Scientific Committee on Consumer Safety

SCCS

OPINION ON

Tetrabromophenol Blue, 4,4'-(4,5,6,7-tetrabromo-1,1-dioxido-3H-2,1-benzoxathiol-3-yliden)bis-2,6-dibromophenol (C183)

The SCCS adopted this Opinion

on 07 March 2017

About the Scientific Committees

Two 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 that may pose an actual or potential threat.

These Committees are the Scientific Committee on Consumer Safety (SCCS) and the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) and are made up of scientists appointed in their personal capacity.

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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All Declarations of Working Group members are available on the following webpage:
http://ec.europa.eu/health/scientific_committees/experts/declarations/sccs_en.htm

This Opinion has been subject to a commenting period of 8 weeks (from 14 April to 9 June 2016) after its initial publication. There was one comment received from the Applicant to enable the SCCS to make the final version of the Opinion. In that communication, the Applicant provided further clarifications on the chemical composition of the substance and on its specification to be used in commercial batches. Therefore the main changes refer to Section 3.1.4 (purity and composition).

Keywords: SCCS, scientific opinion, Tetrabromophenol Blue, 4,4'-(4,5,6,7-tetrabromo-1,1-dioxido-3H-2,1-benzoxathiol-3-yliden)bis-2,6-dibromophenol (C183), Regulation 1223/2009, CAS 4430-25-5

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1. BACKGROUND

The hair dye Tetrabromophenol Blue (C183), with the chemical name 4,4'-(4,5,6,7-tetrabromo-1,1-dioxido-3H-2,1-benzoxathiol-3-yliden)bis-2,6-dibromophenol (CAS 4430-25-5) is intended to be used as a direct dye in oxidative and non-oxidative hair colouring products with a final on-head concentration up to 0.2%.

Submission I and II on hair dye Tetrabromophenol Blue (C183) were transmitted by COLIPA[1] in September 2003 and July 2005 respectively.

The latest safety evaluation on hair dye Tetrabromophenol Blue (C183) was adopted by the Scientific Committee on Consumer Safety (SCCS) in June 2012 with the following conclusions:

"Based on the data provided, the SCCS is of the opinion that the use of Tetrabromophenol Blue with a maximum on-head concentration of 0.2% in non-oxidative hair dye formulations does pose a risk to the health of the consumer due to the low Margin of Safety.
Tetrabromophenol Blue is a mixture of Octa-, Hepta- and Hexa-bromo phenolsulfonphthaleins, and does not contain any Tetrabromo-homologue, therefore the INCI name is misleading. The criteria for meeting the specifications of other batches, similar to the present mixture should be defined.
No acceptable dermal absorption study under oxidative conditions was provided.
An assessment of the use of Tetrabromophenol Blue in oxidative hair dye formulations cannot be performed without an adequate dermal absorption study and stability data in an oxidative environment." (SCCS/1426/11)

In July 2013, Cosmetics Europe (former COLIPA) submitted additional data to address the issues raised by the SCCS in the Opinion of June 2012.

2. TERMS OF REFERENCE

1. In light of the new data provided, does the SCCS consider Tetrabromophenol Blue (C183) safe when used as a direct dye in oxidative and non-oxidative hair colouring products with a final on-head concentration up to 0.2%?

2. Does the SCCS have any further scientific concerns with regard to the use of Tetrabromophenol Blue (C183) in other cosmetic products?

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association

3. OPINION

3.1 Chemical and Physical Specifications

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

Tetrabromophenol Blue

3.1.1.2 Chemical names

This hair dye is a mixture of Octa-, Hepta- and Hexa-bromo phenolsulfonphthaleins (see section 3.1.4. below). The chemical name below corresponds to the Octabromo-derivative only, while the chemical structure of the other homologues is not provided.

- Phenol, 4,4'-(4,5,6,7-tetrabromo-1,1-dioxido-3H-2,1-benzoxathiol-3-ylidene)bis[2,6-dibromo- (CA Index name, 9CI)

Other Names:
- Phenol, 4,4'-(4,5,6,7-tetrabromo-3H-2,1-benzoxathiol-3-ylidene)bis[2,6-dibromo-,S,S-dioxide;Tetrabromophenol blue (CA Index name, 6CI)
- 3',3'',5',5''-Tetrabromophenol-4,5,6,7-tetrabromosulfonephthalein (TSCAINV - EPA Chem. Sub. Inventory)

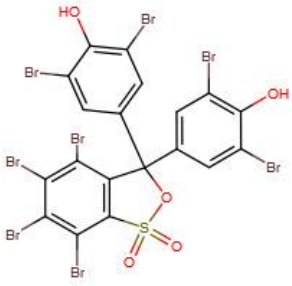
3.1.1.3 Trade names and abbreviations

Gardex Royal Blue (Wella)
Royal Blue (Wella)

3.1.1.4 CAS / EC number

CAS: 4430-25-5
EC: /

3.1.1.5 Structural formula



3.1.1.6 Empirical formula

Formula: $C_{19}H_6Br_8O_5S$

3.1.2 Physical form

Yellowish grey powder

3.1.3 Molecular weight

Molecular weight: 985.55 g/mol

3.1.4 Purity, composition and substance codes

Chemical characterisation was performed using NMR, IR, LC-MS, and UV-Vis spectroscopy.
This hair dye is a mixture of Octa-, Hepta- and Hexa-bromo phenolsulfonphthaleins. The relative composition (HPLC-peak area method at 210nm, 254nm and 615nm) is provided for the batch TBFB3/02/30.

