Scientific Committee on Consumer Safety

SCCS

OPINION ON

Bismuth citrate

The SCCS adopted this opinion at its 4th plenary meeting

on 12 December 2013

About the Scientific Committees

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

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

SCCS

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

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1. BACKGROUND

The chemical Bismuth Citrate (CAS 813-93-4) (EC 212-390-1) is a new substance in connection with the hair dye strategy.

The first submission for this substance was received by end of January 2009.

The present submission I includes a complete dossier according to the hair dye strategy and the applicant ask for its safety evaluation when used in non-oxidative , but progressive hair dye formulation at a concentration up to 2.0% in the finished cosmetic product.

Progressive hair dyes work - according to the dossier - gradually, with colour build-up over a period of two to three weeks of daily application. With such application, the hair gradually darkens until the required shade is achieved. Thereafter, colour is maintained by up to 3 applications per week. The product is intended for use by middle-aged and older people, principally men.

Bismuth preparations are commonly used to treat a variety of gastrointestinal disorders, including peptic ulcers and dyspepsia.

According to the current US Code of Federal Regulation[1], bismuth citrate may be safely used in cosmetics intended for colouring hair on the scalp, subject to the following restrictions:

- the amount of bismuth citrate in the cosmetic shall not be in excess of 0.5% (w/v);

- the cosmetic may not be used for colouring eyelashes, eyebrows or hair on parts of the body other than the scalp.

2. TERMS OF REFERENCE

1. *Does SCCS consider that the use of bismuth citrate as an hair dye substance in cosmetic products is safe for the consumers when used in a concentration up to maximum 2.0 % taken into account the provided scientific data?*

2. *Does SCCS have any other scientific concerns for the safe use of bismuth citrate in finished cosmetic products?*

[1] 21CFR73, 2110, April 1, page 375, 2002

3. OPINION

Preamble
The quality of the dossier is poor and contains the following major shortcomings:

- Although a concentration of 2% of bismuth citrate in cosmetic formulations is applied for, all of the studies submitted for local toxicity testing were performed with a concentration of 0.5%. In addition, supporting data for the concentration claimed was not provided.

- Data on identity and/or characterization of bismuth citrate in batches, lots, and formulations was often missing or inadequate.

- Data on solubility and/or stability of bismuth citrate in solvents was missing, partly conflicting or inadequate.

- The list of references was poorly sorted, titles of the pdf files were partly misleading (e.g., ref. 58), and several copies were only partly legible.

- The only certificate of analysis (CoA) provided was only partly legible.

3.1 Chemical and Physical Specifications

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

Bismuth citrate

3.1.1.2 Chemical names

2-Hydroxy-1,2,3-propanetricarboxylic acid bismuth salt
Bismuth(3+) 2-hydroxy-1,2,3-propanetricarboxylate
1,2,3-propanetricarboxylic acid, 2-hydroxy-, bismuth(3+) salt (1:1)

3.1.1.3 Trade names and abbreviations

Bismuth citrate
Citric acid bismuth salt

Batches or Lots:
Bismuth Citrate BPC 40, 48 or 49(?) (CoA partly not readable, see 3.1.4), batch 97/90137/000, purity 99% based on based on 51.74% bismuth dry weight
Ref. 42, Ref. 43, Ref. 59

Bismuth Citrate, (internal laboratory No Haskell 26337; sponsor reported 99% purity, no CoA) Ref. 41, Ref. 45

Bismuth Citrate, batch No. RM070 (purity based on Bi content 91%) Ref. 40

Formulations:
Formulations were claimed to contain the concentrations indicated of bismuth citrate according to the dossier.
Grecian Formula 16 (0.5%) Ref. 32

MKT 79 (0.5%) Ref. 20, Ref. 28, Ref. 34

MKT 92 (Liquid) (0.5%) Ref. 21
MKT 92, Bismuth Grecian Formula (liquid) (0.5%) Ref. 29, 35

MKT 109 (0.5%) Ref. 31, 37
MKT 109, Bismuth Grecian Formulation (liquid) Ref. 22

MKT 121 (0.5%) Ref. 38
MKT 121, (0.5% bismuth preparation, Batch: Q050048 (analysis result 2.037%), declared in the dossier) Ref. 75
MKT 121, Bismuth Grecian Formulation (liquid) (0.5%) Ref. 23, 27, 75

MKT 122, MKT 123, MKT 124 (no information in the dossier and the study report as well) Ref. 75

MKT 138 (0.5%) Ref. 30, Ref. 36
MKT 138 (bismuth hair cream) (0.5%) Ref. 24

MKT 394 (Bismuth citrate hair coloring preparation (0.5%), declared in the dossier) Ref. 77
MKT 395 (0.5%) Ref. 77
Bismuth citrate hair coloring preparation MKT 395 (0.5%) (p. 27)

Bismuth citrate formulation: SCP 2716 Ref. 39
(according to the applicant's dossier equivalent to RD4165=Grecian Bismuth Liquid Citrate 2%)

Grecian Liquid Formulation (with 2% bismuth citrate equivalent to 1.10% bismuth)
Batch No. RD4165 Ref. 40

1087, bismuth citrate (0.5%, formulation) Ref. 44
Bismuth citrate
97/90137/000 (purity 99%, based on 51.74% bismuth dry weight based on CoA (see 3.1.4)

Grecian Bismuth Liquid Citrate 2%, batch No. RD4165 Ref. 40
Grecian Bismuth Liquid Citrate 2%, batch #16B: SCP2717, RD4165 Ref. 74

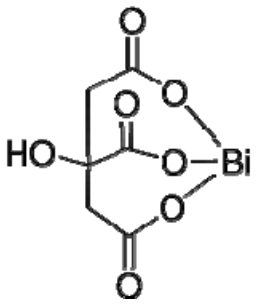
Bismuth citrate hair colouring preparations containing 0.5% bismuth citrate: (p. 27)
R&D 1230#617, batch R8GO6D, liquid
R&D 1237#898, batch R8H03D, cream
R&D 1229#412, batch R8G05D Ref. 76

SCCS comment
Identity, purity and other characteristics of the test item bismuth citrate as batches or bismuth citrate formulations were not described in several studies. Some of the required information such as concentrations of bismuth citrate has been provided in the applicant's dossier but supporting material to the studies has not been provided.
In the formulations, information on the identity of bismuth citrate and/or transformation to other bismuth species is required because bismuth citrate may be unstable under various conditions and for instance different complex compounds of bismuth and citrate or bismuth and thiol groups may be formed. Depending on the study type, information on the pH or the vehicles of the formulations is needed.
No information on the compositions of the formulations was provided.

3.1.1.4 CAS / EC number

CAS: 813-93-4
EC: 212-390-1

3.1.1.5 Structural formula



3.1.1.6 Empirical formula

Formula: $BiC_6H_5O_7$

Comments on chemical identity
Bismuth citrate should be distinguished from different complex compounds of bismuth and citrate such as:

- **Bismuth subcitrate**, a complex of citrate and bismuth at a molar ratio of 2:1, commercially available as tri-potassium bismuth subcitrate (CAS 57644-54-9), molecular formula $C_{12}H_{10}BiK_3O_{14}$. In particular, this substance is sometimes mixed up with bismuth citrate in the open literature and also in the dossier (p. 11) and some of the studies provided.
Synonyms reported: de-nol; de-noltab; bismuth subcitrate; duosol (ulcer treatment); Bismuth citrate [basic]; Bismuth Potassium Citrate; Potassium bismuth dicitrate; Colloidal Bismuth Subcitrate; Bismuth subcitrate potassium; Tripotassium dicitrato-bismuthate.

- **Colloidal bismuth citrate** or **colloidal bismuth subcitrate (CBS)**, a polyanionic structure of $[Bi(cit)_2Bi]n^{2n-}$, which is commercially used in pharmaceutical preparations (see Functions and Uses) (Ref. 1).
The reported bismuth content of CBS is **35.5%** (Ref. 65, Tab.2); in another reference, 36.0% bismuth content was found (ref 63).

- **Ranitidine bismuth citrate** (Tritec® and Pylorid® GSK), a pharmaceutical preparation consisting of colloidal bismuth subcitrate and ranitidine molecules encased in the crystal latter.

3.1.2 Physical form

White powder

3.1.3 Molecular weight

Molecular weight: 398.10 g/mol
Bi 209, citrate 189

3.1.4 Purity, composition and substance codes

The theoretical bismuth content is 52.50%.

In the dossier, for several studies, one Certificate of Analysis (CoA) of the test substance was provided which was difficult to read: CoA — 3 May 2001, Bismuth Citrate BPC 48(?), batch No 97/90137/000 (as specified in the dossier, apart from that not readable), expiry date Sept 2007(?).

Purity: 99% (based on 51.74% bismuth dry weight)

Ref. 42 (CoA in Appendix A, pp. 163 f.)
Ref. 59, CoA in Appendix A, pp.34-35

SCCS comment
Any other information on purity and certificates of analysis were not provided.

3.1.5 Impurities / accompanying contaminants

No known impurities.

According to the above COA provided for various batches and lots in various studies:

lead	<20 ppm
copper	<50 ppm
silver	<25 ppm
arsenic	<0.5 ppm

SCCS comment
Any other information on impurity and COA (certificate of analysis) was not provided.

US. FDA requires according to good manufacturing practice:
Bismuth citrate, not less than 97 percent.
Mercury (as Hg), not more than 1 part per million.
Arsenic (as As), not more than 3 parts per million.
Lead (as Pb), not more than 20 parts per million.
Volatile matter, not more than 1 percent. Ref. 137

3.1.6 Solubility

Insoluble or sparingly soluble in water, alcohol and ether (general information)
Partly soluble in cold water –according to a supplier (ref. 138)

- Solubility in sodium citrate buffer (10 mM, pH 11) with a maximal solubility of 2 mg/ml was reported (Ref. 43).

The "highest possible" (final) concentration of bismuth citrate reported in a cell culture medium was 20 µg/ml (ref. 45). A final concentration of 50 µg/ml *in vitro* was also reported in a culture medium (ref. 46).

- Solubility of 100 µg/ml in Hank's Balanced Salt Solution (HBSS) has been reported (Ref. 68).

- Solubility of up to 50% in DMSO has been reported (LLNA test, Ref. 33) whereas the solubility in DMSO was described in another study report as "very limited" (Ref. 43).

- According to ref. 52 and 54, 16 mg bismuth citrate were dissolved in 500 µl 1 M NH_3 and diluted with the culture medium up to 100 ml to obtain a 400 µM stock solution of bismuth citrate (containing 5 mM NH3).

- Bismuth citrate was dissolved in physiological saline (37°C) to obtain a saturated solution at about 170 mg/L (ref. 65).

SCCS comment
Water solubility has not been determined by EU Method A.8.

Solubility of bismuth citrate (100 µg Bismuth/ml) in receptor fluid for dermal absorption study has not been documented (based on Bismuth analysis only, Ref. 40). However, bismuth is considered the active ingredient systemically available.
In various solvents and at various pH values mentioned in the studies, data on solubility and stability of bismuth citrate is needed.

3.1.7 Partition coefficient (Log P_{ow})

Log P_{ow}: /

3.1.8 Additional physical and chemical specifications

Melting point:	decomposition
Boiling point:	not applicable
Flash point:	not applicable
Vapour pressure:	not relevant
Density:	3.5 g/cm³ (25 °C)
pH:	not applicable
UV-Vis spectrum (200-800 nm):	Bismuth Citrate solution shows absorption in the UV region having maxima at 260 nm

3.1.9 Homogeneity and Stability

The substance has been reported to be light sensitive and has to be protected from light during storage. Ref. 138

For the determination of homogeneity of test substance preparations in methylcellulose or stability of the test substance during repeated dose studies, the preparations or the test substance H-26337 (bismuth citrate) were dissolved in concentrated nitric acid and hydrochloric acid and samples were diluted with nitric and hydrochloric acid (1% v/v) in de-ionized water for ICP analysis of bismuth.

Ref. 42, Ref. 59

SCCS comment
It is assumed that the test substance bismuth citrate is not stable under the work-up conditions using concentrated acids for ICP analysis. Only bismuth can be determined under these conditions.
Documentation on the stability of the compound in sodium citrate pH 11, aqueous ammonia and other solvents used in the safety studies on bismuth citrate is required.
Only the concentrations of the element bismuth have been determined by the method ICP-AES (see SCCS comments on the respective studies). This method is not adequate to determine the stability of the Bi citrate complex and/or the transformation to other Bi species.
Stability of bismuth citrate in typical hair dye formulations was not demonstrated.

General SCCS comments on physico-chemical characterisation

- No study concerning purity and impurity(ies) of bismuth citrate was provided. The reported purity of bismuth citrate is 99% (based on 51.74% bismuth content). Thus, about 1% impurities have not been identified.
- Water solubility of bismuth citrate was not determined by EU method A.8
- Log P_{ow} of bismuth citrate was not provided.
- Homogeneity and stability of bismuth citrate in stock and test solutions were not adequately demonstrated because only the element bismuth was analysed.
- Stability of bismuth citrate in typical hair dye formulations was not demonstrated.

3.2 Function and uses

Bismuth citrate has been in use as a progressive hair dye in the USA for some time and Grecian Bismuth was launched in Europe in 2005 (approximately 6 million units sold) and in Canada in 2008 (approximately 175,000 units sold). Bismuth citrate will be incorporated in progressive hair dye formulations at a maximum concentration of 2.0%. In the USA up to 0.5% are permitted.

Progressive hair dyes work gradually, with colour build-up over a period of two to three weeks of daily application. This hair dye changes the colour of hair from grey to darker shades by reacting with the sulphur of hair keratin as well as oxidizing on the hair surface. With such application, the hair gradually darkens until the required shade is achieved. Thereafter, colour is maintained by up to 3 applications per week. This product is intended for use by middle-aged and older people, principally men.
The principle of coloration from bismuth citrate has been described in section 1, Background.

Bismuth is one of the medicinal drugs referenced in Pharmacopoeias, with bismuth compounds used topically or orally for indications such as minor stomach-aches, ulcers and venereal diseases for over 200 years.
Bismuth compounds have been used as antimicrobial agents for:
- syphilis (e.g. sodium/potassium bismuth tartrate, bismuth quinine iodide, iododbismol, bismuth chloride)
- colitis (e.g. bismuth citrate, bismuth subnitrite),
- wound infection (e.g. bismuth oxide),
- quartan malaria (e.g. sodium bismuth thioglycolate),
- dyspepsia (e.g. bismuth subsalicylate, bismuth subnitrate) and
- peptic ulcers (e.g. colloidal bismuth subcitrate, bismuth citrate, bismuth subnitrate).

Three bismuth compounds, bismuth subsalicylate (Pepto-Bismol ® Procter and Gamble), colloidal bismuth citrate (De-Nol®, Gist-Brocade, Yamanouchi, Lizhudele®) and ranitidine bismuth citrate (Tritec® and Pylorid® GSK) have been in extensive use worldwide to treat gastrointestinal diseases, including those related to infection of Helicobacter Pylori which causes gastric or duodenal ulcers (ref. 1).
Bismuth subgallate is no longer used as a topical treatment for eczema and other skin complaints but bismuth subnitrate and carbonate are still used in surgical dressings (ref. 2, ref. 3, ref. 4).
Currently, the major medicinal use of bismuth compounds is focussed on two fields: antimicrobial and anticancer (ref. 1).

Occupational exposure to bismuth occurs in the manufacture of alloys, catalysts, and ceramics, polymers for bone implants, cosmetics, semiconductors and X-ray contrast media, in dental prosthetic devices or in medical devices (scanners, implants, sutures, catheters...)

(ref.5 - 9). In cosmetics, exposure occurs to bismuth citrate, triphenyl bismuth or bismuth oxychloride which is used as a pearlescent pigment or lubricating agent in formulations of lipstick, nail polish, eye shadows and facial powders. Bismuth is included in hair dyes to add colour and to deodorize (ref. 4).

3.3 Toxicological Evaluation

3.3.1 Acute toxicity

3.3.1.1 Acute oral toxicity

Acute oral toxicity studies were performed in rats by gavage, with prototype cosmetic **formulations** containing bismuth citrate at **0.5%**, as follows:

Study	**Product tested**	**Results**	**GLP compliance**	**Ref**
Consumer Product Testing 7954-3, March 2, 1979	MKT 79 bismuth Grecian Form. (liquid)	LD_{50} > 40 g/kg bw	Not stated	20
MBRL, Inc., MB 80-5044 A 12/17/80	MKT 92 bismuth Grecian Form. (liquid)	LD_{50} > 5.0 g/kg bw	Yes	21
MBRL, Inc., MB 81-5429 A 08/04/81	MKT 109 bismuth Grecian Form. (liquid)	LD_{50} > 30 ml/kg bw	Yes	22
MBRL, Inc., MB 81-5433 A 08/04/81	MKT 121 bismuth Grecian Form. (liquid)	LD_{50} > 14 g/kg bw	Yes	23
MBRL, Inc., MB 81-5613 A 8/10/81	MKT 138 bismuth hair cream	LD_{50} > 5.5 g/kg bw	Yes	24

SCCS comment

MKT 79, MKT 92, MKT 109, MKT 121 and MKT 138 were characterized only as a liquid formulation, partly with data on specific gravity.

With MKT 79, two deaths were observed at the highest dose (1 F, 1 M) 4 and 7 days after treatment (Ref. 20). With the other formulations, no deaths were observed. Isolated instances of diarrhea, ptosis, chromodacryorrhea and chromorhinorrhea were noted.

The studies (ref. 21-24) were claimed to comply with US GLP regulations of 1979 and 1980.

As no information is available in the study reports or accompanying documents on the content of Bi citrate in the formulations, the acute oral toxicity of the studies cannot be evaluated.

A single oral dose of 0, 0.5, 1.0, 2.0 or 5.0 mg/kg **colloidal bismuth citrate (CBS)** was administered to Sprague-Dawley rats, 8 weeks old, weighing 150-200g (21 animals per group). Blood parameters were evaluated in weeks 12, 16 and 20 post dosing. Significant decreases in RBC, Hb, Ht, MCV, MCH, MCHC. PLT and WBC in all groups and time intervals compared to controls indicated a CBS induced microcytic/hypocromic anemia. Results indicated that the effects of CBS were not dependent on dose and time.

Ref. 25

SCCS comment
The study cannot be evaluated due to the lack of a dose-response relationship.

3.3.1.2 Acute dermal toxicity

One acute dermal toxicity study was conducted in 10 (6 male and 4 female) albino rabbits with a bismuth Grecian Formula (0.5% bismuth citrate). The test product was kept in occluded contact with intact and abraded skin for 24 h (about 10% of the body surface was exposed). Abrasions were performed in two males and 3 females and were sufficiently deep to remove the stratum corneum, but not deep enough to produce bleeding. The results are summarized below:

Study	Product tested	Results	GLP compliance	Ref
MBRL, Inc., MB 81-5433 B Aug 4, 1981	MKT 121 Bismuth Grecian Formula	LD_{50} > 2 g/kg bw	Yes	**27**

SCCS comment
Pre-guideline study. Four of the five animals with abraded skin exposed to the test item showed reduced body weight after 14 days by about 8-15% whereas body weight increase was observed in the majority of the animals with normal skin exposed. This may be a hint that the test substance was absorbed by the animals with abraded skin in an amount that caused body weight loss.

3.3.1.3 Acute inhalation toxicity

No data available

3.3.1.4 Acute intraperitoneal toxicity

Colloidal bismuth subcitrate (CBS) was administered by intraperitoneal injection to male Sprague–Dawley rats, 8 wk old, weighing 150–200 g at 0, 100, 200, 400, 500, and 1000 µg/L (21 animals per group). Levels of serum enzymes were determined 24, 48, and 72 h later. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and creatine kinase (CK) levels significantly increased after all CBS treatments compared to controls without dependence on time and dose. All doses significantly affected the lactate dehydrogenase (LDH) in serum after 72 h.

Ref. 26

SCCS comment
Dosage related to bodyweight is unclear and there was no dose-response relationship. The study cannot be used for risk assessment.

