Scientific Committee on Consumer Products

SCCP

OPINION ON

Citric acid (and) Silver citrate

The SCCP adopted this opinion at its 19th plenary of 21 January 2009

About the Scientific Committees
Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Products (SCCP), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

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

SCCP
Questions concerning the safety of consumer products (non-food products intended for the consumer).
In particular, the Committee addresses questions related to the safety and allergenic properties of cosmetic products and ingredients with respect to their impact on consumer health, toys, textiles, clothing, personal care products, domestic products such as detergents and consumer services such as tattooing.

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TABLE OF CONTENTS

1. BACKGROUND

Submission I for the preservative with the official INCI-name Citric acid (and) Silver Citrate and with the code name FAT81'034 was submitted by December 2006.

The ELINCS number 460-890-5 has been assigned to the substance. No CAS number has been provided or applied for.

2. TERMS OF REFERENCE

1. *Does SCCP consider Citric acid (and) Silver Citrate safe for consumers when used in cosmetic products as a preservative in a concentration up to 0.2%, taken into account the scientific data provided?*

2 *And/or does the SCCP recommend any further restrictions with regard to the use of Citric acid (and) Silver Citrate in cosmetic products?*

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1.	Chemical identity

Citric acid (and) silver citrate is a stable mixture of citric acid monohydrate and silver dihydrogen citrate monohydrate (FAT 81'034). The aqueous preparation of FAT 81'034 is called TINOSAN® SDC (FAT 81'033) and is a mixture of silver ions, citric acid and water. Axenohl is a mixture of silver ions, citric acid, water and sodium lauryl sulfate acting as surfactant.

3.1.1.1.	Primary name and/or INCI name

INCI: Citric acid (and) Silver citrate
Primary name: mixture of citric acid monohydrate and silver dihydrogen citrate monohydrate

3.1.1.2.	Chemical names

1,2,3-Propanetricarboxylic acid, 2-hydroxy-, monohydrate (9CI) and 1,2,3-Propanetricarboxylic acid, 2-hydroxy-, silver(1+) salt, monohydrate

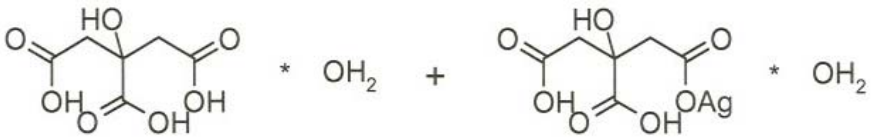
3.1.1.3.	Trade names and abbreviations

TINOSAN® SDC Active
TINOSAN® SDC lyophilisate
TINOSAN® SDC
FAT 81'034
FAT 81'033
Axenohl
C-1390

3.1.1.4.	Registry numbers

CAS: /
ELINCS: 460-890-5

3.1.1.5.	Structural formula



according to NMR

3.1.1.6.	Empirical formula

$C_6H_8O_7.H_2O$ and $C_6H_7O_7Ag.H_2O$

3.1.2. Physical form

White crystallized powder

3.1.3. Molecular weight

210 (citric acid monohydrate) and 317 (silver dihydrogen citrate monohydrate)

3.1.4. Purity, composition and substance codes

The aqueous trade product called TINOSAN® SDC (also FAT 81'033), produced by electrolysis in a citric acid/water solution, is placed on the European market as a liquid preparation containing following ingredients:

- 0.72% of 1,2,3-Propanetricarboxylic acid, 2-hydroxy-, silver(1+) which represents about 2400 ppm Ag+
- 22.4% of 1,2,3-Propanetricarboxylic acid 2-hydroxy-
- 76.88% water.

The lyophilized active substance defined chemically as a "mixture of 1,2,3-Propanetricarboxylic acid, 2-hydroxy-, monohydrate and 1,2,3-Propanetricarboxylic acid, 2-hydroxy-, silver(1+) salt, monohydrate" is dissociated and corresponds to 1-fold of "1,2,3-Propanetricarboxylic acid, 2-hydroxysilver(1+) which represents about 2400 ppm Ag+" and a 47-fold excess of "1,2,3-Propanetricarboxylic acid 2-hydroxy-," in the aqueous trade product called TINOSAN® SDC (FAT 81'033).

Ref. 35

3.1.5. Impurities / accompanying contaminants

See 3.1.4

3.1.6. Solubility

884.4 g/L at 20°C, based on determination of silver in the saturated solution, pH 1.1 (OECD 105)

Ref. 4

3.1.7. Partition coefficient (Log P_{ow})

Log $P_{o/w}$: -1.3 at 21°C, pH of aqueous phase 3.4 (calculated, OECD 107)

Applicant's justification for the calculation of log P_{ow}:
"The partition coefficient was calculated related to the Ag^+ concentration measured in the aqueous phase. The determination of Ag^+ concentration in octanol phase was not possible using the ICP-OES method. Due to its ionic character Ag^+ is assumed not to be soluble in octanol. Therefore no further quantification of the silver concentration in the octanol phase was performed. The log P_{ow} was calculated on an assumed concentration of 0.05 mg/L for the octanol phase".

Ref. 5

3.1.8. Additional physical and chemical specifications

Melting point: 60-149 °C (OECD 102)

FAT 81 034/B melted in the range of 60°C - 149°C (Wet point 60°C, Shrinking point 60.5°C, Collapse point 144.5°C, Liquefying point 145.9°C and Final stage of melting 149°C)

Ref. 6

Boiling point: 484.49 °C (decomposes prior to boiling, calculated using Miller's method)

Ref. 7

Vapour pressure: 1.74 x 10^3 Pa at 25 °C (Calculated using the Modified Watson Correlation method; OECD 104 and further estimation methods)

Ref. 8

Flammability: Citric acid (and) Silver citrate is not considered as flammable.

Ref. 9

Explosive properties: Based on the chemical composition, Citric acid (and) silver citrate is not expected to pose an explosive hazard.

Ref. 10

Hydrolysis as a function of pH: Test item is considered to be hydrolytically stable.

Ref. 10

Particle size distribution: Test item has been sieved through a 125 µm sieve. Less than 1% of the test item passed a 125 µm sieve and less than 0.2% passed a 75 µm sieve.

Ref. 11

The liquid preparation Tinosan SDC does not contain solid particles in the range of 1.0 to 3000 nm, as shown by dynamic light scattering.

Ref. 35

3.1.9. Stability and homogeneity

The stability and homogeneity of Citric acid (and) Silver citrate suspended in solvents or mixed to form an anti-bacterial hand cream (W/O Type) or a deodorant-emulsion (O/W Type), has been assessed in each study enclosed in this dossier.
FAT 81'034 is stable in water, at room temperature for >180 days

Ref. 12

3.2. Function and uses

Citric acid (and) silver citrate is a preservative system based on a stabilised silver complex, effective against gram-positive and gram-negative bacteria as well as against yeasts and moulds. It is to be used in aqueous leave-on and rinse-off cosmetic products up to a maximum concentration of 0.2%. In addition, Citric acid (and) Silver citrate is also intended to be used as an active ingredient in deodorants. Oral care products and products intended to be applied in the vicinity of the eyes are excluded from this application.

3.3. Toxicological Evaluation

The applicant has performed a series of (sub-) acute toxicological and genotoxicity studies with Citric acid (and) Silver citrate, all according to OPPTS and/or OECD guidelines. Considering the low amount of citric acid and sodium lauryl sulphate used in cosmetic formulation, both are recognized as substances with low toxicity and are considered of no concern with respect to human health. Therefore the presence of citric acid (which has been affirmed as generally recognized as safe (GRAS) by the United States Food and Drug Safety Authority (FDA)) and that of sodium lauryl acid in Axenohl, is considered of no relevance with regard to the toxicological evaluation.

Ref. 12

3.3.1. Acute toxicity

3.3.1.1 Acute oral toxicity

The acute oral toxicity of Axenohl, (batch 8995, purity 21.34% Citric acid (and) Silver citrate) was estimated according to OPPTS 870.1100 Health Effects Test Guidelines (1998), Acute Oral Toxicity, in Sprague-Dawley rats. A single oral gavage dose of 5000 mg/kg body weight was given to 5 male and 5 female Sprague-Dawley rats. All animals survived and showed no clinical signs of adverse effects and behavioural abnormalities. With the exception of one male that showed loss in body weight between days 7 and 14, all animals gained body weight over the 14-day observation period. Gross abnormalities were not apparent at necropsy. Pursuant to the OPPTS Health Effects Test Guideline for acute toxicity, a maximum dose level of 5000 mg/kg body weight was used instead of the limit dose of usually 2000 mg/kg body weight according to EEC Directive.

Conclusion
The oral LD_{50} is greater than 5000 mg/kg body weight according to these study results. On the basis of these results TINOSAN® SDC is considered to be non-toxic by oral exposure. The active ingredient Citric acid (and) Silver citrate is considered to be maximum moderately toxic.

Ref. 13

Comment
In the acute oral toxicity test, Axenohl was tested, which contains only 21.34% of the test compound Citric acid (and) Silver citrate. Five thousand mg/kg Axenohl is equivalent to approximately 1000 mg/kg FAT81'034/D, the pure test compound. The concentration used is too low for a limit test for classification.

