Scientific Committee on Consumer Safety

SCCS

OPINION ON

Quaternium-15 (cis-isomer)

COLIPA n° P63

The SCCS adopted this opinion at its 13th plenary meeting

of 13-14 December 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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TABLE OF CONTENTS

1. BACKGROUND

Quaternium-15 (INCI name) with the chemical name Methenamine 3-chloroallylochloride and EC n° 223-805-0 and CAS n° 4080-31-3 is currently regulated as a preservative in Annex VI, entry 31 of the Cosmetic Directive in a concentration up to 0.2%.

Submissions I and II were provided by Colipa [1] in 1983 and 1984, respective to support the continued use of this up to then provisionally allowed preservative.

An opinion was expressed by the Scientific Committee on Cosmetology in 1986 with the statement that "*a short–term oral study with dose-levels sufficiently high to induce a systemic effect, information on dermal absorption through human skin, and a chromosomal aberration test is needed to evaluate this substance. Information is also requested on the purity of the substance and on the presence of the trans-isomer.*

However, the Committee sees no objections to maintaining the use of this substance as preservative in cosmetic products".

From the above opinion it could be inferred that the Quaternium-15 is a mixture of isomers, where the *cis*-form is the dominant form and where the *trans*-form is the minor component present as an impurity.

Recently, the *cis*-form has been classified as a CMR substance with the classification toxic to reproduction, category 2 (GHS). This classification only concerns the cis-isomer:

Cis-1-(3-chloroallyl)-3,5,7-triaza-1-azoniaadamantane chloride (EC n° 426-020-3; CAS n° 51229-78-8) referred to as *cis*-CTAC.

Whereas the current entry in the Cosmetics Directive refers to:

Methenamine 3-chloroallylochloride (EC n° 223-805-0; CAS n° 4080-31-3), which designates the mixture of *cis-/trans*-CTAC.

A new dossier was submitted to support a continuation of the use of Methenamine 3-chloroallylochloride as a preservative. The current submission only covers the *cis*-isomer, which, according to the information provided by the applicant, is also the one traditionally sold for cosmetic preservation. Consequently, should the SCCS establish the safety of this ingredients at the intended use concentration, it is planned to change the entry for the Methenamine 3-chloroallylochloride to cover only the cis-form with the name: *Cis*-1-(3-chloroallyl)-3,5,7-triaza-1-azoniaadamantane chloride (EC n° 426-020-3; CAS n° 51229-78-8).

2. TERMS OF REFERENCE

- *Based on the scientific data provided, does the SCCS consider that Cis-1-(3-chloroallyl)-3,5,7-triaza-1-azoniaadamantane chloride is safe for the consumers, when used as a preservative in a concentration up to 0.2% in cosmetic products?*

- *Does the SCCS have any scientific concerns for the continued use or any modification in the specifications for the substance?*

[1] Colipa – European Cosmetics Association

3. OPINION

Preamble
According to information received from the applicant, it is exclusively cis-CTAC which is used in cosmetic products. Cis-CTAC has been classified as a CMR substance with the classification toxic to reproduction, category 2 (GHS). However, for toxicity testing, in many studies, especially in the newer ones, the applicant uses a mixture of cis-/trans-CTAC instead of using cis-CTAC, referring to a study of Rodan and Clark 1987, who claim that "the two isomers are very similar". This argument is not relevant in this context. Principally the state of the art demands to use that substance for testing that is in question, not a surrogate. Moreover there is no reason to use a mixture of cis-/trans-CTCA instead of cis-CTAC because Cis-CTAC is readily available. The SCCS therefore does not accept to use a mixture of cis-/trans-CTAC for toxicity testing.

The studies where a mixture of cis-/trans-CTCA are taken may be considered for information and may be supportive, if the results of these studies are interpreted with care taking into account that only the cis-isomer may be the active compound.

Therefore, this evaluation is based on data obtained with cis-CTAC. Data generated with a mixture of cis- and trans-CTAC, which also were submitted by the applicant, are included in the annex to the opinion

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Quaternium-15 (INCI-name, refers to the mixture of cis-/trans-isomers)

3.1.1.2. Chemical names

cis-1-(3-chlorallyl)-3,5,7-triaza-1-azoniaadamantane chloride (cis-CTAC)

3.1.1.3 Trade names and abbreviations

DOWICIL™ 200 Preservative
DOWICIL™ 150 Antimicrobial
cis-CTAC

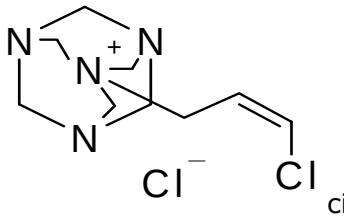
Similar products cis-/trans-isomer mixture
DOWICIL™ 75 Preservative
DOWICIL™ 100
CTAC

COLIPA P63

3.1.1.4 CAS /EC number

CAS: 51229-78-8 (cis-CTAC)
4080-31-3 (cis-/trans-CTAC)EC: 426-020-3 (cis-CTAC)
223-805-0 (cis-/trans-CTAC)

3.1.1.5 Structural formula



cis-CTAC

3.1.1.6	Empirical formula

Formula: $C_9H_{16}N_4Cl_2$

3.1.2	Physical form

Off-white powder at room temperature

3.1.3	Molecular weight

Molecular weight: 251.2 g/mol

3.1.4	Purity, composition and substance codes

DOWICIL™ 150 Antimicrobial, ASG 10387427: The identity of the test substance has been confirmed using IR, FTIR, 1H and 13C NMR, HRMS and HPLC-MS. The spectra/chromatograms were supplied (Ref. 29).

DOWICIL 200, Lot SL2001QT2P: the identity of the test substance has been examined using IR-spectroscopy

Comments

Only for DOWICIL™ 150 Antimicrobial, ASG 10387427, but not for DOWICIL 200, Lot SL2001QT2P, state of the art identification data has been submitted.

DOWICIL™ 150, ASG 10387427(Lot QT091306B1-2)
According to applicant:

98.6% cis-1-(3-chlorallyl)-3,5,7-triaza-1-azoniaadamantane chloride (HPLC)
0.7% trans-1-(3-chlorallyl)-3,5,7-triaza-1-azoniaadamantane chloride (HPLC)
0.3 ± 0.1% hexamethylenetetramine (HMTA) (HPLC)
0.42 ± 0.06% water (Karl Fischer coulorimetric titration)
< 0.02%Benzoic acid

DOWICIL 200, Lot SL2001QT2P
The applicant analyzed the purity using Karl-Fischer titration and HPLC.

Purity:	98.6% cis isomer
Impurities:	0.651% trans isomer
	0.029% hexamethylenetetramine
	0.047% methyl-3,5,7-triaza-1- azoniaadamantane chloride(area)
	1.06 ± 0.01% water
	<LOD Benzoic acid

CTAC lot 157-9-4:
81.0% cis-/trans-CTAC (NMR)
6.9% HMTA HCl (NMR)
92.2% CTAC (titration)
3.7% HMTA (titration)

Ref.: 24

In 1996 the applicant analyzed 5 batches of CTAC using titration, HPLC and GC to test for purity and impurities (Ref. 45)

Descriptive summary with conclusions of the applicant:
Preliminary analysis of five representative batches of DOWICIL 150 Antimicrobial samples (Lot#s WP 950330 684B, WP950323 668B, WP 950408 700B, WP950411 705B and WP950419 721B) were conducted as part of the data requirements for registration with EPA. The samples were analysed by acidity and chloride titration, high pressure liquid chromatography (HPLC), gas chromatography (GC) and Karl Fisher titration. The concentration ranges of analytes as determined by the stated methods are listed below:

Acidity / chloride titration:	active ingredient (cis-CTAC) 97.38 – 97.65 wt%
HPLC:	cis-CTAC 95.7 - 96.4 wt%: trans-CTAC 0.20 – 0.26 wt%; benzoic acid 0.59 - 0.67 wt%; methyl quat 0.14 – 0.24 wt%; HMTA 0.77 – 0.94 wt%; cis-allyl < 0.005 wt%; trans-allyl < 0.005 wt%
GC:	no volatile organic impurities were detected in any of the samples at a detection limit of < 0.001 wt% for any individual component
Headspace Karl Fisher Titration:	water 0.51 – 0.62 wt%

Table 1: Information on test materials from submission 2009.

Reference	Test material & purity	Lot no.	Characterisation report or certificate of analysis (CoA) available	NMR/FTIR	Solubility & Stability in test matrices over duration of testing
Hansen 2008 (Ref. 23)	DOWICIL™ 75 (cis-/trans-CTAC) Non-Radiolabeled: 31.3% cis-1-(3-Chloroallyl)-3,5,7-triazo-1-azoniaadamantane chloride, 32.5% trans-1-(3-Chloroallyl)-3,5,7-triazo-1-azoniaadamantane chloride, 33% sodium bicarbonate, 3.1% hexamethylene-tetramine Radiolabeled DOWICIL™ 75 PRESERVATIVE-RL, 62.3% Radiolabeled DOWICIL™ 75 PRESERVATIVE-SC, 89.9%	Non-Radiolabeled-Lot SH2701QT1P Radiolabel- ^{14}C-cis-/trans-CTAC-R Lot # 358100R5 ^{14}C-cis-/trans-CTAC-SC Lot # ELM020907	Yes (Non-Radiolabeled)	Yes (Non-Radiolabeled)	Stability-Yes (Non-Radiolabeled)
Murli 1994	cis-CTAC (Dowicil™ 200) 98.1% by NMR and 97.0%	Lot	Yes	Yes	Solubility-

Reference	Test material & purity	Lot no.	Characterisation report or certificate of analysis (CoA) available	NMR/FTIR	Solubility & Stability in test matrices over duration of testing
(Ref. 38)	by titration	WP930510470B			Yes Stability-Not reported
Day 2000 (Ref. 15)	cis-CTAC (Dowicil™ 200) 98.7%	Lot NE2801QT1P	Yes	Yes	Not reported
Cifone 2002 (Ref. 11)	cis-CTAC (Dowicil™ 200) 99.3%	Lot NH1901QT1P	Yes	Yes	Not reported
Carney 2006 (Ref. 8)	ccis-/trans-CTAC purity 62.9% active ingredients, comprised of 30.9% (area normalized) cis-1-(3-chloroallyl)-3,5,7-triaza-1-azoniaadamantane chloride and 32.0% (area normalized) Trans-1-(3-chloroallyl)-3,5,7-triaza-1-azoniaadamantane chloride	Lot SH2701QT1P	Yes	Yes	Stability-Yes
Carney 2005 (Ref. 7)	Dowicil™ 200- 98.9%	Lot SL2001QT2P	Yes	Yes	Stability-Yes
Carney 2008 (Ref. 6)	cis-/trans-CTAC (Dowicil™ 75) 31.3% cis-CTAC, 32.5% trans-CTAC	Lot SH2701QT1P	Yes	Yes	Stability-Yes

Comment
The original analytical data for the substances in the table, like chromatograms etc., have not been supplied.

3.1.5 Impurities / accompanying contaminants

See 3.1.4.

3.1.6 Solubility

The test substance is soluble in all proportions from 10% to over 50% in distilled deionised water at 10°C, 20°C and 30°C and in pH 5 and pH 9 buffers at 20°C.

3.1.7 Partition coefficient (Log P_{ow})

Log Po/w: at neutral pH: <-2 at 25 °C ± 1 °C
pH 5: <-2 at 25 °C ± 1 °C
pH 9: <-2 at 25 °C ± 1 °C

The test substance was not detected in the octanol layers. The log P_{ow} was set at the lower limit of the partition coefficient test according to OECD TG 107. EC Method A.8

Ref.: 29

3.1.8 Additional physicochemical specifications

Melting point:	192 °C (decomposition)
Boiling point:	Boiling point cannot be measured; the test material decomposes before melting (Ref. 29).
Flash point:	/
Vapour pressure:	9.0 x 10-5 Pa at 25°C (estimated vapour pressure; Watson Correlation) < 1 x 10-5 Pa at 25°C (theoretical calculation)
Density:	mean apparent density = 0.41 g/ml at ambient temperature CIPAC MT33 (Ref. 29))
Viscosity:	/
pKa:	/
Refractive index:	/

3.1.9. Stability

After 2 weeks storage at 54 +/-2°C, the test substance DOWICIL™ 150 l, ASG 10387427, showed a recovery of 98.8% (w/w) +/- 3.5% (HPLC).

Dependent on pH (7 and 9), 4% (w/w) aqueous solutions of CTAC decreased in concentration between 2.9 and 20% within 24 h.

The solutions of CTAC used to investigate reproductive toxicity were shown to be stable and homogenous in the time between preparation and application.

3.2. Function and uses

Quaternium-15 (according to the applicant only the cis-isomer) is used as a preservative in a number of rinse-off and leave-on cosmetic products. According to the current EU legislation, the preservative is allowed in cosmetics up to 0.2%[1].