(Batch TBFB3/02/30)	**210 nm**	**254 nm**	**615 nm**
Octabromo-homologue (corrected values)*	37.9 % (38.2 %)*	45.2 % (45.1 %)*	47.3 % (47.6 %)*
Heptabromo-major homologue Heptabromo-minor homologue	38.7% 7.1%	34.8% 6.8%	40.0% 4.6%
Hexabromo-homologue	12.9%	10.7%	6.8%
Sum of Octa-, Hepta- and Hexabromo (corrected values)*	**96.6 %** **(96.7 %)***	**97.2 %** **(97.5 %)***	**98.7 %** **(98.8 %)***
Number of UV-absorbing impurities Content of UV-absorbing impurities (% HPLC peak area)	13** 3.4	8 2.8	7 1.3

* Corrected values are reported, but without any information about the correction method.
** According to the Applicant: "*The 13 impurities detected in the HPLC at the wavelength of 210 nm consist of the three major impurities, all of lower brominated derivatives of Tetrabromophenol Blue. Two of the major impurities are Heptabromo derivatives with 38.7 and 7.1 area %. The third major impurity is a Hexabromo derivative of Tetrabromophenol Blue with 12.9% area. The other three to nine impurities are all below 1.1 area%. The Tox testing was performed with this batch and therefore covers also this quality of Tetrabromophenol Blue*".

It should be noted that all the above values are percentages relative to the total amount of only the UV-absorbing organic components. The absolute content of the test substance could not be determined using ^{1}H-NMR spectroscopy owing to signal interferences in consequence of all homologues. By using a quantitative HPLC-method with external calibration, the absolute Tetrabromophenol Blue content (i.e. the Octabromo-homologue content) yields 42.2 %, and the total content of all homologues (including Tetrabromophenol Blue) was found to be 96.6 % (for the batch TBFB3/02/30). Thus,

The content of the batch TBFB3/02/30 (as sum of Octa-, Hepta-, and Hexabromo-phenolsulfonephthaleins): 96.6%
Loss on drying: 0.9%
Water content: 0.8%
Sulfated ash: 1.1%

Another HPLC-DAD analysis of the same batch (TBFB 3/02/30) found the following peaks (no details regarding the identity of the peaks are provided):

Retention time (min)	Relative peak areas
3.98	39.0%; 34.4%; 40.8% Mean: 38.1%
5.38	37.18%; 44.0%; 47.1% Mean: 42.8%
7.32	13.2%; 10.9%; 6.6% Mean: 10.2%
11.28	6.6%; 7.3%; 4.1% Mean: 6.0%

Analysis of two other batches shows the following peaks:

Batch (MM-0573520001)

Retention time (min)	Relative peak areas
5.21	64.1%; 65.4%; 78.2% Mean: 69.3%
10.66	34.3%; 33.4%; 21.4% Mean: 29.7%

Batch (MM-0573520001/14)

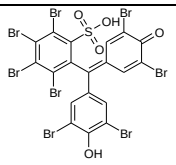
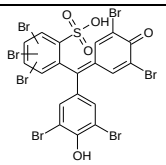
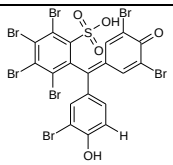
Retention time (min)	Relative peak areas
5.21	73.4%; 74.8%; 84.9% Mean: 77.7%
10.66	25.0%; 24.4%; 14.8% Mean: 21.4%

SCCS comment

Details of the analytical procedure used for material characterisation have not been provided. Files containing HPLC-DAD profiles of different batches have been provided without any explanation of the identity of the observed peaks. Despite this, different batches show a large variation in regard to the test material composition, and the concentration of Tetrabromophenol and other homologues in each batch appears to be different. As such, it is not clear whether

any of the batches would meet the same mixture specifications as the one used in the toxicity testing described in this Opinion. The Applicant should therefore provide exact specifications of the material they intend to use in hair dye formulations in regard to the composition of Tetrabromophenol Blue and other homologues. The Applicant should process the additional data provided, explaining the identity of the observed peaks and mentioning the purity calculations for these batches.