3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

Guideline: /
Species/strain: Albino New Zealand Rabbits
Group size: 6 (both sexes)
Test substance: MKT 79
Batch: /
Purity: /
Vehicle: /
pH: /
Dose level: /
Dose volume: 0.5 ml
GLP: /
Study period: February 13, 1979 to March 2, 1979

0.5 ml of the test material was applied to clipped areas of intact and abraded skin. Applications were made under occlusive patches (2" x 2" gauze, covered by adhesive tape). Following application of the test material the entire trunk of each animal was covered with an impermeable occlusive wrapping. The wrapping and test material were removed 2 hours following application. The sites were individually examined and scored separately for erythema and oedema at 24 and 72 hours. The mean scores for 24 and 72 hour grading were averaged to determine final irritation indices.

Results
No irritation was observed under the conditions of the test.

Conclusion
Bismuth citrate is not an irritant to the rabbit skin under the study conditions.

Ref. 28

SCCS comment
Information on bismuth citrate as the a.i. is missing in the study report. The concentration of bismuth citrate claimed by the applicant was 0.5% (see preamble and 3.1.1.3).

Guideline: /
Species/strain: Albino New Zealand Rabbits
Group size: 6 (males)
Test substance: MKT 92 (Liquid)
Batch: /
Purity: /
Vehicle: /
pH: /
Dose level: /
Dose volume: 0.5 ml
GLP: in compliance with FDA 1979
Study period: December 1980

Six rabbits were dosed once dermally at one abraded and one intact site/animal. 0.5 ml was applied to each site beneath 2.5 cm square gauze patches. The patches were secured with adhesive tape and the trunks were wrapped with impervious material. The test material was kept in contact with the skin for 24 hours.

Results
At 24 hours, erythema was slight at all 12 sites (mean score: 1.00) and oedema was slight at 5/12 sites (mean score: 0.33/0.50 for intact/abraded skin, respectively). At 72 hours, all sites were free from signs of dermal irritation. The primary Irritation Index was 0.71.

Conclusion
The test article is a non-irritant under the study conditions.

Ref. 29

SCCS comment
Information on Bismuth citrate as the a.i. is missing in the study report. The concentration of bismuth citrate claimed by the applicant was 0.5% (see preamble and 3.1.1.3).The data indicate that the test substance has a slight irritant potential to the skin of the rabbit under the conditions of the experiment.

Guideline:	/
Species/strain:	Albino New Zealand Rabbits
Group size:	6 (males)
Test substance:	MKT 138
Batch:	/
Purity:	/
Vehicle:	/
pH:	/
Dose level:	/
Dose volume:	0.5 ml
GLP:	in compliance with FDA 1979

Study period: October 1981

Six albino rabbits were dosed dermally with MKT 138. 0.5 ml of the test article was applied to 1 intact and 1 abraded site on the clipped back. The sites were occluded for 24 hours. The skin reactions were evaluated by the Draize technique at 24 and 72 hours after dosing. The Primary Irritation Index was calculated.

Results
Erythema, generally slight at 24 hours, was minimal at 72 hours.
Oedema was generally slight at 24 hours and essentially absent at 72 hours.
At 24 hours, erythema was observed in all animals (mean score: 1.67/1.17 for intact/abraded skin, respectively) and oedema in about half of the animals (mean score: 1.00/0.67 for intact/abraded skin, respectively). At 72 hours, erythema was still observed in some animals (mean score: 0.50/0.17 for intact/abraded skin, respectively) and oedema in one animal (mean score: 0.17 for abraded skin).
There appeared to be no difference between abraded and intact skin reactions.

Conclusions
The test article is a non-irritant.

Ref. 30

SCCS comment
Information on Bismuth citrate as the a.i. is missing in the study report. The concentration of bismuth citrate claimed by the applicant was 0.5% (see preamble and 3.1.1.3).
Bismuth citrate induces slight skin irritation under the study conditions.

Guideline:	/
Species/strain:	Albino New Zealand Rabbits
Group size:	6 (5 females, 1 male)
Test substance:	MKT 109
Batch:	/
Purity:	/
Vehicle:	/
pH:	/
Dose level:	/
Dose volume:	0.5 ml
GLP:	in compliance with FDA 1979
Study period:	June 1981

Six albino rabbits were dosed dermally with MKT 109. 0.5 ml of the test article was applied to 1 intact and 1 abraded site on the clipped back. The sites were occluded for 24 hours. The skin reactions were evaluated by the Draize technique at 24 and 72 hours after dosing. The Primary Irritation Index was calculated.

Results
Slight Erythema (score 1) was observed in only one animal with intact skin at 24 hours, but was absent at all other sites and time points.
No oedema was present. There appeared to be no difference between abraded and intact skin reactions.

Conclusion
The test article is a non-irritant.

Ref. 31

SCCS comment
Information on Bismuth citrate as the a.i. is missing in the study report. The concentration of bismuth citrate claimed by the applicant was 0.5% (see preamble and 3.1.1.3).
The test article is a non-irritant under the study conditions.

Guideline:	/
Species/strain:	Albino New Zealand Rabbits
Group size:	6
Test substance:	Grecian Formula 16
Batch:	/
Purity:	/
Vehicle:	/
pH:	/
Dose level:	/
Dose volume:	0.5 ml
GLP:	/
Study period:	October 1974

0.5 ml of the test material was applied to clipped areas of intact and abraded skin. Applications were made under occlusive patches (1" x 1" gauze, covered by adhesive tape). Following application of the test material the entire trunk of each animal was covered with an impermeable occlusive wrapping. The wrapping and test material were removed 2 hours following application. The sites were individually examined and scored separately for erythema and oedema at 24 and 72 hours. The mean scores for 24 and 72 hour grading were averaged to determine final irritation indices.

Results
All the animals presented slight erythema (score 1) at 24 and 72 hours. Oedema (score 1) was observed in 3 animals at 24 hours.

Conclusion
The test article is a non-irritant.

Ref. 32

SCCS comment
Information on Bismuth citrate as the a.i. is missing in the study report. The concentration of bismuth citrate claimed by the applicant was 0.5% (see preamble and 3.1.1.3).
The test article is a slight skin irritant under the study conditions.

Overall SCCS comments on skin irritation
Five different studies were performed between 1974 and 1981 with different formulations. The applicant informs that all formulations contained 0.5% of bismuth citrate, but no information of the test item concentration was reported in either of the studies.

A safety dossier has been submitted requesting approval for a 2% bismuth citrate concentration in cosmetic formulations, but the formulations tested contained 0.5% of bismuth citrate as claimed by the applicant.

Three studies were performed under GLP conditions according to FDA 1979 but were conducted prior to the adoption of the OECD test guideline 404 (in 1981).

Slight erythema (with or without oedema) was observed with three of the five formulations. The applicants classified the formulations as non-irritants; nevertheless the results indicate that the formulations tested are slightly irritant to the skin.

3.3.2.2 Mucous membrane irritation / Eye irritation

Guideline:	/
Species/strain:	Albino New Zealand Rabbits
Group size:	9 (both sexes)
Test substance:	MKT 79
Batch:	/
Purity:	/
Vehicle:	/
pH:	/
Dose level:	/
Dose volume:	0.5 ml
GLP:	/
Study period:	13 February to 2 March 1979

Nine rabbits, mixed sex, received 0.1 ml single administration in the right eye, followed by No Wash for 24 hours (3 rabbits), 4 seconds Wash (3 rabbits), and 30 seconds Wash (3

rabbits). Material used as received. Readings facilitated by hand-held lenses were made 1, 2, 3 and up to 7 days after treatment.

Results
No signs of irritation were observed in any animals.

Conclusions
The test substance was not an ocular irritant to rabbit eyes under conditions of the test.

Ref. 34

SCCS comment
Information on Bismuth citrate as the a.i. is missing in the study report. The concentration of bismuth citrate claimed by the applicant was 0.5% (see preamble and 3.1.1.3).

Guideline:	/
Species/strain:	Albino New Zealand Rabbits
Group size:	9 (6 males, 3 females)
Test substance:	MKT 92 (Liquid)
Batch:	/
Purity:	/
Vehicle:	/
pH:	/
Dose level:	/
Dose volume:	0.1 ml
GLP:	in compliance with FDA 1979
Study period:	December 1980

0.1 ml of the test article was placed once into the conjunctival sac of one eye of each of nine rabbits. The lids were held together briefly to ensure adequate distribution of the test article. The untreated eye of each rabbit served as a control. The treated eyes of three animals remained unwashed. The treated eyes of three animals were washed at 4 seconds with 20 ml of distilled water. The treated eyes of three animals were washed 30 seconds after dosing with 20 ml of distilled water. The ocular reactions of the cornea, iris, and conjunctiva were graded at 1, 2 and 3 days after dosing. At 24 hours, the eyes were examined with sodium fluorescein. The eyes were graded by the Draize scoring system.

Results
No signs of irritation were observed in any animals.

Conclusions
The test substance was not an ocular irritant to rabbit eyes under conditions of the test.

Ref. 35

SCCS comment
Any information on Bismuth citrate as the a.i. is missing in the study report. The concentration of bismuth citrate claimed by the applicant was 0.5% (see preamble and 3.1.1.3).

Guideline:	/
Species/strain:	Albino New Zealand Rabbits
Group size:	9 (6 males, 3 females)
Test substance:	MKT 138
Batch:	/

Purity: /
Vehicle:
pH: /
Dose level: /
Dose volume: 0.1 ml
GLP: in compliance with FDA 1979
Study period: October 1981

Nine healthy albino rabbits, free from evidence of ocular irritation were dosed with MKT 138. 0.1 ml was placed into the conjunctival sac of one eye of each rabbit. Three eyes remained unwashed, three eyes were washed at 4 seconds and three eyes were washed at 30 seconds. The eyes were scored by the Draize technique on Days 1, 2 and 3.

Results
In all 3 animals with unwashed eyes conjunctival redness, chemosis and discharge (1 animal with score 2 for chemosis, otherwise score 1) were observed at 24 hours; signs of conjunctival irritation (score 1) remained in two animals at 48 hours and in one animal at 72 hours. Conjunctival discharge (score 1) was observed at 24, 48 and 72 hours in 2/3 animals with their eyes washed after 4 seconds; no other signs of conjunctival irritation were observed in these animals. In all 3 animals with eyes washed after 30 seconds, conjunctival redness, chemosis and/or discharge (score 1) were observed at 24 and 48 hours; signs of conjunctival irritation (chemosis, score 1) remained in one animal at 72 hours. No corneal opacity or iritis were observed in any animal.

Conclusion
The test article does not appear to be irritating.

Ref. 36

SCCS comment
The SCCS considers MKT 138 as slightly irritant to the rabbit eyes because of signs of slight conjunctival irritation in unwashed and washed eyes.
Information on Bismuth citrate as the a.i. is missing in the study report. The concentration of bismuth citrate claimed by the applicant was 0.5% (see preamble and 3.1.1.3).

Guideline: /
Species/strain: Albino New Zealand Rabbits
Group size: 9 (5 males, 4 females)
Test substance: MKT 109
Batch: /
Purity: /
Vehicle:
pH: /
Dose level: /
Dose volume: 0.1 ml
GLP: in compliance with FDA 1979
Study period: June 1981

0.1 ml of MKT 109 was placed into the conjunctival sac of one eye of each rabbit. Three eyes remained unwashed, three eyes were washed at 4 seconds and three eyes were washed at 30 seconds. The eyes were scored by the Draize technique on Days 1, 2 and 3.

Results
In 2 animals with unwashed eyes conjunctival redness, chemosis and/or discharge (score 1) were observed at 24 hours; signs of conjunctival irritation (chemosis, score 1) remained in one animal at 48 hours. No signs of conjunctival irritation were observed in animals with

eyes washed after 4 and 30 seconds. No corneal opacity or iritis were observed in any animal.

Conclusion
The test article appears to be a non-irritant in all eyes.

Ref. 37

SCCS comment
The SCCS considers MKT 109 as slightly irritant to the rabbit eyes because of signs of slight conjunctival irritation in unwashed eyes.
Information on Bismuth citrate as the a.i. is missing in the study report. The concentration of bismuth citrate claimed by the applicant was 0.5% (see preamble and 3.1.1.3).

Guideline:	/
Species/strain:	Albino New Zealand Rabbits
Group size:	9 (6 males, 3 females)
Test substance:	MKT 121 (0.5% bismuth citrate)
Batch:	/
Purity:	/
Vehicle:	/
pH:	/
Dose level:	/
Dose volume:	0.1 ml
GLP:	in compliance with FDA 1979
Study period:	July 1981

0.1 ml of MKT 121 was instilled onto everted lower lid of the right eye of rabbits to assess its ocular irritation potential. Three groups of three rabbits each were established. The treated eyes of three animals remained unwashed. The treated eyes of three rabbits were flushed for one minute with lukewarm water beginning 30 seconds after instillation of the test article. The treated eyes of three animals were similarly washed beginning 4 seconds after instillation. All eyes were scored for ocular irritation at 24, 48 and 72 hours and at 4 and 7 days.

Results
In all 3 animals with unwashed eyes conjunctival redness and chemosis (score 1) were observed at 24 hours; signs of conjunctival irritation (score 1) remained in two animals at 48 and 72 hours, but not after 96 hours. Conjunctival redness and chemosis (score 1) was observed at 24 hours in 1/3 animals with their eyes washed after 4 seconds; no other signs of conjunctival irritation were observed in these animals. In all 3 animals with eyes washed after 30 seconds, conjunctival redness (score 1) was observed at 24 hours; conjunctival redness (score 1) remained in one animal at 48, 72 and 96 hours. No corneal opacity or iritis were observed in any animal.

Conclusion
When instilled onto the rabbit eye, the test article produced no greater than transient (reversible) grade 1 redness and chemosis.

Ref. 38

SCCS comment
The SCCS considers MKT 121 as slightly irritant to the rabbit eyes because of signs of slight conjunctival irritation in unwashed and washed eyes.
Information on Bismuth citrate as the a.i. is missing in the study report. The concentration of bismuth citrate claimed by the applicant was 0.5% (see preamble and 3.1.1.3).

Overall SCCS comments on eye irritation studies *in vivo*:

Five different studies were performed from 1979 to 1981 with different formulations. The applicant informs that all formulations contained 0.5% of bismuth citrate, but no information of the item concentration was reported in neither of the studies.

Three of the studies (using MKT 109, MKT 121 and MKT 138) showed slightly conjunctival irritation in the eyes of rabbits under the study conditions.

Four studies were performed under GLP conditions according to FDA 1979 but were conducted prior to the adoption of the OECD test guideline 405 (in 1981).
Any information on Bismuth citrate as the a.i. is missing in all study reports.

A safety dossier has been submitted requesting approval for a **2%** bismuth citrate concentration in cosmetic formulations, but the formulations tested contained only **0.5%** of bismuth citrate, as claimed by the applicant.

***In vitro* eye irritation study**

Guideline:	/
Species/strain:	Chorioallantoic membrane of White Leghorn eggs (14 days incubated)
Group size:	20
Test substance:	SCP 2716
Batch:	/
Purity:	/
Vehicle:	/
pH:	/
Dose level:	undiluted and 50% suspension in distilled water
Dose volume:	40 µl
GLP:	in compliance
Study period:	March 2005

The chorioallantoic membrane (CAM) of twenty White Leghorn eggs, incubated for 14 days, was dosed with 40 µl of SCP 2716. A total of two concentrations (undiluted and 50% suspension in distilled water) were used, one per group. The dosed eggs were then incubated for another 30 + 5 minutes after which the CAM was observed for signs of vascular haemorrhage, capillary injection, or ghost vessels.
The percentage of CAM'S responding to each dilution was plotted on 3 cycle log-probit paper and an RC50 (the calculated concentration theoretically producing a reaction in 50% of the treated eggs) with 95% Confidence Limits. The irritation potential was determined based on the calculated RC50, generally within the following thresholds:
RC50> 3.0% = Non-irritant
RC50> 1.0% <3.0% = Indeterminate
RC50< 1.0% = Irritant

Results
There are 30% positive responses (capillary injection) for the undiluted formulation and no positive responses for the 50% diluted formulation

Conclusion
As RC50 is >100%, the formulation is not an eye irritant.

Ref. 39

SCCS comment
Any information on Bismuth citrate as the a.i. is missing in the study report.

The information about the composition of the formulation tested (bismuth citrate formulation: SCP 2716 (equivalent to RD4165=Grecian Bismuth Liquid Citrate 2%) is only present in the summary of the applicant but not in the study report (see preamble and 3.1.1.3).
The method does not adherea guideline and it is not validated. The criteria of identification of eye irritation are not those normally used. The endpoint evaluated is based on the time of haemorrhage, coagulation and lysis. In this study only the presence or absence of these signs is considered. The accepted alternative method uses eggs incubated for 10 days instead of 14 days because it is considered the maximal period not inducing pain to embryo.

Overall SCCS comments on both skin and eye irritation studies

No information on Bismuth citrate as the a.i. is available in the study reports. Documented information on the identity and characterization of the test substance is mandatory in such studies.

Due to these shortcomings, the conclusions from the studies on local irritation are not considered valid. However, the test formulations already at 0.5% Bismuth citrate showed skin and eye irritation potential.

3.3.3 Skin sensitisation

Local Lymph Node Assay (LLNA)

Guideline:	OECD 429
Species/strain:	Mouse: Female CBA/J
Group size:	5 mice per group (two groups per concentration)
Test substance:	Bismuth citrate
Batch:	97/90137/000
Purity:	99%
Vehicle:	Dimethylsulfoxide
pH:	
Concentration:	10%, 25%, 50%
Positive control:	Chlorpromazine 1%
GLP:	not in compliance (see comment)
Study period:	August 2005

Separate groups of five healthy female CBA/J mice were treated with increasing concentrations of Bismuth Citrate (two groups per concentration) by topical application to the dorsum of each ear, once daily for three consecutive days. Five days following the initial dose, and five hours prior to sacrifice, the mice were injected with the thymidine analog 5-bromo-2'-deoxy-uridine (BrdU), and at sacrifice the auricular lymph nodes were isolated and single-cell suspensions of lymph node cells (LNC) were generated. For each animal, the LNC suspension was analysed for BrdU incorporation and the total number of lymphocytes (LNC) by flow cytometry. The amount of proliferating (#BrdU+) LNC was determined as a measure of the proliferative response of the local lymph node. The stimulation index (SI) was calculated by dividing the proliferative response of each test article group by the proliferative response of the vehicle control group. Test articles that yielded a SI > 3 were characterized as sensitizing substances.
For each test concentration or control group, one of the two paired groups of five mice was irradiated with UVA 15-30 minutes after topical application of the test articles using a Honle SOL-500 solar simulator with an H-1 filter to cut off UVB and UVC. The test article was not wiped off the ear prior to UVA irradiation. The distance between the source and the mice was adjusted to give an irradiance of between 1.7 and 3.5 mW/cm² and the period of irradiation was adjusted to yield a total UVA dose of 10 J/cm² for 50 minutes.

Results
The SI values for the test article, bismuth citrate, + UVA at 10%, 25% and 50% were all below the threshold of 3.0 (i.e. 0.7, 0.8 and 1.5, respectively; and 1.3, 1.6 and 0.7, respectively, without UVA), indicating the test article is not a photo-sensitiser.

Conclusion
Topical application of the test article bismuth citrate, Lot #97/90137/000, at 10%, 25% and 50%, with or without UVA, resulted in a stimulation index of less than 3 (SI < 3.0), and therefore this test article is neither a dermal sensitiser nor a dermal photo-sensitiser in the Photo-Local Lymph Node Assay.

Ref. 33

SCCS comment
GLP compliance: The QA statement is missing.
In this experiment, no appropriate positive control for sensitisation was used (Chlorpromazine was normally used as a positive control in photosensitisation only).
The high solubility of Bi citrate in DMSO up to 50% is questionable as in the mutagenicity tests the solubility was characterized in another study as "very limited" (Ref. 43). The solubility of Bi citrate in DMSO requires clarification before the test can be accepted as valid. Thus, sensitisation potential of Bismuth citrate cannot be excluded.