3.3.1.2. Acute dermal toxicity

The acute dermal toxicity of Axenohl, (batch 8995, purity 21.34% Citric acid (and) Silver citrate) was estimated according to OPPTS 870.1200 Health Effects Test Guidelines (1998), Acute Dermal Toxicity, in Sprague-Dawley rats. A single dose of 5000 mg/kg body weight was applied evenly by dermal patch for 24-hours to approximately 10% of the body surface area to 5 male and 5 female Sprague-Dawley rats. All animals survived, gained weight and were without clinical signs of adverse effects and behavioural abnormalities. Gross abnormalities were not apparent at necropsy.

Conclusion
The acute dermal LD_{50} is greater than 5000 mg/kg. On the basis of these results TINOSAN® SDC is considered to be non-toxic by dermal exposure. The active ingredient Citric acid (and) Silver citrate is considered to be maximum moderately toxic.

Ref. 14

Comment
In the acute dermal toxicity test, Axenohl was tested, which contains only 21.34% of the test compound Citric acid (and) Silver citrate. 5000 mg/kg Axenohl is equivalent to approximately 1000 mg/kg FAT81'034/D, the pure test compound. The concentration used is too low for a limit test for classification.

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

An acute dermal irritation/corrosion study was performed in three male New Zealand White rabbits according to OECD guideline 404. Doses of 0.5 g Citric acid (and) Silver citrate (FAT 81034/B, batch 2005.0001, purity 98.9% Citric acid (and) Silver citrate) ground to a fine powder were placed on a pad moistened with purified water, which was then applied to intact skin under semi-occlusion for 4 hours. In one animal a slight erythema (grade 1) was noted on day 1 only; the 2 other animals did not show erythema or oedema during the study. Corrosion and adverse signs of toxicity did not occur through an observation period of 72-hours after patch removal.

Conclusion
Citric acid (and) Silver citrate is classified as non-irritant according to the Council Directive 67/548/EEC (as amended).

Ref. 16

3.3.2.2. Mucous membrane irritation

Study 1

An acute eye irritation /corrosion study was performed in one male New Zealand White rabbit according to OECD guideline 405.
Citric acid (and) Silver citrate (FAT 81034/B, batch 2005.0001, purity 98.9% Citric acid (and) Silver citrate) ground to a fine powder was administered by placing 0.1 g of the test article into the conjunctival sac of the left eye of one male New Zealand White rabbit. The lower and upper eyelids were held together for about 1 second. The right eye served as the untreated control. The eye of the animal remained not rinsed after instillation of the test article. A marked chemosis, a moderate or severe redness of the conjunctiva and a clear or whitish purulent discharge and red discharge were noted in the animal on day 1 and 2. An iritis and severe corneal opacity were recorded on days 1 and 2. On day 2, the animal was sacrificed for ethical reasons.

Ref. 17

Study 2

The irritation and corrosion potential of the trade product TINOSAN® SDC aqueous formulation of Citric acid (and) Silver citrate containing 79.6% water, 20.2% citric acid and 2500 ppm silver has been evaluated (FAT 81'033/A, batch 2004-190-0001, purity 20.45% Citric acid (and) Silver citrate).
An acute eye irritation /corrosion study was performed in three male New Zealand White rabbits according to OECD guideline 405.
A single dose of 0.1 ml as 0.5% (w/w) or as 5% (w/w) solution of FAT 81'033/A in purified water, adjusted to pH 6±0.5 using sodium hydroxide, was administered into the

conjunctival sac of the left eye of male New Zealand White rabbits (three animals for each concentration). The lower and upper eyelids were held together for about 1 second. The eye of the animals remained not rinsed after administration of the test item.
At the concentration of 0.5%, a slight chemosis (grade 1) and a slight redness of the conjunctivae (grade 1) were noted in 1/3 animals on day 1 only. No ocular reactions were observed on the two other animals. Mean scores calculated for each animal over 24, 48 and 72 hours for chemosis, redness of the conjunctiva, iris lesions and corneal opacity were all 0.0.
At the concentration of 5%, a slight chemosis (grade 1) and a slight redness of the conjunctivae (grade 1) were noted in 1/3 animals on day 1, a slight chemosis (grade 1) persisted in this animal on day 2. No ocular reactions were observed on the two other animals. Mean scores calculated for each animal over 24, 48 and 72 hours were 0.3, 0.0 and 0.0 for chemosis, 0.0, 0.0 and 0.0 for redness of the conjunctiva, 0.0, 0.0 and 0.0 for iris lesions and 0.0, 0.0 and 0.0 for corneal opacity.

Conclusion on mucous membrane irritation
The powdered form of Citric acid (and) Silver citrate is severely irritating to the eye when applied directly at full strength, therefore the classification as irritant (Xi) and the risk phrase R41, Risk of serious damage to eyes, will apply. An aqueous solution of TINOSAN® SDC up to a concentration of 5% adjusted to pH 6 (±) 0.5 has some irritant effect but is not classified as irritant according to the EU classification system. The applicant stated that TINOSAN® SDC is not intended to be applied in the vicinity of the eyes and that pH adjustment of cosmetic formulations is expected to further reduce any irritant effect.

Ref. 18

3.3.3. Skin sensitisation

Delayed skin contact hypersensitivity of Citric acid (and) Silver citrate, (FAT 81034/E, batch 2006.0003, purity 100.0% Citric acid (and) Silver citrate) was determined in mice by the Local Lymph Node Assay according to OECD Guideline 429 (April 2002). Female CBJ/A mice were assigned to five groups of 4 mice per group and given ethanol/water (7/3, v/v) vehicle, alpha-hexylcinnamaldehyde positive control substance, or Citric acid (and) Silver citrate at doses of 5, 10 and 25% in ethanol/water (7/3 v/v). 25% was the highest technically achievable concentration in the chosen vehicle. The vehicle selection was based on results of a non-GLP solubility pre-test.
Neither clinical / local signs nor other findings were observed in any animals of the control group or any animals treated with 5% or 10% of the test item. About three hours after the first topical application, a slight ear erythema was observed at both dosing sites in all mice of the highest dose group, persisting for a total of four days. The stimulation index (SI) was below 3 for each group treated with Citric acid (and) silver citrate Citric acid (and) silver citrate. No dose-response relationship was observed. The ear erythema signs observed in combination with an SI close to 3 (2.9) suggest an irritation response to the test item. The results are summarized in the following table. The HCA positive control group showed the test system was robust and sensitive for the assay.

Table 1: SI values of different concentrations of FAT 81034/E in the LLNA test

Group % FAT 81034/E	SI value
5	2.9
10	2.3
25	2.6
25% HCA	6.2

Conclusion
On the basis of these results, Citric acid (and) Silver citrate is not considered a skin sensitizer.

Ref. 19

3.3.4. Dermal / percutaneous absorption

The absorption and distribution of Citric acid (and) Silver citrate, (FAT 81'034/E, batch 2006.0003, purity 100.0% Citric acid (and) Silver citrate), was evaluated in pig dermatomed skin preparations ex vivo following OECD guideline 428 and SCCNFP opinion 0750/03.

Date of study:	August, 2006
Guideline(s):	OECD 428, SCCNFP 0750/03
GLP/QUA statement:	Signed statements available
Test system:	Dermatomed pig skin (750 µm)
Contact time:	24 hours
Test substance:	Citric acid (and) Silver citrate, FAT 81'034/E
Test formulation:	a) Hand Cream water/oil (W/O type) formulation b) Deodorant oil/water (O/W type) formulation c) Aqueous solution of Citric acid (and) Silver citrate Each of the formulations/solution containing 0.1 % (w/w) Citric acid (and) silver citrate, with a silver content of 1.2 % by weight
Control substance:	Merck ICP Multi-element standard solution VI CertiPUR1.10580.0100 ("MerckVI")
Application:	20 mg/cm^2 (240 ng Ag/ cm^2)
Receptor fluid:	Approximately isotonic phosphate buffer, pH 7.0, consisting of 3.63 g KH_2PO_4 + 7.13 g $Na_2HPO_4*H_2O$ per L water

Six (integrity checked) skin preparations from the back and the flanks of two young pigs, dermatomed to 0.75 mm, were used in each experiment. As the concentrations of the test item in permeate were assumed to be very low the test was performed in a static diffusion cell system to enhance analytical sensitivity. Skins were inserted in static penetration cells (Franz-cells) with an application area of 1.0 cm².
An additional blank experiment was performed with a reduced number of samples (n=2) to analyse the background level of silver in reagents and in the leaching of glass ware. The test substance was analysed by determining the silver content with inductively coupled plasma mass spectrometry (ICP/MS).

Application of test substance
After thawing the deep-frozen (-20 °C) skin, the integrity of each skin membrane was checked by determining the transcutaneous electrical resistance (TER). Only skin samples with a TER of at least 7 kΩ were accepted and used in the test. The test substance formulations were applied topically to the horny layer of the skin in nominal quantities of 20 mg/cm^2, which corresponded to nominally 20 µg test substance or 240 ng silver per cm^2. A non-occlusive exposure under temperature controlled conditions (32° C) was performed and the formulations were left on the skin for 24 h. The receptor fluid consisted of approximately isotonic phosphate buffer, pH 7.0, consisting of 3.63 g KH_2PO_4 + 7.13 g $Na_2HPO_4*H_2O$ per L water. Usual used buffers like phosphate buffered saline were avoided to prevent a precipitation of silver ions by chloride.