Amongst others, Quaternium-15 is used in barrier cream, hair cream, body shower product, shampoo, hair conditioner, body lotion, body milk, after sun lotion and moist toilet tissues, sunscreen, face powder, hairspray, baby shampoo, baby body lotion, baby bath preparation.

Cis-CTAC is also used as a biocide in several product categories according to the EU Biocide Directive[2]: as in-can preservative (product type (PT) 6), fibre, leather, rubber and polymerised preservative (PT 9) slimicide for paper and pulp (PT12) and metal working-fluid preservative PT13).

CTAC is known to be a formaldehyde releaser. Therefore a consumer product containing CTAC may also contain free formaldehyde.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline:	not in compliance
Species/strain:	rat/CDF
Group size:	6/sex/dose
Test substance:	Dowicil 200 (cis-CTAC)
Batch:	Lot 12158001OB
Purity:	94%
Dose:	200, 400, 800, 1600, 3200, 6300 mg/kg bw, in water
Application route:	single oral gavage
Observation period:	14 days
GLP:	not in compliance
Study date:	1981

Rats were evaluated for signs of toxicity periodically throughout the 14-day observation period. Each animal was weighed before the study, at the day of treatment, and after one and two weeks. Necropsy and gross pathological examination was performed on all survivors.

Results
At a dose of 3200 mg/kg bw 5 male and 5 female animals died. At the highest dose all (12) animals died. The following in-life observations were noted: lethargy, diarrhoea, palpebral closure, lacrimation, exudate staining of the nares, and body tremors. At the scheduled necropsy, no treatment-related changes were observed. There were no significant effects on body weights of the survivors.

[1] Council Directive 76/768/EEC, Annex VI, entry 31

[2] Commission Regulation EC/1451/2007

The acute oral median lethal dose was calculated by the moving average method. The LD_{50} was calculated to be 2664 mg/kg (95% confidence intervals, 1836-3512 mg/kg).

Conclusions
It was concluded that DOWICIL 200 is of low acute oral toxicity in rats.

Ref.: 9

3.3.1.2. Acute dermal toxicity

Guideline:	not in compliance
Species/strain:	rabbit
Group size:	2/sex/dose
Test substance:	Dowicil 200 (cis-CTAC)
Batch:	Lot 12158001OB
Purity:	94%
Dose:	160, 320, 630, 1300, 2500, 5000 mg/kg bw test material with 5 ml water 250, 500, 2000 mg/kg bw as 50% aqueous solution
Application route:	dermal
Observation period:	14 days
GLP:	not in compliance
Study date:	1981

The test material was applied under a heavy-gauge Saran film sleeve held in place with rubber bands. The animals were placed in individual holding cages with free access to food and water. After 24 hours, the sleeves were removed, and the skin was washed with soap and water. The animals were observed for the following 2 weeks for signs of toxicity. Body weights were recorded before and after exposure and at 1 and 2 weeks post treatment. All rabbits were submitted to gross pathological examination.

Results
Application of the test material together in 5 ml water produced external lesions at the site of application in all dose groups. Deaths occurred in all dose groups except the lowest. Internal lesions were not observed for the lower dose groups up to 1300 mg/kg bw. The kidneys from surviving animals of the 2500 mg/kg bw dose group were pale and mottled. The surviving animals of the 5000 mg/kg bw dose group exhibited a decreased amount of abdominal adipose tissue and thymic atrophy In the experiment using a 50% aqueous solution, deaths occurred in all dose groups. Gross examination of surviving rabbits at time of necropsy revealed no evidence of systemic lesions attributed to treatment with the test material.

The acute percutaneous LD_{50} calculated by moving average method was 923 mg/kg bw (86.8-9972 mg/kg bw; 95% confidence interval).

The acute percutaneous LD_{50} after testing with 50% aqueous preparations was calculated to be 605 mg/kg bw (102- 1559 mg/kg bw; 95% confidence interval)

Ref.: 9

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline:	not in compliance
Species/strain:	rabbit/New Zealand White
Group size:	6 (1 male and 5 females)
Test substance:	DOWICIL™ 200 Preservative (cis-CTAC)
Batch:	Lot# 121580010B
Purity:	94%
Dose:	0.5 grams of undiluted test material was applied
Observation period:	24 and 48 hours after the beginning of the dosing period and up to 72h after termination of exposure
GLP:	not in compliance
Study date:	Sept. 1981

The undiluted test material was applied to an intact and abraded site of the back of each animal and the sites were covered with gauze. Patches were covered with Saran film to retard any evaporation.

Results
Application of the test material to intact and freshly abraded skin on the backs of the rabbits resulted in slight (2/6) to moderate redness (2/6), and slight (5/6) to moderate swelling (1/6) of the skin. There was no complete resolution of the dermal irritation signs 72 h after termination of exposure. The Draize Primary Irritation Score was calculated to be 1.2 out of a possible 8.0.

Conclusions
It was concluded that the test material is slightly irritating when placed on the intact and abraded backs of rabbits.

Ref.: 9

3.3.2.2. Mucous membrane irritation

Guideline:	not in compliance
Species/strain:	rabbit/New Zealand White
Group size:	9
Test substance:	DOWICIL™ 200 Preservative (cis-CTAC)
Batch:	Lot # WP121580010B
Purity:	94%
Dose:	0.1 g
Observation period:	days 1, 2, 3, 4, and 7
GLP:	not in compliance
Study date:	September 1981

The test material (0.1 g) was instilled into the conjunctival sac of the right eye of 6 rabbits, and the left eye served as the control. The test material (0.1 g) was instilled into the conjunctival sac of the right eye of the remaining 3 rabbits, rinsed with tap water after 30 seconds; the left eyes served as controls.

Results
Instillation of the test material into the unwashed eyes of 6 rabbits resulted in slight (3/6) or moderate (1/6) redness, and slight discharge (1/6). Corneal opacity was not observed. Signs of eye irritation were absent in all animals by 72 hours post exposure.

Instillation of the test material into the subsequently washed eyes of 3 rabbits resulted in slight redness (2/3). Corneal opacity was not observed. Signs of eye irritation were absent in all animals by 48 hours post exposure.
Draize scoring was 0-4 out of 110 possible. The average for the unwashed eyes was 1.4, and 1.3 for the washed eye.

Conclusion
It was concluded that the test material is slightly irritating to the eyes of rabbits.

Ref.: 9

3.3.3. Skin sensitisation

Guideline:	/
Species/strain:	guinea pigs/Hartley
Group size:	10/group
Test substance:	DOWICIL™ 200 Preservative (cis-CTAC)
Batch:	Lot # WP121580010B
Purity:	94%
Dose levels:	Induction: 10% solution of the test material Challenge: 0.1 mL of a 10% solution of the test material
Vehicle:	Dowanol DPM/Tween 80 (9:1)
Adjuvant:	0.2 mL Freund's adjuvant injected intradermally
Route:	Challenge: Both flanks were clipped and challenged with the test material & solvent or positive control. Challenge sites were unoccluded
Positive control:	10 animals treated with DER 331 epoxy resin
Negative control:	in the challenge phase the vehicle only was applied on one of the clipped flanks
Observation period:	Each time the test material was applied and 24 and 48 hours following the challenge application.
GLP:	not in compliance
Study date:	September 2001

Induction:
Ten guinea pigs received 4 applications of the test material preparation within 8 days during the insult phase of the test. An additional group of 10 guinea pigs were treated with DER 331 epoxy resin as a positive control. Each insult application was 0.1 mL test material or positive control applied to a gauze square and held in place with adhesive tape.

The first insult was in place 24 hours, and then removed for another insult immediately. At the time of the third insult, 0.2mL Freund's Adjuvant was injected intradermally to the insult site. Two days later, the patch was removed, and a fourth insult applied for 48 hours. Each time the test material was applied, observations of irritation effects were recorded. Animals were allowed a 2 week rest period prior to challenge application.

Challenge:
Both flanks of the animals were clipped and challenged with the test material, with the solvent only or the positive control. Challenge sites were left unoccluded.

Results
The positive control DER 331 sensitized 7 of 10 animals. The test material resulted in sensitisation of 1 of 10 animals.

Conclusions
It was concluded that standardised treatment with *cis*-CTAC did not cause allergic sensitization in a Guinea pig sensitization study which employed repeated dermal dosing

with a 10% solution and co-administration of adjuvant. The test material was not considered to have a significant potential as a human skin sensitizer

Ref.: 9

Human data

Guideline:	not in compliance
Method:	Human-Repeat-Insult-Patch-test (HRIPT)
Group size:	189 adults (>18 years) completed the study
Test substance:	Dowicil 200 (cis-CTAC)
Batch:	/
Purity:	/
Dose levels:	challenge: 0.6% Dowicil 200 in petrolatum
Vehicle:	petrolatum
GLP:	not in compliance
Observation period:	72 and 96h after application
Study date:	1999

The method used is a modification of the Draize technique for humans.
200 adult volunteers were screened and deemed free of active skin pathology prior to study.
Induction exposures occurred during the first 3 weeks, applied three times weekly for 48 to 72 hours to the same site each time. The test patches were moistened by 0.2 g Dowicil 200 and adequately secured by means of occlusive bandage. Application sites were scored after removal of the patch.
Approximately 2 weeks after induction, challenge applications were made to a naive site. Patches were prepared with a 0.6% solution of test material in white petrolatum. Patches were affixed to the upper arms or backs of subjects, and secured to the skin with a bandage.
Patches were removed after 72 hours. Challenge sites were scored at 72 and 96 hours. 189 subjects completed the study.

Results
One subject reacted on first exposure, and continuously thereafter, and was judged to be pre-sensitized. No additional compound-related effects were noted in any of the remaining panellists.

Conclusions
It was concluded that DOWICIL200 preservative at 0.6% in petrolatum did not induce allergic contact dermatitis in this Human-Repeat-Insult-Patch-Test

Ref.: 36

Guideline:	/
Method:	HRIPT
Group size:	160 adults
Test substance:	DOWICIL 200 (cis-CTAC)
Batch:	lot 07127010
Purity:	/
Dose levels:	induction: 1% DOWICIL 200 in water challenge: 0.1; 0.3; 1% DOWICIL 200 in water
Vehicle:	water
GLP:	not in compliance
Observation period:	48 and 96h after application
Study date:	1978/1979

A human panel comprised of 160 males and females were exposed to nine applications of the test material as a 1% solution in distilled water under a 24-hour occluded patch to the upper right arm during a period of 3 weeks. After a 2-week rest period, challenge applications were made to a naive site on each subject, and these sites scored for reactions indicative of contact sensitization. The test subjects were challenged with the test material as 1%, 0.3%, 0.1% solutions in distilled water

Results
No positive sensitization response was produced in subjects challenged with 0.1% and 0.3% aqueous Dowicil® 200. However, 7 subjects challenged with the 1% solution were considered sensitised and 4 subjects were thought to need a confirmatory test. Of these 11 subjects, 10 were re-challenged with the 1% solution, together with 8 concurrent control subjects. The sensitized state of 7/10 subjects was confirmed, one was reclassified as non-sensitized, and 2 remained inconclusive.

These subjects (as 2 were unavailable, only 9 were tested), as well as 11 control subjects who did not exhibit a sensitisation response to DOWICIL 200, were subsequently challenged with a 0.01% solution of formaldehyde, a degradation product of CTAC suspected of contributing to its sensitising properties. The challenge applications were again made to a naïve site. These applications failed to elicit any response considered as evidence of contact sensitisation. Therefore, it was concluded that formaldehyde at the concentration tested is not likely to be the component of DOWICIL 200 which causes the skin contact sensitisation response.

Conclusions
It was concluded that Dowicil® 200 was a potential human skin sensitizer under these test conditions.

Ref.: 25

Guideline:	/
Method:	modified Draize
Group size:	47 adults (>18 years) free of skin pathology
Test substance:	DOWICIL 200 (cis-CTAC)
Batch:	/
Purity:	/
Dose levels:	induction: 0.6%
Vehicle:	petrolatum
Observation period:	96h after application
GLP:	not in compliance
Study date:	1996

Adult subjects (18 years of age and older) were recruited for this skin sensitization study 47 of 52 subjects completed the exposure phase of the study with Dowicil 200. Patches were prepared with 0.2 g of test material at concentrations of 0.6% in white petrolatum. Patches were affixed to the upper arms or backs of subjects, and secured to the skin with a bandage. Induction exposures occurred during the first 3 weeks, applied three times weekly for 48 to 72 hours each to the same site each time. Readings were scored. Approximately 2 weeks after the induction, the challenge applications were made to a naive site. Patches were removed after 72 hours. Challenge sites were scored at 72 and 96 hours.

Results
One study subject (1/47) showed positive visual scores, erythema and oedema on final elicitation. A repeat test revealed macular erythema only. A subsequent provocative use test, i.e. two applications to the cubital fossa per day, was negative. Therefore this response was interpreted as irritation.