In reply to the above SCCS concerns, the Applicant provided new data in which the composition of 2 batches, produced using an optimised manufacturing process and representative of current market quality (MM-0573520001/2-1 and MM-0573520001/14), was compared with the composition of the previously analysed batch that was used in the toxicity test described in this Opinion (TBFB2/02/30):

Purity at 254nm	Octabromo homologue	Heptabromo homologue (A)	Heptabromo homologue (B)	Hexabromo homologue	Sum of homologues
					
Initial specs 2003	40 – 50%	n.d	n.d.	n.d.	95-100%
Revised Specs. 2012	>50%	0%		0%	95-100%
Revised specs.2016	**59 – 90%**	**0%**	**10 – 39%**	**0%**	**.> 98.5%**
HLPC retention time #	5.2min	3.9min	10.6min	7.3min	
TBFB2/02/30	44.0%	34.4%	7.3%	10.8%	
MM-0573520001/2-1	65.4%		33.4%		98.8
MM-0573520001/14	74.8%		24.4%		99.2

n.d.: not defined

The Applicant concludes that better control of the reaction conditions in large-scale production results in a higher purity of the Octabromo- and Heptabromo-homologue B. The Heptabromo-homologue A and Hexabromo-homologue could be removed. The improved manufacturing process has led to an increased overall purity of > 98.5%.

Ref.: 2a-d

SCCS comment on the new submitted data

SCCS notes that in the two commercial batches MM-0573520001/2-1 and MM-0573520001/14, the percentage of the main Octabromo-homologue has increased from 44% to 65-74%, the Heptabromo-homologue B form increased from 7.3% to 24.4-33.4% compared to the batch that was used for toxicological testing (TBFB2/02/30), whilst the Heptabromo-homologue A and Hexabromo-homologues were removed. This indicates that the batches intended for commercial use have more than 40% difference in mixture specification compared to the batch

that was used for the toxicological testing and considered in this Opinion. In addition, from the provided chromatograms (Ref.2a-c), it appears that an impurity is still present in the batches MM-0573520001/2-1 and MM-0573520001/14 that elutes at 12.05 minutes and in the batch TBFB2/02/30 at 11.86 minutes. Upon SCCS request for further clarification on the chemical characterisation of these impurities, the Applicant clarified that the impurity eluting at 12.05 min in batch MM-0573520001/2-1 (representative of the market quality) is also present in batch TBFB2/02/30 used for the toxicological testing. This impurity was characterised as the Hexabromo-homologue of C183 (Ref.3a). The impurity at 11.86 min present in batch TBFB2/02/30 is not identical with the impurity eluting at 12.05 min, and is no longer present in the current market quality batches of C183 (Ref.3a).
SCCS notes that contrary to the Applicant's declaration that the Hexabromo-homologue was removed in commercial batches, it still appears to be present as an impurity (between 0.6 and 0.8 %).

3.1.5 Impurities / accompanying contaminants

Potential impurities:
9 UV-absorbing materials of unknown identity have been reported: 3.4 % (HPLC peak area)

Heavy metals content:

Bromide:	< 5 %
Iodide:	< 0.1 %
Lead:	< 20 ppm
Mercury:	< 1 ppm
Arsenic:	< 3 ppm
Iron:	< 100 ppm

Solvent Residues: No solvents such as methanol, ethanol, isopropanol, n-propanol, acetone, ethyl acetate, cyclohexane, methyl ethyl ketone and monochlorobenzene were detected.

3.1.6 Solubility

In water: 0.159 g/L at 20°C and pH 3.54 by EC Method A.6
In acetone / water 1:1 (pH 2.6): 0.9 weight %
In DMSO: > 10 weight %

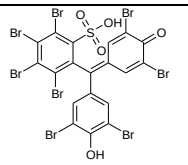
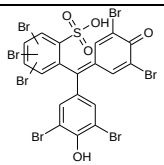
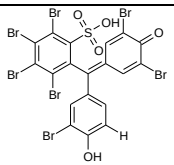
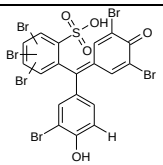
New data provided by the Applicant (Ref.3c) indicates that the water solubility of the individual Octabromo- and Heptabromo homologues of batches of current market quality at a physiological pH (6 to 7) is in the range between >25.2 (%w/v) and >30.2 (%w/v) and is comparable to the water solubility of > 28.2 (%w/v) determined for batch TBFB2/02/30 used for the toxicological testing.

3.1.7 Partition coefficient (Log P_{ow})

Log Pow: 3.71 (pH 4.0, room temperature) by EC Method A.8

Log Pow: 5.98 ± 0.20 (calculated for pure Tetrabromophenol Blue-most acidic)

The Applicant provided the following new data on the logP values for the different homologues of Tetrabromophenol Blue (Ref.2d):

	Octabromo homologue	Heptabromo homologue (A)	Heptabromo homologue (B)	Hexabromo homologue
				
Physical property ALogP*	8.7658	7.974	7.974	7.1822

*calculated by Biovia Draw 4.2

3.1.8 Additional physical and chemical specifications

Melting point: 203°C (decomposition)
Boiling point: /
Flash point: /
Vapour pressure: /
Density: 1.857 g/ml (20°C)
Viscosity: /
pKa: /
Refractive index: /
UV_Vis spectrum (200-800 nm): λmax at 224nm, 299 nm and 610 nm

3.1.9 Homogeneity and stability

The dyestuff dissolved in acetone (2%, w/v), DMSO (2%, w/v) and phosphate buffer pH 7.5 (1%, w/v) was found to be stable after keeping the solutions for 7 days at room temperature, protected from light (recoveries >98% for all homologues).