Determination of penetration and absorption of test substance
Samples of the receptor fluid were drawn through before and 0.5, 1, 2, 4, 6 and 24 h after application. Twenty-four hours after application, the test substance formulations were removed by rinsing. The skin membranes were consecutively stripped until the stratum corneum was removed from the skin membrane. The sum of absorbed and penetrated quantities was considered the overall amount of bioavailable test substance.

Results
A summary of the results is given in the tables below.

First the data on the applied silver concentration as well as the mass of the applied formulation and test substance are given for the different experiments (a, b, c and blank):

	Citric acid (and) silver citrate in			Blank experiment
	Hand Cream Formulation (W/O type)*	Deodorant Formulation (O/W type)	Aqueous solution	
Concentration of silver in the applied formulation [mg/g]	0.01214	0.01145	0.00972	-
Mass of applied formulation [mg/cm²]	20.7	21.0	21.8	-
Mass of applied test substance [µg/cm²]	20.9	20.0	17.7	-

* Data without an outlier, only 5 samples were used in experiment A (W/O formulation)

In the following table a summary is given on the mean values of the different parameters.

	Experiment A		Experiment B		Experiment C		Blank exp.
Parameter*	**% of dose**	**[µg/cm²]**	**% of dose**	**[µg/cm²]**	**% of dose**	**[µg/cm²]**	**[µg/cm²]**
Skin rinsings	**68.3**	14	**70.6**	14	**31.9**	5.64	0.8
Adsorption after 24 h	**35.97**	7.54	**19.25**	3.83	**56.2**	9.92	0.09
Absorption after 24 h	**3.22**	0.67	**6.03**	1.19	**13.8**	2.43	0.07
Penetration 0-24 h	**0.92**	0.19	**0.76**	0.15	**2.46**	0.43	0.3
Bioavailability after 24 h**	**4.14**	0.86 (sd 0.20)	**6.78**	1.34 (sd 0.5)	**16.2**	2.86 (sd 0.72)	0.4
Mass balance	109.1		98.7		109.1		-

* values for the test substance not corrected for the background level of silver as determined in the blank experiment
** highest values: experiment A: 1.11; experiment B: 2.36; experiment C: 3.71(all in µg/cm²);

At the end of the 24 h exposure period, the major part of applied test substance was still present on the skin membrane (percentage adsorbed on SC + amount in skin rinsings), accounting for 104.3%, 89.9% and 88.1% for the hand cream formulation, deodorant formulation and the aqueous solution of the test substance, respectively. The bulk of the applied test item could be washed off after the exposure period of 24 hours. The majority of these amounts were detected in the pads that were used to dry the skin after the washing. The test item remaining after skin rinse occurred in the stratum corneum (tape strips) and amounted to 36 %, 19.3% and 56.2 % of the dose for the hand cream formulation, deodorant formulation and the aqueous solution of the test substance, respectively.
After tape stripping, respectively 3.2%, 6.0% and 13.8% of the dose for the hand cream formulation, deodorant formulation and the aqueous solution of the test substance were detected in the remaining skin membrane, the dermis and the residual epidermis, including remaining hair shafts. This is the amount of test substance absorbed after 24 hrs.
The cumulative percutaneous penetration at 24 h p.a. was low in each experiment. It was within the background, as detected in the blank experiment performed for determination of the background level of silver in reagents and in the leaching of glass ware. The presented data for the silver solution are therefore an upper limit of the penetration.
The bioavailable amount of test substance (= absorption + penetration) is highest when the aqueous solution of Citric acid (and) Silver citrate is being tested.

Conclusion
Citric acid (and) Silver citrate, applied to pig skin membranes, penetrated at a low rate and to a limited extent through the skin membranes, with 3.2% and 6.0% of the dose from

cosmetic formulations and 13.8% of the dose from an aqueous solution being absorbed in the dermis and epidermis.

Ref. 24

Comments

- The bioavailable fraction of the test substance was 4.1% and 6.8% of the dose from cosmetic formulations and 16.2% of the dose from an aqueous solution.
- Only one concentration of the test substance per formulation has been tested. The tested concentration was too low, since the intended use concentrations of Citric acid (and) Silver citrate is 0.2% in the finished cosmetic product and the present study was carried out with formulations containing only 0.1%.
- An excessive amount of formulation (20 mg/cm²) has been applied.
- The number of samples per experiment is 6 coming from 2 donors, while the SCCP Basic Criteria for the *in Vitro* Assessment of Dermal Absorption of Cosmetic Ingredients ask for the use of 6 samples per donor coming from 3 different donors. In addition, only five samples were evaluated in experiment A, because of one outlier after application of the test substance.
- Reference compound data are lacking (reference substance for validation in phosphate buffer is named in report, but no data are available on that). There are no data reported on the validity of the method (e.g. data obtained with caffeine or benzoic acid).This study is hampered by relative high background levels of silver.
- The analytical limit of quantification in e.g. the aqueous rinsing samples was 0.2 % of the applied substance; the limit of determination in the aqueous risings caused by the background level of silver, was estimated from the mean + 2 SD of the blank samples and was 4.4% of the applied substance.

Given the shortcomings in the study, an MOS cannot be calculated.

3.3.5. Repeated dose toxicity

3.3.5.1. Sub-acute oral toxicity/ repeated dose toxicity

The objective of this study was to evaluate the potential toxicity of the test item, (FAT 81'034/D, batch number 2006.0002, purity 97.8% Citric acid (and) Silver citrate) following daily oral administration (gavage) to rats for 4 weeks. On completion of the treatment period, designated animals of control and high dose groups were held for a 2-week treatment-free period in order to evaluate the reversibility of any findings. The dose-levels used in this 4-week study were selected on the basis of the results of a 5-day dose range-finding study (CIT/Study No. 31269 TSR) in which adverse effects did not occur after daily oral gavage dosing with 75, 300, or 1000 mg/kg/day. The study was designed to comply with guideline OECD 407.

Two groups of five male and five female and one group of 10 male and 10 female rats (Sprague-Dawley rat, Rj Han: SD) were treated once daily, by oral gavage with FAT 81'034/D at dose-levels of 75, 300 or 1000 mg/kg/day for 4 weeks, respectively. The test item was administered as a solution in the vehicle (purified water) adjusted to pH 5 to 6 with sodium hydroxide, at a constant dosage-volume of 10 ml/kg/day. One control group of 10 males and 10 females received the vehicle, purified water, alone under the same experimental conditions. A second control group (placebo group) of 10 males and 10 females received purified water with sodium hydroxide and citric acid to mimic the adjustments to pH made in the treated groups. The actual concentrations of the test item in the dosage forms were determined in weeks 1 and 4 during the administration period, using a validated analytical method.

At the end of the treatment period, the animals of each group were sacrificed, except five animals of each sex in the control groups and in the high dose group, which were kept for a 2-week treatment-free period to observe any changes.

Animals were checked at least once daily for mortality and clinical signs and a detailed clinical examination was performed once a week. Body weights were recorded once during the pre-treatment period, on the first day of treatment and then once a week. Food consumption was recorded once a week during the study. Haematological and blood biochemical investigations as well as urinalysis were performed on all animals at the end of the treatment period and on animals in both control groups and in the high-dose group at the end of the treatment-free period.

A Functional Observation Battery was performed on all animals at the end of the treatment period. Motor activity was assessed over a 1-hour period after the Functional Observation Battery was finished. On completion of the treatment or treatment-free period, the animals were sacrificed and a full macroscopic post-mortem examination was performed. Designated organs were weighed and selected tissue specimens were preserved. A microscopic examination was performed on designated tissues from animals of the control group (group 1) and high-dose group and on all macroscopic lesions.

A satisfactory agreement was observed between the nominal and actual concentrations of the test item in the administered dosage forms analyzed (deviations from nominal concentration in the range of ± 5%).
There were no unscheduled deaths during the study and clinical signs considered related to the test article did not occur. There were no clear effects of treatment on body weight, body weight gain, development or food consumption. No abnormal behaviour was observed during the functional observation battery. Males given 1000 mg/kg/day had statistically significantly higher urea, alkaline phosphatase and alanine aminotransferase. All parameters were comparable with the controls at the end of the treatment-free period. There were no treatment-related effects on haematology and organ weights and no compound-related macroscopic and microscopic findings.

At the end of the treatment period, both males and females given 1000 mg/kg/day had statistically significantly higher specific gravity and pH of the urine and higher urine protein content when compared to the control group 1. Differences between the 1000 mg/kg/day dose group and the control group 2 (placebo group) were negligible. The pH of the urine was also higher than of the control group 1 for the males given 75 or 300 mg/kg/day and the females given 300 mg/kg/day. As all observed effects at 1000 mg/kg/day were similar to effects noted in the placebo group, these changes may be attributed to the pH and citric acid content of the dosage formulations. All parameters were considered to be comparable with the control groups at the end of the treatment-free period.