Conclusions
It was concluded that Dowicil 200 at 0.05 M (1.256%) did not induce or elicit allergic contact dermatitis under the conditions of this test.

Ref.: 46

Comment
The SCCS considers HRIPT studies to be unethical.

Human patch test data

The submission contained a number of publications describing patch test results for Quaternium-15, mainly from the 1980s and 90s (Refs. 1, 12, 16, 22, 28, 31, 39, 40, 50). As a recent review on contact allergy to quaternium-15 is available (Ref. AR1), these reports are not described in detail here.
Quaternium-15 in petrolatum is included in most baseline patch testing series including the European baseline series. Experience with routine testing is summarized in the Table (adapted from AR1.). Test concentrations have included 1% and 2% in petrolatum.

As with formaldehyde, there are major differences in the frequencies of sensitization between USA and European studies. In the multicentre studies from the USA frequencies of sensitization have ranged from 7.1% to 9.6% (mean, adjusted for sample size: 8.8%) (AR2-7, AR14). In the European studies, prevalences were consistently lower, ranging from 0.6% to 1.9% (mean, adjusted for sample size: 1.1%) (AR8, 9, 10, 11, 12, 13, 15–19). In other non-European countries such as Israel and Turkey, equally low rates were observed (AR20–22). Relevance was established or considered 'probable' in 29–90% of the positive patch test reactions. In the USA, the 2% petrolatum test substance detected more cases of sensitization than the 1% patch test material, but direct comparisons in the same populations are lacking and the 1% preparation was used in one study only (AR5).

Country	Years of study	Number of patients	Test concentration & vehicle	Positive (%)			Current relevance %	Comments/setting
				All	Women	Men		
Denmark	1985–2005	14 993	1% pet.	0.9	1.1	0.5	NS	One centre, Copenhagen
United Kingdom	2004–2005	6958	1% pet.	1.9	2.2	1.1	NS	Multicentre study
USA	2001–2005	3841	1% pet.	8.1			76	Mayo Clinic, three locations
Israel	1998–2004	2156	1% pet.	0.8			NS	One centre, Tel Aviv
Turkey	1992–2004	1038	1% pet.	0.8	1.2	0	NS	One centre, Ankara
Europe	2004	7454	1% pet.	1.4			NS	31 departments, 11 countries, ESSCA
Europe	2002–2003	5845	1% pet.	1.2			NS	17 centres in nine countries, ESSCA
USA	2001–2002	4910	2% pet.	8.4			29/56[b]	Multicentre study, NACDG
Finland	2000–2002	11 802	1% pet.	0.8			NS	Multicentre study
Czech Republic	1997–2001	7642	1% pet.	0.7	0.8	0.5	NS	Multicentre study
United Kingdom	2000	3063	1% pet.	1.3			90	Relevance (90%) = current *and* past relevance in one centre (674 patients) only-
Sweden	2000	3790	1% pet.	1.2			NS	Multicentre study
Europe	1996–2000	26 210	1% pet.	1.3	1.5	1.0	NS	10 centres, seven countries, EECDRG
Israel	1999–2000	943	1% pet.	0.6	0.7	0.5	NS	One centre, Petah Tiqwa
USA	1998–2000	5832	2% pet.	9.2			35/52[b]	Multicentre study, NACDG
USA	1996–1998	3436	2% pet.	9.0			89[c]	Multicentre study, NACDG
USA	1988–1997	927	1% pet.	7.1			NS	One centre, Boston
USA	1994–1996	3110	2% pet.	9.2			58/27[b]	Multicentre study, NACDG
Finland	1995–1996	9364	1% pet.	1.1			NS	Multicentre study
USA	1992–1994	3500	2% pet.	9.6			78[c]	Multicentre study, NACDG
Austria	1992–1993	11 516	1% pet.	0.6	0.7	0.5	NS	Multicentre study
Switzerland	1989–1990	2295	1% pet.	1.0			NS	Multicentre study

EECDRG, European Environmental and Contact Dermatitis Research Group; ESSCA, European Surveillance System on Contact Allergies . NACDG, North American Contact Dermatitis Group; NS, not stated.
[a]Data provided back to 1990.
[b]Definite or probable relevance (first number)/possible relevance (second number).
[c]Percentage includes 'possible relevance'.

General comment on sensitisation

The above data demonstrates that Quaternium 15 is an established and important contact allergen in man.

3.3.4. Dermal / percutaneous absorption

In vitro studies on skin penetration are not available. Information on skin penetration only can be obtained indirectly from two in vivo studies on pharmacokinetics and metabolism. These studies are described in chapter 3.3.9. on toxicokinetics.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (30 days) dermal toxicity

Guideline:	/
Species/strain:	rabbit/New Zealand White
Group size:	7/group
Test substance:	DOWICIL™ 200 Preservative (cis-CTAC)
Batch:	Lot TA- 07127010
Purity:	no data supplied
Dose levels:	0, 25, 50 or 100 mg/kg bw, 5 days a week
Vehicle:	water
Dose route:	dermal
Exposure period:	30 days
GLP:	not in compliance
Study date:	1978

Groups of 7 male rabbits per dose level were treated topically with a 20% aqueous solution of DOWICIL™ 200 preservative 5 days per week over a 30 day period. Parameters evaluated included topical skin response, body weight, food consumption, haematological data, clinical chemistry determinations, organ weights and organ/body weight ratios of the major organs, and gross and microscopic pathological evaluations

Results
Food consumption was significantly decreased at the 100 mg/kg/day level throughout the study when compared with the control. Food consumption was also decreased, though not significantly, in the 50 mg/kg/day dose group.
Body weights were significantly decreased at the 100 mg/kg/day level throughout the study when compared with the control.
Repeated, uncovered application of DOWICIL™ 200 preservative to clipped, abraded backs of rabbits resulted in a dose-related occurrence of chronic inflammation, degeneration, and necrosis of the epidermis and dermis at the site of application.
Sporadic changes in haematology were noted, but not considered treatment-related by the study authors.
Liver weights for the top two doses were decreased. There were no other statistical differences in the organ weights.

Conclusion
The authors concluded that there were no signs indicative of systemic toxicity at any dose level, whereas local irritation effects at the dermal test site were significant. Decreased liver weights were attributed to decreased food consumption. A NOAEL of 25 and a LOAEL of 50 mg/kg bw/day has been derived for CTAC after dermal application.

Ref.: 35

Comments
The applicant's conclusions that liver weights were attributed to decreased food consumption are in line with the fact that there were no findings at necropsy indicating target organ toxicity.

3.3.5.2. Sub-chronic (90 days) dermal toxicity

Additional studies have been performed with a mixture of cis-/trans-CTAC (see Annex, A.1).

Guideline:	/
Species/strain:	rabbit/New Zealand White
Group size:	5/sex/group
Test substance:	Cinaryl 200; according to DOW company (2009) identical to DOWICIL™ 200 Preservative
Vehicle:	0.5% cetyl alcohol, 1% lanolin, 2-3% stearic acid, 2% glycerine, 0.75% triethanolamine in H2O
Batch:	no data supplied
Purity:	no data supplied
Dose levels:	0, 1.04, 10.5 and 31.3 mg/kg bw/day, 5 days a week
Dose route:	dermal
Exposure period:	91 days
GLP:	not in compliance
Study date:	1969

Groups of adult New Zealand white rabbits received doses of Cinaryl™ 200 in a lotion formulation for 13 weeks corresponding to 1.04, 10.5, 31.3 mg/kg bw/day. The test material was applied to clipped intact skin representing 10-15% of the total body surface. One group received a water negative control application with the same frequency as test animals (5 times weekly). The other control group received the lotion formulation without Cinaryl 200. Applications were made daily 5 times per week, and the residual material was removed with a damp sponge at the end of each treatment day. The study was terminated after 91 days, with a total of 62 dermal applications.

Mortality and symptoms of toxicity and skin reactions were noted daily. Body weights were taken weekly. Food intake was recorded throughout the experimental period. Haematological studies and clinical chemistry evaluations were made on all animals pre-test and on days 24 and 91. Gross examination of all animals was performed at necropsy. The heart, liver, kidney, spleen, testes, and brain were removed and weighed; they were examined histologically along with sections of the lungs, urinary bladder, thymus, pancreas, adrenal, skin, skeletal muscle, thyroid, ovary, and uterus.

Results
According to the authors of the study there were no treatment related effects on any of the parameters examined. Slight erythema at the test site had been observed in all animals, including those in the control group. These however were resolved by the end of the 91-day test period.

Conclusion
In the absence of effects even at the highest dose the authors concluded a NOEL of 31.3 mg/kg bw/day for CTAC after dermal application

Ref.: 37

General comments on repeated dose toxicity
Six studies have been performed to investigate subchronic toxicity, four with dermal application and two studies with oral application. None of these studies was in compliance with a guideline.

The majority (four) of the studies, including the two oral studies, used mixtures of cis-/trans-CTAC (see Annex) and are principally not suitable for the toxicological evaluation of

cis-CTAC. The results of these studies may be supportive, if it is taken into account that only the cis-isomer might be the effective substance.

In dermal studies, effects of CTAC on the skin at the application site were observed at all dose levels. These effects were characterized as moderate or severe chronic ulcerative dermatitis, degeneration, and necrosis of the epidermis and dermis. No systemic effects were observed in these studies.

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

Bacterial Reverse Mutation Assay

Guideline:	/
Species/Strain:	*S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100 *E. coli WP2 uvrA*
Replicates:	duplicate cultures
Test substance:	Quaternium-15 (cis-CTAC)
Batch:	WP930510 470B
Purity:	97.4%
Vehicle:	distilled water
Concentration:	10, 20, 39, 78, 156 and 313 µg/plate for *S. typhimurium* strains without S9-mix 39, 78, 156, 313, 625 and 1250 µg/plate for *S. typhimurium* strains with S9-mix 156, 313, 625, 1250 and 5000 for *E. coli* without and with S9-mix
Treatment:	pre-incubation method with 20 min pre-incubation and at least 48 h incubation
Control:	Positive controls varied by strain, and included 2-(2-Furyl)-3-(5-nitro-2-furyl)acetate, sodium azide, ICR-191, 2-aminoanthracene, and benzo(a)pyrene.
GLP:	not in compliance
Study date:	December 1993

Test concentrations were based on the results of a range-finding study with Quaternium-15 at concentrations of 1.2, 4.9, 20, 78, 313, 1250 and 5000 µg/plate both in the absence and presence of metabolic activation measuring both growth inhibition and mutant frequency. Liver S9 fraction from phenobarbital/5,6-benzoflavone-induced rats was used as exogenous metabolic activation system. In the main test, test concentrations were set at 1.2, 4.9, 20, 78, 313, 1250 and 5000 µg/plate.
The pre-incubation method was used with 20 minutes pre-incubation and at least 48 h incubation. If a significant, reproducible increase in the number of revertants (2x solvent control) was detected in at least one of the test points, or a reproducible dose-related increase occurred over more than one time point, the results were considered positive.

Results
In the range-finding study growth inhibition was found without metabolic activation at >313 µg/plate in *S. typhimurium* strains and at >2500 µg/plate for *E. coli*, and with activation at >1250 µg/plate for *S. typhimurium* and at 5000 µg/plate for *E. coli*. Accordingly, for the definitive test dose levels were set at 1.2, 4.9, 20, 78, 313, 1250 and 5000 µg/plate.

A dose dependent increase in the number of revertant colonies by more than 2-fold was observed in *S. typhimurium* strains TA98 and TA100 and in *E. coli* with and without activation. Sterility tests were performed on both the test material and the S9-mix, and indicated that neither bacterial growth nor bacterial contamination was a factor in the results. The positive control responded as expected, and the test is considered valid.

Conclusion
Under these experimental conditions used, Quaternium-15 was genotoxic (mutagenic) in the bacterial reverse mutation assay.

Ref.: 32

An ***in vitro*** **Mammalian Cell Gene Mutation Test** has been performed with a mixture of cis-/trans-CTAC (see Annex A.2.).