3.3.4 Dermal / percutaneous absorption

Guideline:	OECD 428
Tissue:	Dermatomed pig back skin (females) (thickness 500 ± 50 µm)
Group size:	Number of animals unknown
Diffusion cells:	6 cells
Skin integrity:	Transepidermal water loss measurement (Tewameter TM210)
Method:	Franz type diffusion cells
Test substance:	Bismuth citrate
Batch:	RM070
Purity:	Bi content was 47.64% (ICP analysis) corresponding to 91% purity of the a.i. Bismuth citrate
Formulation:	Grecian Liquid Formulation (with 2.1% bismuth citrate equivalent to 1.10% bismuth)
Batch:	RD4165
Dose applied:	10 µl (2 mg/cm^2) formulation (corresponding to 105 µg/cm^2 or 55 µg/cm^2 bismuth, when assuming a density of 1.0 of the formulation)
Receptor fluid:	phosphate-buffered saline solution (with 1% w/w bovine serum albumin and 0.04% w/w gentamicin sulphate)
Solubility receptor fluid:	100 µg bismuth/ml (determination of the bismuth content of the Grecian Liquid Formulation in the receptor fluid)
Method of Analysis:	Inductively Coupled Plasma (ICP) methodology
GLP:	/
Study period:	2006

The tested formulation containing about 2% of Bismuth Citrate was applied to pig skin membranes (skin thickness about 500 µm) mounted in Franz type diffusion cells at a target dose of about 2 mg/cm^2. After 24 hours, the diffusion cells were dismantled, the skin surface wiped with a specific washing procedure and the receptor fluid collected. The stratum corneum of the skin was then tape stripped 8 times using D-Squame adhesive tapes. The epidermis was separated from the dermis by a heat procedure. In particular, the skin compartments (stratum corneum, rest of epidermis and dermis) were extracted with a solvent (1% HNO_3 solution) in order to obtain acceptable recoveries of bismuth contained in

each sample. The surface excess, the receptor fluid, tape strips, the epidermis and dermis samples were analysed for their Bismuth content and a full mass balance calculated.

Results
After 24 hours, the average total amount of Bismuth recovered from the wash, tape strips, epidermis, dermis and receptor fluid, represented an overall recovery of 96.42% ± 5.13 for Grecian Liquid Formulation. The majority of the applied material was recovered in the washing of skin surface (about 94%). The amount of Bismuth found in the Stratum Corneum was about 1.09 µg/cm^2. With regards to epidermis and dermis, the content of Bismuth was 0.35 ± 0.10 and 0.07 ± 0.04 µg/cm^2, respectively. Bismuth was also analytically quantified in the receptor fluid and the amount found was 0.07 ± 0.05 µg/cm^2.

Table 1: Individual results on percutaneous absorption of bismuth incorporated in a Grecian bismuth liquid formulation (24h exposure)

µg/cm^2	Samples						
	FCB 7	FCB 8	FCB 9	FCB 10	FCB 12	FCB13	Mean ± SD
Surface	51.98	59.16	59.34	54.94	54.88	52.67	55.50 ± 3.14
Stratum corneum	1.08	0.97	1.01	1.05	1.14	1.32	1.09 ± 0.12
Epidermis	0.29	0.35	0.40	0.24	0.29	0.51	0.35 ± 0.10
Dermis	0.14	0.04	0.07	0.10	0.070.02	0.07	0.07 ± 0.04
Receptor Fluid	0.16	0.02	0.09	0.10	0.05	0.03	0.07 ± 0.05
Total Recovery	53.65	60.54	60.90	56.44	56.43	54.55	57.08 ± 3.02
Percutaneously absorbed	0.58	0.41	0.55	0.45	0.41	0.56	0.49 ± 0.08
Total Applied	59.22	59.22	59.22	59.22	59.22	59.22	

Conclusion
The amount systemically available after percutaneous absorption of **Bismuth** contained in the tested formulation (Grecian Bismuth Liquid Formulation) may be considered to be 0.49 ± 0.08 µg/cm^2 or 0.83 ± 0.13 % of the applied dose.

Ref. 40

SCCS comment
The study was not done under GLP.
The test substance was dissolved in aqueous ammonia (conc. of ammonia and pH of the test material are not reported). Evidence for the stability of the test substance in aqueous ammonia has not been provided.
Evidence for the solubility of bismuth citrate in the receptor fluid has not been provided as only the bismuth content of the formulation was determined by ICP analysis.
The absorption data is referred to bismuth as element (ICP analysis). The absorption of the test item bismuth citrate has not been determined. It is not clear whether bismuth citrate and/or other bismuth species were absorbed.
Conflicting results were depicted in Annex 3 of the study (see table above). Assuming that an absorption value of 0.07 µg/cm^2 for dermis of sample FCB 12 was achieved, the mean value of 0.07 for µg/cm^2 for dermis is not correct and should read 0.085. The mean value of the percutaneously absorbed dose should then read **0.50 µg bismuth/cm^2** and this value will be used for further calculations.

Few cells have been used and the number of donors is unknown, then the Mean + 2 SD of Bismuth (0.66 µg/cm^2) corresponding to 1.26 µg/cm^2 of Bismuth citrate could be used to calculate the MoS.

3.3.5 Repeated dose toxicity

3.3.5.1 Repeated Dose (14 days) oral toxicity

Guideline:	/
Species/strain:	Crl: CD® (SD)IGS BR rats
Group size:	5 animals per dose and gender
Test substance:	bismuth citrate (Haskell 26336, internal laboratory No)
Batch:	/
Purity:	purity reported by the sponsor was 99%.
Vehicle:	test substance suspended in 0.5% methylcellulose in deionized water
Dose levels:	0, 100, 300, and 1000 mg/kg bw/day
Dose volume:	
Route:	oral
Administration:	gavage
GLP:	/
Study period:	October 2004 (unpublished data: protocol date Sept 20, 2004)

Based on the reported results of a published developmental toxicity study in rats (Ref. 60), dose levels of 0, 100, 300, and 1000 mg/kg/day were selected for this range-finding study.

Results
No adverse effects were observed at any dose on body weight or nutritional parameters, clinical observations, clinical pathology or gross pathology. Statistically significant reductions in some clinical chemistry parameters were observed in all dose groups, including BUN and potassium in males and bilirubin in females. None of these changes was considered adverse, based on the direction and magnitude of change, but they may have been related to exposure to the test substance. Black staining of the faeces was noted in 1000 mg/kg bw/day males and females and in 300 mg/kg bw/day females. This was considered to be a typical response to exposure to bismuth-containing compounds and not adverse as it was not associated with any clinical or pathological effects.

Ref. 41

SCCS comment
Only the study plan and a rangefinder summary table are available. The test substance was not adequately characterized (identity and purity not proved, no CoA). Differences in clinical chemistry parameters between dosed and control groups were only qualitatively reported (blood urea nitrogen, K+, bilirubin, creatinine, albumin reported to be lower in mid and high dose groups than controls). The study is of limited value and cannot be used for risk assessment.

3.3.5.2 Sub-chronic (90 days) toxicity (oral)

Guideline:	OECD 408
Species/strain:	Rats Crl:CD (IGS)BR (between 6 and 8 weeks of age at the study start)
Group size:	10 per sex per dose
Test substance:	bismuth citrate
Batch:	97/90137/000
Purity:	99% (Bi content 51.74% according to CoA)
Vehicle:	test substance suspended in 0.5% methylcellulose in de-ionized water

Dose levels:	0, 30, 300, 1000 mg/kg bw/day
Dose volume:	5 mL/kg bw
Route:	oral
Administration:	gavage
GLP:	in compliance
Study period:	Experimental phase Nov 2004 to Jan 2005; study completion date July 18, 2005

Results
Test substance-related reductions in body weight, weight gain, and food efficiency were observed in male and female rats dosed with 1000 mg/kg bw/day, compared to controls. No effect on food consumption was observed at any dose. One high dose male was killed in extremis on day 56, due to noisy breathing. All other rats survived to termination of the study. Noisy breathing was also observed in several high dose male and female rats which survived to the end of the study, which was attributed to test substance-related histopathological changes of the nasal turbinate. Black staining of faeces was observed in all rats dosed at 300 or 1000 mg/kg bw/day and was attributed to test substance exposure; this was considered to be a typical, well-documented response to dosing with a bismuth compound, and was therefore considered not adverse.
No ophthalmological lesions were attributed to test substance exposure and there were no effects on any neurobehavioral parameters (forelimb or hindlimb grip strength, duration of movement, number of movements, behavioural parameters evaluated in the functional observational battery). No adverse, test substance-related effects were observed on haematology, clinical chemistry or coagulation parameters.
A few changes were observed in high-dose male and female relative organ weights but were considered to be related to reductions in body weight at that dose. Large caeca, filled with dark ingesta, were observed during gross necropsy of all surviving males and females treated at 300 or 1000 mg/kg/day, but no microscopic lesions were associated with this observation. Inflammation of the nasal turbinate and/or maxillary sinus was also observed in animals from these groups and was attributed to reflux of material (test substance and/or gastric fluid) from the oesophagus or stomach. These lesions were considered to be secondary to test substance effects on the gastrointestinal tract, and not direct effects on nasal tissue. Mild degeneration/necrosis of renal tubular epithelium was observed in one male and one female dosed with 1000 mg/kg bw/day, but this was not associated with any effects on renal clinical chemistry.

The no observed effect level (NOEL) was considered to be 30 mg/kg bw/day in males and females, based on effects at 300 and 1000 mg/kg bw/day which included ingesta-filled caeca and nasal histopathology changes at 300 and 1000 mg/kg/day.

Conclusion
A NOEL of 30 mg/kg bw/day can be derived for this 90-day oral toxicity study.

Ref. 42

SCCS comment
The synonym bismuth subcitrate in the section Study information of the report (p. 10) is not correct.
The purity of the test substance bismuth citrate has been determined based on the bismuth content alone (see CoA).The citrate moiety of the substance has not been determined.
The stability of the test substance and its stability in the test formulations during study conduct were determined by a work-up procedure with concentrated acids whereby the test substance is destroyed. The analytical method ICP-AES is capable of determining Bi as an element but not suited for the determination of the test item bismuth citrate and its stability. Therefore, the stability of the test item in dosing formulations has not adequately been proved.

As the bismuth species systemically available is/are unknown and systemic bismuth is considered the toxic agent, a NOAEL of 16 mg/kg bw/day for bismuth could be used for the calculation of the MoS.

3.3.5.3 Chronic (> 12 months) toxicity

No data available

3.3.6 Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial Reverse Mutation Assay

Guideline:	/
Species/Strain:	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538 and *Saccharomyces cerevisiae* D4
Replicates:	single cultures in a single experiment
Test substance:	1087, bismuth citrate formulation (0.5%) described as a "suspension of yellow granules in a clear base". Hand-written amendment on the title page: "International formula #7, 0.5% bismuth citrate".
Batch:	/
Purity:	/
Solvent:	/
Concentrations:	0, 1, 10, 100 and 500 µl/plate without and with S9-mix
Treatment:	direct plate incorporation with 48 - 72 h incubation, without and with S9-mix
GLP:	/
Study period:	November - December 1975

1087 was investigated for the induction of gene mutations in *Salmonella typhimurium* and *Saccharomyces cerevisiae* (Ames test). Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. The experiment was performed with the direct plate incorporation method. Negative and positive controls were included.

Results
The highest concentration (500 µl/plate) was slightly toxic to the cells.
A biologically relevant increase in revertant colonies was not found in any tester strain, for any dose without and with S9 metabolic activation.

Conclusion
Under the experimental conditions used, the test item 1087 was not mutagenic in this gene mutation test in bacteria.

Ref. 44

SCCS comment
The test was performed before the adoption of the OECD test guidelines. The protocol is significantly different from the present standard protocol described in the respective OECD test guideline. Vital information (characterization of the test item, batch nr, purity and solvent) is incomplete or lacking. Under the test conditions, the identity and concentration of the test compound is not clear: The information on the identity and concentration of the a.i. (0.5%, free bismuth or bismuth citrate?) is hand-written and has been added later.

It is not known to which Bismuth species (Bismuth ion or Bismuth citrate) bacteria were exposed (if any due to the low solubility of bismuth citrate at physiological pH). The value of this test is very limited.

Bacterial Reverse Mutation Assay

Guideline:	OECD 471 (1997)
Species/Strain:	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *Saccharomyces cerevisiae* WP2*uvr*A
Replicates:	duplicate or triplicate cultures in two independent experiments
Test substance:	Bismuth citrate
Batch:	97/90137/000
Purity:	99 % (based on 51.74% bismuth dry weight)
Solvent:	sodium citrate buffer (10 mM, pH 11)
Concentrations:	initial toxicity-mutation experiment: 0, 1, 3.3, 6.7, 10, 33, 67, 100 and 200 µg/plate without and with S9-mix confirmatory mutagenicity test: 0, 10, 33, 67, 100 and 200 µg/plate without and with S9-mix
Treatment:	direct plate incorporation method with approximately 48 h incubation, without and with S9-mix
GLP:	in compliance
Study period:	10 May 2004 – 27 May 2004

Bismuth citrate was investigated for the induction of gene mutations in *Salmonella typhimurium* and *Saccharomyces cerevisiae* (Ames test). Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. The experiment was performed with the direct plate incorporation method. Toxicity was evaluated on the basis of a clearing of the bacterial background lawn. Negative and positive controls were in accordance with the OECD guideline.

Results
Toxic effects evident as a clearing of the bacterial background lawn were not observed up to the highest concentration without and with S9-mix in all strains. A biologically relevant increase in revertant colonies due to exposure to bismuth citrate was not found in both experiments without and with S9-mix in any tester strain.

Conclusion
Under the experimental conditions used, the test item was not mutagenic in this gene mutation test in bacteria.

Ref. 43

SCCS comment
In the study report, the synonym Bismuth subcitrate in the section 'Study information' of the report (p. 7) is not correct. It should read Bismuth citrate.
The identity and purity of the test substance has been determined based on the Bi content alone (see CoA). The citrate moiety of the test substance has not been determined.
Evidence that the test substance is stable in 10 mM sodium citrate, pH 11 has not been provided. Analysis of the test substance has been performed by a work-up procedure with concentrated acids whereby the test substance is destroyed. The analytical method ICP-AES is capable of determining Bi as an element but not suited for the determination of the test item bismuth citrate and its stability. Therefore, the stability of the test item in 10 mM sodium citrate, pH 11 has not adequately been proven. Under these test conditions, the identity of the test compound is not clear and may have changed (bismuth citrate or other bismuth species). As toxicity at high concentrations was not reached, the required maximum concentration of the test substance was not achieved. It is not clear whether bismuth citrate or any other bismuth species formed has entered the bacteria because

evidence that bismuth citrate (or another bismuth species formed) is soluble at high concentrations used in the test system is missing. This evidence is important, as the solubility of bismuth citrate and/or other bismuth species formed (e.g., bismuthyl ion BiO+) is very low at physiological pH. The value of this test for risk assessment is very limited.

DNA Damage and Repair/Unscheduled DNA Synthesis in Mammalian Cells *in vitro*

Guideline:	OECD 482 (1986)
Species/strain:	primary hepatocytes from male Crl:CD® (SD) rats
Replicates:	duplicate cultures in a single experiment
Test substance:	Bismuth citrate
Batch:	97/90137/000
Purity:	99 % (based on 51.74% bismuth dry weight)
Solvent:	sodium citrate buffer (10 mM, pH 11)
Concentrations:	0, 0.05, 0.1, 0.5, 1, 5, 10, 25 and 50 µM
Treatment:	/
GLP:	in compliance
Study period:	20 July 2004 – 18 August 2004

Bismuth citrate was investigated in an *in vitro* unscheduled DNA synthesis (UDS) test in hepatocytes of male Crl:CD® (SD) rats. Test concentrations were based on the results of a preliminary toxicity test measuring the release of LDH from damaged cells. A toxicity test was also conducted in parallel with the UDS assay. The vehicle used was sodium citrate buffer. Since this vehicle is not commonly used, 8 concentrations of bismuth citrate were included in the UDS test.
In the main test, after an attachment period of 2 h, the hepatocytes were exposed to bismuth citrate in the presence of ^{3}H-thymidine. The number of silver grains above the nucleus and the number of grains above two nuclear-sized cytoplastic areas adjacent to the nucleus were counted. UDS is reported as the net nuclear grain count (nuclear grain count minus the average cytoplasm grain count). Additionally, the percentage of cells in repair (cells with ≥5 net nuclear grains) is reported. Unscheduled DNA synthesis was determined on 2 slides in 50 randomly selected hepatocytes/slide. Negative and positive controls were in accordance with the OECD guideline.

Results
In the preliminary toxicity test, cytotoxicity was indicated by released LDH. Measurements of LDH release indicated relative cytotoxicity above 34% for cultures treated with 50 µM and above. Microscopic evaluation of the cultures at termination of treatment indicated normal cell morphology at all concentrations. Based on these results, the top concentration for use in the UDS test was 50 µM.
In the concurrent toxicity assay parallel to the UDS test, precipitation was not observed. Measurement of LDH release demonstrated relative cytotoxicities in all concentrations tested. Microscopic evaluation of the cultures at termination of treatment indicated normal cell morphology at all concentrations.
A biologically relevant increase in mean net nuclear grain count as compared to the untreated control was not found in hepatocytes at any concentration tested. A biologically relevant increase in the % of cells in repair was not found, either.

Conclusion
Under the experimental conditions reported, the test item did not induce DNA-damage leading to unscheduled DNA synthesis in hepatocytes and, consequently, is not genotoxic in this *in vitro* UDS test.

Ref. 46

SCCS comment
The exposure time of the primary rat hepatocytes to bismuth citrate is not reported.

In the study report, the synonym Bismuth subcitrate in the section 'Study information' of the report (p. 7) is not correct. It should read Bismuth citrate.
The identity and purity of the test substance has been determined based on the bismuth content alone (see CoA).The citrate moiety has not been determined.
Evidence that the test substance is stable in 10 mM sodium citrate, pH 11 has not been provided. Analysis of the test substance has been determined by a work-up procedure with conc. acids whereby the test substance is destroyed. The analytical method ICP-AES is capable of determining Bi as an element but not suited for the determination of the test item bismuth citrate and its stability. Therefore, the stability of the test item in 10 mM sodium citrate, pH 11 has not adequately been proven.
Under these test conditions, the identity of the test compound is not clear and may have changed (bismuth citrate or other bismuth species). The value of this test is limited.

In vitro Mammalian Chromosome Aberration Test

Guideline:	OECD 473 (1997), ICH (1996, 1997)
Species/strain:	CHO K_1 cells
Replicates:	duplicate cultures in a single experiment
Test substance:	Bismuth citrate
Batch:	97/90137/000
Purity:	99 % (based on 51.74% bismuth dry weight)
Solvent:	sodium citrate buffer (10 mM, pH 11)
Concentrations:	main experiment: 0, 1.25, 2.5 and 5 µg/ml without and with S9-mix 0, 1, 2.5 and 5 µg/ml with S9-mix
Treatment	4 h treatment without and with S9-mix; harvest time 20 h after the start of treatment 20 h treatment without S9-mix; harvest time 20 h after start of treatment.
GLP:	in compliance
Study period:	3 August 2004 – 1 September 2004

Bismuth citrate has been investigated for the induction of chromosomal aberrations in CHO cells both in the absence and presence of metabolic activation. Liver S9-fraction from Aroclor 1254-induced rats was used as an exogenous metabolic activation system. Test concentrations were based on the results of a preliminary toxicity assay on cell growth inhibition relative to growth inhibition in the solvent control in order to determine the cytotoxicity of bismuth citrate. CHO cells were exposed to 9 concentrations, ranging from 0.36 up to 20 µg/ml, the highest possible concentration, due to the solubility of bismuth citrate. The experimental conditions in this preliminary toxicity assay were identical to those of the main test.
In the main test, cells were treated for 4 h (without and with S9-mix) or 20 h (without S9-mix) and harvested 20 h after the start of treatment. Approximately 2 h before harvest, each culture was treated with colcemid (0.1 µg/ml culture medium) to block cells at metaphase of mitosis. Chromosome (metaphase) preparations were stained with Giemsa and examined microscopically for structural and numerical chromosomal aberrations. The percentage of cells in metaphase (mitotic index) was determined per 1000 cells scored. A concurrent cytotoxicity test determining total cell growth inhibition (%) relative to the solvent control was conducted for all assays and testing conditions. Negative and positive controls were in accordance with the OECD guideline.

Results
In the preliminary toxicity assay the pH and the osmolality of the highest concentration were not significantly different from the values for the solvent control.
Substantial toxicity (at least a 50% reduction in relative cell growth) was observed at concentrations ≥ 10 µg/ml in the 4 h exposure conditions and at concentrations > 5 µg/ml

in the 20 h non-activated condition. Based on these findings, the top concentration chosen for the chromosome aberration test was 5 µg/ml for all test conditions.
In the main test, sufficient relative growth inhibition (at least a 50% reduction) was observed in the tests without S9-mix. With S9-mix a sufficient level of reduction was not reached. However, in the test with S9-mix the mitotic index was approximately 63 % reduced whereas without S9-mix the mitotic index was not different from the solvent control. Apparently the exposure of the CHO cells to bismuth citrate was sufficient.
A biologically relevant increase in the number of cells with chromosomal aberrations was not found at any of the concentrations evaluated, without and with S9-mix and both after 4 and 20 h exposure.