Long-term stability of the dyestuff in a common market formulation (90% recovery) is reported on the basis of a single determination of the dye content after storage for 10 months at 25°C and comparison with the "theoretical content".

The stability in the presence of hydrogen peroxide and persulfate was provided in additional data. In these tests, stability was monitored over 45 minutes at ambient temperature using HPLC/DAD in a 1:2 mixture of the cream formulation and Welloxon Perfect 12%. The recovery of Tetrabromophenol Blue was 101% (t=15min), 96% (t=30min) and 93% (t=45min). The data indicated that the material is stable (>90%) over a period of 45 minutes in the presence of hydrogen peroxide and persulfate. According to the Applicant, this demonstrates sufficient stability of the hair dye under use conditions.

SCCS comment
The applicant should explain the drift in retention time of Royal blue 1 from 6.62 min in the calibration standard 3 to 7.56 min in the samples solution after 45 min of degradation.

General comments on physicochemical characterisation
Submission I and II

- The test material is not composed of a single substance, but of different homologues. Analysis of different batches shows a large variation in homologue mixture composition of the test material intended for commercial use versus the batch that was used for toxicity testing and considered in this Opinion.
- For the batch used for the toxicity tests, the information provided on the compound is incomplete concerning the chemical identity of the 9 organic impurities identifiable by HPLC which may comprise up to 3.4% of the test material. SCCS notes that because of an optimized manufacturing process the impurity present at 11.86 min is no longer present in the current market quality batches of C183. With respect to the batches intended to be used in hair dye formulations, the information provided shows the presence of the Hexabromo-homologue (between 0.6 and 0.8%).
- The analytical data provided by the Applicant suggests that the substance is sufficiently stable (>90%) during storage, and also under oxidative conditions during use.

3.2 Function and uses

C183 is used in oxidative as well as in non-oxidative hair dye formulations at a maximum concentration of 0.2% on the scalp.

3.3 Toxicological evaluation

3.3.1 Acute toxicity

3.3.1.1 Acute oral toxicity

No data submitted

3.3.1.2 Acute dermal toxicity

No data submitted

3.3.1.3 Acute inhalation toxicity

No data submitted

3.3.1.4 Acute intraperitoneal toxicity

No data submitted

3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

Taken from SCCNFP/0797/04, SCCS/1426/11

Guideline:	OECD 404 (1992)
Species/strain:	Albino Rabbit, New Zealand White, (SPF-Quality)
Group size:	3 (same sex/male)
Test item:	Tetrabromophenol Blue
Batch:	TBFB3/02/30
Purity:	96.7 – 98.8%
Dose:	0.5 g
GLP:	in compliance

Three rabbits were exposed to 0.5 g of the test item (moistened with 0.25 ml water), applied onto clipped skin (150 square centimetres) for 4 to 5 hours using a semi-occlusive dressing. Observations were made 1, 24, 48 and 72 hours after application.

Results

No skin irritation was caused by 4 or 5 hours exposure to the test item. After 1 hour, no scoring of erythema and/or oedema was possible in two animals due to (light) blue staining of the test substance.
(Light) blue staining of the treated skin by the test item was observed throughout the observation period. Dry remnants of the test item were noted on the skin of one animal up to 48 hours after removal of the bandage.

Conclusion

Based on these results the test item is not a skin irritant.

Ref.: 13

3.3.2.2 Mucous membrane irritation / Eye irritation

Taken from SCCNFP/0797/04, SCCS/1426/11

<u>Study 1, neat substance</u>

Guideline:	OECD 405 (1998)
Species/strain:	Albino Rabbit, New Zealand White, (SPF-Quality)
Size:	3 males
Test item:	Tetrabromophenol Blue
Batch:	TBFB3/02/30
Purity:	96.7 – 98.8%
Dose:	67 mg of powdery test item (a volume of approximately 0.1 ml)
GLP:	in compliance

Single samples of approximately 67 mg of the test item (a volume of approximately 0.1 ml) were instilled into one eye of each of three rabbits. The eyes of each animal were examined 1, 24, 48 and 72 hours after instillation of the test sample.

Results

Instillation of the test item resulted in effects on the cornea, iris and conjunctivae. Corneal injury was seen as opacity (maximum grade 4) and epithelial damage (maximum 50 % of the corneal area). Iridial irritation (grade 1) was observed in all animals from the 24- or 48-hour observation period onwards. Irritation of the conjunctivae was seen as redness, chemosis and discharge.

Grey/white discolouration of the eyelids (sign of necrosis) and reduced elasticity of the eyelids were observed in all animals after 48 and 72 hours. Based on the severity of the corneal injury, the study was terminated after the 72-hour observation.

Blue staining of (peri) ocular tissues and of the fur on the head and paws by the test item was noted during the observation period. This staining prevented scoring of corneal injury, iridial irritation and conjunctival redness after 1 hour, and scoring of the lower eyelid, nictitating membrane and sclera after 24 hours among all animals. Scoring of iridial irritation was hampered by corneal damage (opacity) in two animals at 48 and 72 hours after instillation. Also, remnants of the test item were present in the eyes of all animals at 1 and 24 hours after instillation.