In vitro **Mammalian Chromosome Aberration Test**

Guideline:	/
Species/strain:	Chinese hamster lung fibroblast cell line (CHL cells)
Replicates:	duplicate cultures
Test substance:	Quaternium 15 (cis-CTAC)
Batch:	WP930510 470B
Purity:	97.4%
Vehicle:	saline
Concentrations:	10, 12.5, 15, 17.5 and 20 µg/ml treatment24 and 48 h 9.5, 19, 38 and 76 µg/ml treatment 6 h
Treatment:	24 or 48 h treatment without S9-mix; harvest time 24 or 48 h after the start of treatment. 6 h treatment without and with S9-mix; harvest time 24 h after the start of treatment.
Positive controls:	mitomycin C or dimethylnitrosamine
GLP:	not in compliance
Study date:	November 1993 –December 1994

A growth inhibition test with 10 serially diluted concentrations ranging from 0.01 – 5 mg/ml was performed prior to the main assay to estimate an appropriate test concentration range. Cells were exposed either for 2 days (without S9-mix) or 6 h (with S9-mix) The growth inhibition test without S9-mix was repeated with doses ranging from 7.5 -20 µg/ml. Liver S9 fraction from phenobarbital/5,6-benzoflavone-induced rats was used as exogenous metabolic activation system.
Based on these results in the chromosomal aberration test the top dose without S9-mix was 20 µg/ml and with S9-mix 76 µg/ml. In the chromosomal aberration test cells were treated for 24 or 48 h without S9-mix and harvested immediately after the end of treatment or for 6 h and harvested 24 h after the start of treatment both without and with S9-mix. Two h prior to cell harvest colcemid was added to the culture medium at a final concentration of 0.2 µg/ml to block cells at metaphase of mitosis. Chromosome (metaphase) preparations were stained with 1.7% Giemsa and examined microscopically for chromosome aberrations. Two-hundred well-separated metaphase cells (100 for each culture) were examined by light microscopy for morphological abnormalities of the chromosome. Polyploid cells with more than 37 chromosomes were recorded.

Results
In the growth inhibition test the 50% inhibition concentration was approximately estimated as 12 µg/ml without S9-mix (48 h exposure) and 38 µg/ml with S9-mix (6 h exposure).

Both in the absence as well as presence of S9-mix an increase in the number of cells with chromosomal aberrations was observed in the cultures treated for 6, 24 and 48 h. The increase was clearly dose dependent in the cultures treated for 24 h without S9-mix and for 6 h with S9-mix.
Under all treatment conditions but predominantly after 24 and 48 h without S9-mix, also increases in the number of polyploid cells were observed.

Conclusion
Under the experimental conditions used, Quaternium 15 was genotoxic (clastogenic) to CHL cells.

Ref.: 33

Comment
In the growth inhibition test inhibition was only measured after 6 and 48 h exposure whereas in the chromosome aberration test exposure time of 24 h was used. It can not be excluded that the positive findings were found at concentration with high toxicity. In the main test cytotoxicity (e.g. inhibition of proliferation inhibition or mitotic index) was not measured.

Guideline:	OECD 473	
Species/strain:	Rat (Sprague-Dawley) whole blood rat lymphocytes	
Replicates:	duplicate cultures in 2 independent tests	
Test substance:	cis-CTAC	
Batch:	WP930510 470B	
Purity:	98.1+/- 0.8% (NMR)	
Vehicle:	distilled water	
Concentrations:	first test:	5, 16.7 and 50 µg/ml without S9-mix 1.67 and 5 µg/ml with S9-mix
	confirmatory test:	5, 16.7 and 50 µg/ml without S9-mix 24 h harvest 50 µg/ml without S9-mix 48 h harvest 0.5, 1.67 and 5 µg/ml with S9-mix 24 h harvest 5 µg/ml with S9-mix 48 h harvest
Treatment	first test:	4 h treatment without and with S9-mix; harvest time 24 h after the start of treatment.
	confirmatory test:	4 h treatment without and with S9-mix; harvest time 24 h and 48 after the start of treatment.
Positive control:	Mitomycin C for the non activation systems, and cyclophosphamide in the assays with activation	
GLP:	not in compliance	
Study date:	January- May 1994	

Whole blood lymphocytes were obtained from 5 young adult male Sprague-Dawley rats. After isolations lymphocyte cultures were cultured with phytohaemagglutinin at a concentration of 0.012 mg/ml to stimulate the lymphocytes to divide. After 48 h culture whole blood rat lymphocytes were treated with and without metabolic activation at concentrations of 1.67, 5.00, 16.7, 50.0, 167, 500, 1670, 5000 µg/ml for 4 hours, and were harvested approximately 24 or 48 hours (confirmatory trail only) prior to treatment. The 3 concentrations for analysis were chosen on the basis of the mitotic indices; the top dose showing a decrease of about 50% compared to the mitotic index found for the control cultures. Mitotic index was assessed in 1000 cells, and was expressed as a percentage of cells analyzed. Liver S9 fraction from Aroclor^{TM} 1254-induced rats was used as the exogenous metabolic activation system.
Approximately 2.5 h before harvest, colcemid was added to each culture (final concentration 0.2 µg/ml) to block cells at metaphase of mitosis. Chromosome (metaphase) preparations were stained with 5% Giemsa and examined microscopically for chromosome aberrations and mitotic index.

Results
Both in the initial assay and in the confirmatory assay no significant increase in the number of cells with chromosomal aberrations was observed at any of the concentrations tested. Likewise, no increase in the number of polyploid cells was observed. The positive control chemicals induced significantly higher incidences of abnormal cells.

Conclusions
Under the experimental conditions used, cis-1-(3-chloroallyl)-3,5,7-triaza-1-azoniaadamantane chloride was not genotoxic (clastogenic) to rat whole blood lymphocytes.

Ref.: 38

***In vitro* unscheduled DNA Synthesis Test**

Guideline:	/
Cells:	rat hepatocytes; male CDF Fischer 344
Replicates:	triplicate
Test item:	Dowicil 200 (cis-CTAC)
Batch:	lot 061600212
Purity:	96.3%
Vehicle:	Williams Medium E (WE)
Concentrations:	0.01, 0.1, 1, 10, 100, 1000, 10000, 20000 and 50000 µg/ml
Treatment	18 h treatment
Positive control:	2-acetylaminofluorene
GLP:	not in compliance
Study date:	July 1980- December 1980

Hepatocytes were prepared by perfusion with collagenase of livers of approximately 14 weeks old male CDF Fischer 344 rats. Cell cultures were exposed to Dowicil 200 or the solvent/negative control at the end of the attachment period with ^{3}H-thymidine (10 µCi/ml) for 18 h. Thereafter the cells were washed for 30 minutes with unlabeled thymidine. Slides were then progressed for autoradiography. Evaluation of autoradiography was done after 10 days exposure and hematoxilin and eosin staining.
UDS was measured by counting the number of grains in each nucleus and subtracting the average number of grains present in 3 equal-sized adjacent cytoplasmic areas (net nuclear grain). A mean of 6 grains per nucleus or higher, and statistical significance relative to the control are required for an unequivocal positive result.

Results
Dowicil 200 was toxic to hepatocytes at concentrations of 100 µg/ml and above.
Dowicil 200 did not induce an increase in net nuclear grain (UDS) counts in the hepatocytes isolated from rat at all concentrations tested. The positive control did elicit a dose-related increase in DNA repair when compared to the control.

Conclusion
Under the experimental conditions used Dowicil 200 did not induce unscheduled DNA synthesis and, consequently, is not genotoxic in rats in this *in vitro* UDS test.

Ref.: 17

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

***In vivo* Mammalian Erythrocytes Micronucleus Test**

Guideline:	OECD 474
Species/strain:	male outbred CD-1 (1CR)BR mice (CD-1)
Group size:	4/sex/dose for dose finding study

	6 males/dose in the main study (phase I and phase II)
Test substance:	cis-CTAC
Batch:	NE2801QT1P
Purity:	98.7% HPLC
Vehicle:	corn oil
Dose level:	phase I experiment: 0, 500, 1000, 2000 mg/kg bw/day phase II experiment: 0, 250, 500, 1000 mg/kg bw/day
Route:	gavage on 2 consecutive days
Sacrifice times:	24 h after the second treatment
Positive controls:	cyclophosphamide-monohydrate (CP) dissolved in distilled water
GLP:	in compliance
Study date:	November 1999- March 2000

The purpose of the study was to determine the micronucleus-inducing capability of cis-CTAC (cis-1-(3-Chloroallyl)-3,5,7-triaza-1-azoniaadamantane chloride) in mouse bone marrow polychromatic erythrocytes. Dose selection was based on the result of a dose range-finding test for toxicity. Groups of 4 mice were treated with various doses of cis-CTAC on 2 consecutive days and observed for at least 72 h for any signs of toxicity. In the main experiment mice were treated by oral gavage on 2 consecutive days at 24 h intervals with 0, 500, 1000, 2000 mg cis-CTAC /kg bw/day. The mice were observed for positive signs of toxicity at least once on the day of dosing and subsequent days, and were weighed on the day of their scheduled sacrifice in order to assess the extent of stress experienced by the animals following treatment. The relative body temperatures of the treated animals in the micronucleus test were monitored using programmable transponders immediately prior to each dosing, approximately 2 and 6 hours after each dosing, and prior to sacrifice. Approximately 24 hours after the second administered dose, bone marrow samples were obtained from both femurs. Two thousand PCEs were examined from each animal. Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and normochromatic erythrocytes (PCE/NCE ratio). Negative and positive controls were in accordance with the OECD guideline.

Results

Observations in the range-finding test included decreased faeces and perineal soiling described as faecal and/or urine.

In the phase I experiment, 4 animals were found dead prior to necropsy. There was also a substantial decrease in body weights as well as a remarkable decrease in relative body temperature, predominantly in the surviving animals treated with 2000 mg/kg bw/day as compared to the control animals. Based on these finding and since Asanami and Shimono (1998) demonstrated that decreased body temperature (i.e., hypothermia) can increase the incidence of micronuclei in the mouse bone marrow, it was concluded that this highest dose of 2000 mg/kg bw/day exceeded the MTD. Consequently, the study was repeated using a lower dose range.

In the repeat (phase II) experiment, various mice predominantly those treated with 1000 mg/kg bw/day, showed decreased faeces and in one mice of the 1000 mg/kg bw/day dose group faecal soiling was observed. Only the body weights of animals treated with 1000 mg/kg bw/day were lower at the time of necropsy as compared to dosing weights of control animals. There was a noticeable decrease in the relative body temperature of all the animals after treatment but all animals appeared to be recovering at 6 hours after the second administered dose and were near normal at the time of necropsy with the exception of animals treated with 1000 mg/kg bw/day.

A more or less dose dependent but not statistically significant decrease in the PCE/NCE ratio was observed. In addition, systemic availability of cis-CTAC was also indicated by the clinical signs observed.

In comparison to the concurrent vehicle controls, there was no biologically relevant or statistically significant increase in the number of erythrocytes with micronuclei in the animals treated with 250, 500 and 1000 mg/kg bw/day cis-CTAC over two consecutive days.

Conclusion
Under the experimental conditions used cis-CTAC did not induce micronuclei in erythrocytes of treated mice and, consequently, cis-CTAC was not genotoxic (clastogenic and/or aneugenic) in erythrocytes of mice.

Ref.: 15

Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *In Vivo*

Guideline:	OECD 486
Species/strain:	male Fischer 344 rats
Group size:	4-7 animals/group
Test substance:	Dowicil 150 (cis-CTAC)
Batch:	NH1901QT1P
Purity:	98.7%
Vehicle:	distilled water
Dose level:	0, 750, 1500 mg/kg bw
Route:	oral, gavage
Sacrifice times :	2-4 and 12-14h after dosage
Positive controls:	dimethylnitrosamine (DMN)
GLP:	in compliance
Study date:	July 2001 - November 2001

The objective of this assay was to detect DNA damage caused by cis-CTAC, or an active metabolite, by measuring unscheduled DNA synthesis (UDS) in rat primary hepatocytes. Dose selection was based on the result of a dose range finding study for toxicity with concentrations of test material in water at 500, 1000, 1500, 1750 and 2000 mg/kg bw by oral gavage to three male and three female rats per group. Dose range finding animals were observed within 0.5, 2 to 4 hours of dosing, and daily for three days. In the UDS assay, the test material was administered as a single oral gavage dose of 750 and 1500 mg/kg bw in water. The hepatocytes were harvested 2 to 4 or 14 to 16 hours after dosing. After an attachment period of 1.5-2 h, the cultures were labelled with 10 µCi/mL ^{3}H-thymidine for 4 hours followed by 16-20 h incubation with 0.25 mM unlabelled thymidine. Evaluation of autoradiography was done after 8 days.
UDS was reported as net nuclear grain: the nuclear grain count subtracted with the average number of grains in 3 nuclear sized areas adjacent to each nucleus. Unscheduled synthesis was determined in 50 randomly selected hepatocytes on triplicate slides per rat. Negative and positive controls were in accordance with the OECD guideline.

Results
In the dose range finding study, one male dosed at 1750 mg/kg bw and one dosed at 2000 mg/kg bw, and one female dosed at 1750 mg/kg bw and all three dosed at 2000 mg/kg bw were found dead by day 3 post dose. Signs of toxicity were observed at 1000 mg/kg bw and higher in both sexes.
In the UDS test, signs of toxicity were limited to red crusts around the nose and/or mouth in most animals dosed at 1500 mg/kg bw at both time points. The test material did not produce any statistically significant or biologically relevant effects in either treatment group at either time point. Neither treatment group met criteria for a positive response at any time.