Conclusion
Based on blood biochemical and urinalysis effects observed at 1000 mg/kg/day, under the experimental conditions, the No Observed Effect Level (NOEL) is concluded to be 300 mg/kg/day and the No Observed Adverse Effect Level (NOAEL) is concluded to be 1000 mg/kg/day.

Ref. 15

3.3.6 Mutagenicity/Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial reverse mutation testing

Citric acid (and) Silver citrate, (FAT 81034/B, batch number 2005.0001, purity 98.9% Citric acid (and) Silver citrate), was tested according to OECD guideline 471 (1997) in the Ames test using four *Salmonella typhimurium* strains (TA98, TA100, TA102, TA1535, and TA1537) and *Eschericia coli* strain WP2*uvr*A. After a preliminary toxicity assay, the substance was tested in two independent experiments in the absence or presence of the

metabolic activation (liver S9 fraction from Aroclor-induced rats). Both experiments were performed according to the direct plate incorporation method except for the second test with S9 mix, which was performed according to the pre-incubation method (60 minutes, 37°C). Positive control substances appropriate to each test system component were used concurrently in the test item experiments.

Since the test item was toxic in the preliminary toxicity test, the choice of the highest dose-level was based on the level of toxicity, according to the criteria specified in the international guidelines. The selected treatment-levels in the main test in the absence of S9 mix ranged from 2.44 up to 625 µg/plate for the *Salmonella* strains and from 2.44 up to 78.13 µg/plate for the *E. coli* strain. In the presence of S9 mix, the dose-levels with the *Salmonella* strains and *E. coli* ranged from 39.06 up to 2500 µg/plate. No precipitate was observed when scoring the revertants. Depending on the tester strain, moderate to marked toxicity was noted at dose-levels ≥39.06 µg/plate without S9 mix, and at dose levels ≥1250 µg/plate with S9 mix.

Citric acid (and) Silver citrate did not induce an increase in the number of revertants, both with or without S9 mix, in any of the six strains, in either experiment. The number of revertants for the vehicle and positive controls was as specified in the acceptance criteria and the study was considered valid.

Conclusion
Under the applied experimental conditions, Citric acid (and) Silver citrate did not show mutagenic activity with and without S9 activation in the bacterial reverse mutation test with *Salmonella typhimurium* and *Escherichia coli.*

Ref. 28

In vitro Mammalian Chromosome Aberration Test

An *in vitro* Chromosome Aberration Assay was conducted according to OECD Guideline N° 473 (1997) with human lymphocytes exposed to Citric acid (and) Silver citrate (FAT 81034/B, batch number 2005.0001, purity 98.9% Citric acid (and) Silver citrate). Three independent experiments were performed with and without exogenous rat liver microsomal (S9 mix) activation. The test item was dissolved in water. Human lymphocytes were prepared from whole blood samples obtained from two healthy donors (one male and one female for each experiment). For each culture, heparinized whole blood was added to culture medium containing phytohemagglutinin (a mitogen to stimulate lymphocyte division) and incubated at 37°C for 48 hours. The highest dose-level for treatment in the first experiment was selected on the basis of pH, osmolality and solubility. For selection of the dose-levels for the second experiment, any toxicity indicated by the reduction of mitotic index (MI) in the first experiment was also taken into account.

In the first experiment, lymphocyte cultures were exposed with or without S9 mix for 3 hours Cells were harvested 20 hours after the beginning of treatment.
In the second experiment, exposure time was 3 or 44 hours without S9 mix, and 3 hours with S9 mix; cell harvest time was 20 hours and 44 hours after the beginning of treatment.
A confirmatory experiment (experiment 3) was performed without S9 mix with an exposure time of 3 or 20 hours and harvest time of 20 hours after the beginning of treatment.
At 1.5 hours before harvest each culture was treated with a colcemid solution to block cells at the metaphase-stage of mitosis.
Mitomycin C and Cyclophosphamide were used as the positive controls
The treatment-levels in the different experiments were from 78.13 µg/ml to 5000 µg/ml with S9 mix, and from 39.06 µg/ml to 2500 µg/ml without S9-mix.

Results

No precipitate was observed at the end of the treatment period, at any dose-level. Due to the decrease in pH observed in the treated dose-levels, pH measurements were performed in treated medium at a time equivalent to the end of the treatment period (first and second experiments) or in the supernatant harvested following the centrifugation (confirmatory experiment).

Experiments without S9 mix:

I) Cytotoxicity:
* 3-hour treatment, a slight to strong toxicity at dose-levels ≥ 1250 µg/ml (33-100% decrease in mitotic index).
* 20-hour treatment, a slight to strong toxicity at dose-levels ≥ 500 µg/ml (36-96% decrease in mitotic index).
* 44-hour treatment, a marked to strong toxicity at dose-levels ≥ 1250 µg/ml, (66-100% decrease in mitotic index).

II) Metaphase analysis:
The dose-levels selected for metaphase analysis induced marked toxicity (up to 62% decrease in mitotic index) at the highest concentrations tested.

Following the 3-hour treatment, a dose dependent increase in the number of cells with structural chromosomal aberrations was noted. Among the structural aberrations, mainly chromatid deletions were observed, some chromosome deletions were also noted. Statistical significant increases in cells with chromosomal aberrations were seen at the highest and strongly cytotoxic doses, only. However, in the third confirmatory assay, where high cytotoxic levels were not observed, an increase in the number of cells with chromosomal aberrations was not found.
Following the 20-hour treatment, a dose-related increase in the frequency of aberrant cells was observed.
Following the 44-hour treatment, no noteworthy increase in the frequency of aberrant cells was observed at 625 µg/ml.
The dose-levels selected and those showing clastogenic activity did not result in severe decrease in pH (at the highest dose-level, in treatment medium harvested after centrifugation, pH shifts ranged from 0.58 to 1.07 in comparison to the vehicle control cultures), however since even weak deviations may result in significant increase in induced frequency of aberrant cells, a contribution of pH shift to the positive result obtained without S9 mix (3-hour and 20-hour treatments) could not be excluded.

Experiments with S9-mix:

I) Cytotoxicity:
* 20-hour harvest time, a slight to severe decrease in mitotic index at dose-levels ≥ 1875 µg/ml (38-100% decrease).
* 44-hour harvest time, a moderate to severe decrease in mitotic index at dose-levels ≥ 1250 µg/ml (44-97% decrease).

II) Metaphase analysis:
In the first experiment with S9-mix the highest dose-level selected for metaphase analysis induced marked toxicity (up to 62% decrease in mitotic index). In the second experiment the decrease in mitotic index was conform the OECD guideline.

No significant increase in the frequency of cells with structural chromosomal aberrations which could be considered as relevant was noted at either harvest time.

Conclusion
Under the experimental conditions, Citric acid (and) Silver citrate induced chromosome aberrations in cultured human lymphocytes in the absence of metabolic activation but not in

presence of S9 activation. This increase in the frequency of aberrant cells was associated with a slight decrease in pH; therefore a contribution of pH shift to the positive result cannot be excluded.

Ref. 29

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

In vivo Bone Marrow Micronucleus Test in mice

The *in vivo* genotoxicity of Citric acid (and) Silver citrate *(*FAT 81034/B, batch number 2005.0001, purity 98.9% Citric acid (and) Silver citrate) was evaluated in mice (5 animals/sex/group) receiving an intraperitoneal administration of the test substance in water, adjusted to pH 5 to 6 with sodium hydroxide, at the dose-levels of 75, 150 and 300 mg/kg/day over a 2-day period. The study was conducted according to OECD guideline N° 474. One group of five males and five females received the vehicle (water for injections, adjusted to pH 5-6 with citric acid and sodium hydroxide) under the same experimental conditions, and acted as control group. An additional group of five males and five females received the vehicle (water for injections) without any adjustment of pH. For proof of systemic exposure, supplementary animals (three males and three females) were used at the high-dose group in order to determine plasma levels of the test item. One group of five males and five females received the positive control test item (Cyclophosphamide) once by oral route at the dose-level of 50 mg/kg. The top dose-level for the cytogenetic test was selected according to the criteria specified in the international guidelines; since toxic effects were observed in a preliminary toxicity test, the choice of the top dose-level was based on the level of toxicity. The highest dose-level of 300 mg/kg/day was the maximum tolerated dose since immediately higher dose-levels induced mortality.

Mean ± SD plasma levels of the test item obtained 4 hours following the second treatment in animals treated at 300 mg/kg/day were 355 ± 72 µg/g and 116 µg/g, for males and females respectively. Standard deviation was not calculated for females since one female out of three showed a value below of the limit of quantification and only two values were available.
Achieved plasma levels clearly demonstrated the systemic exposure to the test item. Animals of the treated and vehicle control groups were sacrificed 24 hours after the last treatment, animals of the positive control group were killed 24 hours after the single treatment and the bone marrow cells were collected for micronuclei analysis. For micronuclei, 2000 polychromatic erythrocytes (PCEs) per animal were scored. The polychromatic and normochromatic (NE) erythrocyte ratio was established by scoring a total of 1000 erythrocytes.

The test item did not exert any cytotoxic effect in the bone marrow as indicated by unchanged ratios of normochromatic erythrocytes (NCEs) to PCEs in treated animals compared to controls. However, plasma levels pointed to sufficient systematic exposure. Statistically significant increases in the frequency of micronucleated polychromatic erythrocytes did not occur in the Citric acid (and) Silver citrate treated groups at any preparation interval and with any dose level used.