Conclusion

Based on the degree and persistence of the corneal injury, it was concluded that ocular corrosion had occurred by instillation of the pure test item into the rabbit eye in all three animals. The test item (pure substance) poses a risk of serious damage to eyes.

Ref.: 14

<u>Study 2, diluted substance</u>

Guideline:	OECD 405 (1998)
Species/strain:	Albino Rabbit, New Zealand White, (SPF-Quality)
Group size:	3 male animals
Test item:	Tetrabromophenol Blue
Batch:	TBFB3/02/30
Purity:	96.7 – 98.8%
Dose:	0.1 ml of 2 w/w% solution in phosphate buffer
GLP:	in compliance

Single samples of 0.1 ml of a 2 w/w% solution of the test item in phosphate buffer were instilled into one eye of each of three rabbits. Observations were made 1, 24, 48 and 72 hours after instillation.

Results

Instillation of the test substance resulted in irritation of the conjunctivae, which was seen as redness and/or discharge. The irritation had completely resolved within 24 hours in all animals.

No iridial irritation or corneal opacity was observed. Treatment of the eyes with 2% fluorescein, 24 hours after test substance instillation revealed no corneal epithelial damage in any of the animals.

Blue staining of the fur on the head and paws, caused by the test substance, was noted during the observation period.

Conclusion

Tetrabromophenol Blue in a dilution of 2% is not irritant for the eyes.

Ref.: 15

3.3.3 Skin sensitisation

Taken from SCCNFP/0797/04, SCCS/1426/11

Local Lymph Node Assay (LLNA)

Guideline:	OECD 429 (2000)
Species/strain:	Mouse: CBA/J
Groups size:	5 females per concentration
Test item:	Tetrabromophenol Blue
Batch:	TBFB3/02/30
Purity:	96.7 – 98.8%
Dose:	0, 0.2, 0.5, 1.5 and 2% (w/v) in DMSO
GLP:	in compliance

Tetrabromophenol Blue was tested in different concentrations (0, 0.2, 0.5, 1.5, 2.0% (w/v)) in DMSO (vehicle). On days 0, 1 and 2 the animals received 25µl of the test item formulation, positive control or vehicle on the dorsal surface of each pinna. Each concentration was tested on one animal group, which consisted of 5 animals.
Morbidity/mortality checks were generally performed twice daily. Clinical examinations were performed daily. Individual body weights were recorded on days -1 and 5. All animals were sacrificed on day 5. The cell proliferation was assessed by measuring the 3H-methyl thymidine incorporation in the cell suspension prepared from the lymph node of each animal.

Results
No mortality was observed during the study. There were no treatment-related clinical signs. There were no treatment-related effects on body weight or body weight gains. The positive control (p-phenylenediamine) induced a positive response, as it elicited at least a 3-fold increase in isotope incorporation relative to the vehicle. The mean stimulation index was 3.9 at the concentration of 1%.
The test substance induced a negative response, as it did not elicit at least a 3-fold increase in isotope incorporation relative to the vehicle. The mean stimulation indices were 0.6, 0.8, 1.0 and 1.1 at the concentrations of 0.2 %, 0.5%, 1.5% and 2%, respectively.

Conclusion
Based on these results, the test substance is not a skin sensitiser under the defined experimental conditions.

Ref.: 16

3.3.4 Dermal / percutaneous absorption

***In vitro* percutaneous absorption under non-oxidative conditions**

Guideline:	OECD TG428 (2004)
Test system:	frozen dermatomed human skin (380 - 400 µm)
Membrane integrity:	tritiated water method
Replicates:	12 replicates (5 donors)
Method:	flow-through diffusion cells
Test substance:	Tetrabromophenol Blue
Batch:	TBFB3/02/30 SAID (non-radiolabelled), CFQ40843 (radiolabelled)
Purity:	96.32% (non-radiolabelled), 99.4% (radiolabelled)

Test item:	0.2 % (w/w) [^{14}C]-Tetrabromophenol Blue in a typical hair dye formulation under non-oxidative conditions (test preparation 1)
Dose applied:	20 mg/cm^2 of the test item (approx. 40 µg Tetrabromophenol Blue/cm^2)
Exposed area:	0.64 cm^2
Exposure period:	30 minutes
Sampling period:	72 hours
Receptor fluid:	Minimum Essential Medium Eagle with 6.00% (w/v) polyethylene 20-oleyl ether, 1% (w/v)glucose, 0.01% (w/v) sodium azide, penicillin-streptomycin solution (100 units/mL and 0.1 mg/mL, respectively)
Solubility in receptor fluid:	33.71 mg/l
Mass balance analysis:	provided
Tape stripping:	yes (20)
Method of Analysis:	liquid scintillation counting
GLP:	in compliance
Study period:	2 December 2011 - 3 April 2012