Conclusion
Under the experimental conditions used cis-CTAC did not induce unscheduled DNA synthesis and, consequently, is not genotoxic in rats in the *in vivo* UDS test.

Ref.: 11

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive and developmental toxicity

3.3.8.1. Reproductive toxicity

A study has been performed in Crl:CD (SD) rats with a mixture of cis-/trans-CTAC (see Annex A.3.1).

3.3.8.2. Developmental toxicity

An additional study has been performed in rabbits with a mixture of cis-/trans-CTAC (see Annex A.3.2.).

Developmental toxicity study with cis-CTAC in rats, study 1

Guideline:	/
Species/strain:	rats Fischer 344
Group size:	33/34 females/dose
Test substance:	cis-CTAC
Batch:	WP121580010B
Purity:	97.9%
Vehicle:	water
Dose levels:	0; 5; 25; 75 mg/kg bw/ day
Dose volume:	?
Route:	oral, gavage
Administration:	days 6 through 15 of gestation
GLP statement:	not in compliance
Study date:	July - September 1981

Animals were observed daily for indications of toxicity. Body weights were recorded daily during the period of dosage. Food and water consumption were recorded at three day intervals. Maternal liver weights were recorded at the time of caesarean section.

Test animals were killed by CO_2 inhalation on day 21 of gestation. The following data were recorded: (1) number and position of live and dead foetuses (2) number and position of resorption sites (3) number of corpora lutea (4) sex, body weight and crown rump length (5) gross external alterations. The foetuses were examined for skeletal alterations. The incidence of pregnancy was determined. One half of the litter was examined for soft tissue alterations. The heads of rat foetuses were examined by the serial sectioning technique of Wilson.

Results
In the highest dose group there was a significant decrease in body weight and body weight gain and an increase in absolute and relative liver weight. During the first three days of dosing animals of the dose group 25 mg/kg bw/day gained significantly less body weight than controls. The rats in the highest dose group (75 mg/kg bw/day) consumed significantly less food than controls. In this group water consumption was significantly different from that of controls. In the middle dose (25mg/kg bw/day) group the animals consumed less food during days 9 through 14. Liver weights in this dose group were not significantly different from controls. In the group exposed to 75 mg/kg bw/day, the incidence of resorptions was significantly increased compared to controls. In the same dose group the foetuses weighed significantly less than controls.

None of the above effects were found in the lowest dose group of 5 mg/kg bw/d.

The incidence of total major malformations of foetuses was significantly higher in dose groups 25 and 75 mg/kg bw/day than in controls. The majority of the malformed foetuses exhibited anomalies of the eye, microphthalmia or anophthalmia. 5/145 (2% of foetuses, 17% of litters) and 6/137 foetuses (2% of foetuses and 19% of litters) exhibited microphthalmia at 25 and 75 mg/kg bw/day, respectively.
Though not significant, 2 rats in 2 litters in the low dose group (5 mg/kg bw/day) showed major malformations compared to 1 in 1 litter of the control group. In the 5 mg/kg bw/day one foetus exhibited micrognathia and anophthalmia. Another foetus exhibited polydactyly. A third foetus who died in utero showed exencephaly. This case was not included in the statistical evaluation.
One case of anophthalmia was also found in the control group. Neither micrognathia nor polydactyly or exencephaly was observed in the controls. However, in the treated groups, no dose-response relationship was observed for these effects. There is no information about historical controls for these malformations at that time.

Conclusion
The applicant deduced a NOEL of 5 mg/kg bw/day for maternal and foetal toxicity.

Ref.: 27

Comment
The decreased weight gain which was observed during the first days at a dose of 25 mg/kg bw/day may be considered as the first effects of maternal toxicity.

Developmental toxicity study with cis-CTAC in rats, study 2

Guideline:	OECD 414
Species/strain:	rats Fischer 344, female, mated
Group size:	33
Test substance:	cis CTAC
Batch:	SL2001QT2P
Purity:	98.9 ± 0.8 wt. %
Vehicle:	water
Dose levels:	0, 25, 75 mg/kg bw/day
Dose volume:	4 ml/kg
Route:	oral, gavage
Administration:	days 6 through 15 of gestation
GLP statement:	?
Study date:	January 2005 – February 2005 (final report 2007)

23 years later the above study (Ref. 27) was repeated with the aim to determine whether the ophthalmic malformations observed in the first study were the result of a genetic cluster effect or cis-CTAC-related. Only two instead of three doses were applied.

Results
The animals showed similar decreases in maternal body weight, body weight gains, and feed consumption as those seen in the first study. Decreased mean foetal body weight was also seen at 75 mg/kg/day.
In the repeat study, 2/251 (0.8% of foetuses, 6.3% of litters) and 2/209 foetuses (1.0% of fetuses, 6.4% of litters) exhibited microphthalmia and/or anophthalmia in the 25 and 75 mg/kg/day groups, respectively.

Conclusions
The applicant concluded that the incidence of microphthalmia and/or anophthalmia is similar to historical control incidence for Fischer 344 rats, and is considerably lower than the

incidence of eye defects in the first study. Furthermore they concluded that the known propensity of Fischer 344 rats for foetal eye defects suggests that the original study findings were likely related to a spontaneously occurring genetic cluster effect, rather than a specific consequence of DOWICL 200 exposure. Moreover they underline that there was no dose response relationship with respect to microphthalmia and/or anophthalmia.

Ref.: 7

Comment

The repeat study in 2005, intended to establish whether the malformations of foetuses observed in the first study (1981) were cis-CTAC related, is hampered by the fact that the dose of 5 mg/kg bw/day, at which some malformations had been observed in the first study, has not been included. However, there were indeed a lower number of eye malformations in the repeat study compared to the first study. Furthermore the applicant could show that in historical controls the incidence of eye malformation is comparably high in this rat strain used.

In the repeat study, malformations other than microphthalmia and/or anophthalmia like micrognathia, polydactyly or exencephaly have not been observed.

Effects of cis-CTAC on maternal weight and weight gain were confirmed in the second study. Effects on liver have not been reported.

General comments on reproductive, developmental and maternal toxicity

The strain of rats (Fischer 344) used in the above studies is considered inappropriate by the SCCS due to the high and variable background incidence of spontaneous eye abnormalities (personal communication, Buschmann 2011).

The decrease of bodyweight and body weight gain in dams observed in the first study in the mid and high dose group leads to a NO(A)EL of 5 mg/kg bw/day for maternal toxicity.

Despite the limitation stated above, due to the severity of the developmental effects observed at the low dose of 5 mg/kg bw/d, these effects give rise to considerable concern. This means that the dose of 5 mg/kg bw/d should be considered rather a LOAEL than a NOAEL. However, on the basis of the animal studies supplied this question cannot be decided.

In summary, taking into account the results and the shortcomings of both studies using cis-CTAC, the developmental effects at 5 mg/kg bw/d in the first study cannot be considered as a reliable basis for a LOAEL or NOEL with regard to developmental toxicity.

Two additional studies on reproductive/developmental toxicity are available, which have been performed with a mixture of cis-/trans-CTAC (see Annex), while only cis-CTAC is used in cosmetic products. These studies using a mixture of cis-/trans-CTAC are therefore not suitable for the estimation of a NOEL for cis-CTAC.

3.3.9. Toxicokinetics

An additional study has been performed with a mixture of cis-/trans-CTAC (see Annex A.4.)

Guideline:	/
Species/strain:	rats female Fischer 344
Group size:	27
Test substance:	^{14}C DOWICIL 200 ring-labelled (cis-CTAC) (97.8% radiochemical purity) ^{14}C DOWICIL 200 side chain labelled (cis-CTAC) (94% radiochemical purity)
Batch:	lot WP121580010B
Purity:	97.9%
Dose levels:	5, 75 mg/kg bw as 1% or 50% aq. respectively
Route:	dermal; oral; intravenous (i.v.)
Dose volumes:	oral: 4ml/kg bw Dermal: 0.5 to 0.15 µL /kg bw to provide dose i.v.: 4ml/kg bw
GLP	in compliance
Date	1983

Twenty-four female Fischer 344 rats received either a dermally-applied or orally-administered dose of Dowicil® 200, with targeted doses of 5 or 75 mg/kg bw. The experiments were conducted under (early) GLP. Radio-labelled material was used for the study. The material was labelled either uniformly in the hexamethylenetetramine (HMTA) ring-structure or in the number 2 carbon of the chloroprene side-chain of the molecule. For both experiments with either the ring-labelled or the side chain-labelled substance, twelve rats were used to determine the dermal and oral pharmacokinetics (3/dose/route). Three rats received an intravenous dose of 5 mg/kg of ring-labelled cis-CTAC in order to compare the pharmacokinetic and metabolic fate of the material following i.v. administration.

Dermal Dosing
Doses were applied as aqueous solutions, with target dose volumes of 0.5 and 0.15 µL for the 75 and 5 mg/kg dose levels, respectively for each of the two labelled samples. Doses were applied to an area on the clipped back of the animal. They remained in contact with the skin for 48 hours under an impervious patch held in place with a cloth rodent jacket.

Oral Dosing
Oral doses were also given as aqueous solutions by gavage.

Intravenous Dosing
Doses were administered via the in-dwelling cannulae as aqueous solutions.

Sample Collection and Analysis
Immediately following the dose administration, rats were returned to metabolism cages for collection of urine, faeces, and expired air. Blood samples were collected (orally- and dermally-exposed animals) at 0.5, 1, 2, 4, 6, 12, 24, 48 hours post-dosing. Blood samples of i.v.-treated rats were taken at 10 minutes, 0.5, 1, 2, 4, 6, 12, 24, 48 hours post-dosing. Blood was analyzed using liquid scintillation counting. Urine and faeces were collected at 12-hour intervals and analyzed by liquid scintillation. Volatile organics in the expired air were adsorbed on activated charcoal columns and counted by liquid scintillation. Animals were sacrificed at 48 hours, and the liver, kidneys, carcass, spleen, eyes, ovaries, a portion of the backbone, and samples of fat were removed. In dermally-applied animals, the wrappings and skin from the test site were also collected. Radioactivity in aliquots of pulverised tissues

was quantified as $^{14}CO_2$ using a Biological Materials Oxidizer and liquid scintillation spectrometry.

Urinary metabolites were separated by liquid chromatography from selected urine samples of animals dosed orally or dermally, and analyzed by ion exclusion chromatography and reverse-phase HPLC. Pooled or individual samples were concentrated and reconstituted with deionised water, and injected onto the column without further treatment. Fractions of the eluent from the column were also evaluated by liquid scintillation counting.

Results
The toxicokinetic study in female 344 Fischer rats conducted with dermal, oral and i.v. application, respectively, partly revealed marked differences in the disposition, excretion and metabolic profile of cis-CTAC in urine, depending on the exposure route, the doses applied, and the position of the ^{14}C-labelling in the molecule. Dermal absorption was low compared to oral exposure. Apart from formiate, the other three or four urinary metabolites could not be identified.

In order to derive a MoS if possible, mainly dermal exposure is considered in the following. After dermal application the following data was obtained:

Dose /label	**Group size**	**Recovery %**	**% dose absorbed**
5 mg/kg ring	2	93.8 ± 5.1	1.98 ± 0.062
5 mg/kg side chain	3	91.4 ± 4.8	0.38 ± 0.03
75 mg/kg ring	3	106 ± 4.8	1.416 ± 1.065
75 mg/kg side chain	3	78.9 ± 1.9	1.068 ± 1.069

Ring-labelled Dowicil 200:
The total radioactivity in all excreta, tissues, naive skin, and remaining carcass was 2.0±0.06% (2 animals) and 1.42±1.07 (3 animals) of the dermally administered dose of 5 and 75mg/kg bw respectively.

Side-chain-labelled Dowicil 200:
The total radioactivity in all excreta, tissues, naive skin, and remaining carcass was 0.38±0.03 (3 animals) and 1.07± 1.07% (3 animals) for the 5 and 75 mg/kg dermally administered dose levels respectively.

Orally administered ^{14}C-DOWICIL™ 200 was extensively absorbed, metabolized and the metabolites rapidly excreted primarily in the expired air as ^{14}C-CO_2, and in urine as four or five different metabolites. Analysis of the urine by ion-exclusion liquid chromatography revealed that one of the urinary metabolites of ring-labelled 14C-DOWICIL™ 200, co-eluted with a standard solution of formic acid. Similar results were obtained after i.v. application of 5 mg/kg of the ring-labelled substance.

Ref.: 52

Comments
The values given for dermal uptake are average values, including the value 2% of skin penetration, which was used by the applicant for the calculation of MoS. The recoveries in part of the study were poor.