Conclusion
Under the experimental conditions Citric acid (and) Silver citrate is considered to be not mutagenic in the in vivo mouse micronucleus assay.

Ref. 30

General comment on mutagenicity
Only 2 *in vitro* mutagenicity tests were submitted where the SCCP Notes of Guidance requires 3 *in vitro* mutagenicity tests. No scientific justification for this omission has been given. In the absence of positive *in vitro* studies, an additional *in vivo* test is not considered

appropriate. However, an *in vitro* mammalian cell gene mutation test is required to exclude gene mutation potential.

3.3.7 Carcinogenicity

No data submitted

3.3.8 Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2 Teratogenicity

The objective of this study was to evaluate the potential toxic effects of the test item Citric acid (and) Silver citrate (FAT 81'034/D, batch 2006.0002, purity 97.8% Citric acid (and) Silver citrate), on the pregnant female and on embryonic and foetal development following daily oral administration (gavage) to pregnant female rats during the period of organogenesis (from implantation to closure of the hard palate: day 6 to day 20 post-coitum inclusive). The study design was based on U.S. EPA OPPTS Prenatal Developmental Toxicity Study Testing Guideline (OPPTS 870.3700, August 1998) and OECD 414.
Three groups of 24 mated female rats of the Sprague-Dawley strain received the test item FAT 81'034/D, adjusted with sodium hydroxide (10% solution) to a pH between 5 and 6, by daily oral administration at 75, 300 or 1000 mg/kg body weight/day at a constant dosage volume of 10 ml/kg/day; the once-daily doses were administered from day 6 to day 20 post-coitum (p.c.). Two groups, each including 24 mated females of the same strain served as the control groups. One control group received the vehicle alone (purified water), the "placebo control" group received the vehicle with citric acid and sodium hydroxide under the same experimental conditions. Dosages were selected based on a dose-range finding study with doses of 75, 300, and 1000 mg/kg/day in which test item treatment did not elicit any maternal or foetal toxicity at any dose-level and none of the pregnancy parameters were affected by the treatment. The dose formulation was prepared daily and shown by analytical verification to remain stable under refrigeration for this length of time.
Clinical signs and mortality were checked daily. Body weight and food consumption were recorded at designated intervals. On day 21 p.c., the dams were sacrificed and subjected to a macroscopic post-mortem examination. The gravid uteri were weighed and the foetuses were removed by hysterectomy. The following litter parameters were recorded: numbers of corpora lutea, number and distribution of dead and live foetuses, of early and late resorptions, of uterine scars and of implantation sites.

Following a technical problem at evisceration of the foetuses before staining of the skeletons, less than 20 litters were available for skeletal evaluation. In order to have 20 litters per group for skeleton evaluation, a complementary study phase was performed. Sufficient numbers of pregnant females per group from the complementary study phase to achieve 24 females and 20 litters were selected for inclusion in the main study.
The foetuses were weighed, sexed and subjected to external, soft tissue or skeletal examinations. Uterine horns without visible implantation site were immersed when appropriate in an aqueous solution of ammonium sulphide to reveal the presence of uterine scars. The placentas were examined for grossly observable changes.

Results
Unscheduled deaths did not occur in any of the dosed pregnant females of any group. Body weight, body weight change, and food consumption were not affected by the test material. The gravid uterine weights in the test material-dosed dams were not significantly different

from those in the dams of the control groups. No abnormal treatment-related clinical signs were observed in any female given the test item.

Litter data. The numbers of corpora lutea and implantation sites were equivalent between test item-treated and both control groups. At 1000 mg/kg/d, the percentage of the live foetuses was slightly lower than in the control group. This was mainly due to one dam with a high proportion of early and late resorptions. Excluding this dam, all values of litter parameters were similar to those of the placebo control group, thereby excluding a relationship with test item treatment. Sex ratio and mean foetal body weight were not affected by treatment with the test item.
Two and one foetuses, each from a single litter in groups given 75 or 300 mg/kg/day, respectively showed several external malformations and variations. These included cleft palate, cleft lip, mandibular cleft, protruding tongue, sirenomelia, gastroschistis, arhinia, malrotated paw, anal atresia and short tail. However, these findings were considered to be spontaneous in origin, as no malformations were observed in the high-dose group and as these malformations are seen in similar frequency in historical control foetuses from this laboratory.
Soft tissue malformations and variations including dilated renal pelvis, absent kidney, absent or dilated ureter, malpositioned testis and absence of bladder were either limited to the lowest dose-level (sometimes including the control group) and not present in the highest dose group, or were within the range of the laboratory's historical control data. Therefore none of them were considered to be attributable to the test material.
The only skeletal malformation observed was absence of lumbar vertebra in one foetus from the 1000 mg/kg/day dose group. As this finding was not correlated with other skeletal observations and due to its limited incidence, the relationship to treatment with the test item was not established. The general ossification of the foetuses was not affected by the test item treatment. No variations were considered to be significantly increased in comparison with the controls.

Conclusion
Based on the study results and in the absence of test material-related malformations and effects on dams, embryo and foetus, it is concluded that for Citric acid (and) Silver citrate , the NOEL of 1000 mg/kg body weight/day, the highest dose used, can be set for maternal toxicity, embryotoxicity and foetal effects.

Ref. 23

Comment
This study included two groups of rats (due to insufficient number of pregnant females). The results of these groups were combined.

3.3.9 Toxicokinetics

No data submitted

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

The cutaneous phototoxic potential of Citric acid (and) Silver citrate (FAT 81034/B, batch 2005.0001, purity 98.9% Citric acid (and) Silver citrate) dissolved in purified water was evaluated in Hartley Crl: (HA) BR Guinea pigs. The photoirritant potential of the test item was evaluated after the first treatment and/or irradiation performed on day 1 in the animals of all groups. Cutaneous reactions were scored before and 1 hour, 4 and 24 hours after the single application and/or irradiation. For assessment of the photosensitizing potential, six topical applications and/or UV A + UV B irradiation were performed on the anterior area of the right and left flanks of the animals of all groups (including that for photoirritant potential

assessment). Following a rest period of 13 days without treatment and irradiation, on day 22, a challenge phase was performed by topical application and/or UV A + UV B irradiation to the posterior area of the right and left flanks of the animals. Cutaneous reactions were scored before and 1 hour, 4, 24 and 48 hours after the challenge application and/or irradiation.
Purified water was used as vehicle. Irradiation was performed thirty minutes after the treatment phase (infra-erythematogenic doses, first irradiation with UV B, 0.1 Joule/cm^2, and then irradiation with UV A, 9 Joule/cm^2). The non-irradiated region (back and flanks) was protected from the ultra-violet irradiation.
Two preliminary tests were performed in order to determine the maximal non-photoirritant concentration of the test item. As coloration induced by irradiation was observed, the maximal concentration of the test item for which the coloration induced by irradiation did not mask the evaluation of the erythema was also evaluated in a preliminary test.
Two main tests were performed.

Experiment 1

Study design

Group	Number of Animals	Induction phase (6 applications days 1 to 8)*		Challenge phase (day 22)	
		Anterior left flank	Anterior right flank	Posterior left flank	Posterior right flank
1	10	5% or 10% Test item	None	5% Test item	2.5% Test item
2	10	5% or 10% Test item + UV	Vehicle + UV	5% Test item + UV	2.5% Test item + UV
3	5	Vehicle + UV	UV	5% Test item + UV	2.5% Test item + UV

*The concentrations of the test item in the induction phase of the first experiment were 10% (w/w) on days 1 to 3, 5% (w/w) on day 6, and 10% (w/w) on days 7 and 8.

Results
Photoirritant potential
No cutaneous reactions were noted on either flank of the group 1 animals.
The cutaneous reactions (questionable or discrete erythema; grade 0.5 or 1) observed on both flanks of the group 2 and 3 animals remained within the range of a local reaction at an infra-erythematogenic irradiation dose, except for a moderate erythema (grade 2) observed in one animal of the vehicle control group (group 3). No cutaneous reactions persisted at day 2.
In all animals of group 2, a brown coloration of the skin was noted on the left flank. Due to the coloration masking the evaluation of the erythema, it was not possible to conclude on the photoirritant potential of the test item.

Photoirritant potential of FAT 81034/B in the first experiment

Group	Number of animals	Scorings of skin reactions*							
		Day 1				Day 2			
		LF		RF		LF		RF	
1	10	0	(10/10)	0	(10/10)	0	(10/10)	0	(10/10)
2	10	1/C C1 C2	(1/10) (1/10) (8/10)	0 0.5 1	(7/10) (2/10) (1/10)	0/C C1 C2	(1/10) (8/10) (1/10)	0	(10/10)
3	5	0.5 1 2	(2/5) (2/5) (1/5)	0 0.5 1	(1/5) (3/5) (1/5)	0	(5/5)	0	(5/5)

* Evaluation of photosensitizing reactions from day 1 and 2

Photosensitizing potential:
A discrete erythema was noted on the left and right flanks of the group 1 animals at day 22. No cutaneous reactions persisted thereafter. In group 2, a brown coloration of the skin was noted on the left and right flanks of all animals at day 22 and persisting at the 48-hour reading. A discrete or moderate erythema (grade 1 or 2) was observed on the left flank, discrete erythema persisting at the 24-hour reading in 2/10 animals. On the right flank, a discrete or moderate erythema was observed only at day 22. In group 3, a brown coloration of the skin was noted on the left and right flanks of all animals. A discrete or moderate erythema was observed on both flanks, on the left flank a discrete erythema persisting at the 48-hour reading in one animal. On the right flank, discrete or moderate erythema was noted at the 24-hour reading in all animals. The skin reactions were attributed to a photoirritant potential of the test item.
Due to the similar reactions observed in groups 2 and 3 it was not possible to conclude on the photosensitizing potential of the test item.