Human abdominal and breast skin samples were obtained from five different donors. The skin was dermatomed (380 - 400 µm) and then the split-thickness membranes were stored frozen, at approximately -20° C, wrapped in aluminium foil until use. Dermatomed skin membranes (12 skin membranes from 5 donors) were thawed and checked for integrity by the tritiated water method prior to use. Only skin samples within the acceptable range of <0.6% were used. Skin samples were mounted into flow-through diffusion cells (exposed surface area: 0.64 cm^2). The receptor fluid was pumped through the receptor chambers at 1.5 ± 0.15 ml/h. The samples were maintained at a constant temperature (32 ± 1 °C). Radiolabelled Tetrabromophenol Blue was incorporated into a typical hair dye formulation at approximately 0.2% (w/w). The dose was applied under occlusive conditions for a period of 30 minutes at a nominal rate of 20 mg/cm. Absorption of Tetrabromophenol Blue was evaluated by collecting receptor fluid in 30 min fractions from 0 to 1h post dose, then in hourly fractions from 1 to 6h post dose and then in 2-hourly fractions from 6 to 72h post dose. At 30 min post dose, the parafilm occluding the chambers was removed and retained for analysis. The skin was washed with water, sodium dodecyl sulphate (SDS) solution (2% w/v) and then with water again. The skin was dried with tissue paper swabs. At 72h post dose, the skin surface was washed and dried in the same manner as described for the 30 min wash. The underside of the skin was rinsed with receptor fluid. The skin was then removed from the flow-through cells and dried. Skin under the cell flange (unexposed skin) was cut from the exposed area using scissors and forceps. The skin was divided into exposed and unexposed skin. The *stratum corneum* was removed by tape stripping. The exposed epidermis was then heat-separated from the dermis. Skin compartments were extracted separately. The radioactivity was quantified by liquid scintillation counting.
The stability of the test item over the exposure period was assessed. The concentration of radiodiluted [^{14}C]-Tetrabromophenol Blue remained above 100% over the course of the exposure period.

Results

The total recovery was within the range of 100 ± 10% of the applied dose for all skin samples and therefore confirmed the validity of the test. The majority of the applied dose of Tetrabromophenol Blue was rinsed off from the skin surface at 30 min post application, representing 65.77%. At 72h, 9.54 ± 3.07 µg/cm^2 (22.43 ± 7.22%) of Tetrabromophenol Blue was recovered from the *stratum corneum*. This amount was not considered bioavailable. From the dermis 0.02 ± 0.02 µg/cm^2 (0.06 ± 0.05%) and from the epidermis 1.62 ± 1.96 µg/cm^2

(3.82 ± 4.57%) were recovered. A maximum amount of 0.03 ± 0.01 µg/cm^2 (0.07 ± 0.02%) Tetrabromophenol Blue passed through the skin and was recovered in the receptor fluid during 72h exposure. The results are summarised in the Table below:

Amount of Tetrabromophenol Blue in:	0.2% (w/v) Tetrabromophenol Blue in typical non-oxidative hair dye formulation	
	µg equiv./cm^2 (n=12)	% of applied dose (n=12)
30 min Dislodgeable dose*	27.96 ± 1.56	65.77 ± 3.67
Total Dislodgeable Dose**	30.55 ± 1.72	71.86 ± 4.04
Unabsorbed Dose	40.09 ± 2.37	94.30 ± 5.58
Epidermis	1.62 ± 1.94	3.82 ± 4.57
Dermis	0.02 ± 0.02	0.06 ± 0.05
Stratum corneum	9.54 ± 3.07	22.43 ± 7.22
Absorbed Dose	0.03 ± 0.01	0.07 ± 0.02
Dermal Delivery	1.68 ± 1.96	3.94 ± 4.61
Mass Balance	41.77 ± 1.05	98.25 ± 2.47

* sum of: skin wash, tissue swab, pipette tips and parafilm after 30 min of exposure
** sum of: skin wash, tissue swab, pipette tips, donor chamber wash after 72h incubation
Epidermis = epidermis + cling film+ epidermis inadvertently removed during tape stripping

Conclusion

All samples recovered from the receptor fluid were below the limit of reliable measurement (3.07 ng/cm^2) and most samples recovered after 66h were below the limit of detection (0.1 ng/cm^2). Therefore, a depot effect from the epidermis can be excluded. Under the described test conditions, a total amount of 0.05 ± 0.02 µg/cm^2 Tetrabromophenol Blue is obtained by summing up the amounts present in the receptor fluid and in the dermis. Consequently, this amount is considered as bioavailable.

Ref.: 1c

SCCS comment

As no movement of the dye from the skin reservoir to the receptor fluid occurred after 72 h, SCCS is willing to accept that the amount in the epidermis may be excluded as dermally absorbed.