General comments on toxicokinetics
No *in vitro* dermal absorption studies are available. The toxicokinetic study partly revealed marked differences in the disposition, excretion and the metabolic profile of cis-CTAC in urine, depending on the exposure route, the doses applied, and the position of the ^{14}C-labelling in the molecule. Dermal absorption was low compared to oral exposure. Whereas the dermal low dose experiment apparently revealed a statistically significant difference between the ring-labelled and the side chain-labelled substance, no differences regarding the position of the labelling and the variability of the dermal absorption was observed in the dermal high dose group. Taken together, the large differences and the variability of the dermal absorption of cis-CTAC cannot be explained by different dose or fate of the radio-labelled substance and hence do not allow drawing any firm conclusions on the dermal absorption in this study.

In addition to Ref. 52, a similar study has been performed with cis-/trans-CTAC (see Annex). This study more or less is a repetition of Ref. 52. After dermal application, the absorbed relative amounts of radioactivity in tissues, faeces, urine and of ^{14}C-CO_2 from this study add up to averages of 6.5% (^{14}C-CTAC labelled in the side chain) and 8.1% (ring-labelled ^{14}C-CTAC). Maximum values add up to 10%.

In the summary of their submission, the applicant states that the results of this study should not be used for risk assessment of cosmetic applications, as cis-/trans-CTAC has been used, whereas in the marketed products cis-CTAC is used. They suggest using the results (2% absorption) of Ref. 52, for cis-CTAC only.

The SCCS is of the opinion that, in the light of the highly variable data on dermal absorption and the in part poor quality of the study with cis-CTAC and the discrepancy in absorption values obtained from the two studies, the available results are not suitable for a MoS calculation.

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

Guideline:	not in compliance
Species/strain:	human subjects
Group size:	50 adults: 25 males, 25 females
Test substance:	Dowicil 200 (cis-CTAC)
Batch:	not reported
Positive control:	2% solutions of Tetrachloro salicylanilide (TCSA) in methanol and petrolatum
Dose levels:	1% Dowicil 200 in water; 2% Dowicil 200 in methanol 0.1; 0.25; 0.7% Dowicil 200 in various cosmetic preparations
Dose volume	0.1 ml
Application and irradiation:	30 sec ;12 inches distance; 5 days a week; 6 weeks
Contact area:	0.5 inches diameter
UV source:	Fischer quartz sunlamp model 88
GLP:	not in compliance
Study date	1970

Fifty individuals, 25 males and 25 females, were selected to participate in this study. None of the subjects had any history of previous photosensitization. Ages ranged from 21 to 35 years. The panel was divided into three groups. Group 1 consisted of 30 individuals and groups 2 and 3 consisted of 10 individuals each.

Preliminary to the study each individual's susceptibility to the U.V. lamp used in this study was tested. None of the individuals responded to the series of exposures of 30-second duration which, was selected for the experimental procedure during the study.
Contact with the materials was limited to discrete areas of the skin on the anterior surface of the forearm. After application of 0.1 ml of test material, the material was allowed to soak in for 60 seconds and then exposed to the U.V. lamp for 30 seconds at a distance of 12 inches. Following the U.V. treatment the skin was blotted dry to remove residual wet samples. This procedure was used to bring about contact with the test materials and to effect U.V. exposure throughout the study. If any of the skin sites showed irritation, exposure to the test material was discontinued at that site. Close examination of the irritation was performed daily in order to determine the severity of progression and clinical course so that a diagnosis could be made of primary irritation sensitization. During the sixth week of the study, individuals in Groups II and III were followed-up daily for delayed reactions. After the challenge application, each individual in this study was followed-up for delayed reactions during the summer months.

Results
In the positive control group the procedure successfully produced a state of photosensitization in 9/10 and 8/10 individuals to 2% solutions of Tetrachloro salicylanilide (TCSA) in methanol and petrolatum, respectively.
Dowicil in 1% concentrations of aqueous based formulations showed no irritant or sensitizing effects. Dowicil in a concentration of 2% methanol elicited some response in three individuals who were concurrently in a state of hypersensitivity to TCSA. This irritative phenomenon was *trans*ient and could not be elicited again on the challenge applications. Exposure to ambient sunlight throughout the summer months did not elicit any signs of photosensitization to Dowicil.

Conclusion
The study authors concluded that Dowicil® 200 in concentrations of 1% or lower in aqueous-based formulations does not produce photosensitisation under the test conditions.

Ref.:49

Comment
Phototoxicity studies in the absence of relevant absorption spectra are considered unjustified. Such studies are considered unethical.

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

No data submitted

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

A safety evaluation including the calculation of a MoS is not possible, as no appropriate dermal absorption study is available. No *in vitro* dermal absorption studies are available. Dermal absorption and toxicokinetics were investigated in two studies in rats. However, the SCCS is of the opinion that the values of both studies are not suitable for MoS calculation. In addition, in view of the shortcomings of the toxicity studies, no sound NOAEL could be established.

3.3.14. Discussion

Quaternium-15 is used in rinse-off and leave-on cosmetic products, including baby products

Introductory remark on test substances used in submitted studies
In many studies, especially in the newer ones, the applicant uses a mixture of cis-/trans-CTAC for toxicity testing instead of cis-CTAC, which is, according to the submission, used in cosmetic formulations. Moreover, only cis-CTAC is classified as toxic to reproduction (cat 2 GHS). Using a mixture of cis-/trans-CTAC, the applicant refers to a study of Rodan and Clark 1987, who claim that "the two isomers are very similar". This argument, however, is not relevant in this context. Principally the state of the art demands to use that substance for testing that is under evaluation, not a surrogate. Moreover, there is no reason to use a mixture of cis-/trans-CTCA instead of cis-CTAC because cis-CTAC is readily available. For these reasons, the SCCS does not accept the data for mixture of cis-/trans-CTAC and bases its assessment on the available data on cis-CTAC.

The studies using a mixture of cis-/trans-CTAC may be considered as supportive information, but the results of these studies need to be interpreted with care taking into account that only the cis-isomer may be the active compound. Studies performed with cis-/trans-CTAC as the test substance are described in the Annex to this opinion.

Physico-chemical specification
For many of the submitted studies, data on the identity and purity of the test substances used has not been provided. Chemical identification data has been submitted only for DOWICIL™ 150 Antimicrobial, ASG 10387427, but not for DOWICIL 200, Lot SL2001QT2P, which was used in some studies.

Irritation, sensitisation
The test substance has irritant potential for both the skin and eye. Chronic exposure to quaternium-15 resulted in dermal effects (see below) in animal studies. Quaternium-15 is well recognised as a contact sensitiser in man.

Toxicokinetics / dermal absorption
A dedicated *in vitro* dermal absorption study is not available.
Dermal absorption and toxicokinetics were investigated in two studies in rats. In one study with cis-CTAC (1983), which is not in compliance with a guideline and is poorly documented, the highest average dermal uptake measured was 2% +/-0.06%. Primary data for the experiments were not provided and the group sizes are partly inadequate. Moreover, in part of this study, recoveries of dermally applied doses are poor.
The second study (2008) was similar in design and in compliance with the guideline, but a mixture of cis-/trans-CTAC has been used instead of the cis-isomer only. Here, maximum values of 10% dermal uptake of CTAC were found. The SCCS does not consider such a study, using an isomer mixture, suitable to be used in the risk assessment.
The SCCS is of the opinion that neither of these two studies are suitable for MoS calculation. To get a valid basis for the estimation of dermal penetration the SCCS is of the opinion that in vitro studies on dermal absorption should be performed

Toxicity
The acute oral median lethal dose (LD_{50}), calculated by the moving average method, was 2664 mg/kg bw (95% confidence intervals, 1836-3512 mg/kg). The acute dermal toxicity, characterized as LD_{50}, had been calculated with values of 923 mg/kg bw and 605 mg/kg bw, respectively, for two different preparations of cis-CTAC.

None of the six studies on subchronic toxicity is in compliance with a guideline.

In the two dermal studies with cis-CTAC, dose-dependent effects on the skin were observed. These effects were characterized as moderate or severe chronic ulcerative dermatitis, degeneration, and necrosis of the epidermis and dermis. No systemic effects were observed in these studies.

In the other two dermal studies mixtures of cis-/trans-CTAC were used as test substances. These are therefore not suitable for a toxicological evaluation of cis-CTAC.

Two studies on oral toxicity are available, where mixtures of cis-/trans-CTAC have been used instead of cis-CTAC only. The NOEL after oral administration (7.5 mg/kg bw /day of cis/-trans-CTAC; beagle dogs, Ref. 47) is governed by systemic effects on liver, heart and possibly the haematopoietic system. The findings at this dose level reported in Ref. 26 do not contradict this NOEL, as they are considered to be caused by decreased food consumption due to inpalatability of the test substance. The NOEL has to be corrected for the amount of cis-isomer.

Mutagenicity
Overall, the genotoxicity of Quaternium-15 is sufficiently investigated in valid genotoxicity tests for the 3 endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy. Quaternium-15 induced mutations in S. typhimurium and E. coli WP2uvrA bacteria with and without metabolic activation. Two studies investigating chromosomal aberrations showed contradictory results. Increased numbers of cells with structural chromosomal aberrations were observed in CHL cells in the presence as well as in the absence of induced rat liver enzymes. Negative findings were observed in a chromosome aberration test with rat whole blood lymphocytes. An *in vitro* unscheduled DNA synthesis assay in the rat, which was conducted to assess the potential of Quaternium-15 to damage DNA, was negative.
The positive findings found in the *in vitro* tests were not confirmed in adequately performed in vivo tests. Quaternium-15 did not induce an increase in cells with micronuclei in an *in vivo* micronucleus assay nor did it cause DNA damage leading to unscheduled DNA synthesis in rat hepatocytes.
Consequently, Quaternium-15 can be considered to have no *in vivo* genotoxic potential and additional tests are unnecessary.

Reproductive toxicity
The strain of rats (Fischer 344) used in the studies with cis-CTAC is considered inappropriate by the SCCS due to the high and variable background incidence of spontaneous eye abnormalities (pers. communication Buschmann 2011).
Despite these limitations, the severity of the developmental effects observed at the low dose of 5 mg/kg bw/d give rise to considerable concern. This means that the dose of 5 mg/kg bw/d should be considered rather a LOAEL than a NOAEL. However, on the basis of the animal studies supplied this question cannot be decided. In summary, taking into account the results and the shortcomings of both studies using cis-CTAC, the developmental effects at 5 mg/kg bw/d in the first study cannot be considered as a reliable basis for a LOAEL or NOEL with regard to developmental toxicity.
The decrease of bodyweight and body weight gain in dams observed in the mid and high dose group leads to a NO(A)EL of 5 mg/kg bw/day for maternal toxicity.

Two additional studies on reproductive toxicity have been performed with a mixture of cis-/trans-CTAC.

Carcinogenicity
No data submitted

4. CONCLUSION

Based on the scientific data provided, does the SCCS consider that Cis-1-(3-chloroallyl)-3,5,7-triaza-1-azoniaadamantane chloride is safe for the consumers, when used as a preservative in a concentration up to 0.2% in cosmetic products?

The SCCS cannot assess the safety of *Cis-1-(3-chloroallyl)-3,5,7-triaza-1-azoniaadamantane chloride* because

- The available dermal absorption values are not sufficiently reliable to calculate the dermal uptake of cis-CTAC, and
- Appropriate toxicity studies are lacking to establish a reliable NOAEL.

Therefore, a MoS cannot be calculated

*Does the SCCS have any scientific concerns for the continued use or any modification in the specifications for the substance?*Taking into account the CMR classification of *Cis-1-(3-chloroallyl)-3,5,7-triaza-1-azoniaadamantane chloride* and considering the absence of relevant toxicological data, the SCCS considers its continued use in cosmetic products may not be safe for the consumers.

5. MINORITY OPINION

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Submission 2009

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Annex: Toxicological data obtained with cis-/trans-CTAC

A.1. Repeated dose toxicity

Sub-chronic (90 days) oral / dermal toxicity

Oral studies

Guideline:	/
Species/strain:	rat/ Sprague- Dawley
Group size:	10/ sex / dose
Test substance:	DOWICIL 100 Preservative (cis-/trans-CTAC)
Batch:	Lot 188718
Purity:	91 %
Dose levels:	0, 7.5, 15, 30, 60 mg/kg bw/day
Vehicle:	?
Dose route:	oral, diet
Exposure period:	90 days
GLP:	not in compliance
Study date:	1972

Groups of male and female Sprague-Dawley rats were maintained for 90 days on diets containing sufficient Dowicil™ 100 (cis-/trans-CTAC) to provide doses of 0, 7.5, 15, 30, 60 mg/kg bw/day. Parameters evaluated included: appearance and demeanour, mortality, body weights, food consumption, haematological determinations, urinalyses, serum level of urea nitrogen, alkaline phosphatase and glutamic pyruvic transaminase, final organ weights and organ/body weight ratios, and gross and histopathological examination of tissues.