Conclusion
Under the experimental conditions used, the test item was not genotoxic (clastogenic) in this chromosome aberration test in CHO cells.

Ref. 45

SCCS comment
In the study report, the synonym Bismuth subcitrate in the section 'Study information' of the report (p. 7) is not correct. It should read Bismuth citrate.
The identity and purity of the test substance has been determined based on the bismuth content alone (see CoA).The citrate moiety has not been determined.
Evidence that the test substance is stable in 10 mM sodium citrate, pH 11 has not been provided. Analysis of the test substance has been performed by a work-up procedure with conc. acids whereby the test substance is destroyed. The analytical method ICP-AES is capable of determining Bi as an element but not suited for the determination of the test item bismuth citrate and its stability. Therefore, the stability of the test item in 10 mM sodium citrate, pH 11 has not adequately been proven.
Under these test conditions, the identity of the test compound is not clear and may have changed (bismuth citrate or other bismuth species.
It is not known to which Bismuth species (Bismuth ion or Bismuth citrate) cells are exposed. The value of this test is limited.

3.3.6.2 Mutagenicity / Genotoxicity *in vivo*

Bone marrow chromosome aberration test in mice

Guideline:	/
Species/strain:	Swiss albino mice
Group size:	5 male mice/group
Test substance:	bismuth trioxide
Batch:	/
Purity:	/
Vehicle:	distilled water
Dose levels:	0, 400, 666.67 and 1000 mg/kg bw/day as a suspension in distilled water
Route:	orally, daily for 7, 14 or 21 days
Sacrifice times:	at day 7, 14 or 21 of treatment
GLP:	/
Study period:	/

Bismuth trioxide has been investigated for the induction of chromosomal aberrations in bone marrow cells of male mice. Male mice were exposed daily to oral doses of 0, 400, 666.67 and 1000 mg/kg bw/day. Approximately 1.5 h before death, each mouse was treated i.p. with colchicine (4 mg/kg bw) to block cells at metaphase of mitosis. Bone marrow preparations were stained with diluted Giemsa and examined microscopically for chromosomal aberrations. To evaluate the effect of treatment on cellular proliferation,

indicating to exposure of the target cells, the percentage of dividing cells per treatment group was scored. Negative and positive controls were not included.

Results
The mitotic index was reduced by bismuth trioxide treatment indicating to exposure of the target cells.
Independent of the treatment time, a dose dependent increase in the number of bone marrow cells with chromosome aberrations was observed compared to the untreated control group. A statistically significant trend test (ANOVA) was only found after 21 days of treatment.

Conclusions
Under the experimental conditions used, bismuth trioxide induced an increase in the number of bone marrow cells with chromosome aberrations and, consequently, is genotoxic (clastogenic) in bone marrow cells of mice.

Ref. 58

SCCS comment
The data are from a publication in the open literature. Very little detail on the performance of the test was reported. Batch number and purity are lacking. The test was not conducted in compliance with GLP or OECD guidelines. The performance of the test does not comply with the present standard requirements.
Bismuth trioxide is insoluble in aqueous media.
The test has only limited value and can only be used for confirmation purposes.

Sperm head abnormality test in mice

Guideline:	/
Species/strain:	Swiss albino mice
Group size:	5 male mice/group
Test substance:	bismuth trioxide
Batch:	/
Purity:	/
Vehicle:	distilled water
Dose levels:	0, 400, 666.67 and 1000 mg/kg bw/day as a suspension in distilled water
Route:	orally, daily for 7, 14 or 21 days
Sacrifice times:	at day 7, 14 or 21 of treatment
GLP:	/
Study period:	/

Bismuth trioxide has been investigated for the induction of sperm head abnormalities in sperm obtained from the epididymis of mice. The production of abnormal sperm heads is considered to indicate changes in the genetic component controlling the process of spermatogenesis. Male mice were exposed daily to oral doses of 0, 400, 666.67 and 1000 mg/kg bw/day. Epididymal sperm preparations were stained with diluted Giemsa and examined microscopically for sperm head abnormalities. Negative and positive controls were not included.

Results
A biologically relevant increase in the frequency of sperm head was not observed compared to the untreated control group.

Conclusions
Under the experimental conditions used, bismuth trioxide is not genotoxic in this sperm head abnormality test in mice.

Ref. 58

SCCS comment
The data are from a publication in the open literature. Very little detail on the performance of the test was reported. Batch number and purity are lacking. The test was not conducted in compliance with GLP. The performance of the test does not comply with the present standard requirements. Bismuth trioxide is insoluble in aqueous media. It is questionable whether a sufficient internal dose was achieved for reaching the target organ. The test has only very limited value.

3.3.7 Carcinogenicity

Bismuth oxychloride (BiOCl)

BD rats, groups of 20 males and 20 females (100 days old at the start of the experiment) were fed a diet containing 1, 2, or 5% BiOCl. A group of 30 males and 30 females served as the untreated control. The daily intake of the mash was 50 g for the males and 40 g for the females. After a feeding period of 2 years the treatment was terminated and surviving animals were transferred to the basic diet and observed until their natural death. Body weight was recorded monthly. At autopsy all important organs were examined and tissues were fixed for histological investigations.

The mean survival varied from 810 to 890 days. The mean body weights of the test groups did not differ significantly from those of the controls. No macroscopic or histological findings could be attributed to the BiOCl treatment. One mammary carcinoma was found in the control group. No malignant tumours were found in the groups treated with BiOCl.

Ref. 47

Bismuth subcarbonate (Bi_2O_3 . CO_2 . H_2O)

BD rats, a group of 20 males (100 g at start of experiment) were fed a diet containing 2% bismuth subcarbonate. Mean survival was 753 days. No tumours attributable to bismuth subcarbonate were found.

Ref. 50

Tris(dimethyldithiocarbamato)bismuth

(C57BL/6 X C3H/Anf)F1 and (C57BL/6 X AKR)F1 hybride mice received 10 mg/kg bw Tris(dimethyldithiocarbamato)bismuth in 0.5% gelatine by gavage for 21 days. The treatment started when the mice were 7 days old. After this treatment the mice received 34 ppm tris(dimethyldithiocarbamato)bismuth in the diet for 17 months. A large number of different control groups involving more than 100 mice participated in the study.
No significant elevation of tumour incidence in any of the treated mice was found.

Ref. 51

Bismuth dextran

40 mice received subcutaneous injections with bismuth dextran. No tumours were found at the site of injection.

Ref. 49

SCCS comment
No carcinogenicity studies have been performed with bismuth citrate. Some bismuth compounds have been studied in long term studies with rats and mice. These studies with the exception of the study on bismuth oxychloride are old (from 1960 – 1969) and incompletely reported. None of the studies reported tumour induction by bismuth compounds.

3.3.8 Reproductive toxicity

3.3.8.1 Two generation reproduction toxicity

No data available

3.3.8.2 Other data on fertility and reproduction toxicity

No definitive studies on the effects of bismuth citrate administration on male or female fertility and early embryonic development have been conducted and the literature search did not identify any such studies of any bismuth salt. However, experimental studies on **bismuth citrate** (ref. 52) and **bismuth subnitrate** (ref. 53, ref. 55, ref. 56, ref. 57) were identified in the literature review:

The histochemical silver amplification technique auto-metallography (AMG) was used to trace bismuth in the testis of Wistar rats after i.p. administration of **bismuth subnitrate**. Groups of 4 male Wistar rats were treated with an overdose of 500 mg/kg bismuth subnitrate intraperitoneally and allowed to survive for 2 weeks and 8 weeks, respectively. A group of four rats served as control and received an intraperitoneal injection of 0.9% saline. The reason for choosing this high dose was that bismuth subnitrate is rather insoluble, and very high doses of bismuth subnitrate are needed to get bismuth into the bloodstream, compared to other bismuth compounds. In both treatment groups, in the seminiferous tubules, bismuth was located in lysosomes of Sertoli cells. Leydig cells showed large amounts of AMG-bismuth in their lysosomes pointing at a possible effect of bismuth on testicular function and male reproductive capability.

Ref. 57

In a subsequent study, the authors used the same technique to trace bismuth in the testis and pituitary glands of Wistar rats after i.p. treatment (500 mg/kg bw) with **bismuth subnitrate** and survival for 2 weeks. Again, large amounts of bismuth AMG grains were presentin the lysosomes of Leydig cells. Serum testosterone levels were reduced when compared with controls. No histochemical traces of bismuth were found in the anterior lobe of the pituitary gland. Compared with their corresponding controls, neither follicle-stimulating hormone nor luteinizing hormone were affected. According to the authors, the selective uptake of bismuth in Leydig cells, followed by decreased testosterone levels, emphasizes a potential hazard of bismuth-provoked male reproductive impairment.

Ref. 56

Studies have demonstrated that bismuth overdose results in a lowered serum testosterone level but the mechanisms involved are unknown. The effect of **bismuth subnitrate** was therefore investigated on Leydig cells isolated from rats.

10 male Wistar rats were treated with one i.p. injection of 500 mg/kg bismuth subnitrate and allowed to survive for 2 weeks. 10 rats served as control and received an intraperitoneal injection of 0.9% saline. Under the experimental conditions applied, bismuth

was observed in Leydig cells, with a subsequent reduction in serum testosterone levels. Stereological procedures were used to estimate the number of Leydig cells in the right testis from retained rats used in a previous study. The mean number of Leydig cells in the control group was estimated to be 18.7 x 10^6, which was comparable to previous estimations. In the group exposed to bismuth, the mean was 15.5 x 10^6. The observed 17% difference between the two groups was statistically significant. The inter-individual variation was largest in the bismuth-exposed group. Testis weight and body weight were not significantly reduced after bismuth exposure. No signs of overt toxicity were observed but a reduction in the number of Leydig cells in testes was demonstrated. These findings support the hypothesis that bismuth has a direct toxic effect on rat Leydig cells under the experimental conditions used and suggests a potential risk of bismuth to male reproduction.

Ref. 53

Recent studies suggest that bismuth accumulates in Leydig cells. In addition, a reduced level of serum testosterone and a statistically significant reduction of Leydig cells have been observed. It was therefore hypothesized that Bi has a direct toxic effect on rat Leydig cells. A more recent study performed in Wistar rats injected intraperitioneally with 500 mg/kg of **bismuth subnitrate** employed a combination of autometallography for bismuth tracing and immunohistochemistry for macrophage localization. By use of this method for double labelling of bismuth and ED-2 (a marker for testicular macrophages) it was shown that the heavily bismuth-loaded cells in rat testis, originally interpreted as being Leydig cells, are testis loaded macrophages. Consequently, the data suggest a modified hypothesis regarding bismuth-induced interactions between testicular macrophages and Leydig cells.

Ref. 55

SCCS comment

The study data suggest that high internal doses of bismuth achieved by i.p. application may lead to damage of Leydig cells and consequently serum testosterone levels may be reduced compared with controls. The data shows a potential hazard to male reproduction at high internal doses that may also be otherwise toxic. Any conclusions on risk assessment of male fertility and reproduction with regards to bismuth citrate or other bismuth species formed after dermal or oral absorption are not possible on the basis of such studies.

In vitro study

Effects of bismuth citrate on the viability and function of Leydig cells and testicular macrophages isolated from rats were investigated. Bismuth citrate was dissolved in 500 µl 1 M aqueous ammonia and diluted with the culture medium up to 100 ml to obtain a 400 µM stock solution of bismuth citrate (containing 5 mM NH_3). No change in viability or secretion of testosterone were observed in Leydig cells treated for 24 h with increasing doses of bismuth citrate (1, 10, 100 µM). However, a significant effect on testicular macrophages was observed under the experimental conditions applied. No influence on the production of TNF-α, known to be inhibitory to Leydig cells and produced by macrophages when activated, was noted.

Given the previously observed effects of bismuth on testosterone *in vivo*, it was concluded that bismuth has no direct effect on Leydig cells but lowers testosterone levels by destroying testicular macrophages, thereby interrupting their local paracrine influence on Leydig cells through factors other than TNF- α.

Ref. 52

SCCS comment
The stability of bismuth citrate in 1 M aqueous ammonia is not clear and the pH of the bismuth citrate stock solution was not reported. The test item seems to exert similar effects *in vitro* compared to bismuth subnitrate *in vivo* after high doses suggesting that bismuth ions or unknown bismuth species formed *in vitro* or *in vivo* are the toxic agents. The mechanism of action remains unclear.

3.3.8.3 Developmental Toxicity

The following study data were obtained from a summary of a scientific review (report not available):
Passage of bismuth into amniotic fluid and the human foetus has been reported after intake of bismuth containing drugs but information on effects upon foetal development appear limited. In order to establish levels of bismuth which could be tolerated during pregnancy, preliminary studies were performed in pregnant AHA rats and Dutch rabbits.
Bismuth citrate was formulated as solutions in aqueous ammonium hydroxide.

a) Rabbits: Dosages of 50, 100 or 200 mg/kg were administered once daily by oral gavage to 10 rabbits from Days 8-20 of pregnancy inclusive (day of mating is Day 1 of pregnancy). Plasma bismuth levels were determined on Days 8 and 20 of pregnancy. On Day 30, the rabbits were subjected to autopsy and foetuses examined for external, visceral and skeletal abnormalities. Maternal toxicity, manifest as a marked reduction in bodyweight, was apparent in the rabbits (dose not reported). No adverse effects upon pre- or post-implantation loss, numbers of viable foetuses or foetal development were observed. Results obtained indicated that plasma bismuth levels in the rabbits of up to 420ng/g were not linearly related to dose and that there was evidence of accumulation.
b) Rats: Doses of 300, 600, and 1200 mg/kg/day were administered on days 7-16 of gestation. No effects on maternal toxicity, fetal toxicity, or postnatal development were observed in rats at any dose. Rat post-natal development was also unaffected. Maximum bismuth levels in rat plasma (495 ng/g) were similar to those of the rabbits while there was evidence of a linear relationship to dose but with no obvious accumulation.

Ref. 60

SCCS comment
The study cannot be evaluated as the study report is not available. In addition, stability of bismuth citrate after dissolution in aqueous ammonium hydroxide is not reported.

Guideline:	OECD 414 (22nd January, 2001)
Species/strain:	Rat Crl:CD (SD)IGS BR
Group size:	22 mated females
Test substance:	bismuth citrate
Batch:	97/90137/000
Purity:	99% according to the bismuth content of % (CoA from 1997)
Vehicle:	5% aqueous methylcellulose (M_n *ca.* 86,000)
Dose levels:	0, 100, 300, 1000 mg/kg/day
Dose volume:	5 ml/kg bw
Route:	oral
Administration:	gavage
Positive control:	/
GLP statement:	in compliance
Study period:	Experimental phase Nov 2004

Doses were selected based on the above described developmental toxicity study in rats (ref. 60). Rats were dosed once daily during days 6-20 of gestation. Maternal clinical

observations, body weights, and food consumption data were recorded until day 21 of gestation when all dams were killed and subjected to caesarian section. Fetuses were examined for external, visceral, and skeletal alterations. There were no deaths and no treatment-related findings at gross necropsy of the dams. Clinical observations were limited to dark feces at 300 and 1000 mg/kg bw/day which were considered test substance-related but not adverse. Maternal toxicity was observed at 300 and 1000 mg/kg bw/day indicated by statistically significant reductions in maternal body weights and/or weight gain. Transient effects on food consumption were observed at 1000 mg/kg bw/day. There was no evidence of maternal toxicity at 100 mg/kg bw/day. No developmental toxicity was observed at any dose level. The mean number of implantation sites, resorptions, live fetuses, mean fetal weight and sex ratio were comparable across all groups. There were no test substance-related fetal abnormalities observed.
The no-observed-effect level (NOEL) for maternal toxicity was identified as 100 mg/kg bw/day, based on reductions in body weight /body weight gain at 300 and 1000 mg/kg bw/day. The NOEL for developmental toxicity was 1000 mg/kg bw/day.

Ref. 59

SCCS comment
The synonym Bismuth subcitrate in the "Study information" of the report (p. 7) is not correct.
The purity of the test substance has been determined based on the Bismuth content alone (see CoA). The citrate moiety has not been determined.
The stability of the test substance and its stability in the test formulations during study conduct have been determined by a work-up procedure with conc. acids whereby the test substance is destroyed. The analytical method ICP-AES is capable of determining Bismuth as an element but is not suited for the determination of the test item bismuth citrate and its stability. Therefore, the stability of the test item in dosing formulations has not adequately been proven.

3.3.9 Toxicokinetics

3.3.9.1 Toxicokinetics in laboratory animals

Rats

Study 1
A pharmacokinetic study of "bismuth$^{205/206}$ citrate" in the Wistar rat established a model with two open compartments. The animals were either orally dosed by gavage or received an intravenous injection into the caudal vein at the dose of 0.2 µCi/250 g of "bismuth citrate" per animal. Blood samples were taken at 1, 2, 4, 8, 16, 24 and 48 hours after dosing. In the nervous system, the highest concentration of bismuth was in the spina medulla and a descending gradient was noted in the central nervous system. After distribution, the concentrations of bismuth were highest in the kidney, followed by lung, liver, gut and spleen.

Concentrations of a bismuth$^{205/206}$ citrate-like substance in µg/g tissues for an injected quantity of 0.2 µCi/250 g/animal:

		1 hr	**2 hr**	**4 hr**	**8 hr**	**16 hr**	**24 hr**	**48 hr**
Lung	i.v.	33	160	24	9	8	6	4
	p.o.	1	1	1	1			
Liver	i.v.	72	73	70	68	15	14	8
	p.o.	-	1	6				

Spleen	i.v.	30	39	41	35	23	18	10
	p.o.	4	5	24	7	3	2	1
Kidney	i.v.	163	216	181	176	141	133	41
	p.o.	2	20	106	2	1		
Duodenum	i.v.	42	36	29	26	18	7	2
	p.o.	14	12	17	8	1		
Colon	i.v.	44	45	32	27	16	10	4
	p.o.	1	1	1	6	1	1	
Brain	i.v.	1.006	1.117	0.902	0.741	0.555	0.535	0.189
	p.o.	0.671	0.500	0.496	0.266	0.183	0.126	0.073
Cerebellum	i.v.	1.954	2.040	1.098	1.071	0.879	0.786	0.420
	p.o.	0.892	0.855	0.802	0.750	0.610	0.407	0.327
Spine medulla	i.v.	2.860	5.008	2.255	2.215	2.115	1.625	1.102
	p.o.	5.788	1.055	1.243	1.784	1.433	1.215	0.783

Ref. 62

SCCS comment
The synthesis and purification of the radiolabelled bismuth substance was not described. A soluble salt of "Bi citrate" was used (see introduction of the publication). It cannot be excluded that a commercially available bismuth salt, **Bi subcitrate**, potentially the potassium salt was used.
The study cannot be used for quantitative evaluation because of the unclear identity and characterization of the Bi substance. Moreover, the copy of the publication provided was in part not readable so that possible important information on toxicokinetic parameters such as half-lives of the substance could not be retrieved.

Study 2
The bioavailability of ^{205}bismuth from various labeled salts, most of them used in pharmaceutical commercial oral preparations used in peptic ulcer therapy (**basic bismuth citrate – see SCCS comment, water soluble bismuth citrate, colloidal bismuth subcitrate, basic bismuth nitrate, salicylate, gallate and bismuth aluminate**) was studied in female Wistar rats. Synthesis of the labeled substances and their characteristics were described (see table).