In vitro percutaneous absorption under oxidative conditions

Guideline:	OECD TG428 (2004)
Test system:	frozen dermatomed human skin (380 - 400 µm)
Membrane integrity:	tritiated water method
Replicates:	12 replicates (5 donors)
Method:	flow-through diffusion cells
Test substance:	Tetrabromophenol Blue
Batch:	TBFB3/02/30 SAID (non-radiolabelled), CFQ40843 (radiolabelled)
Purity:	96.32% (non-radiolabelled), 99.4% (radiolabelled)
Test item:	0.2 % (w/w) [^{14}C]-Tetrabromophenol Blue in a typical oxidative hair dye formulation (test preparation 2)
Dose applied:	20 mg/cm^2 of the test item (approx. 40 µg Tetrabromophenol Blue/cm^2)
Exposed area:	0.64 cm^2
Exposure period:	30 minutes
Sampling period:	72 hours
Receptor fluid:	Minimum Essential Medium Eagle with 6.00% (w/v) polyethylene 20-oleyl ether, 1% (w/v)glucose, 0.01% (w/v) sodium azide, penicillin-streptomycin solution (100 units/mL and 0.1 mg/mL, respectively)
Solubility in receptor fluid:	33.71 mg/l
Mass balance analysis:	provided
Tape stripping:	yes (20)
Method of Analysis:	liquid scintillation counting
GLP:	in compliance
Study period:	2 December 2011 - 3 April 2012

Human abdominal and breast skin samples were obtained from five different donors. The skin was dermatomed (380- 400 µm) and then the split-thickness membranes stored frozen, at approximately -20° C, wrapped in aluminium foil until use. Dermatomed skin membranes (12 skin membranes from 5 donors) were thawed and checked for integrity by the tritiated water method prior to use. Only skin samples within the acceptable range of <0.6% were used. Skin samples were mounted into flow-through diffusion cells (exposed surface area: 0.64 cm^2). The receptor fluid was pumped through the receptor chambers at 1.5 ± 0.15 ml/h. The samples were maintained at a constant temperature (32 ± 1 °C). Radiolabelled Tetrabromophenol Blue was incorporated into a typical hair dye formulation at approximately 0.2% (w/w). The dose was applied for a period of 30 minutes at a nominal rate of 20 mg/cm. Absorption of Tetrabromophenol Blue was evaluated by collecting receptor fluid in 30 min fractions from 0 to 1h post dose, then in hourly fractions from 1 to 6h post dose and then in 2-hourly fractions from 6 to 72h post dose. At 30 min post dose, the skin was washed with water, sodium dodecyl sulphate (SDS) solution (2% w/v) and then with water again. The skin was dried with tissue paper swabs. At 72h post dose, the skin surface was washed and dried in the same manner as described for the 30 min wash. The underside of the skin was rinsed with receptor fluid. The skin was then removed from the flow-through cells and dried. Skin under the cell flange (unexposed skin) was cut from the exposed area using scissors and forceps. The skin was divided into exposed and unexposed skin. The *stratum corneum* was removed by tape stripping. The exposed epidermis was then heat-separated from the dermis. Skin compartments were extracted separately. The radioactivity was quantified by liquid scintillation counting.

The stability of the test item over the exposure period was assessed. The concentration of radiodiluted [^{14}C]-Tetrabromophenol Blue remained above 100% over the course of the exposure period.

Results

The total recovery was within the range of 100 ± 10% of the applied dose for all skin samples and therefore confirmed the validity of the test. The majority of the applied dose of Tetrabromophenol Blue was rinsed off from the skin surface at 30 min post application, representing 95.31%. The results are summarised in the Table below:

Amount of Tetrabromophenol Blue in:	0.2% (w/v) Tetrabromophenol Blue in a typical oxidative hair dye formulation	
	µg equiv./cm^2 (n=12)	% of applied dose (n=12)
30 min Dislodgeable dose*	44.89 ± 1.35	95.31 ± 2.77
Total Dislodgeable Dose**	45.13 ± 1.33	95.82 ± 2.82
Unabsorbed Dose	45.48 ± 1.29	96.56 ± 2.74
Epidermis	0.05 ± 0.06	0.10 ± 0.13
Dermis	< 0.00 ± 0.00	0.01 ± 0.00
Stratum corneum	0.34 ± 0.18	0.73 ± 0.38
Absorbed Dose	0.02 ± 0.02	0.05 ± 0.04
Dermal Delivery	0.07 ± 0.06	0.16 ± 0.12
Mass Balance	45.55 ± 1.29	96.72 ± 2.73

* sum of: skin wash, tissue swab and pipette tips after 30 min of exposure
** sum of: skin wash, tissue swab, pipette tips, donor chamber wash after 72h incubation
Epidermis = epidermis + cling film+ epidermis inadvertently removed during tape stripping

Conclusion

All samples recovered from the receptor fluid were below the limit of reliable measurement (3.07 ng/cm^2) and most samples recovered after 66h were below the limit of detection (0.1 ng/cm^2). Therefore, a depot effect from the epidermis can be excluded. Under the described test conditions, a total amount of 0.02 ± 0.02 µg/cm^2 Tetrabromophenol Blue is obtained by summing up the amounts present in receptor fluid and in the dermis. Consequently, this amount is considered as bioavailable.

Ref.: 1c

SCCS comment

As no movement of the dye from the skin reservoir to the receptor fluid occurred after 72 h, SCCS is willing to accept that the amount in the epidermis may be excluded as dermally absorbed.