Photosensitizing potential of FAT 81034/B in the first experiment

Group	Number of animals	Scorings of skin reactions*					
		Day 22		Day 23		Day 24	
		LF	RF	LF	RF	LF	RF
1	10	1 (7/10)	1 (3/10)	0 (10/10)	0 (10/10)	0 (10/10)	0 (10/10)
2	10	C1 (4/10) 1/C (1/10) 2/C (5/10)	0 (1/10) C1 (3/10) 1/C (5/10) 2/C (1/10)	0 (3/10) 0/C (5/10) 1/C (2/10)	0 (7/10) 0/C (3/10)	0 (7/10) 0/C (3/10)	0 (9/10) 0/C (1/10)
3	5	C1 (1/5) 1/C (1/5) 2/C (3/5)	C1 (2/5) 2/C (3/5)	1 (2/5) 1/C (1/5) 2/C (2/5)	1 (3/5) 1/C (1/5) 2/C (1/5)	0 (1/5) 0/C (3/5) 1/C (1/5)	0 (3/5) 0/C (2/5)

* Evaluation of photosensitizing reactions from day 22 to day 24

Experiment 2

In the second experiment, a slightly lower dose of UV irradiation and a lower concentration of the test item for the induction and challenge phase were used.

Study design

Group	Number of animals	Induction phase (6 applications days 1,2,3,6,7, 8)		Challenge phase (day 22)	
		Anterior left flank	Anterior right flank	Posterior left flank	Posterior right flank
1	10	5% Test item	None	1% Test item	None
2	10	5% Test item + UV	Vehicle + UV	1% Test item + UV	Vehicle + UV
3	5	Vehicle + UV	UV	1% Test item + UV	UV

Results
Photoirritant potential:
In group 1, a discrete erythema was observed on the left flank of the animals at the first day only.
In group 2, a brown coloration of the skin and a questionable or discrete erythema was noted on the left flank of the animals. On the right flank, the cutaneous reactions (questionable or discrete erythema) observed remained within the range of a local reaction at an infra-erythematogenic irradiation dose. In group 3, the cutaneous reactions (questionable or discrete erythema) observed on both flanks of the animals remained within the range of a local reaction at an infra-erythematogenic irradiation dose. No cutaneous reactions persisted at the 24-hour reading. The cutaneous reactions in the group

2 being at most similar to those noted in the group 1, it was not possible to conclude on the photoirritant potential of the test item.

Photoirritant potential of FAT 81034/B in the second experiment

Group	Number of animals	Scorings of skin reactions*			
		Day 1		Day 2	
		LF	RF	LF	RF
1	10	1 (6/10)	0 (10/10)	0 (10/10)	0 (10/10)
2	10	0.5/C (3/10) 1/C (3/10) C1 (4/10)	0 (4/10) 0.5 (6/10)	0 (2/10) 0/C (1/10) C1 (1/10) C2 (1/10) 1 (4/10) 1/C (1/10)	0 (10/10)
3	5	0.5 (5/5)	0 (2/5) 0.5 (3/5)	0 (5/5)	0 (5/5)

* Evaluation of photoirritant reactions on days 1 and 2

Photosensitizing potential:
In group 1, no cutaneous reactions were noted except a discrete erythema on the left flank of 1/10 animals at the 24-hour reading. In group 2, a brown coloration of the skin was observed on the left flank at the 1-hour reading only. On both flanks, questionable erythema was observed. No cutaneous reactions were noted at the 48-hour reading. The cutaneous reactions remained within the range of a local reaction at an infra-erythematogenic irradiation dose. In group 3, cutaneous reactions observed were limited to a brown coloration of the skin at the 1-hour reading and a questionable erythema at the 24- and 48-hour readings, both on the left flank. The cutaneous reactions remained within the range of a local reaction at an infra-erythematogenic irradiation dose. No cutaneous reactions which could be attributed to a photosensitizing effect of the test item were observed.

Photosensitizing potential of FAT 81034/B in the second experiment

Group	Number of animals	Scorings of skin reactions*					
		Day 22		Day 23		Day 24	
		LF	RF	LF	RF	LF	RF
1	10	0 (10/10)	0 (10/10)	0 (9/10) 1 (1/10)	0 (10/10)	0 (10/10)	0 (10/10)
2	10	0 (3/10) 0/C (3/10) 0.5 (4/10)	0 (9/10) 0.5 (1/10)	0 (8/10) 0.5 (2/10)	0 (8/10) 0.5 (2/10)	0 (10/10)	0 (10/10)
3	5	0 (4/5) 0/C (1/5)	0 (5/5)	0 (4/5) 0.5 (1/5)	0 (5/5)	0 (4/5) 0.5 (1/5)	0 (5/5)

* Evaluation of photosensitizing reactions from day 22 to day 24

Overall, based on the results of both experiments, erythema noted in animals following application of Citric acid (and) Silver citrate at a concentration of 2.5% and 5% during the challenge phase of the first experiment were attributed to a photoirritant potential of the test item. No cutaneous reactions that could be attributed to photoirritant reactions were noted after application of Citric acid (and) Silver citrate at a concentration of 1%.

Conclusion
Under the experimental conditions, topical applications of Citric acid (and) Silver citrate followed by UV irradiation did not induce any photosensitizing reactions, but induced photoirritation reactions in guinea pigs when applied at concentrations of 2.5% and above. On the basis of these results, Citric acid (and) Silver citrate applied at concentrations up to 1% is not considered photoirritant.

Ref. 20

Comment
Difficult to assess because it is not clear whether an applicable test method is used (no guidelines available for in vivo test). The "3t3 NRU PT" in vitro test has been recommended by the SCCP for the assessment of cosmetic ingredients that are used as UV filters (SCCNFP/0690/03), however, this test has not been performed for FAT 81'034/B.

3.3.10.2 Phototoxicity / photomutagenicity / photoclastogenicity

Photo-mutagenicity in Bacteria

This study was performed to investigate the potential of Citric acid (and) Silver citrate (FAT 81´034/C, batch No. 2006.0001, purity 96.9% Citric acid (and) Silver citrate) dissolved in deionised water) to induce gene mutations under irradiation with artificial sunlight according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the *Salmonella typhimurium* strains TA 1537, TA 98, TA 100, and TA 102. These strains were chosen since they tolerate relatively high doses of UV irradiation used to assess the possible photomutagenic potential of sunblockers.
The irradiation was performed with a Xenon-lamp that emits a continuous spectrum of simulated sunlight. The intensity of irradiation was 0.1 – 0.3 mW/cm^2 and each bacterial strain received its respective amount of tolerable UVA and UVB exposure.
The assay was performed in two independent experiments. Each concentration, including the controls, was tested in triplicate. The test item was tested at the following concentrations:

Pre-Experiment: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate
Experiment I and II: 0.3; 1; 3; 10; 33; 100; and 333 µg/plate

Toxic effects, evident as a reduction in the number of revertants, occurred in all experiments at higher concentrations. The plates incubated with the test item showed reduced background growth in all strains used in the pre-experiments and experiment I.
No substantial increase in revertant colony numbers of any of the four tester strains was observed following treatment with Citric acid (and) Silver citrate under irradiation with artificial sunlight at any dose level. There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.
Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies. In conclusion, it can be stated that under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Conclusion
Citric acid (and) Silver citrate is considered to be non-mutagenic in this Salmonella typhimurium photomutagenicity assay.

Ref. 31

Photo-clastogenicity assay with V79 Chinese Hamster cells in vitro

Citric acid (and) Silver citrate (FAT 81´034/C, batch No. 2006.0001, purity 96.9% Citric acid (and) Silver citrate), dissolved in deionised water, was assessed for its potential to induce structural chromosomal aberrations in V79 Chinese Hamster cells in the absence and presence of artificial sunlight in three independent experiments.

The light source was an Atlas Suntest CPS, a xenon burner with an additional special filter glass, emitting visible light and UVA/UVB light (ratio: about 30:1) > 290 nm. In this study,

the cultures were pre-incubated with the test item for 30 minutes where after they were exposed to 125 mJ/cm^2 UVA (Exp. I and II) or 200 mJ/cm^2 UVA (Exp. II). Three hours after start of treatment, the cultures were washed. Corresponding cultures with the test item were kept in the dark for the 3 hrs exposure period. The chromosomes were prepared 18 hrs (Exp. IA and IB) and 28 hrs (Exp. II) after start of treatment with the test item.