Table 1. Bismuth content and solubility (21° C) of ^{205}Bi labelled compounds used in this study

^{205}Bi compound	Formula	mw	Bismuth content		Solubility (µmol/l)	
			calculated	found (AAS)	Artificial gastric juice	Artificial duodenal juic
Basic bismuth citrate	$BiC_6H_7O_8$	419	50.2%	50.2%	48	30
Colloidal bismuth subcitrate	?			36.0%	41	35
Water soluble bismuth citrate				37.5%	43	40
Basic Bi salicylate	$BiC_7H_5O_4$	364	57.7%	57.5%	19	1.1
Basic bismuth gallate	$BiC_7H_7O_7$	412	50.7%	49.8%	22	1.2
Bismuth aluminate	$Bi_2(Al_2O_4)_3 10\ H_2O$	952	43.9%	44.8%	20	1.1
Basic bismuth nitrate	$4\ Bi(OH)_2NO_3BiO(OH)$	1461	71.5%	72.0%	27	1.5

Intestinal absorption, determined in groups of 10 rats each and calculated from ^{205}bismuth whole body retention and accumulated ^{205}bismuth urinary excretion, was generally low, but significantly higher (0.26-0.33% of dose) from oral bismuth citrates (basic bismuth citrate,

water soluble bismuth citrate, colloidal bismuth subcitrate) compared to basic bismuth nitrate, salicylate, gallate, and bismuth aluminate (0.04-0.11% of dose). For all compounds, more than 99% of the label was excreted in the feces. The low solubility of most bismuth compounds (with the exception of bismuth citrates) at neutral pH has been suggested as the main factor for low intestinal absorption. Solubility of the bismuth citrate compounds in an artificial gastric juice was only about two-fold higher than the solubility of the water-insoluble bismuth substances, whereas marked differences between both substance groups were observed when using an artificial duodenal juice (see table).

Table 2. Accumulated faecal and urinary excretions, and whole body retention (WBR) of ^{205}Bi from oral ^{205}Bi-labelled compounds (6–8 mg, 1.9–3.9 μCi) in rats (n = 10), 7 days after administration. The results are expressed in % of dose (mean ± SD)

^{205}Bi compound	Faecal excretion	Urinary excretion	Whole body retention	Absorption (urinary excretion + WBR)
Basic bismuth citrate	99.5±0.5	0.27±0.06	0.06±0.01	0.33±0.06
Colloidal bismuth subcitrate	99.4±0.4	0.30±0.05	0.05±0.01	0.35±0.05
Water soluble bismuth citrate	99.1±0.6	0.22±0.02	0.04±0.01	0.26±0.03
Basic bismuth salicylate	99.8±0.7	0.07±0.03	0.01±0.01	0.08±0.03
Bismuth aluminate	99.7±2.2	0.03±0.02	0.01±0.02	0.04±0.03
Basic bismuth gallate	99.6±0.6	0.10±0.06	0.01±0.02	0.12±0.07
Basic bismuth nitrate	99.9±0.6	0.06±0.01	0.01±0.01	0.07±0.01

The biological half-life and the tissue distribution of bismuth was studied following single administration of colloidal bismuth subcitrate (20 mg, 150 μCi) to rats by gastric intubation. Twenty days after oral application 0.008-0.017% of the radioactivity, i.e. 2-5% of the dose absorbed was still retained in the body. Retained bismuth was mainly in the kidney, followed by bone, red blood cells and the lung.
The ^{205}Bi activity in blood was mainly attributed to the red cell fraction, whereas the serum concentration was almost negligible. This might be of importance in view of the fact that the safety of bismuth therapies has been often monitored by measuring the plasma concentration.

Table 3. ^{205}Bi-activity in organs of rats (n = 4, mean ± SD) 20 days after oral administration of ^{205}Bi labelled colloidal bismuth subcitrate (20 mg, 150 μCi). WBR = whole body retention

Tissue	% of dose ($\times 10^{-5}$/g tissue)	Percentage of ^{205}Bi-WBR	
		Organ	g tissue
Kidney	29.43±3.01	8.81±2.57	
Bone (femur)	13.71±0.67		2.64±0.49
Red blood cells	8.72±3.89		1.42±0.60
Lung	1.70±0.51	0.34±0.04	
Spleen	1.07±0.13	0.13±0.01	
Heart	1.07±0.15	0.14±0.06	
Liver	1.05±0.62	1.31±0.46	
Muscle	0.83±0.14		0.19±0.12
Brain	0.47±0.12	0.17±0.10	
Serum	0.05±0.05		0.01±0.01
Fat	0.04±0.04		0.01±0.01

Whole body retention, fecal and urinary excretions of ^{205}bismuth was described as a three-compartment model. Biological ^{205}bismuth half-lives of 10, 36 and 295 h were found in the rat. Ref. 63

SCCS comment
"Basic bismuth citrate" in this study is the monohydrate of bismuth citrate, $BiC_6H_7O_8$, MW 416, Bi content 50.2%.
"Water soluble bismuth citrate" in this study is probably not the potassium salt of bismuth subcitrate (which has a molecular weight of 704,47 and a Bi content of 29.7%, not 37.5% as in the table) (see 3.1.1.6).
According to this study, liver is apparently not a major organ of Bi retention in rats 20 days after oral uptake, in contrast to humans (see next section, toxicokinetics in humans).

Study 3
After 15 days of twice daily oral gavage with bismuth subcitrate at 13.7 mg/kg bw/day to eight rats, deposition of bismuth was found in all tissues studied, especially the kidney (30.8 +/- 8.6 µg/g dry weight). Bismuth was detected in kidney, brain, lung and liver but deposition was not influenced by gastric pH.
A similar pattern of distribution and tissue concentrations was found when bismuth subcitrate was given with ranitidine hydrochloride 8.6 mg/kg bw/day to another eight rats. However, this combination resulted in lower brain levels (3.1 +/- 1.3 µg/g dry weight) than after administration of bismuth subcitrate alone (4.8 +/- 1.0 µg/g dry weight).
When six rats were given ranitidine bismuth citrate by gavage at 22.8 mg/kg bw/day for 15 days, kidney levels were lower (4.2 +/- 1.8 µg/g dry weight) compared to an equivalent dosing with bismuth subcitrate, and brain levels were below detection limits. Blood levels correlated poorly with deposition in organs. Bismuth could not be detected in any of the organs examined at 30 days post-dosing but was found in the urine.

Ref. 64

SCCS comment
Bismuth subcitrate in combination with ranitidine hydrochloride or given as a ranitidine bismuth citrate complex apparently reduces the absorption and/or the deposition of bismuth in critical organs of the rat. However, this study is available only as a summary.

Study 4
Intestinal absorption of bismuth from bismuth subnitrate (**BSN**), bismuth subsalicylate (**BSS**), colloidal bismuth subcitrate (**CBS**), bismuth chloride (**$BiCl_3$**) or bismuth citrate (**BCit**) was investigated by use of an *in vivo* perfusion of rat small intestine in combination with systemic blood sampling in female Wistar rats (200-220 g bw). The objective of the study was to clarify whether the absorption of bismuth behaves differently compared with oral intake and passage via the stomach.
Perfusate solutions in isotonic saline were prepared containing the equivalent of 1 g of elemental Bi per liter for five Bi compounds: BSS (containing 56% elemental Bi), BSN (71% Bi), CBS (35% Bi), BiCl3 (66% Bi), and BCit (51% Bi). The solutions were stirred and heated at 37°C for 1 h directly before the experiment to allow the suspensions to reach equilibrium. The perfusate solution of CBS, which forms an unstable colloidal solution, was prepared directly before starting the experiment. Eight rats were perfused with CBS and four with each of the other compounds. The isotonic Bi-containing medium was recirculated at 37°C through the small intestine (duodenum, ileum, and jejunum; total length, about 1 m) at a perfusion rate of 10 ml/min. Osmolality was controlled to limit interference of water and salt transport with the absorption of Bi. Samples of perfusate and blood were collected directly before the experiment (t = 0) and for 60 min at 15-min intervals during the experiment and analyzed by electro-thermal atomic absorption spectrometry (EAAS).
The dose dependency of Bi absorption was studied for CBS because it was the Bi compound that was most easy to handle. $BiCl_3$ was chosen as a reference compound, but because of its low pH in isotonic saline (pH = 1.7), citrate buffer (0.1 M, pH 6.3) was added. At least two rats were perfused with each concentration.

Results

The Bi concentrations in the perfusates prepared with CBS, $BiCl_3$ and BCit were higher than those in the perfusates prepared with BSN and BSS (see table 1). The initial pH of the $BiCl_3$ perfusate was significantly lower (pH = 1.7 ± 0.1) than in all other perfusates, and the pH in the BCit and BSS perfusates (pH = 4.3 ± 0.2 and 5.3 ± 0.6, respectively) was lower than in the BSN and CBS perfusates ($p < 0.001$; pH = 6.6 ± 0.1 and 6.7 ± 0.3, respectively). Osmolality and pH at the end of the perfusion were comparable between the compounds, with the exception of $BiCl_3$, which had a significantly lower pH ($p < 0.001$; pH = 3.4 ± 0.8) and a higher osmolality compared with the other compounds at the end of the perfusion. In all perfusates, pH increased during perfusion due to mixing of gastrointestinal fluids with the unbuffered perfusate.

The absorption of Bi (BiB_{60}) after 60 min was dependent on the type of compound in the perfusate. Absorption was higher from CBS- and BCit-containing perfusates (see table 1) than from BSN, BSS, and $BiCl_3$. (Differences in the time course of absorption were also observed between the two groups of substances.)

Table 1—Bi Concentration in Perfusate Before (BiP_0) and After 60 min (BiP_{60}) and Bi Concentration in Blood after 60 min (BiB_{60}) of Perfusion with Several Bi Compounds

Compound	Aspect	BiP_0 (mg/L)	BiP_{60} (mg/L)	BiB_{60} (μg/L)
$BiCl_3$	White suspension	308 ± 127	140 ± 78	25.0 ± 7.5
BCit	White suspension	172 ± 74	75 ± 10	489 ± 206
CBS[a]	Clear solution	826 ± 152	1278 ± 278	1045 ± 586
BSN	White suspension	0.5 ± 0.3	0.3 ± 0.1	63.8 ± 25.6
BSS	White suspension	2.2 ± 1.2	0.3 ± 0.1	70.8 ± 20.2

NOTE: Bi compounds at concentrations equivalent to 1 g of elemental Bi in isotonic saline; values are expressed as mean ± SEM; $n = 4$. [a] $n = 8$.

Dose dependency of Bi absorption: For both compounds studied (i.e., CBS and $BiCl_3$ in citrate buffer), the perfusate concentrations of Bi increased with the amount of Bi added to the perfusate in a dose-dependent manner (see table 2).

Table 2—Bi Concentration in Perfusate Before (BiP_0) and After 60 min (BiP_{60}) and Bi Concentration in Blood after 60 min (BiB_{60}) of Perfusion with $BiCl_3$ in Citrate Buffer and CBS in Isotonic Saline Solution

Compound	*n*	Dose (mg/L)	BiP_0 (mg/L)	BiP_{60} (mg/L)	BiB_{60} (μg/L)
Blank	4	0	ND	ND	ND
$BiCl_3$	5	10	10.2 ± 2.2	7.5 ± 1.6	298 ± 173
	8	100	92.8 ± 3.6	87.3 ± 3.5	567 ± 164
	4	1000	631 ± 165	499 ± 145	1213 ± 325
CBS[a]	3	10	1.2 ± 0.8	1.9 ± 0.8	2.7 ± 1.7
	5	100	21.8 ± 5.2	16.0 ± 5.2	52.8 ± 31.0
	2	1000	290 ± 30	175 ± 65	83.0 ± 1.0
	4	10000	2925 ± 63	2775 ± 193	1623 ± 397
	4	100000	32750 ± 479	30867 ± 1659[b]	NA

NOTE: Values are expressed as mean ± SEM; ND = not detectable; NA = not analyzed, because animals died before $t = 60$ min (BiB at death was $40.8 \cdot 10^3$–$243 \cdot 10^3$ μg/L). [a] CBS contains 35.5% Bi. [b] At $t = 30$ min.

Ref. 65, Ref. 91

SCCS comment
The study data indicates that the *intestinal* absorption of bismuth compounds is dependent on their water solubility (table 1) even if the solubility is low and also strongly depends on the concentration of the bismuth compound in the perfusate (table 2), meaning that acute toxic, up to lethal Bi concentrations in blood may be achieved in case of circumvention of the gastric passage. Gastric passage after oral uptake may change the bismuth compound, for instance by formation of insoluble BiOCl under the acidic conditions and presence of hydrogen chloride in the stomach.
The authors recognized in their discussion that bismuth citrate may have been formed at least in part in the $BiCl_3$ solution after enhancing the pH by addition of citrate buffer. However, no details have been reported.
For a detailed discussion of the study results and their implications for oral absorption of bismuth compounds in rats and humans see **Annex 2**.

Study 5
The effect of liver disease on the distribution of bismuth in the body was studied in normal and experimental cirrhotic rats to test the hypothesis that diseases of the liver could predispose to bismuth accumulation and potential intoxication. Cirrhosis was induced in the rats using a phenobarbitone and CCl_4 regime. Normal rats used as a comparison group received phenobarbitone alone in drinking water. Two weeks after the treatment, the animals were given bismuth as an intramuscular injection at a dose of 630 µg/kg-bw twice a week for a period of 70 d.
Excretion and tissue distribution of bismuth were investigated in animals administered **bismuth subcitrate** by the intramuscular route for 70 days. Plasma bismuth in normal rats reached an apparent steady state of 31.89 ± 4.15 µg/l by day 28–35. The plasma profile in cirrhotic rats resembled that of normal rats until day 42 after which bismuth concentrations became significantly elevated. At day 70 mean plasma bismuth concentration was 63.68 ± 9.68 µg/l in cirrhotic rats compared with 32.68 ± 4.24 µg/l in control rats ($p < 0.05$). Total urinary excretion of cirrhotic animals closely paralleled that of controls; however, urinary bismuth clearance was significantly reduced beyond 42 d, as was faecal excretion. In both groups, deposition of bismuth was highest in the kidney, followed by liver and bone. Much lower amounts were found in lungs, spleen, brain and heart. Bismuth concentration in the liver, bone, spleen, lungs and heart of the cirrhotic rats was significantly higher (about 1.5-3 fold), with no change in the kidney.

Ref. 66

SCCS comment
Determination of bismuth in plasma is a method of limited value for the assessment of the internal load of bismuth as bismuth levels in whole blood are considerably higher (ref. 63).

Mice

The distribution of bismuth in the gastrointestinal tract and other organs was studied after single oral exposure in BALBc/a female mice with **bismuth citrate** or **ranitidine bismuth citrate** corresponding to bismuth concentrations in the range of 0.5 to 15 mg (i.e., 25-750 mg bismuth/kg for a 20 g mouse). Cryostat tissue sections from all animals were examined.
Cultured murine peritoneal macrophages were exposed to bismuth citrate at concentrations of 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, and 128 µM or pure medium for 24, 48 or 72 hours. The bismuth accumulation was examined over time.
Bismuth was absorbed and was present in gastrointestinal epithelial cells shortly after exposure. Deposits of bismuth were found in lymph node macrophages, liver, spleen and kidney as well as in macrophages in the gastrointestinal *lamina propria* for at least 9 weeks. At the subcellular level, bismuth was found exclusively in lysosomes, primarily in

macrophages and dendritic cells. Lysosomal accumulation was shown to be time and dose dependent.
Ranitidine bismuth citrate was more readily absorbed than bismuth citrate and this was attributed to a pH effect, as ranitidine increases the intra-gastric pH.
No signs of morphological changes in the examined tissues or cell cultures and no signs of adverse effects were noted in experimental animals.

Ref. 67

Rabbits

The content of bismuth in blood, serum, kidneys and brain of rabbits was determined after twice daily oral administration of **colloidal bismuth subcitrate, potassium salt (Ventrisol)** and its chemical analogue, **bismuth citrate** (dissolved in ammonia). The daily dose of Ventrisol was equivalent to 28.8 mg/kg bw/day of Bi203 (corresponding to 25.8 mg Bi/kg bw/day) during the four week treatment period (4 times higher than human dosage). The dosage of bismuth citrate was not mentioned.

Higher bismuth content in kidneys was observed suggesting the excretion of both pharmaceuticals in urine. The very low values obtained from bismuth dosages in blood serum of rabbits before and after repeated administration, and the lack of statistical differences, showed that bismuth was not absorbed from pharmaceuticals under investigation. These observations were confirmed by the analysis of bismuth content in kidneys and brain of the rabbits.

Ref. 61

SCCS comment
High levels and high variations of bismuth in blood (5-25 µg/L) were observed in the control groups so that no statistically significant difference could be obtained after 4 weeks of dosage. It is not clear whether the solution of bismuth citrate in ammonia may change the bismuth species. The dosage of "bismuth citrate" was not mentioned but was apparently equivalent.
The study cannot be evaluated because of severe shortcomings.

3.3.9.2 Toxicokinetics in humans

Introduction

There are only few studies reported on the toxicokinetics of bismuth citrate in humans and even in these cases the identity of bismuth citrate as test item is not always certain. Plenty of toxicokinetic studies in humans, published clinical safety studies and case reports exist which were performed or reported when using bismuth substances of pharmacological interest. These substances include a wide range of bismuth salts but also bismuth substances with organic ligands and thus different properties such as higher lipophilicity. Only studies on Bi substances will be referred to here that are considered similar to bismuth citrate in their chemical properties, in particular chemical complex formation of bismuth and citrate such as **bismuth subcitrate** (mostly the water soluble tripotassium salt, also termed tripotassium dicitrato bismuthate) or the colloidal form derived from this salt, **colloidal bismuth subcitrate (CBS)** or **ranitidine bismuth citrate**, a complex formed from ranitidine and bismuth subcitrate (see section 3.1.1.6). For more general information on the toxicokinetics of bismuth in humans see **Annex 1**.

Studies reported

Study 1
An exploratory study was conducted in one healthy male subject aged 49 years (bw 83 kg) in order to establish the relationships among systemic uptake, blood levels, and excretion of bismuth. The volunteer received an intravenous injection of **^{207}bismuth as a citrate compound** (5 ng of bismuth dissolved in in 0.01 M nitric acid with an equal volume of 2% (wt/vol) trisodium citrate to give an activity concentration of about 10 kBq ^{207}Bi/ml). The quantity injected was about 9 kBq. Levels of the tracer in blood and in excretion samples, and its retention and distribution within the body were determined by measurement of radioactivity.
Under the experimental conditions applied, levels in whole blood samples fell very rapidly, with only 1% of the injection remaining at 7 h and only 0.1% at 18 days suggesting rapid distribution and/or excretion. There was a rapid initial excretion, with 55% lost during the first 47 h, principally in urine. However, long-term losses were much slower. Whole body retention 25 days after intake was about 8%, and 0.6% remained in the body at 924 days, when the contemporary rate of loss implied a biological half-life of 1.9 years.

Table 6 Activity balance

Days after intake	*Whole body retention (%)*	*Cumulative urine (%)*	*Cumulative faeces (%)*	*Whole body+ excretion (%)*
0.25	75.7	20.8	0	96.5
0.95	56.8	34.5	3.1	94.4
1.95	45.1	42.4	6.8	94.3
2.95	37.9	46.6	10.2	94.7
3.97	31.7	49.3	13.0	94.0
8.00	20.2	55.3	19.1	94.6
11.3	14.8	57.6	21.3	93.6
18.3	10.0	60.3	23.4	93.7
25.0	7.9	61.7	24.7	94.4

Ref. 70

SCCS comment
The identity of the bismuth citrate compound has not been proven.

Study 2
Labelled compounds in human volunteers demonstrated oral bioavailability values of 0.043%±0.008% for **colloidal bismuth subcitrate**, 0.039%±0.001% for **basic bismuth subgallate** and <0.005% for **basic bismuth nitrate**, **basic bismuth subsalicylate** and **bismuth aluminate**.
Oral administration of 108 mg Bismuth (**colloidal bismuth subcitrate**) to human volunteers gave a maximum blood concentration of between 4.7 and 21 µg/l after 15 to 60 minutes. The intake of 216 mg Bismuth (colloidal bismuth subcitrate) gave C_{max} values ranging between 25 and 300 µg/l after 30 minutes.
As citrate increased intestinal absorption of bismuth from several compounds, formation and absorption of a bismuth citrate complex was suggested.

Ref. 71

SCCS comment
This summarized information was taken from a review in a handbook which is not available to the SCCS. However, an initial peak concentration (C_{max}) of bismuth in blood after the first administration during a medical therapy using bismuth pharmaceuticals was also observed in other studies. After that, bismuth concentrations in blood mostly declined resulting in lower levels (reviewed by Tillman *et al.* 1995, ref.85).