Results
Significantly decreased food consumption correlated with decreased body weights (up to about 20%) in all treatment groups and in both sexes. A significant increase in brain/body weight ratios was observed in males and females in all dose groups as well as a significant increase of the ratio testis/body weights in all dose groups. Increased liver/body weight ratios were observed in male and female rats at the high dose (60 mg/kg bw/day).
Hepatocellular swelling in some male rats at the high dose was observed.
Male animals showed significantly elevated serum urea nitrogen levels and significantly decreased alkaline phosphtase concentrations at the high dose. Males moreover had significantly lower SGPT concentrations than controls in all dose groups down to 15 mg/kg bw/day.

Conclusions
The applicant suggested a LOEL of 30 and a NOEL of 15 mg/kg bw/day.

Ref.: 26

Comment
The original study report describes significant effects on relative testes weights down to the lowest dose group. In male rats significant decreases of SGPT concentrations were observed down to 15 mg/kg bw/d. These effects were not mentioned in the applicant's submission. The deduction of LOEL and NOEL as suggested by the applicant cannot be retraced, as the effect on which the applicant bases the decision is not mentioned in the submission summary.

Guideline:	/
Species/strain:	Beagle dogs
Group size:	4/sex/dose
Test substance:	DOWICILE 100 Preservative (cis-/trans-CTAC)
Batch:	Lot No 257736
Purity:	96.28%
Dose levels:	0, 7.5, 15, 30 mg/ kg bw/ day
Vehicle:	gelatine capsules
Dose route:	oral
Exposure period:	91-92 days
GLP:	not in compliance
Study date:	1976

Male and female Beagle dogs were given Dowicil™ 100 (*cis*-/*trans*-CTAC) orally in gelatin capsules for 91-92 days at dose levels of 0, 7.5, 15, or 30 mg/kg bw/day. Measurements of body weight and food consumption were made throughout the study, and the dogs were examined daily for overt signs of toxicity. Haematological and clinical chemistry parameters were monitored. Gross and microscopic pathological examinations of the tissues were conducted. The weights of the brain, heart, liver, kidneys, and testes were recorded and organ/body weight ratios were calculated.

Results
One female animal of the high dose group was sacrificed on day 84 of the study because of poor physical conditions.
The absolute weights of heart were lower in male and female dogs at the intermediate and high dose levels. This in part also applies to the relative heart weights of both sexes.
The absolute and the relative weight of the liver among female at the high dose level was significantly greater than that of controls. Among male dogs, a similar trend toward an increase in liver weight at the high dose level was observed. Microscopic examinations of the tissues revealed changes in the liver of males and females at the high-dose, as well as one female at the mid-dose. These changes included obliterative vasculitis and perivasculitis of the hepatic blood vessels, moderate perivascular and pericholantiolar infiltration of mononuclear cells in the liver, and hyperplasia of the reticulendothelial cells of the hepatic sinusoids in the liver.
Alterations in the heart tissue were observed in one male dog at the high dose level (multifocal myocardial degeneration, necrosis and inflammation)
A statistically significant depression in packed cell volume red and white blood cell counts and percent haemoglobin was observed among the male dogs at the high dose level. A depression of packed cell volume, red blood cell counts and percent haemoglobin was observed in female dogs at the two higher dose levels, but this difference was not significant.
Statistically significant depressions of SGOT and SGPT in males and females of the two highest dose levels were seen.

Conclusions
The applicant draws the conclusion that these results indicate that the liver and possibly the heart were the primary target organs for the toxic effects of DOWICIL 100. They suggest a NOEL of 7.5 mg/kg bw/day.

Ref.: 47

Comment
The fact that there were no decreases in body weight, as it has been reported in Ref. 26, might be due to the application of CTAC in form of capsules which do not impair the palatability of food. So the effects in this study are not influenced by decreased intake of food. The applicant bases the NOEL on effects on heart and liver. The haematological effects, which also would lead to the same NOEL, were not considered by the applicant.

Dermal studies

Guideline:	/ (similar to guideline OECD 411)
Species/strain:	rabbit/ New Zealand White
Group size:	10/ sex / dose
Test substance:	DOWICIL 100 preservative (cis-/trans-CTAC)
Batch:	Lot GW-21-87-47 Lot GW-21-87-92
Purity:	94.85%; 90.2%
Dose levels:	0, 50, 200, 1000 mg/kg bw/ day
Vehicle:	water
Dose route:	dermal, occlusive
Exposure period:	91 days
GLP:	in compliance
Study date:	Oct 1987- June 1988

Groups of 20 adult New Zealand white rabbits (10/sex/dose) received 0, 2.9, 11.8, 60% (w/v) doses 5 days/week for 13 weeks corresponding to 0, 50, 200 and 1000 mg/kg bw/day cis-/trans-CTAC. The test material was applied to clipped skin using a blunt-tipped needle, covered by an absorbent gauze and cotton for a 6 hour exposure. Rabbits were observed daily for signs of toxicity. The condition of the skin at the application site was evaluated daily prior to application of the test material according to the modified Draize system recommended by the OECD (1983).
Haematology and clinical chemistry was evaluated. Gross pathology included weights of the heart, liver, brain, kidneys, adrenals, and testes. Complete histopathology was performed on a standard tissue set on animals in the control and high dosage groups. Microscopic examinations of tissues from other dose groups were limited to the skin from the test site and a naive site, as well as any gross lesion.

Results
Signs of irritation observed at the test sites were slight to severe erythema, oedema and scaling, slight fissuring, scabbing, and scarring, mainly limited to areas of abrasion from clipping. The onset and degree of skin changes were dose-related.
There were no significant changes in haematological parameters for males, however there was an increase in white blood cell count in females at the high dose and an increase in platelets of the same group.

There were likewise no clinical chemistry changes in males, but sporadic decreases in serum potassium and blood urea nitrogen, and an increase in cholesterol.

Gross pathological findings at necropsy were limited to the skin at the test sites, consisting of scab encrusted foci ranging in size and frequency. These effects were observed in all dose groups except controls. Histopathology showed an inflammatory reaction limited to focal involvement of the epidermis and dermis at the application site, characterized as moderate or severe chronic ulcerative dermatitis. Microscopic necrotic debris was present in several animals.

Conclusions
The authors claim that haematological and clinical chemical changes are within historical control ranges and therefore considered them not to be toxicologically significant. Histological observations across the dose groups in several tissues are interpreted as not treatment related. The applicant derived an NOEL of 1000 mg/kg bw/day *cis*-/*trans*- CTAC for systemic toxicity following dermal exposure.

Ref.: 13

Comment
Some effects described in the study report have not been considered to be toxicologically relevant by the study authors. The SCCS, however, is of the opinion that the haematological changes seen in this study should attract attention, even if those seen after oral application of CTAC are different from those found here. Also the segmental degeneration of the seminiferous tubules in the testes of 3 of the 10 top dose males compared to 1/10 of controls should be noted.

Guideline:	/
Species/strain:	mice
Group size:	10/sex/ dose
Test substance:	DOWICIL 100 (cis-/ trans-CTAC)
Batch:	GW-16-94-57A
Purity:	91.3 ± 1.3% (NMR)
Dose levels:	0, 100, 400, 1200 mg/kg bw/ day
Vehicle:	distilled water
Dose route:	dermal,
Exposure period:	90 days
GLP:	in compliance
Study date:	1995-1996

Male and female mice were treated with cis-/trans-CTAC applied to an absorbent pad of a bandage at 0, 2.5, 10, and 30 mg CTAC/mouse/application (equivalent to dermal doses of 100, 400 or 1200 mg/kg bw/day). The bandage was placed on the clipped, depilated backs of the mice three times each week for a 6-hour exposure. After the application interval, the bandage was removed and sites were evaluated for any treatment-related effects. An additional 10 animals per sex were added to the control and high-dose group; these animals were maintained for a 4-week recovery period following the final dermal dose. Clinical pathology and clinical chemistry were evaluated. At necropsy, the brain, heart, kidneys, liver and testes were weighed for each mouse. The ratio of organ weight to terminal body weight was calculated. The dermal test site and a naive site were evaluated histopathologically, as well as the liver, kidneys, and lungs.

Results
According to the applicant, there were no findings indicative of systemic toxicity in the study at the highest dose level tested (1200 mg/kg bw/day) in either sex. A NOEL of > 1200 was suggested

Comment
No physico-chemical data have been submitted for this batch, and the ratio of cis-/trans-CTCA in this material is not known.

Ref.: 42

A.2. Mutagenicity / Genotoxicity

Mutagenicity / Genotoxicity *in vitro*

***In vitro* Mammalian Cell Gene Mutation Test**

Guideline:	/
Species/strain:	Chinese hamster Ovary (CHO-K_1-BH_4) cells
Replicates:	5 replicates
Test substance:	Dowicil 100 (cis-/trans-CTAC)
Batch:	GW- 21-87-47
Purity:	94.85%

Vehicle:	culture medium	
Concentrations:	initial experiment:	16, 32, 62.5, 125 and 150 µg/ml both without and with metabolic activation
	repeat experiment:	60, 80, 100 and 125 µg/ml with metabolic activation
Treatment:	4 h treatment without and with S9-mix; expression period 8 days; selection period of 7-9 days without and with metabolic activation	
Positive controls:	ethyl methane sulfonate (non-activating system) and 20-methylcholanthrene (activating system)	
GLP:	in compliance	
Study date:	November 1987 - March 1988	

Cultures of Chinese hamster ovary cells were treated with Dowicil 100 for 4 hours both without and with S9-mix. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. After treatment, cells were cultured for 8 days to allow the fixation of the DNA damage into stable *hprt* mutations. Thereafter cells were cultured on selection media to determine *hprt*-mutant colonies and cloning efficiency. The experiment with S9-mix was repeated with other concentrations to confirm the positive effect found in the initial experiment. Toxicity was measured in the main experiments as relative cell survival of the treated cultures relative to that of the solvent control cultures.

Results
In the non-activated assay the relative cell survival of cultures treated in the toxicity assay with 125 µg/ml was 9.9%, and 22.2% in the activation assay. This concentration was selected as highest concentration for the mutagenicity assay.
There were no significant increases in the frequencies of 6-thioguanine resistant mutants (representing the *hprt*-genotype) in cultures treated with the test material in the absence of S9-mix. However, in the presence of S9-mix, both in the initial and the repeat experiment a biological relevant, dose dependent and statistically significant increase in the mutant frequency was observed.

Conclusion
Under the experimental conditions used, Dowicil 100 was genotoxic (mutagenic) in this *hprt* test.

Ref.: 34

Comments
The applicant commented on the results of this test that there were deviations from the current guideline test method:

- in the absence of S9-mix, there was no concentration in the recommended 10-20% survival range compared to the negative control,
- there was no repeat of the assay in the absence of S9-mix with no justification,
- duplicate cultures were not used,
- and there were not more than 8 analyzable concentrations in the absence of duplicate cultures.

The applicant moreover argued, that an activation of cis-/trans-CTAC by the S9 enzymes may not only result in the mutagenic form of the test material, but may also accelerate the decomposition of S9-mix into mutagenic species, which could be able to increase mutation frequency.
So the applicant concluded, that based solely on this in vitro test, a possible genotoxicity of cis-/trans-CTAC seems to be ambiguous, until the nature of the interaction between S9-mix and the test chemical is elucidated.

A.3. Reproductive and developmental toxicity

A.3.1. Reproductive toxicity

Guideline:	OECD 422
Species/strain:	rats, Crl:CD(SD)
Group size:	10/sex/dose
Test substance:	cis-/trans-CTAC
Batch:	
Purity:	30.9% cis-CTAC 32.0% trans-CTAC 33.6% sodium bicarbonate 3.1% Hexamethylenetetramine (HMTA) 0.4% water
Vehicle:	distilled water
Dose levels:	0, 75, 225, 750 mg/kg bw/day; dose levels have been corrected for purity of cis-/trans CTAC
Dose volume:	1 ml/kg bw
Route:	dermal (occluded)
Administration:	6 hours/day Females: from 4 weeks prior to breeding till the end of lactation Males: 10 weeks starting four weeks prior to breeding F1 offspring: 1 week following weaning
GLP statement:	in compliance
Study date:	December 2005 – March 2006

The study was based on the OECD 422 study guideline (Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test, 1996), but was enhanced by extended dosing of the parental and F1 offspring rats to provide additional characterization of reproductive toxicity by the dermal route.

Groups of 10 Crl:CD(SD) rats/sex/dose group received 0, 75, 225, or 750 mg/kg/day of cis-/trans-CTAC applied to the skin (occluded) for 6 hours/day. Females were dosed for 4 weeks prior to breeding, through breeding, gestation and lactation. The adult males were dosed for 10 weeks starting four weeks prior to breeding. F1 offspring were dosed for one week following weaning. An additional naive control group was included to assess the impact of dermal wrapping.