In the cytogenetic experiments for each experimental group two parallel cultures were set up. Per culture at least 100 metaphase plates were scored for structural chromosome aberrations, except for the positive control in Exp. II after irradiation (200 mJ/cm² UVA), where 50 metaphase plates were scored due to strong genotoxicity.
Clear toxic effects were observed after treatment with 9.8 µg/ml and above with and without
irradiation in a pre-test. Precipitation was observed at concentrations of 625 µg/ml and above. Dose selection for the cytogenetic experiments was performed considering the toxicity data.
The test conditions and applied concentrations are summarized in the following Table:

Table 9. Test conditions and applied concentrations of FAT 81´034/C in photo-clastogenicity assay

Exp.	Exposure Period	Preparation Interval	Concentrations (µg/ml)				
IA	3 hrs	18 hrs	0.625	1.25	2.5		
IB	3 hrs	18 hrs	2.5	3.125	3.75	4.375	5.0
II	3 hrs	28 hrs	1.25	2.5	5.0		
IA	3 hrs	18 hrs	0.625	1.25	2.5		
II	3 hrs	28 hrs	1.25	2.5	5.0		
			200 mJ/cm^2 UVA and 6.5 mJ/cm^2 UVB				
II	3 hrs	28 hrs	1.25	2.5	5.0		

In Experiment IA, clastogenicity was not observed at the concentrations evaluated, neither with nor without irradiation. In contrast, in the confirmatory Experiment IB, in the absence of irradiation, and in Experiment II, with or without irradiation with 125 and 200 mJ/cm², clastogenicity was observed after treatment with the test item. In addition, in Experiment II, in the absence and the presence of irradiation the frequencies of polyploid metaphases and of endoreduplications were distinctly increased beyond the historical control data range. Appropriate mutagens were used as positive controls. They induced statistically significant increases ($p < 0.05$) in cells with structural chromosome aberrations.

In conclusion, it can be stated that under the experimental conditions reported, TINOSAN SDC active induced structural chromosome aberrations in the presence and absence of artificial sunlight as determined by the chromosomal aberration test in V79 cells (cell line from the lung of the Chinese Hamster).

Conclusion
Citric acid (and) Silver citrate is considered to be clastogenic in this chromosomal aberration test.

Ref. 32

3.3.11 Public literature on the toxicity of silver

Some reviews on the toxicity of silver are cited in the dossier. The applicant concluded that in general, silver is of limited concern for adverse toxicological effects. However, silver causes argyria, a permanent discoloration of the skin, after chronic high level exposure, irrespective of the route of exposure. This effect is a result of irreversible accumulation of

silver in the skin, which upon exposure to light leads to deposition as silver complexes with sulphide or selenium. The accumulated or deposited silver is not known to interfere with organ or tissue functions. Based on argyria, the US-EPA established a reference dose (RfD) of 0.005 mg/kg bw/day, which is considered sufficient to prevent argyria from a life-time oral exposure to silver. The study on which this reference dose is based (from 1935) reports one individual who developed argyria following an iv dose of 1 g silver (4 g silver arsphenamine). Other individuals did not respond until levels five times higher were administered.

Ref. 1

Comment of the SCCP
The human study the US-EPA reference dose was based on is very old (1935) and offers only information regarding the total dose of silver injected over a stated period of time.
Only (syphilis) patients developing argyria are described, and no information is provided on patients who received the same dose of silver arsphenamine without developing argyria. Therefore, it is difficult to establish a NOAEL. Furthermore, the patients suffering from syphilis may have been of compromised health. The studies used to support the RfD were not controlled studies in which the amount of ingested silver is determined.

Ref. 34

The WHO (2003, silver in drinking water) concludes that there have been no reports of argyria or other toxic effects resulting from the exposure of healthy persons to silver. On the basis of present epidemiological and pharmacokinetic knowledge, a total lifetime oral intake of about 10 g of silver can be considered as the human NOAEL. This corresponds to 0.39 mg Ag/person/day or 0.0065 mg/kg bw/day. As the contribution of drinking-water to this NOAEL will normally be negligible, the establishment of a health based guideline value is not deemed necessary. On the other hand, special situations may exist where silver salts are used to maintain the bacteriological quality of drinking-water. Higher levels of silver, up to 0.1 mg/litre (a concentration that gives a total dose over 70 years of half the human NOAEL of 10 g), could then be tolerated without risk to health.

Ref. 36

EFSA has evaluated silver-based preservatives for the use in food contact materials on the basis of human and animal data and has derived a group restriction limit of 0.05 mg Ag/kg food, which corresponds to a worst case exposure of 0.05 mg Ag/person/day or 0.00083 mg/kg bw/day.

Ref. 37

3.3.12 Safety evaluation (including calculation of the MoS)

A margin of safety cannot be calculated.

3.3.13 Discussion

Systemic toxicity
In an acute dermal and oral LD_{50} a 21% solution of citric acid (and) silver citrate dosed at 5000 mg/kg was non-toxic. As a result, the active ingredient citric acid (and) silver citrate is considered to be maximum moderately toxic.
In an oral 28-day study in rats, the No Observed Adverse Effect Level (NOAEL) was 1000 mg/kg/day. No 90-day study was performed.
In a teratogenicity study the NOEL for maternal toxicity, embryotoxicity and foetal toxicity was 1000 mg/kg bw/ day, the highest dose tested.

Local toxicity
Citric acid (and) silver citrate was not irritant to skin. The powdered form of citric acid (and) silver citrate is severely irritating to the eye when applied directly. An aqueous solution of TINOSAN® SDC up to a concentration of 5% adjusted to pH 6 (±) 0.5 has some irritant effect. pH adjustment of cosmetic formulations is expected to further reduce any irritant effect.
Citric acid (and) silver citrate did not show a sensitizing potential in an LLNA test. The substance was photoirritant in a Guinea pig test at concentrations of 2.5% and above, it showed no photoirritant effects when tested at a concentration of 1%. The substance was not a photosensitizer in the Guinea pig.

Genotoxicity
Citric acid (and) silver citrate was not mutagenic to bacteria *in vitro* with and without UV irradiation. It was clastogenic to human lymphocytes *in vitro* in the absence but not in the presence of metabolic activation. Citric acid (and) silver citrate was also clastogenic in V79 Chinese Hamster cells *in vitro* with and without UV irradiation, but it was not mutagenic in an *in vivo* micronucleus test in mice. The submitted *in vitro* mutagenicity tests do not fulfil the SCCP requirements for genotoxicity testing of cosmetic ingredients. An *in vitro* mammalian cell gene mutation test is required to exclude gene mutation potential.

Dermal absorption
An ex vivo study has been conducted on pig skin showing low penetration rates and low to medium amounts of test substance being absorbed in the dermis and epidermis. Oil/water and water/oil formulations gave similar results (a mean of 4.1 and 6.8% of the applied dose bioavailable, respectively), while a mean of 16.2% for an aqueous solution of Citric acid (and) Silver citrate became bioavailable after 24 hour contact to the ex vivo skin. However, the SCCP noted several shortcomings of this study, which raised doubts about the reliability of the results. These include a low number of samples used, the inadequate concentration of Citric acid (and) Silver citrate in the formulations tested, and on the excessive amount of formulation applied on the skin, which may have influenced the dermal absorption values. Therefore this study cannot be used for the calculation of the margin of safety.

Discussion on risk assessment
In light of the shortcomings of the dermal absorption study, a data-based margin of safety cannot be calculated.

However, using worst case assumptions for dermal penetration (100% absorption) the resulting exposure to citric acid (and) silver citrate (SED 0.6 mg/kg bw/day) would lead to a high MoS when compared to the NOAEL of 1000 mg/kg bw/day of citric acid (and) silver citrate from the oral 28 day study provided. This NOAEL is in agreement with the low systemic toxicity that both citric acid and silver have demonstrated in previous studies when tested individually.

The oral 28-day study from which the NOAEL was derived, is, due to its short duration, not relevant for argyria, which is generally considered the most critical endpoint for silver compounds.

With regard to argyria, various regulatory limits have been set for exposure to silver. These are based on values from old case reports of patients with argyria.
These limits are:
- a US EPA RfD of 0.005 mg/kg bw/d
- a WHO human lifetime NOAEL of 10 g silver (0.39 mg Ag/person/day, 0.0065 mg/kg bw/d)

- an EFSA group restriction limit of 0.05 mg Ag/kg food, (worst case exposure of 0.05 mg Ag/person/day, 0.00083 mg/kg bw/d)

Taking the above limits, the daily exposure to silver from citric acid (and) silver citrate as a preservative in cosmetics, considering 100% absorption (arriving at 0.0072 mg silver/kg bw /day) is in the same order of magnitude as the EPA RfD and the WHO human lifetime NOAEL and an order of magnitude higher than the EFSA group restriction limit.

Calculating the daily exposure to silver from citric acid (and) silver citrate as a preservative in cosmetics based on a worst case assumption from the dermal absorption study provided (16.2%, from experiment C, aqueous solution), the resulting SED is below the US EPA and WHO lifetime NOAEL, and only slightly exceeds the EFSA group limit; the latter takes into account exposure to silver from additional sources.

