys. Data indicate that approximately 96% and 56% of radioactivity from radiolabelled chloroacteamide can be systemically bioavailable after oral and dermal dosing, respectively. Concerning metabolism, there is information available, that chloroacetamide binds to glutathione.

Human data
Repeated application of a 0.1% solution of CA 24 (corresponding to 0.07% chloroacetamide) to the skin of human volunteers for 14 days did not induce skin irritation. However, the concentration might have been too low to elicit skin irritating effects. Chloroacetamide was well tolerated in the eyes of 5 humans when applied as 0.2% solution;

when applied as a 5% solution of CA 24 (corresponding to 0.35% chloroacetamide), discomfort, lacrimation and blurred vision was observed. Thus, at least at higher concentrations, chloroacetamide has a mucous membrane irritating potential in humans.
Numerous reports are available, which demonstrate that chlorocetamide can elicit contact allergy in humans. It has been shown for many products that chloroacteamide is the causative agent for contact allergies and that allergic reactions can be caused at concentrations lower than those intended to be used in cosmetic products. Retrospective surveys reveal, that the percentage of chloroacetamide allergy is around 1%.

Special investigations
In one in vitro and two in vivo studies it was demonstrated that chloroacetamide caused glutathione depletion and lipid peroxidation. These events are key events in the formation of cell damage and of morphological changes in the liver of animals treated i.p. with chloroacetamide.

4. CONCLUSION

On the basis of the data available, the SCCS comes to the conclusion that 2-chloroacetamide is not safe for consumers when used under the current use conditions of 0.3% in cosmetic products.

Human data demonstrate that allergic reactions can be elicited at concentrations lower than 0.3% (use conditions in cosmetics products).

5. MINORITY OPINION

Not applicable

6. REFERENCES

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