Parameters evaluated included clinical observations, dermal grading, body weight, feed consumption, clinical chemistry, urine parameters, haematology, gonadal function, mating behaviour, conception, development of the conceptus, parturition and postnatal growth and survival. In addition, a gross necropsy of the adults was conducted with histopathologic examination of tissues

Results
Due to the severity of the skin lesions, the high-dose group (750 mg/kg bw/day) was terminated on TD 17.
In-life observations showed no treatment-related effects on behaviour or demeanour
Dermal effects like scaling, erythema, and oedema were seen in the dose group 225 mg/kg bw/day. These effects were minor following exposure to 75 mg/kg bw/day
Body weights of males from the 225 mg/kg bw/day group tended to be slightly lower (5.8%) than controls by the end of the study. Female final body weights were statistically significantly decreased (8.1%) at 225 mg/kg bw/day. There were no effects of treatment with 75 mg/kg/day on male or female body weight during the study.
Feed consumption of the 225 mg/kg/day males was significantly lower than that of controls. Feed consumption of the 225 mg/kg/day females was significantly decreased

relative to the controls throughout the four-week pre- mating period. There were no effects of treatment with 75 mg/kg/day on male or female feed consumption during the study.
Reproductive indices, pup survival, and sex ratio (mating, conception, fertility, gestation indices, time to mating, gestation length, post- implantation loss, pup survival, or pup sex ratio) showed no treatment-related effects at any dose level.
In the 225 mg/kg/day group, mean male and female F1 pups body weights seemed to be decreased (7.5-14.7%) relative to the controls throughout the lactation phase. The mean female pup weights were statistically significantly decreased relative to the controls on PND 21.
There were no treatment-related **haematological effects** in males or females at any dose level.
Clinical chemistry data showed that in the 75 mg/kg bw/day group the AST of males and ALP activity of females were higher and lower, respectively, than controls. However at a dose of 225 mg/kg bw/day the values for AST and ALP did not differ from that of controls. The triglyceride level of males exposed to 225 mg/kg bw/day was lower than controls, and seems to be dose related.

Chloroallylamine (CAA), the **metabolite of CTAC**, was found in urine of treated rats. 2.82 mg/l CAA correspond to the dose of 75 mg/kg bw/day CTAC, 9.11 mg/l CAA to a dose of 225 mg/kg bw/day.
Gross pathologic observation: Multifocal scabs at the dermal test site of 2 females given 225 mg/kg/day were considered to be treatment-related.
Histopathological observations showed multifocal crusts at the dermal test site of one female given 225 mg/kg bw/day, and very slight multifocal epithelial erosions at the dermal test site of another female given 225 mg/kg bw/day. Other histopathological observations were interpreted to be spontaneous alterations.
Weanlings
Daily in-life observations showed no treatment-related effects on behaviour or demeanour at any dose level.
Dermal grading data for male and female F1 weanling rats showed one male weanling exposed to 225 mg/kg bw/day which exhibited slight scaling on treatment days TD 5-7.
F1 males at 225 mg/kg bw/day had **body weights** on TD 4 and 7 that were statistically significantly lower than that of controls. F1 female pup weights at 225 mg/kg bw/day were not significantly different from controls. However, the weights remained low during the weanling one-week dosing period. Body weights of F1 males and females at 75 mg/kg bw/day were lower than controls, but this effect was not statistically significant.
Feed consumption of the 225 mg/kg/day males was significantly decreased. There were no significant differences in the amount of feed consumed by males at 75 mg/kg bw/day or by females in any of the treated groups when compared to controls.

Conclusions
Males and females given 225 mg/kg bw/day exhibited dermal effects, comprised of scaling, scabs, erythema and/or oedema, along with multifocal crusts or epithelial erosions found microscopically in two females. Body weights and feed consumption also were decreased at 225 mg/kg bw/day.

Effects of 75 mg/kg bw/day were limited to very minor, transient skin effects in two males and one female.
Urine CTAC was increased in a dose-related manner, verifying absorption and systemic exposure to the test material.
There were no effects on adult reproductive function.
Treatment-related effects on the offspring were limited to slight decreases in pup and weanling body weights at the 225 mg/kg/day dose level, accompanied by slightly decreased feed consumption.
Dermal effects in the F1 weanlings were limited to slight scaling in one male rat.

The no-observed-effect level (NOEL) for both general systemic toxicity and reproductive toxicity suggested by the applicant was 75 mg/kg bw/day.
A NOEL for dermal effects was not suggested because slight skin effects were seen at 75 mg/kg bw/day.

Ref.: 8

Comments
A mixture of cis- (31.3%) and trans- (32.5%) CTAC has been applied. Dose levels and results have been corrected for purity of test substance (i.e. 63.8%). However, the sum of cis- and trans-isomer has been used for calculation.
Because of the (slight) effects (skin, body weight) seen at a dose of 75 mg/kg bw/day this seems to be a LOAEL rather than a NOEL as the applicant claims.
Mean pup weights at 75 mg/kg bw/day although being numerically lower than that of controls were considered to be not affected by cis-/trans-CTAC by the authors of the study.

A.3.2. Developmental toxicity

Guideline:	OECD 414
Species/strain:	New Zealand White rabbits; female mated
Group size:	26
Test substance:	cis-/trans CTAC
Batch:	SH2701QT1P
Purity:	31.3% cis-CTAC, 32.5% trans-CTAC, 3.1% Hexamethylenetetramine, 0.1% unknown impurity 33.0% sodium bicarbonate
Vehicle:	water
Dose levels:	0, 2.5, 8, 25 mg/kg bw/day
Dose volume:	1ml /kg bw
Route:	oral, gavage
Administration:	day 7 through 27 of gestation
GLP statement:	
Study date:	August 2007- October 2007

Cage-side examinations included activity, behaviour, vocalization, incoordination/limping, injury, neuromuscular function, altered respiration, skin and mucous membranes, eye injury, faecal consistency, and faecal/urinary quantity. In addition, all animals were observed for morbidity and mortality.

Clinical observations included a hand-held examination of the animal with an evaluation of abnormalities in the eyes, urine, faeces, gastrointestinal tract, extremities, movement, posture, reproductive system, respiration, skin/hair-coat, and mucous membranes, as well as an assessment of general behaviour, injuries or palpable mass/swellings.

In-life parameters evaluated for all groups included: clinical observations, body weight, body weight gain, and feed consumption.

On GD 28, all surviving rabbits (two were euthanized during the study) were euthanized and examined for gross pathologic alterations and changes in liver, kidney, and gravid uterine weight. The number of corpora lutea, uterine implantations, resorptions, and live/dead foetuses were determined.

All foetuses were weighed, sexed, and examined for external and visceral alterations. The visceral examination included observation of the thymus, trachea, oesophagus, lungs, great vessels, heart, liver, gastrointestinal tract, pancreas, spleen, kidney, adrenal glands,

ureters, bladder and reproductive organs. Also, the heads were examined for craniofacial alterations by serial sectioning for approximately one half of the foetuses in each litter, while skeletal examinations were evaluated on all foetuses.

Results
Oral gavage administration of 25 mg/kg/day cis-/trans-CTAC to time-mated female New Zealand White rabbits resulted in treatment-related maternal toxicity in the form of decreased body weight gain and decreased mean feed consumption throughout the entire dosing period.
Lower mean foetal body weights and lower mean gravid uterine weights were observed in the 25 mg/kg/day dose group.
There was no treatment-related maternal or developmental toxicity in animals given 2.5 or 8 mg/kg/day cis-/trans-CTAC.

Conclusions
According to the applicant the NOEL for maternal and developmental toxicity is 8 mg/kg bw/day.

Ref.: 6

Comments
A mixture of cis-(31.3%) and trans-(32,5%) CTAC has been applied. However, only the cis-isomer has been categorized as toxic for reproduction. The applicant states that dose concentrations are not adjusted for purity. Taking into account that only 31,3% of the applied dose is cis-CTAC and that toxicity might be due only to this isoform, the adjusted NOEL for cis-CTAC would be 2.5 mg/kg bw/day.

A.4. Toxicokinetics

Guideline:	USEPA, OPPTS 870.7485 (1998); OECD Guideline 417 (1984); OECD, Guideline 427 (1984); EC, Guideline B.36 (1986)
Species/strain:	Rats (female) Fischer 344
Group size:	4/group; 8 groups
Test substance:	(a): Ring-labelled DOWICIL™ 75 (^{14}C-cis-/trans-CTAC-R (b): Side chain-labelled DOWICIL™ 75 (^{14}C-cis-/trans-CTAC-SC): (c): non-radiolabelled CTAC,
Batch:	(a) Lot # 358100R5 (ring labelled) (b) Lot # ELM020907 (side chain labelled) (c) Lot # SH2701QT1P
Purity:	(a) 62.3% (b) 89.9% (c) 31.3% cis-CTAC; 32.5% trans-CTAC; 33% sodium bicarbonate, 3.1% hexamethylenetetramine
Dose levels:	5; 75 mg/kg bw
Route:	(1) oral; unlabelled for 14 days; side chain labelled for 1 day (2) intravenous (i.v.) unlabelled (3) oral and dermal; ring- and side chain labelled
GLP:	in compliance
Study date:	June 2008

The purpose of the study was to obtain to study absorption, distribution, metabolism, and elimination (ADME) data for cis-/trans-CTAC and to confirm data from a study suggesting a route dependent difference in the metabolic fate of the ring portion of the molecule (Waechter *et al*., 1983, Ref. 52).
Oral doses of ^{14}C-DOWICIL™ 75 targeted at 5 or 75 mg/kg bw were administered by oral gavage as aqueous solution (6-18 µCi) of ^{14}C-DOWICIL™-RL or ^{14}C-DOWICIL™-SC in a target volume of ~4-ml dosing solution/kg bw.

For dermal dose administration the animals were anesthetized with isoflurane and the hair on the back (5 x 5 cm area) of each rat was clipped 48 hours prior to dosing. Dermal doses of ^{14}C-DOWICIL™-RL or ^{14}C-DOWICIL™-SC targeted at 5 mg/kg bw were applied in aqueous solution (8-14 µCi). The dermal dose was allowed to remain in contact with the skin for 48 hrs post-dosing. The site of application was occluded with a bandage impervious to water. Urine was collected at 12, 24 and 48 hours post-dosing. Faeces were collected at 24 and 48 hours post-dosing.
Selected urine and faecal samples were pooled and stored at –80 °C for chemical analysis. Animals were sacrificed at 48 hours post-dosing. Tissues, remaining carcass, and final cage wash were collected and analyzed for radioactivity at sacrifice. ^{14}C-CO_2 was collected at 6, 12, 24 and 48 hrs post-dosing and analyzed for radioactivity.

Results
Oral administration of ^{14}C-CTAC -SC after 14 days of application of unlabelled CTAC resulted in the excretion of 43% of the applied radioactivity in urine, 13% in the faeces and 32% in form of ^{14}C-CO_2.
I.v. administration of ^{14}C-CTAC–RL yielded 30% of the applied radioactivity in urine, 4% in the faeces and 28% as ^{14}C-CO_2. Animals dermally administered 5 mg/kg bw ^{14}C-cis-/trans-CTAC (SC and RL) excreted approximately 3% of the dermally administered dose as ^{14}C-CO_2; and the urine contained 1.6% and 2.2% of the administered dose as ^{14}C-cis-/trans-CTAC-derived radioactivity for SC and RL, respectively.

Selected pooled urine and pooled faecal extracts were analyzed by high-performance liquid chromatography (HPLC) radiochemical detection for metabolite determination There were 13 metabolites detected in the excreta of animals administered 14C-cis-/trans-CTAC-SC. Three peaks in the urine were detected at >5% of the administered dose and were positively identified with authentic standards. These include the cis- and *trans*- isomers of 3-chloroallyl-1-amine and 3,3-dimercapturate of 1-propanol. There were 9 peaks identified in the excreta of animals administered 14C-cis-/trans-CTAC-RL. Two peaks were detected in the urine >5% of the administered dose. No identification was made of these metabolites.

The metabolic fate of ^{14}C-DOWICIL™ 200-RL was route-dependent (oral vs. dermal).Both peaks of cis- and trans-3-chloroallyl amine were >5% of the administered dose in the orally administered animals but were not present in the urine samples of the dermal dosed animals.

Conclusion
The authors concluded that the route dependent differences in the metabolism are consistent with the hypothesis that the toxicity observed following oral administration may not occur following dermal administration.

Ref.: 23

Comments
This study (ref. 23 with cis/trans-CTAC) more or less resembles that described in Ref. 52 (with cis-CTAC). The results for dermal absorption given in the text are not in agreement with the data presented in table 3 of the original study. According to table 3 the absorbed relative amounts of radioactivity in tissues, faeces, urine and of ^{14}C-CO_2 add up to averages of 6.5% (^{14}C-CTAC-SC) and 8.1% (^{14}C-CTAC-RL). Maximum values would add up to 10%.