Silver (1.2% in mixture)	SED
absorption observed in dermal absorption study (16.2 %), experiment C (worst case assumption)	0.0012 mg/kg bw /day
100% absorption of cosmetics applied daily (18g)	0.0072 mg/kg bw /day

In conclusion, exposure assessment calculation made considers exposure to silver from one cosmetic ingredient only and does not take into account the contribution of other sources of silver (food, air, products, biocides etc) to the overall exposure. However, considering that a very conservative dermal absorption assumption was used, the potential risk for the development of argyria from citric acid (and) silver citrate used in cosmetics, at the proposed concentrations, is judged to be low. New data specifically addressing the end point of argyria is necessary to strengthen this judgement based conclusion.

4. CONCLUSION

On the basis of the data submitted, the safety of citric acid (and) silver citrate cannot be assessed. Before a final conclusion can be reached, an *in vitro* mammalian gene mutation assay to exclude gene mutation potential is required.

The critical aspect in the safety evaluation of silver containing compounds, like citric acid (and) silver citrate, is possible long-term effects of the silver released, in particular in relation to argyria.

The available toxicity data in relation to silver, on which various regulatory limits (RfD by US-EPA, lifetime NOAEL by WHO and group restriction limit for food contact material by EFSA) are based, are very limited and old. Using these data and conservative dermal absorption assumptions derived from the dermal absorption study provided, the SCCP considered that consumer exposure to silver from citric acid (and) silver citrate at the proposed concentration in cosmetics amounts to only a fraction of the reference dose for silver. It came to the judgment-based conclusion that the potential risk for the development of argyria due to this exposure is low. On that basis, the SCCP considers that an additional dermal absorption study is not necessary. However, new data specifically addressing the end point of argyria is necessary to strengthen this judgement based conclusion.

The SCCP is aware that additional silver toxicity information has been generated to address other regulatory needs and would, therefore, welcome the opportunity to obtain and review these data.

This opinion is limited to the evaluation of the potential exposure and risks associated with the use of citric acid (and) silver citrate in cosmetic products at the proposed use concentration. It does not concern uses of other silver containing cosmetic ingredients or exposure to silver from other non-cosmetic sources. Evidence in the public domain suggests an increase in the exposure of consumers to silver from sources other than cosmetics (e.g. textiles, cleaning products, medical products). Therefore, the SCCP strongly recommends the consideration of an aggregate exposure and risk assessment.

5. MINORITY OPINION

Not applicable

6. REFERENCES

1. Ciba Dossier (28 December 2006): Toxicology summary and safety assessment for Citric acid (and) Silver citrate (FAT 81'034). A preservative for personal care products
2. Ciba Specialty Chemicals Inc., Service Center Structure Elucidation, CH-4002 Basel Project Numbers 114070502, (14.11.2005), 131010603 (24.02.2006), 131010603 (04.05.2006), 8131010603 (06.06.2006). Reports on analytical certification and characterization of FAT81'034/B, -/C, -/D, -/E
3. Ciba Specialty Chemicals Inc., CH-4002 Basel. Certificate of Analysis of TINOSAN® SDC (FAT 81'033/A) effective from 07.09.2005
4. IBACON GmbH, D-64380 Rossdorf. Study Number 28023185 (December 21, 2005). Determination of the water solubility of FAT 81'034/B
5. IBACON GmbH, D-64380 Rossdorf. Study Number 28021186 (December 20, 2005). Determination of the partition coefficient (n-octanol/water) of FAT 81'034/B by Shake Flask Method
6. IBACON GmbH, D-64380 Rossdorf. Study Number 28022180 (December 02, 2005). Determination of the melting point/melting range of FAT 81034/B
7. Ciba Specialty Chemicals Inc., CH-4002 BASEL. (May 12, 2006). Expert statement on the expected boiling point of FAT 81'034/B
8. IBACON GmbH, D-64380 Rossdorf. Project Number 28021183 (December 02, 2005). Expert statement on the expected vapour pressure of FAT 81'034/B
9. IBACON GmbH, D-64380 Rossdorf. Study Number 28021187 (December 20, 2005). Determination of the flammability of FAT 81'034/B
10. IBACON GmbH, D-64380 Rossdorf. Project Number 28301193/28304191 (September 05, 2005). Expert statement on the expected rate of hydrolysis and on the explosive properties of FAT 81'034/B
11. IBACON GmbH, D-64380 Rossdorf. Project Number 28028360 (September 05, 2005). Estimation of particle size distribution of FAT 81'034/B. Report 7
12. United States Food and Safety Authority (FDA). 21 CFR, Part 184.1033 - Direct food substances affirmed as generally recognized as safe (GRAS) – Citric acid
13. PSL product safety labs, East Brunswick, New Jersey, USA. PSL Study number 8133 (21 October 1999). Axenohl: Acute oral toxicity study in rats - Limit Test
14. PSL product safety labs, East Brunswick, New Jersey, USA. PSL Study number 8111 (21 October 1999). Axenohl: Acute dermal toxicity study in rats - Limit Test
15. CIT F-Evreux, Laboratory Study number 31589 TSR (27 December 2006). 4-week toxicity study by oral route (gavage) in rats followed by a 2-week treatment-free period with FAT 81'034/D
16. CIT F-Evreux, Laboratory Study number 31054 TAL (04 May 2006). Acute dermal irritation study in rabbits with FAT 81'034/B
17. CIT F-Evreux, Laboratory Study number 31055 TAL (12 May 2006). Acute eye irritation study in rabbits with FAT 81'034/B

18. CIT F-Evreux, Laboratory Study number 31345 TAL (12 May 2006). Acute eye irritation study in rabbits with FAT 81'033/A at the concentrations 0.5% and 5%
19. RCC Ltd, CH-4452 Itingen. Study Number A79255 (18 August 2006). FAT 81034/E - Local Lymph Node Assay (LLNA) in Mice (Identification of Contact Allergens)
20. CIT F-Evreux, Laboratory Study number 31056 TSG (14 September 2006). FAT 81'034/B - Photoirritation and Photosensitization by Cutaneous Route in Guinea Pigs
21. Agency for Toxic Substances and Disease Registry (ATSDR) (1990) Toxicological profile for silver. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service
22. Faust R (1992) Toxicity summary for silver. Health and Safety Research Division Oak Ridge National Laboratory, Oak Ridge, Tennessee. Available from internet address: http://www.risk.lsd.ornl.gov/tox/profiles/silver_f_V1.shtml (US Dept of Energy, Risk Assessment Information System, Oak Ridge National Laboratory)
23. CIT F-Evreux, Laboratory Study number 31858 RSR (22 December 2006). FAT 81'034/D - Prenatal developmental toxicity study by oral route (gavage) in rats
24. ARC Seibersdorf Research GmbH, A-2444 Seibersdorf, Austria, Study number CIB8 (31 August 2006). FAT 81'034/E: Dermal absorption / percutaneous penetration in vitro.
25. SCCNFP (Scientific Committee on Cosmetic Products and Non-food products intended for Consumers) (2003a). Opinion concerning "Basic criteria for the in vitro assessment of dermal absorption of cosmetic ingredients". SCCNFP/0750/03. Updated October 2003
26. SCCNFP (Scientific Committee on Cosmetic Products and Non-food products intended for Consumers) (2003b) The SCCNFP's notes of guidance for the testing of cosmetic ingredients and their safety evaluation. 5th revision. SCCNFP/0690/0
27. Bremmer, HJ ; Prud'homme de Lodder, LCH ; Van Engelen, JGM. (2006). Cosmetics Fact Sheet. To assess the risks for the consumer. Updated version for ConsExpo 4.RIVM report 320104001, RIVM Bilthoven, The Netherlands
28. CIT F-Evreux, Laboratory Study number 30974 MMT (14 April 2006). Bacterial reverse mutation test with FAT 81'034/B
29. CIT F-Evreux, Laboratory Study number 30973MLH (04 August 2006). In vitro mammalian chromosomal aberration test in cultured human lymphocytes with FAT 81'034/B
30. CIT F-Evreux, Laboratory Study number 31783 MAS (18 October 2006). Bone marrow micronucleus test by intraperitoneal route with FAT 81'034/B
31. RCC Ltd, CH-4452 Itingen, Study number 1011902 (29 June 2006). Photomutagenicity in a *Salmonella typhimurium* reverse mutation assay with FAT 81034/C
32. RCC Ltd, CH-4452 Itingen, Study number 1011900 (November 01, 2006). Chromosome Aberration test in vitro: Photomutagenicity in Chinese hamster V79 cells with FAT 81'034/C
33. SCCNFP (Scientific Committee on Cosmetic Products and Non-food products intended for Consumers) (2000). Notes of guidance for the testing of cosmetic ingredients and their safety evaluation. 5th revision. SCCNFP/ 0321/00
34. US Environmental Protection Agency (EPA) (1992). Integrated Risk Information System (IRIS). Reference Dose for Chronic Oral Exposure: Silver, CASRN 7440-22-4. Available at http://www.epa.gov/iriswebp/iris/subst/0099.htm. [Last accessed: August 2008]
35. CIBA letter providing clarification on the chemical nature of Citric Acid (and) Silver Citrate. 8. December 2008
36. Silver in Drinking-water Background document for development of WHO Guidelines for Drinking-water Quality (2003)
37. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from the Commission related to a 4th list of substances for food contact materials (2004). The EFSA Journal 65, 1-17