3.3.10 Photo-induced toxicity

3.3.10.1 Phototoxicity / photo-irritation and photosensitisation

Guideline:	OECD 432
Method:	3T3 Neutral red uptake phototoxicity test
Test system:	Balb/c 3T3 mouse fibroblast cell line (ATTC CCL-163)
Test product:	Bismuth citrate
Purity:	99%
Batch:	Not stated
Positive control:	Chlorpromazine (CPZ)
Irradiation:	5 J/cm2
Solvent	5 mg Bismuth Citrate added to 50 mL Hank's Balanced Salt Solution (HBSS) to yield a 100 µg/ml stock.
Concentrations:	3.16, 4.64, 6.81, 10.0, 14.7, 21.5, 31.6, 46.4 µg/ml
GLP:	No
Study period:	June-August 2005

Balb/c 3T3 cells were seeded in the central 60 wells of two 96-well plates and maintained in culture for 24 hours. The duplicate 96-well plates were then pre-incubated with eight different concentrations of bismuth citrate for one hour.
One plate was irradiated for fifty minutes at 1.7 mW/cm² to achieve a UVA dose of 5 J/cm² UVA (with <1% UVB), the remaining plate was kept in the dark (no UVA). After UV irradiation, the treatment medium was replaced with culture medium and, after 24 hours, cell viability was determined by neutral red uptake for 3 hours.

Results
The EC50 values and Photo-Irritant-Factor (PIF) for the test article and CPZ were as follows:

	Concentration range	EC_{50} No UVA	EC_{50} +UVA	PIF
Bismuth citrate	3.16 - 46.4 µg/ml	13.89	12.04	1.15
CPZ	+UVA: 0.0083-31 µg/ml No UVA: 0.027-100 %g/ml	27.62	0.924	29.98

Conclusion
Bismuth citrate was not considered phototoxic (positive results is defined as PIF>5).

Ref. 68

SCCS comment
Evidence on the stability of bismuth citrate in Hank's Balanced Salt Solution (HBSS) has not been provided.
The batch was not identified or characterized. The study was not performed under GLP conditions.

Local Lymph Node Assay (LLNA) photosensitisation study
The study was done according to the methodology previously described in 3.3.3.

Results
The SI values for the test article, bismuth citrate, + UVA at 10%, 25% and 50% were all below the threshold of 3.0 (i.e. 0.7, 0.8 and 1.5, respectively; and 1.3, 1.6 and 0.7, respectively, without UVA), indicating the test article is not a photo-sensitizer.

Conclusion
Topical application of the test article bismuth citrate, Lot #97/90137/000, at 10%, 25% and 50%, with UVA, resulted in a stimulation index less than 3 (SI < 3.0), and therefore this test article is not a dermal photo-sensitizer in the Photo-Local Lymph Node Assay.

Ref. 33

SCCS comment
See also section 3.3.3, Skin sensitization.

3.3.10.2 Photomutagenicity / photoclastogenicity

No data available.

3.3.11 Human data

Clinical safety and dermal absorption of bismuth citrate

Study 1
The clinical safety, tolerance, and systemic absorption of bismuth following application to hair and scalp of a commercial hair colouring preparation containing **0.5%** bismuth citrate was tested using 21 subjects in a double blind study. Ten subjects received placebo, and 11 the active preparation. Daily application of the test substance for 3 weeks was followed by twice weekly application for an additional nine weeks. A four week post-application wash out interval was used.

Results
The product was clinically safe and well tolerated. No changes in physical findings or clinical laboratory tests attributable to study participation were observed.
Serial bismuth serum and urine quantitative testing was done throughout the study. Blood and urine testing for bismuth at sensitivity levels of <0.7µg % and <0.005 ppm respectively was negative in all subjects throughout the study interval.

Under conditions of this study no systemic absorption of bismuth was detected either by assay of serial blood specimens or serial 24 hour urine collections. Similarly, no local adverse reaction was detected in any subject. Mild scalp pruritis in one subject which disappeared as the daily application of the active drug was continued is not considered significant.

It is concluded that the product as applied in this testing was safe, tolerated, and not associated with detectable systemic absorption of bismuth.

Ref. 72

SCCS comment
The terminology is not clear: "Sensitivity levels" probably means limits of detection or limits of quantification. The dimension "<0.7µg %" corresponds to <7 µg/L. No detailed data is available. The information is based only on the abstract supplied.
Normal background levels of Bi in human blood are in the range <1 - 15 µg/L.
The claimed concentration of Bismuth citrate is 0.5% and not 2% as applied for by the applicant. The complete study report is required for evaluation. Supporting documented

evidence on the concentration, purity and stability of bismuth citrate in the formulation is required for evaluation.

Study 2
A test was conducted with an experimental hair formulation (#1041) for determination of bismuth levels in both blood and urine under use conditions.
A control formulation (#1043) was used for comparison purposes. Ten human male subjects (8 test and 2 control) participated in the study.
The test material was applied according to product instructions in the laboratory 5 days per week and at home on the weekends during a 4-week test period. Quantities of test material applied were recorded. Blood and urine samples for bismuth analysis were taken 3 times during pre-test days, -15, -7 and 0; test days 7, 14, 21 and 28; and after 7 days recovery.

Results
The results of the statistical analyses of the urine bismuth data revealed numerous inter-period differences suggesting changes in bismuth levels during the test period. However, variations noted between pre-test days (-15 vs. -7 vs. 0) and the fact that pre-test day 0 and test day 28 show no significant difference, indicate that any contribution of the test material to urine bismuth levels during the test period is questionable. Also, data for the control subjects follow the same pattern as those of the test subjects.
There was no correlation between the amount of test material used and the levels of bismuth noted in the urine during the test period.

No trace of bismuth was found in the blood at any time during the pre-test, test or recovery periods.
No untoward dermal or systemic reactions were observed at any time during the study.

Ref. 73

SCCS comment
Study design, study conduct and methodology do not comply with modern standards. The control group was too small (2 persons). An increase of bismuth concentrations in urine during the application period of 4 weeks compared with pre-exposure and recovery data cannot be excluded. No data on the concentration of bismuth citrate in the formulation or daily applied is available.

Overall SCCS comment
The above studies cannot be evaluated due to major shortcomings in documentation or study conduct.

Sensitization (Grecian Bismuth Liquid Citrate 2%) April 25, 2005

Type of study	Repeated Insult Patch Test (RIPT). A modified predictive patch test that can detect weak sensitizers that require multiple applications to induce a cell-mediated (Type IV) immune response sufficient to cause an allergic reaction .
Subjects	Human volunteers (male and female)
Group size	101 completed study
Test material	#16B: SCP2717, RD4165 (Grecian Bismuth Liquid Citrate 2%)
Batch n°	#16B: SCP2717, RD4165
Purity	Q050048 (analysis result 2.037%)
Dose	0.2 g semi-occlusive (induction) and occlusive (challenge) patches Induction phase: 9 consecutive 48-hours applications Rest period: 2 weeks Challenge phase: 24-hour challenge period; observations after patch removal and at 48, 72, and 96 hours

A total of 101 subjects completed the test; 33 males and 68 females. The subjects range in age from 18 to 70.
Grecian Bismuth Liquid Citrate 2%
Batch: #16B: SCP2717, RD4165
Purity: Q050048 (analysis result 2.027%)

Results
During the induction phase, no reactions were observed. Following challenge, one subject exhibited a low-level, transient reaction.
Under the experimental conditions applied, the test product SCP2717, RD4165 exhibited no evidence of skin sensitization in humans.

Ref. 74

SCCS comment
In the study there is no information about the composition of the batch #16B: SCP2717, RD4165. There is no documented information in the study on bismuth citrate.
Information on purity: Q050048 (analysis result 2.027%) This information is not supplied in the study report but taken from tables in the applicant's dossier. Supporting documented evidence on the concentration, purity and stability of bismuth citrate in the formulation of this study is required.

Safety clinical studies related to prototype liquids and lotions containing 0.5% bismuth citrate

Study 1

Type of study	Repeated Insult Patch Test (RIPT). A modified predictive patch test that can detect weak sensitizers that require multiple applications to induce a cell-mediated (Type IV) immune response sufficient to cause an allergic reaction .
Subjects	Human volunteers (male and female)
Group size	50
Test material	MKT 121 (0.5% bismuth preparation)
Batch n°	Q050048 (analysis result 2.037%)
Dose	0.1 ml under occlusive and a semi-occlusive patch Induction phase: 10 consecutive 48-hours applications Rest period: 11 days Challenge phase: 48-hour challenge period; observations after patch removal and at 24 hours

Apart from MKT 121, three other test items, MKT 122, MKT 123 and MKT 124 were also tested.

Results
Under the experimental conditions applied, the test items (MKT 121, MKT 122, MKT 123 and MKT 124) exhibited no evidence of irritation or sensitization in human volunteers.

Ref. 75

SCCS comment
No information is available in the study report on MKT 121. The information that the test item MKT 121 contained a 0.5% bismuth preparation and the batch number is only mentioned in the applicant's dossier without any further supporting evidence. The claimed concentration is 0.5% and not 2% as applied for by the applicant.
Three other test items, MKT 122, MKT 123 and MKT 124 were tested (not further characterized in the study report) but not mentioned in the applicant's dossier.

Study 2

Type of study	Single-blind design, 3 weeks
Subjects	Human volunteers (male adults)
Group size	98
Test material	Bismuth citrate hair coloring preparations containing 0.5% bismuth citrate: R&D 1230#617, batch R8GO6D, liquid R&D 1237#898, batch R8H03D, cream R&D 1229#412, batch R8G05D, liquid
Purity	*not stated*
Dose	*not stated*

Bismuth citrate hair colouring preparations containing 0.5% bismuth citrate:
R&D 1230#617, batch R8GO6D, liquid
R&D 1237#898, batch R8H03D, cream
R&D 1229#412, batch R8G05D

Results
Under the experimental conditions applied, the three hair colouring preparations of bismuth citrate exhibited no evidence of adverse reactions in human volunteers except in one case where a reversible red rash (not observed at final examination 10 days after initial observation) and itching around the nape of the neck was observed.

Ref. 76

SCCS comment
No analytical information on bismuth citrate and its concentration in the study is available. The claimed concentration is 0.5% and not 2% as applied for by the applicant.

Study 3

Type of study	Semi-occlusive primary irritation patch test, 48 hours
Subjects	Human volunteers
Group size	111
Test material	Bismuth citrate hair coloring preparation MKT 394 (0.5%)
Amount applied	0.2 ml
Purity	*not stated*

Bismuth citrate hair colouring preparations MKT 394 and MKT 395 (both 0.5%).

Results
The strongest reaction observed (at 48 or 96 hour reading) was used to calculate the irritancy score (sum of all numerical equivalents divided by the total number of subjects completing the study x100. A score of>10-<50 is taken as minimal irritant not expected to be clinically significant under normal use conditions.
Under the experimental conditions applied, the hair colouring preparations of bismuth citrate (MKT 394 and MKT 395) both exhibited minimal irritation in humans following topical application to the skin at a concentration of 0.5%.

Ref. 77

SCCS comment
No analytical information on bismuth citrate and its concentration in the study report is available. The claimed concentration is 0.5% and not 2% as applied for by the applicant.
The type of study should be stopped after RIPT

General SCCS comment
HRIPT studies are generally considered unethical by the SCCS.

3.3.12 Special investigations

No data

3.3.13 Safety evaluation (including calculation of the MoS)

Not applicable

3.3.14 Discussion

A recent comprehensive review on all relevant aspects of bismuth has been provided by Fowler and Sexton (2011, ref. 140).

Chemical identity of bismuth citrate
Bismuth citrate should be distinguished from similar complex compounds of bismuth and citrate such as
- **Bismuth subcitrate**, a complex of citrate and bismuth at a molar ratio of 2:1 (ref. 1, ref. 11). In particular, this substance is sometimes mixed up with bismuth citrate in the open literature (e.g., ref. 62) and also in the dossier (p. 11) and some of the studies provided.
- **Colloidal bismuth citrate** or **colloidal bismuth subcitrate (CBS)**, a polyanionic structure of $[Bi(cit)_2Bi]n^{2n-}$ (ref. 1).

Physico-chemical properties

According to the submission, bismuth citrate will be incorporated in progressive hair dye formulations at a maximum concentration of 2.0%.

Identity, purity and other characteristics of the test item bismuth citrate as batches or bismuth citrate formulations were not described in most of the studies on acute and local toxicity. Some of the required information such as concentrations of bismuth citrate has been provided in the applicant's dossier but supporting material to the studies has not been provided.
In the formulations, information on the identity of bismuth citrate and/or transformation to other bismuth species is required. Depending on the study type, information on the pH or the vehicles of the formulations is needed.

It is usually unknown which bismuth species is/are formed when solved bismuth salts or complexes get into contact with biological tissues or fluids. This may depend on the individual bismuth compound considered. Also, equilibria between bismuth species formed and different competing thiol and hydroxyl groups in the biological system are unknown and may differ depending on the bismuth compound and specific conditions such as pH (see section Toxicokinetics and **Annex II**). It is therefore important to note that documented evidence on the identity, purity and stability of bismuth citrate (and not only bismuth) in test solutions and formulations is required for all tests where bismuth citrate gets into contact with biological tissues or fluids and may exert direct effects such as in tests on local toxicity but also in in vitro tests on mutagenicity. On the other hand, it is plausible that trivalent "bismuth" predominantly bound to thiol groups of amino acids, peptides and proteins is considered the toxic agent when systemically available after absorption although the bismuth species are unknown.

One study concerning purity and impurity(ies) of bismuth citrate was provided (see 3.1.4). The reported purity of bismuth citrate is 99%. Thus, about 1% impurity(ies) has not been identified. As progressive hair dyes work gradually and colour builds up over a period of two

to three weeks of daily application, chronic exposure to impurities of other toxic elements including contact allergens such as Ni, Co and Cr in bismuth citrate may be of concern. Analytical data on such impurities is required.

Conflicting data was reported on the solubility of bismuth citrate in aqueous media and organic solvents: Insoluble, sparingly or partly soluble in water, insoluble or sparingly soluble in organic solvents. Bismuth citrate is apparently soluble in alkaline citrate buffer and aqueous ammonia. However, stability of bismuth citrate was not demonstrated in such media. Dissolution of bismuth citrate in strong mineral acids destructs the substance. Conflicting data was reported for the solubility of Bismuth citrate in DMSO: up to 50% (ref. 33) and "very limited solubility" (ref. 43). Water solubility of bismuth citrate was not determined by EU method A.8, and Log Pow of bismuth citrate was not provided.

Homogeneity and stability of test solutions were not demonstrated. Stability of bismuth citrate in typical hair dye formulations was not demonstrated.

In conclusion, inadequate information has been provided by the applicant and in various studies on the solubility and/or stability of bismuth citrate. Therefore the following information is requested, which is considered indispensable for a sound evaluation of the safety studies provided:
- Documented information on Bismuth citrate identity and characterization (purity, amounts of impurities etc.) in all batches and lots mentioned in the applicant's dossier and submitted studies, i.e., certificates of analysis (CoA's) or comparable documents including a readable CoA of batch No 97/90137/000.
- Documented information on the vehicles and pH of the formulations containing Bismuth citrate.
- Solubility of Bismuth citrate in various solvents mentioned in the dossier and submitted studies such as water, DMSO, buffer solutions such as citrate buffer, pH 11, aqueous ammonia, media for in vitro cultures, etc. Determination of the water solubility of bismuth citrate by EU method A.8, and Log Pow is required.
- Stability of Bismuth citrate in various solutions at various pH values, as far as mentioned in the dossier or in the studies submitted (bismuth determination alone is considered not sufficient), in particular when acidic or alkaline conditions were used (e.g., aqueous ammonia).

Function and uses

Bismuth citrate will be incorporated in progressive hair dye formulations at a maximum concentration of 2.0%. In the USA up to 0.5% are permitted. Progressive hair dyes work gradually, with colour build-up over a period of two to three weeks of daily application. Thereafter, colour is maintained by up to 3 applications per week which means an intermittent use on the long-term.
Bismuth salts and complex compounds such as bismuth subcitrate and colloidal bismuth subcitrate (CBS) have been in extensive use worldwide to treat gastrointestinal diseases, including those related to infection of Helicobacter Pylori which causes gastric or duodenal ulcers (ref. 1). Among the many bismuth substances that have been used as (partly historical) pharmaceuticals, bismuth citrate was also mentioned by the applicant but evidence for the use of bismuth citrate has not been provided.

Toxicological Evaluation

Bismuth forms stable trivalent ions and is considered the least toxic element of the 5th group of the periodic system of the elements compared to arsenic and antimony. Due to the high affinity of the Bi^{3+} ion to sulfhydryl- or hydroxyl-containing ligands chemical complexes of bismuth with such ligands are formed. Bismuth citrate is just one example of such chemical complexes of bismuth. Human exposure to bismuth is generally due to medical use. Much less is known about the significance of environmental or occupational human

exposure to bismuth. Therefore, in terms of the risk assessment of bismuth citrate, the knowledge about toxicokinetics, long-term treatment and side effects of related bismuth compounds such as bismuth subcitrate and colloidal bismuth subcitrate (CBS) that are used as drugs in humans may be useful, as bismuth is considered the main toxic component of these substances (at least for their systemic toxicity). For further information on the systemic toxicity of bismuth and its compounds see **Annex I**.

A main feature of these substances as well as bismuth citrate is their low solubility in aqueous media and most organic solvents. Moreover, at low gastric pH and in presence of hydrochloric acid they form insoluble or sparingly soluble bismuth oxide, bismuth hydroxide or bismuth oxychloride (BiOCl) so that the absorption of bismuth from the human gastro-intestinal tract (GIT) and systemic exposure to bismuth is poor (< 1%; refs. 65, 84, 85, 91). Circumvention of the stomach by *in vivo* intestinal perfusion in rats considerably facilitated intestinal absorption of bismuth salts according to their water solubility. However, the bismuth species absorbed (or the mechanism of intestinal absorption) is unknown (see section 3.3.9.1, ref. 65, ref. 91 and **Annex II**). On the other hand, it has been shown that the presence of sulfhydryl compounds such as cysteine given orally to rats may enhance the absorption of bismuth compounds from the GIT and also bismuth toxicity (ref. 141). Therefore, in humans, varying concentrations of thiol groups containing peptides and proteins in the diet may partly explain that serum/blood levels of bismuth have been reported to vary considerably in unexposed healthy individuals and patients not under bismuth treatment, apart from other factors of inter-individual variability of oral absorption (1–9; 8-10; 15 ± 12 µg/L; ref. 85; ref. 91; ref. 128). Baldwin & Marshall (1999) (ref. 101) reported a very low reference value of 0.1 µg/L blood.

Likewise, bismuth concentrations in serum/blood of patients under treatment with bismuth compounds may considerably vary not only depending on the drug, dosage and duration of treatment: In both single- and multiple-dose studies, wide inter-individual variation has been reported in blood bismuth concentrations amongst patients treated with oral bismuth preparations so that in some patients no increase of bismuth in blood was observed whereas in others remarkable increases were found. As reported, toxic concentrations of several hundred µg/L may be reached. Nephropathies, encephalopathies, neurological disturbances and skeletal problems have been reported. In most cases reported, the symptoms were reversible after discontinuation of bismuth treatment (ref. 11; ref. 12). Regulatory action was taken by different countries in relation to bismuth-containing products for medicinal use between 1974 and 1985 (ref. 11). In pragmatic approaches, it has been suggested that concentrations of more than 50 - 100 µg/L bismuth in blood may be considered toxic (ref. 15; ref. 101) whereas up to 50 µg/L can be considered safe (ref. 12) or a warning range (ref. 101). However, these thresholds or ranges of toxicity/safety have not been adequately proven and have not been generally accepted (ref. 85).
The highest concentrations of bismuth orally absorbed in rats and humans were found in kidneys and liver and to a lesser extent in the spleen, brain, and bones (ref. 142). The main excretion route of absorbed bismuth is via urine.

Acute toxicity

Acute oral toxicity studies were performed in rats by gavage, with five prototype cosmetic formulations claimed to contain bismuth citrate at **0.5%** (whereas the concentration applied for is 2%). The limit doses of 5-40 ml/kg bw of the formulation were below the LD-50.
One acute dermal toxicity study with a cosmetic formulation claimed to contain bismuth citrate at 0.5% was conducted in rabbits at a limit dose of 2 g/kg bw. Body weights of the animals were found reduced and no deaths occurred.
As no information is available in the study reports or accompanying documents on the content of Bi citrate in the formulations, the acute oral toxicity of the studies cannot be evaluated.

Local toxicity

Skin irritation:
Five studies were performed between 1974 and 1981 with different formulations. The applicant informs that all formulations contained 0.5% of bismuth citrate, but no information of the item concentration was reported in either of the studies. However, the safety dossier submitted requests approval for a 2% bismuth citrate concentration in cosmetic formulations. Three studies were claimed to be performed under GLP conditions but neither followed an OECD guideline.
Slight erythema (with or without oedema) was observed with three of the five formulations suggesting that formulations with 0.5% bismuth citrate may be slight irritants.
Consequently, adequate *in vitro* skin irritation tests using formulations of 2% bismuth citrate are required.

Eye or mucous membrane irritation:
Five studies were performed from 1979 to 1981 using rabbits with different formulations. The applicant informs that all formulation contained 0.5% of bismuth citrate, but no information of the item concentration was reported in either of the studies. However, the safety dossier submitted requests approval for a 2% bismuth citrate concentration in cosmetic formulations. Two of the studies (using MKT 121 and MKT 138) presented slight eye irritation affecting conjunctiva.
Four studies were performed under GLP conditions according to FDA 1979 but neither followed an OECD guideline. Any information on Bismuth citrate as the a.i. is missing in the study report.
Adequate *in vitro* eye irritation tests using formulations of 2% bismuth citrate are required.

A HET-CAM test was conducted with a formulation claimed by the applicant to contain 2% bismuth citrate. Any information on Bismuth citrate as the a.i. is missing in the study report. The method is not according to a guideline and it is not validated. The criteria of identification of eye irritation are not those normally used.

Overall SCCS comments on both skin and eye irritation studies

No information on Bismuth citrate as the a.i. is available in the study reports. Documented information on the identity and characterization of the test substance is mandatory in such studies.

Due to these shortcomings, the conclusions from the studies on local irritation are not considered valid. However, the test formulations already at 0.5% showed slight skin and eye irritation potential.

Sensitization:
A LLNA test in mice was conducted. Topical application of the test article bismuth citrate, Lot #97/90137/000, at 10%, 25% and 50% in DMSO, with or without UVA, resulted in a stimulation index less than 3 (SI < 3.0). Therefore this test article was considered by the applicant neither a dermal sensitizer nor a dermal photo-sensitizer in the Photo-Local Lymph Node Assay.
The QA statement of the study is missing and hence the GLP compliance is questionable.
In this experiment, no appropriate positive control for sensitisation was used (Chlorpromazine was normally used as a positive control in photosensitisation only).
The high solubility of bismuth citrate in DMSO up to 50% is questionable as in the mutagenicity tests the solubility was characterized in another study as "very limited" (see ref. 43). The solubility of bismuth citrate in DMSO requires clarification before the test can be accepted as valid.

Dermal absorption

A dermal absorption study using dermatomed back skin from female pigs was conducted according to OECD guideline 428 but not under GLP. A formulation containing 2.1% bismuth citrate equivalent to 1.10% bismuth was applied to the skin at a concentration of about 2 mg/cm^2) formulation (corresponding to 105 µg/cm^2 or 55 µg/cm^2 bismuth). In the tested formulation (Grecian Bismuth Liquid Formulation), the amount of bismuth systemically available after percutaneous absorption was considered by the study authors to be 0.49 ± 0.08 µg/cm^2 or 0.83 ± 0.13 % of the applied dose. Due to an error in the study, the mean value of the percutanously absorbed dose should read 0.50 µg/cm^2 and this value will be used for further calculations. Few cells have been used and the number of donors is unknown, then the mean + 2 SD of bismuth (0.66 µg/cm^2) could be used to calculate the MoS.
Bismuth is considered the relevant systemic toxic agent as it is questionable/not probable that the test substance bismuth citrate has been completely absorbed and it is not clear what the systemic bismuth species absorbed constitute(s) (see discussion in Toxicokinetics below and in **Annex II**).

Repeated dose toxicity

Based on the results of a published developmental toxicity study in rats (ref. 60) , dose levels of 0, 100, 300, and 1000 mg/kg/day were selected for a range-finding 14-day study in rats. No adverse effects were observed at any dose on body weight or nutritional parameters, clinical observations, clinical pathology or gross pathology. As only the study plan and a summary table were available, the test substance was not adequately characterized and due to other shortcomings in reporting, the study description cannot be used for risk assessment.

A sub-chronic (90 days) toxicity study with application of 0, 30, 300 and 1000 mg/kg bw by gavage was conducted in rats. Reductions in body weight, weight gain, and food conversion were observed in male and female rats dosed with 1000 mg/kg bw/day, compared to controls. Some changes were observed in high-dose male and female organ weights but were considered to be related to reductions in body weight. Large caeca, filled with dark ingesta, were observed during gross necropsy of males and females treated at 300 or 1000 mg/kg/day, but no microscopic lesions were associated with these observations. Inflammation of the nasal turbinate and/or maxillary sinus was also observed in animals from these groups and was attributed to reflux of material (test substance and/or gastric fluid) from the oesophagus or stomach. These lesions were considered to be secondary to test substance effects on the gastrointestinal tract, and not direct effects on nasal tissue. Mild degeneration/necrosis of renal tubular epithelium was observed in one male and one female dosed with 1000 mg/kg bw/day, but was not associated with any effects on clinical chemistry.
The no observed adverse effect level (NOAEL) was considered to be 30 mg/kg bw/day in males and females, based on observations at 300 and 1000 mg/kg bw/day.
The stability of the test item in dosing formulations has not adequately been proven as only the bismuth content was determined.
As the bismuth species systemically available is/are unknown and systemic bismuth is considered the toxic agent, a NOAEL of 16 mg/kg bw/day for bismuth (corresponding to 30 mg Bismuth citrate per kg bw/day; see also pages 51-52) could be used for the calculation of the MoS.

Mutagenicity

Overall, the genotoxicity of bismuth citrate was tested in 3 *in vitro* genotoxicity tests covering 2 of the 3 endpoints of genotoxicity: gene mutations and chromosome aberrations. No mammalian cell gene mutation test with bismuth citrate has been provided. Exposure to bismuth citrate did not result in an increase in gene mutations in bacteria, nor in an

increase in structural and numerical chromosome aberrations in CHO cells nor in UDS in primary hepatocytes. Based on the conditions of these tests, bismuth citrate seems to have no genotoxic potential. However, one of the two Ames tests was old and differently conducted from the present standard protocol described in the OECD test guideline. Vital information on the test substance is missing. In the second Ames test, toxic concentrations were not reached. Evidence that the test substance (Bismuth citrate or another Bismuth species formed) is soluble at high concentration in the test system is missing. Although in the chromosome aberration test numerical chromosome aberrations were scored, aneuploidy was not optimally covered.

In all these tests, the identity and purity of the test substance has been determined based on the bismuth content alone (see CoA).The citrate moiety has not been determined.
Evidence that the test substance is stable in 10 mM sodium citrate, pH 11 has not been provided. Analysis of the test substance has been determined by a work-up procedure with conc. acids whereby the test substance is destroyed. The analytical method ICP-AES is capable of determining Bismuth as an element but not suited for the determination of the test item bismuth citrate and its stability. Therefore, the stability of the test item in 10 mM sodium citrate, pH 11 has not adequately been proven. Furthermore, at high concentrations in the Ames test medium, the identity and/or concentration of the test substance after addition to the test suspensions may have changed due to the drop of the pH from 11 to physiological pH which may have caused transformation to other Bismuth species and/or precipitation due to the very low solubility of Bismuth citrate or other Bismuth species at physiological pH. Under these test conditions, the identity of the test compound is not clear (bismuth citrate or other bismuth species).
The *in vitro* studies on mutagenicity cannot be evaluated unless the identity and purity of the test substance, and its stability in 10 mM sodium citrate, pH 11 and its solubility after addition to the test media has been proven.

Bismuth trioxide was positive in an *in vivo* chromosome aberration test in bone marrow cells of mice. However, the quality of the test (performance) did not match current standards.
Bismuth trioxide was negative in a sperm head abnormality test in mice.

In conclusion, the mutagenicity of bismuth citrate cannot be assessed due to many shortcomings of the studies.

Carcinogenicity

No carcinogenicity studies have been performed with bismuth citrate. Some bismuth compounds have been studied in long term studies with rat and mice. These studied with the exception of the study on bismuth oxychloride are old (from 1960 – 1969) and incompletely reported. None of the studies reported tumour induction by bismuth compounds.

Reproductive toxicity

Fertility
No data on two-generation reproduction toxicity is available. Other data on fertility toxicity in rats *in vivo* and *in vitro* suggest a potential of bismuth to accumulate in macrophages of the testes, thereby reducing testosterone production or release by Leydig cells by an unknown mechanism at very high internal concentrations which are achieved by high doses of bismuth applied via an un-physiological pathway circumventing normal defence barriers of the body (500 mg/kg bw by i.p. application.).

Developmental toxicity
A summary of a scientific review reporting a study conducted in rats and rabbits with different doses between 50 and 1200 mg/kg bw/day of bismuth citrate and showing only

maternal toxicity in rabbits (body weight reduction) but no other effects cannot be evaluated as the study report is not available.
A guideline study according to OECD 414 in rats with doses of 0, 100, 300, 1000 mg/kg bw/day applied on days 6 to 21 of gestation gave the following results: Maternal toxicity was observed at 300 and 1000 mg/kg bw/day based on reductions in maternal body weights and/or weight gain. Transient effects on food consumption were observed at 1000 mg/kg bw/day. There was no evidence of maternal toxicity at 100 mg/kg bw/day. No developmental toxicity was observed at any dose level. The SCCS notes that the stability of the test substance and its stability in the test formulations during study conduct have not adequately been proven, as only Bismuth and not Bismuth citrate was determined.

Toxicokinetics

Several toxicokinetic studies with bismuth compounds including bismuth citrate conducted predominantly in rats but also mice and rabbits are available. Likewise, many clinical studies investigating the fate of bismuth after intake of pharmaceutical bismuth preparations in humans have been published.
Radio-labelled $Bi^{205/206}$ compounds were used in animal studies. After oral or i.v. application of a bismuth citrate-like substance in rats, the concentrations of bismuth were highest in the kidney, followed by lung, liver, gut and spleen (ref. 62). A similar distribution was also observed in mice and rabbits (ref. 61; ref. 67).

In another study, the bioavailability of ^{205}bismuth from various labeled salts, most of them pharmaceutical commercial oral preparations used in peptic ulcer therapy, was studied in rats. Intestinal absorption was generally low, but significantly higher (0.26-0.33% of the dose) from oral bismuth citrates (basic bismuth citrate, water soluble bismuth citrate, colloidal bismuth subcitrate) compared to basic bismuth nitrate, salicylate, gallate, and bismuth aluminate (0.04-0.11% of dose). For all compounds, more than 99% of the label was excreted in the feces. The biological half-life and the tissue distribution of bismuth was studied following single administration of colloidal bismuth subcitrate (20 mg, 150 µCi) to rats by gastric intubation. Twenty days after oral application 0.008-0.017% of the radioactivity, i.e. 2-5% of the dose absorbed was still retained in the body. Bismuth was mainly retained in the kidney, followed by bone, red blood cells and the lung.
The ^{205}Bi activity in blood was mainly attributed to the red cell fraction, whereas the serum concentration was almost negligible. This might be of importance in view of the fact that the safety of bismuth therapies has been often monitored by measuring the plasma concentration. Biological ^{205}bismuth half-lives of 10, 36 and 295 h were found in the rat. According to this study, liver is apparently not a major organ of Bi retention in rats 20 days after oral uptake, in contrast to humans (ref. 63).

Intestinal absorption of five bismuth compounds of different water solubility including bismuth citrate was investigated in rats by use of an *in vivo* perfusion of rat small intestine in combination with systemic blood sampling. The objective of the study was to clarify whether the absorption of bismuth behaves differently compared with oral intake and passage via the stomach. The authors could demonstrate that intestinal absorption of bismuth is dependent on the water solubility of the bismuth compounds and on their concentrations in the perfusion/blood system. Even lethal concentrations in blood could be achieved by intestinal absorption (ref. 65, ref. 91). This is in contrast to normal oral absorption of these bismuth compounds whereby uniform absorption rates of < 1% are observed as demonstrated in the study above (ref. 63) and also in general in humans (ref. 85). It is assumed that bismuth compounds are transformed in the stomach to less soluble bismuth species such as bismuth oxide, bismuth hydroxide and bismuth oxichloride under the acidic gastric conditions and in presence of hydrochloric acid so that the stomach acts as a barrier for the gastrointestinal absorption of bismuth compounds. For a detailed discussion of the study results and their implications for oral absorption of bismuth compounds in rats and humans see **Annex 2.**

Toxicokinetics of bismuth citrate and related compounds in humans

There are only few studies reported on the toxicokinetics of bismuth citrate in humans and even in these cases the identity of bismuth citrate as test item is not always certain. A plenty of toxicokinetic studies in humans, published clinical safety studies and case reports exist which were performed or reported when using bismuth substances of pharmacological interest. As already mentioned in the introduction part of the discussion, most bismuth compounds are poorly absorbed from the GIT with a bioavailability of less than 1%. However, a broad inter-individual variability of oral absorption has been reported probably due to variable circumstances and conditions of human life and human individuals compared to animal experiments (ref. 65, ref. 85, ref. 91). Multi-compartimental elimination of bismuth and half-lives of about two days up to 2 years have been reported (ref. 70), the latter indicating a bioaccumulation potential of bismuth. For more details see **Annex I**.

Human data

Two studies on dermal absorption under use conditions on the scalp were conducted showing partly variations of Bismuth concentrations in urine but within the normal background levels of Bismuth in human blood and urine. The claimed concentration of Bismuth citrate is 0.5% and not 2% as applied for by the applicant. Moreover, the studies are old and do not comply with modern requirements. The studies cannot be used for risk assessment due to major shortcomings in documentation or study conduct.

Several HRIPTs were reported, one with a formulation containing 2% Bismuth citrate and several others that contained 0.5% Bismuth citrate. No evidence of adverse reactions in human volunteers was observed except in one case where a reversible red rash and itching around the nape of the neck was noted. Two hair colouring preparations of bismuth citrate exhibited minimal irritation in humans following topical application to the skin at a concentration of 0.5% under semi-occlusive conditions for 48 hours. In all these studies, documented evidence on the identity and concentrations of the test substance is missing.

There is a large amount of clinical case reports on pharmaceutical Bismuth preparations containing colloidal bismuth citrate, tripotassium bismuth dicitrate or other bismuth salts. Mostly encephalopathies or nephrotoxicity including acute renal failure was reported after acute or chronic overdosing. These reports are considered not relevant in the context of this opinion, as very high blood concentrations of bismuth (>100 µg/L) are required to exert such effects.

Final considerations on the safety of bismuth citrate in hair dye products

The toxicological knowledge about bismuth and its compounds mainly stems from the development and use of pharmaceutical bismuth preparations for the treatment of a broad spectrum of gastro-intestinal tract diseases. Drugs based on bismuth also include bismuth citrate complexes such as bismuth subcitrate and colloidal bismuth subcitrate that are similar to bismuth citrate. Low oral bioavailability of < 1% in rats and humans has been reported for these and other bismuth substances. Moreover, a warning range of up to 50 µg/L blood for bismuth has been established whereas a concentration of 100 µg/L blood and higher is considered a range that may lead to toxicity in humans.
For the treatment of most gastro-intestinal tract diseases, daily doses of several hundred mg bismuth are used. When taking into account an oral bioavailability of 1%, the daily internal dose of bismuth amounts to several mg. This oral dose absorbed is higher than the dermally absorbed dose of 0.38 mg bismuth from the use of bismuth citrate as progressive hair dye.

4. CONCLUSION

From the data provided by the applicant, SCCS cannot assess the safety of bismuth citrate. Following information is required to evaluate the safety of bismuth citrate.

A complete and adequate physico-chemical characterisation of Bismuth citrate is needed.

Skin and eye irritation studies are required at the concentration of 2% as applied for by the applicant.

A local lymph node assay (LLNA) in mice was claimed to be negative when using 10 to 50% bismuth citrate dissolved in DMSO. Clarification regarding conflicting information on the solubility of Bismuth citrate in DMSO is required before the test can be accepted as valid.

The mutagenicity of bismuth citrate can presently not be assessed given the studies provided. A complete set of *in vitro* studies according to the current Notes of Guidance is required.

5. MINORITY OPINION

None.

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155. See ref. 154, pp 103-116.

Annex 1

General aspects of bismuth exposure and toxicity

Human exposure by food
The dietary intake of bismuth in the general population is estimated to be 5-20 µg/day. The majority of ingested bismuth is not absorbed, but excreted directly in faeces (ref. 85).

Occupational exposure to bismuth occurs in the manufacture of alloys, catalysts, and ceramics, polymers for bone implants, cosmetics, semiconductors and X-ray contrast media, in dental prosthetic devices or in medical devices (scanners, implants, sutures, catheters...) (ref. 5-9). In cosmetics, exposure occurs to bismuth citrate, triphenyl bismuth or bismuth oxychloride which is used as a pearlescent pigment or lubricating agent in formulations of lipstick, nail polish, eye shadows and facial powders. Bismuth is included in hair dyes to add color and to deodorize (ref. 4).

General information on the safety of bismuth salts
Bismuth is considered the safest of heavy metals primarily because of the low solubility of many bismuth salts: It exists in trivalent and pentavalent oxidation states (the trivalent being more abundant and stable) and forms soluble and insoluble, organic and inorganic salts.
In general, insoluble salts such as bismuth citrate and subcarbonate are of low toxicity; neurotoxic effects are associated predominantly with the lipid soluble organic salts eg bismuth subgallate; renal toxicity is associated with the water soluble organic compounds eg bismuth sodium triglycollamate, bismuth subsalicylate, and bismuth sodium tartrate (ref 10).

Serfontein et al. (1979) (ref. 12) subdivided the pharmacologically active bismuth compounds into four groups depending on structure, stability and solubility:
Group 1: Simple inorganic salts and sub-salts of bismuth eg. bismuth subcitrate, bismuth subcarbonate and bismuth subnitrate. These compounds are water- and lipid- insoluble in the absence of complexing agents, with minimal bismuth absorption and virtually no toxicity.**Group 2:** Predominantly lipid soluble organic compounds and complexes of bismuth eg bismuth subgallate. They are absorbed across the intestinal wall leading to high bismuth blood levels and can be neurotoxic.
Group 3: Predominantly water soluble organic compounds and complexes of bismuth, usually used in injectable formulations, and which are stable enough to be absorbed – leading to high bismuth blood levels eg bismuth triglycollamate, bismuth potassium tartrate. Nephrotoxicity is associated with these compounds in man.
Group 4: Water soluble organic complexes of bismuth which decompose (hydrolyze) in the gastro-intestinal system with the ultimate production of simple, insoluble bismuth compounds, eg., bismuth subchlorides and bismuth sulfides. Minimal absorption and low toxicity are characteristic features.

Reports of adverse effects from industrial or cosmetic use are rare, but toxicity from medicinal use of bismuth salts is not uncommon following accidental or deliberate overdosage (ref. 11).
Bismuth citrate is not mentioned in ref. 11 and ref. 12.

Several forms of toxicity have been described in man:

- parenteral administration of various bismuth containing compounds has resulted in an "epithelial-cutaneous" form of toxicity
- oral administration of bismuth subgallate and bismuth subnitrate has led to neurotoxicity in some patients
- severe toxicity was reported when soluble bismuth salts were used in the treatment of syphilis and other parasitic diseases, pre 1950

- an epidemic of over 1000 cases of bismuth myoclonic encephalopathy was reported in the 1980s, with chronic high-dose use of principally bismuth subnitrate (by reduction of nitrate anion into nitrite responsible for methemoglobinemia), orally in daily dosages between 5 and 25g, for 4 weeks to 30 years. (ref. 13). Recovery was observed following discontinuation of treatment.

The main hazard of bismuth is therefore related to high dose, chronic exposure. All organs can be affected: in the skin, a lichen planus-like rash; in the mouth, stomatitis with a blue black gum line; inflammation and inclusion bodies in the liver; renal failure with degeneration and necrosis of the epithelium of the renal proximal tubules; in the brain, encephalitis. Renal toxicity is evident early in acute poisoning because bismuth is excreted mainly by this route, with only 10% appearing in the feces after a parenteral dose. There are no reports of toxicity of bismuth or bismuth salts by the inhalation route.

It has been suggested that the mechanism of action of bismuth drugs includes enzyme inhibition. Bismuth toxicity like other heavy metals is attributable to the existence of trivalent and pentavalent ions, the trivalent ion being the most abundant and stable form, with sulfhydryl groups. Since sulfhydryl groups are components of many vital enzymes, particularly important for brain metabolism, the effect of bismuth is to denature and destroy the function of these enzymes. The inhibition of cytostolic alcohol dehydrogenase (ADH) by bismuth drugs has been shown to suppress the production of acetaldehyde, which is toxic to mucosal cells (ref. 14)**,** although the inhibition mechanism remains unknown (ref. 1)**.**

Specific diagnosis of overdose based on blood levels is difficult because of the presence of extremely low blood levels in normal individuals as well as those overdosed. The normal blood level has been estimated by some authors to be between 4 and 40 µg/L while overdosed subjects are within the range of 50 to 2850 µg/L. Much more reliable is the urine level which averages 20 µg/L in normal individuals and ≥ 400 µg/L in overdosed individuals (ref. 15). Withdrawal of treatment is recommended at plasma levels of ≥100 µg/L for patients receiving bismuth-containing antacids but this has been considered over-cautious by others (ref. 16).

The complete reversibility of bismuth toxicity is evident in man after treatment ceases, as reported by many authors irrespective of the type of bismuth compound concerned (ref. 12, ref. 17). Several studies conducted *in vitro* and *in vivo* showed that dimercaprol, meso-2,3-dimercaptosuccinic acid, and D,L,2,3-dimercapto-propane-l-sulfonic acid were the chelators of choice for treating bismuth poisoning. Other authors affirmed that the use of chelating agents such as dimercaprol may increase elimination of bismuth from the body, but considered it of unproven clinical benefit (ref. 85).

Acute systemic toxicity

Cationic bismuth salts

Intravenous administration of cationic bismuth salts to rats (no other details) was associated with rapid death from colloidoclastic shock and asphyxial convulsions. Toxicity was influenced by the rate at which soluble salts were injected intravenously.
Intravenously injected bismuth salts caused renal injury, albuminuria, diarrhea, and ulcerative stomatitis.

Ref. 78

Summary of acute toxicity data of various Bismuth compounds

Compound	**Species**	**Route**	**Toxicity**	Dosage/kg body weight		
				Compound		Metal
				mg	**mg**	**mM**
Sodium bismuthate $NaBiO_3$	Rat	Oral	LD_{100}	720	552	2.64
	Rat	IM	LD_{100}	250	192	0.92
	Rat	IV	LD_{100}	25	19.2	0.09
	Rabbit	Oral	LD_{100}	510	391	1.87
	Rabbit	IM	LD_{100}	110	84.3	0.40
	Rabbit	IV	LD_{100}	9	6.9	0.033
Potassium bismuth tartrate $KBiO(C_4H_40_6)$	Rabbit	IV	LD_{100}	9.8	5.0	0.024
	Rabbit	IM	LD_{100}	295	150	0.72
Sodium bismuth citrate $NaBi(C_6H_30_7)$	Rabbit	IV	LD_{100}	10.6	5.0	0.024
	Dog	IV	LD_{100}	2.25	1.0	0.0048
Sodium bismuth thioglycollate	Rabbit	IV	LD_{100}	7.36	2.8	0.011
Sobisminol (Na bismuthate complex)	Rabbit	IV	LD_{100}	52.5	10.5	0.050
Bismuth oxychloride Bi0C1	Rat	Oral	LD_{50}	22 000	-	-
Trimethylbismuth $BiMe_3$	Rabbit	Oral	LD_{50}	484	-	-
	Rabbit	SC	LD_{50}	182	-	-
	Rabbit	IV	LD_{50}	11	-	-

Ref. 78, Ref. 79

Bismuth subnitrate

Group size	Not stated
Species/Strain	Rat/CD
Sex	Male
Dietary levels as bismuth	20, 40 or 80 mg/kg
Route	Subcutaneous
Batch n°	Not stated
Treatment period	Single dose

Ultrastructural and biochemical studies were conducted to evaluate the effects of bismuth on organelle structure and heme biosynthetic parameters in rat liver and kidney cells. Bismuth subnitrate ($BiONO_3$) was administered subcutaneously to male rats at 0, 20, 40, or 80 mg/kg doses 16 hr prior to euthanasia.
Electron microscopy revealed swollen mitochondria and distortion of mitochondrial inner membranes in liver and renal proximal tubule cells at 40 and 80 mg/kg. A direct inhibition of specific heme pathway enzymes was also described *in vitro.* These results were consistent with previous data related to the toxicity of other metals with known toxicologic potential. Ref: 80

Repeated dose toxicity of bismuth and bismuth compounds

Bismuth

Oral (gavage) administration of bismuth to male and female rats at 0, 40, 200 or 1000 mg/kg/day for 28 days elicited no significant adverse effects. There were no effects on organ weights (including testis weight) but histopathology was not conducted. Exposure levels were not identified.

No adverse effects were observed in preliminary studies after single doses of up to 2000 mg/kg or after 14 days at 0, 100, 500 or 1000 mg/kg/day.

Ref. 81

Intertracheal administration of bismuth once weekly for 13 weeks at dose levels of 0.8, 4 or 20 mg/kg resulted in pulmonary lesions but no other treatment related lesions. One or two animals died in each group including controls but death was attributed to the administration procedures. There were no significant effects on food intake, body weight gain, clinical observations, hematology, blood chemistry or urinalysis. Exposure levels were not identified. No effects were observed on organ weights, including testis weight. Histopathology was conducted on lung, liver, kidney, spleen, brain and testis in control and high dose animals. Bismuth inhalation was considered to cause dose dependent but nonspecific adverse effects (foreign body inflammation of lungs and physical changes related to pulmonary lesions). Deaths were reported after single intertracheal doses of 100 and 500 mg/kg in a preliminary study. In this study the half-life of bismuth elimination from the lungs was 4.47, 3.25 and 2.10 days for doses of 20, 100 or 500 mg/kg respectively.

Ref. 82

Bismuth trioxide

Group size	4
Species/Strain	Single Corn White Leghorn hens
Dietary levels as bismuth	1, 10, 100, and 1000 ppm
Batch n°	Not stated
Treatment period	8 weeks

Groups of four, individually caged Single Corn White Leghorn hens, 22 weeks of age, were fed a practical corn-soy laying mash supplemented with 4 increasing levels of bismuth trioxide for 8 weeks. Egg production was recorded daily, individual feed intake weekly, body weight bi-weekly, and each bird was artificially inseminated twice weekly. The supplemental dietary levels as bismuth were: 1, 10, 100, and 1000 ppm. Under the experimental conditions adopted, no significant differences in either food intake, egg production or body weight change were noted.

Ref. 83

Bismuth subgallate

Group size	2
Species/Strain	Beagle dog
Dose level	1 g/day
Batch n°	Not stated
Treatment period	8 and 13 days

Oral feeding of bismuth subgallate at 1 g per day to two beagle dogs, for 8 and 13 days respectively, failed to produce toxic concentrations of bismuth. Some absorption was observed in one dog as bismuth blood level increased from 1.08 to 2.66 µg/dl. A more pronounced increase from 1.19 to 15.0 µg/dl was observed in the second dog when given 50mg IM BAL, a systemic chelating agent, for 6 days. These results indicated that BAL was implicated in the elimination of bismuth from the body and in gastrointestinal absorption.

Ref: No reference given in the dossier

Chronic toxicity (> 12 months)

Review of the literature related to long term use of bismuth compounds confirms that in the majority of cases, chronic bismuth intoxication produces alimentary symptoms: the mouth shows bismuth deposition in epithelial cells, turning mucosa blue-gray. Diarrhea, vomiting, anorexia, and digestive pains can be caused by bismuth salts due to embolism of mesenteric capillaries. These symptoms may be preceded by severe headache and may be followed by various skin lesions, nephrotoxicity, and blood dyscrasias.
Chronically ingested cationic salts of bismuth are not toxic, and are, in fact, used therapeutically to treat ulcers by coating the intestinal mucosa. However, bismuth oxynitrate can be reduced to nitrite in the gastrointestinal tract by intestinal microflora, and subsequent absorption of nitrite can cause methaemoglobinemia in children.

Ref. 78

Toxicokinetics of bismuth compounds in humans

Most of the following text has been taken from a review (ref. 85) primarily focused on pharmaceutical bismuth compounds or preparations but also considering general aspects of exposure and ADME of bismuth in humans.

The dietary intake of bismuth in the general population is estimated to be 5-20 µg/day. The majority of ingested bismuth is not absorbed, but excreted directly in faeces. Less than 1% of the dose administered is absorbed following oral dosing with bismuth subsalicylate, tripotassium dicitrato bismuthate (= bismuth subcitrate) or ranitidine bismuth subcitrate (ref. 85).

Bismuth has been found to bind to transferrin in serum in a manner that confers a lower affinity than iron for this protein which may explain the relatively low efficiency of bismuth delivery to cells after administration of bismuth-containing pharmaceuticals. The intracellular binding of bismuth in the kidneys, a major storage organ of bismuth, has been studied with respect to low molecular-weight bismuth-binding proteins that seemed to have some properties distinct from metallothionein. More recent studies by several authors have shown bismuth to be an effective inducer of metallothionein and to bind to this protein (ref. 140).

The permeability to the placenta of bismuth was demonstrated in an old study from 1928 after i.m. injection of potassium bismuth tartrate and sodium potassium tartro bismuthate into pregnant rabbits and cats (ref. 140).

Renal excretion is the primary route of bismuth elimination, although biliary excretion may also be important. The site of bismuth absorption in man has not been determined. Animal studies suggest that absorption takes place in the small bowel, although the rapid appearance of bismuth in blood after oral intake suggests that bismuth can be absorbed from the stomach. Although often below the quantification limit of 1 µg/L, pre-treatment whole blood bismuth concentrations of 1 - 9 µg/L have been reported in some subjects. During continued treatment bismuth is sequestrated in body tissues; studies in animals and man suggest that the prime sites are the kidneys and bone. Bismuth in the blood remains preferentially in the plasma, with an average plasma to blood concentration ratio of 1,55 (ref. 85).[2] .

Factors influencing bismuth absorption include the following:

[2] In rats, however, after oral application of colloidal bismuth subcitrate, the ^{205}Bi activity in blood was mainly attributed to the red cell fraction, whereas the serum concentration was almost negligible (ref. 63).

(i) *Dietary factors:* For example, ingestion of thiol-containing compounds, present in flour, meat and cheese, may enhance absorption of bismuth.
(ii) *Intragastric pH:* Inhibition of gastric acid secretion increases the absorption of bismuth from tripotassium dicitrato bismuthate tablets, but not from bismuth subsalicylate liquid or bismuth subnitrate tablets. It has been suggested that this occurs because bismuth from tripotassium dicitrato bismuthate precipitates at low pH but remains in colloidal suspension at about pH 6.0.
(iii) *Physico-chemical characteristics:* individual bismuth preparations behave differently in the body according to their physico-chemical properties, For example, tripotassium dicitrato bismuthate is highly soluble in water, whilst the aqueous solubility of bismuth subsalicylate and bismuth subnitrate is low.
(iv) *Pharmaceutical formulation:* Two small, comparative studies have reported greater absorption of bismuth from tripotassium dicitrato bismuthate swallow tablets than from the liquid formulation.
(v) *Effect of food:* bismuth absorption is greater from a morning dose of tripotassium dicitrato bismuthate. Administered after an overnight fast, than from an evening dose, administered to fed subjects. This may be due to an effect of food on bismuth absorption (ref. 85).

Bismuth excretion was noted within 8 h of a single dose of tripotassium dicitrato bismuthate. During continued dosing, bismuth excretion varies widely between individuals. When continuous treatment with ranitidine bismuth citrate (highest dose: 800 mg b.d.) is stopped, excretion in the urine continues for up to 3 months: plasma bismuth concentrations in 1688 patients treated for up to 8 weeks had returned to pretreatment levels 12 weeks after stopping treatment. Urinary excretion of bismuth may continue for over 3 months after 6 weeks of treatment with tripotassium dicitrato bismuthate 240 mg b.d. (ref. 85).

Overdosage and treatment of bismuth overdosage (ref. 85)

During the 1970s prolonged use of very high oral doses of (water insoluble) bismuth subnitrate or bismuth subgallate (considered more lipophilic than inorganic Bi salts) was widespread in some countries for treatment of gastrointestinal disorders. Ingestion of excessive amounts of these medicines was associated with neurotoxicity.
In France, for example, many patients took high doses of **bismuth subnitrate** for gastrointestinal disorders, particularly constipation. By 1979 nearly 1000 cases of bismuth-associated encephalopathy had been reported, and 72 of these were fatal. In Australia, health authorities withdrew all oral preparations of **bismuth subgallate** from the market after development of neurotoxicity was reported in 29 patients with colostomies or ileostomies, who had been taking high doses (3-20 g/day) of bismuth subgallate for prolonged periods, to improve stool consistency and reduce odour.
The pathogenesis of bismuth-associated neurotoxicity is unknown, although it has been suggested that conversion of bismuth into soluble neurotoxic compounds by intestinal flora may be involved. This theory is supported by the observation that the patients involved in both the French and Australian epidemics cited above were likely to have had intestinal bacterial overgrowth. However, the precise aetiology of bismuth-associated neurotoxicity remains unclear. In most cases, the condition is fully reversible upon withdrawal of bismuth therapy. Haemodialysis, diuresis and use of chelating agents such as dimercaprol may increase elimination of bismuth from the body, but are of unproven clinical benefit.

Blood bismuth concentrations are generally abnormally high in patients suffering from bismuth-associated encephalopathy. Comparison of published whole blood bismuth concentrations in 63 encephalopathy patients with those in 41 symptom-free patients showed that the mean whole blood bismuth concentration in the encephalopathy patient group was considerably higher than that in the control group. In the affected patient group, the median whole blood bismuth concentration was 690 µg/L, and concentrations exceeded 100 µg/L in all cases. As a result, Hillemand et al. (1977, ref. 15) proposed that whole blood

bismuth concentrations above 100 µg/L be regarded as 'toxic', and bismuth treatment discontinued. Although widely cited, this threshold is an empirical one based on samples obtained from a modest number of cases reported in the literature, and has been criticized to be misleading because of various methodological reasons (selection of patient groups, time points of sample taking, different analytical techniques).

Bismuth intoxication has also been associated with cases of osteoporosis and nephrotoxicity, occurring predominantly amongst patients treated parenterally with bismuth preparations for conditions such as syphilis.

Blood bismuth concentrations in patients receiving recommended doses of bismuth preparations (ref. 85)

Peak plasma bismuth concentrations exceeding 50 µg/L are observed within 1 h of dosing with tripotassium dicitrato bismuthate in some subjects, but fall below 50 µg/L within a few hours. These concentration peaks become less pronounced during repeated dosing, and are not associated with adverse effects. In contrast to treatment with tripotassium dicitrato bismuthate, the plasma bismuth concentrations observed shortly after dosing with preparations such as bismuth subsalicylate, bismuth subgallate, bismuth subnitrate and ranitidine bismuth citrate are generally below 15 µg/L. For example, a comparative study of 48 patients treated for 10 days with either tripotassium dicitrato bismuthate 240 mg b.d. or ranitidine bismuth citrate 1000 mg b.d. showed that, after adjusting for differences in bismuth dose, peak plasma bismuth concentrations were significantly lower in patients treated with ranitidine bismuth citrate than those receiving tripotassium dicitrato bismuthate. The mean peak plasma bismuth concentration was 12 µg/L (range 4-42 µg/L) after treatment with ranitidine bismuth citrate 1000 mg b.d, (equivalent to 301 mg bismuth per dose), compared with 21 µg/L (range 7-247 µg/L) amongst patients treated with tripotassium dicitrato bismuthate 240 mg b.d, (equivalent to 215,5 mg bismuth per dose). During repeated dosing with oral bismuth preparations, trough plasma bismuth concentrations remain below 50 µ/L. In the few studies in which bismuth concentrations were reported for a control group who were not taking bismuth-containing medication, there was considerable overlap in the blood bismuth concentration ranges reported for the two groups.
There are two studies which determined blood bismuth concentrations in patients receiving half-dose maintenance treatment with tripotassium dicitrato bismuthate 120 mg nocte. In one study, after 6 months, median whole blood bismuth concentrations of 20 µg/L (n = 13) were reported while in the second study (n = 34) whole blood concentrations of 15 µg/L or less after 12 months treatment were reported.

Annex 2

Absorption of Bismuth Compounds from small intestine of rats (Ref. 65)

Discussion part

"Some dissolved Bi could be retrieved from suspensions with BSN, BSS, BCit, and $BiCl_3$ (Table 1), although these compounds are said to be nearly insoluble in water (ref. 143). No attempt has been made to solubilize Bi or to control perfusate variables such as acidity because even with a limited number of chemical components in the perfusates, the concentrations of all solutes are constantly changing and no constant factors are present (ref. 144). Only osmolality was controlled to limit interference of water and salt transport with the absorption of Bi. The chemical behavior of Bi compounds in water and in the human gastrointestinal tract, however, is completely unexplored territory. Consequently, the importance of the variation in acidity between the perfusates cannot be assessed. Furthermore, the Bi compound absorbed is usually not the compound that was ingested. For example, passage through the stomach will affect the chemical form of the metal. Most of the Bi from CBS and BSS will precipitate in the stomach as insoluble compounds (e.g., BiOCl) (ref. 145, ref. 146), leaving an unknown, but possibly very small fraction available for absorption.

Instillation of the perfusates directly into the small intestine consequently may have favored absorption from BSS and CBS. The effect of gastric passage on BSN is not known. Even with the optimized conditions of this experiment, the absorption for CBS and BiCl3 in the small intestine was estimated to be very low (0.2-0.6%). This value corresponds to the bioavailability described for CBS in humans (0.2%, ref. 147) that was calculated from urinary elimination. Measurement of urinary bismuth underestimates the absorption because some of the metal will be stored at least temporarily in body compartments.

The relevance of our approach is supported by the similarities between the absorption in the present study and the absorption of Bi in humans. Single-dose experiments with CBS, BSN, and BSS in humans demonstrated a very low absorption of Bi after oral intake of BSN and BSS, whereas CBS had absorption peaks of short duration (ref.145, 148-151). The bioavailability of ^{205}Bi from various pharmaceutical Bi preparations was studied in rats by whole body retention and accumulated urinary excretion methods. The bioavailability for the citrate-containing compounds (CBS, soluble Bi-citrate, and basic Bi-citrate) was significantly higher (0.26-0.33% of the dose) compared with basic Bi nitrate, basic Bi salicylate, basic Bi gallate, and Bi aluminate (0.04-0.11% of the dose) (ref. 63).

In the present experiment, we also found that the absorption from CBS and BCit differed from that from BSN, BiC13, and BSS. Administration of ^{205}Bi-labeled compounds to human volunteers demonstrated that the bioavailability was even lower than in rats: 0.043% +/- 0.008% for CBS, 0.039 +/- 0.001% for basic BSG, and significantly less (<0.005%) for basic Bi nitrate, basic Bi subsalicylate, or Bi aluminate (ref. 152).

Absorption of Bi from BSN in rats has been shown to be increased by sulfhydryl group-containing compounds, such as 3-mercaptopropionic acid and cysteine, and by complexing agents, such as diethyldithio-carbamate (DEDTC) and ethylenediaminetetraacetic acid (EDTA) (ref. 141, 153). Citrate was also shown to increase the intestinal absorption of Bi from several Bi compounds in rats (ref. 154). In humans, addition of citrate to a single oral dose of BSN resulted in absorption profiles for Bi in blood and citrate in serum that were parallel, suggesting the formation and absorption of a Bi-citrate complex (ref. 155). Retrospectively, therefore, the choice of a citrate buffer to make the $BiC1_3$ perfusates more physiologic was unfortunate because we probably studied the absorption of a Bi-citrate complex and not of $BiC1_3$.

The relationship between BiPo and Bas0 is nonlinear for both CBS and $BiCl_3$ in citrate buffer. In both types of perfusate, the formation of an absorbable Bi species may be the key to the absorption. The availability of such a Bi species, most probably a citrate complex, is

dependent on chemical equilibria between large complexes or colloidal particles and a simple, absorbable Bi-citrate molecule. The mechanism of absorption involved could be passive transport, but also active, or facilitated transport across the intestinal mucosa. Further experiments are necessary to evaluate the character of the absorption process and the Bi species involved."