Taken from SCCS/1426/11

Guideline:	OECD 428 (2004)
Tissue:	pig skin, split thickness skin samples from back and flanks (1.12 ± 0.11 mm thick) from three animals (1 male and 2 females)
Method:	permeation chambers (Teflon chambers with 9.1 cm² surface, in-house development)
Integrity:	tritiated water
No. of chambers:	5 chambers with formulation and 1 control
Test substance:	Tetrabromophenol Blue
Batch:	TBFB3/02/30
Purity:	38.2 area% (HPLC) Tetrabromophenol Blue at 210 nm 45.1 area% (at 254 nm) 47.6 area% (at 615 nm) 96.7 area% all brominated homologues (at 210 nm)
Test formulation:	Colour cream formulation (VDE-0026/1) with 0.2% Tetrabromophenol Blue.
Dose	100 mg/cm^2 test formulation
Receptor fluid:	physiological receptor fluid
Solubility in receptor fluid:	2.04 mg/ml (at pH 7.3)
Stability in receptor fluid:	99% recovery after 3 days of a 1 mg/ml solution
Analysis:	HPLC (detection and quantification at 613 nm; LOD = 3.75 ng/ml)
GLP:	in compliance
Date:	24 October 2005 – 3 November 2005

The cutaneous absorption of Tetrabromophenol Blue in a typical hair dye formulation for direct hair dyeing was measured by HPLC with pig skins *in vitro*.

Results

After application of 100 mg/cm² formulation containing 0.2% Tetrabromophenol Blue for 60 minutes on skin samples and subsequent rinse-off with water and shampoo, the recovered Tetrabromophenol Blue was found predominantly in the rinse solution (92.42 ± 1.72% or 184.83 ± 3.45 µg/cm²). Small amounts of Tetrabromophenol Blue were found in the upper skin (1.10 ± 0.45% or 2.20 ± 0.89 µg/cm²). Tetrabromophenol Blue was not detectable in the receptor fluid fractions collected within 72 hours and in the separated lower skin compartments (after 72 hours).

Table 1: Details of the results

	Skin	Integrity-Test	1)		2)		3)		4)		1) + 2) + 3) + 4)	
	No	^3H_2O Permeation (4 hours cumulative)	Receptor fluid (72 hours cumulative)		Lower skin (72 hours cumulative)		Upper skin (72 hours cumulative)		Rinsing solution (after 60 minutes)		Total***	
		[% Dose]	[µg/cm²]	[% Dose]	[µg/cm²]	[% Dose]	[µg/cm²]	[% Dose]	[µg/cm²]	[% Dose]	[µg/cm²]	[% Dose]
Application of 0.2 mg of WR18042 in 100 mg of vehicle* per 1 cm² of skin	2	1.0	BLD** (0.45)	BLD** (0.23)	BLD** (0.06)	BLD** (0.03)	1.45	0.72	180.29	90.15	182.25	91.13
	4	1.2	BLD** (0.45)	BLD** (0.23)	BLD** (0.06)	BLD** (0.03)	1.70	0.85	186.86	93.43	189.07	94.54
	6	1.1	BLD** (0.45)	BLD** (0.23)	BLD** (0.06)	BLD** (0.03)	2.63	1.38	186.08	93.04	189.22	94.61
	8	0.8	BLD** (0.45)	BLD** (0.23)	BLD** (0.06)	BLD** (0.03)	1.64	0.82	182.27	91.14	184.42	92.21
	10	0.9	BLD** (0.45)	BLD** (0.23)	BLD** (0.06)	BLD** (0.03)	3.56	1.78	188.66	94.33	192.73	96.37
Control skin (vehicle only)	12	1.5	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
Mean		**1.1**	**BLD* (0.45)**	**BLD* (0.23)**	**BLD** (0.06)**	**BLD** (0.03)**	**2.20**	**1.10**	**184.83**	**92.42**	**187.54**	**93.77**
± S.D		0.2	-	-	-	-	0.89	0.45	3.45	1.72	4.18	2.09
(n)		(6)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)

*vehicle: (typical hair dye formulation as detailed in the appendix); ** below the limit of detection, taken as 15 ng/injection for the calculation of the mean (lower skin samples: 56.25 ng/cm², receptor fluid samples: 75 ng/cm²); *** Total is corrected with respect to the assumption, that for each fraction below LOD the amount of LOD (absolute LOD = 15 ng/ injection) and for each fraction below LOQ the amount of LOQ (absolute LOQ = 30 ng/injection) for the corresponding fraction is taken for the calculation.

Conclusion

Taking into account the estimates from limits of detection, 2.71 ± 0.89 µg/cm² of Tetrabromophenol Blue was considered as biologically available (n = 5, three donors; receptor fluid (0.45) + lower skin (0.06) + upper skin (2.20) added).

Ref.: 19

SCCS comment

Only 5 chambers were used and the dose of dye was too high.

According to the SCCP Opinion on 'Basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients, update 2006', skin samples that may be used are split-thickness (200-500 µm) or full-thickness (500-1000 µm) skin preparations [Sanco/222/2000]. For pig skin: since it is technically more difficult to obtain intact split-thickness skin, this could justify the use of full-thickness skin.

Taken from SCCNFP/0797/04, SCCS/1426/11

Guideline:	OECD 428
Species/strain:	Pig skin, full thickness skin (1000 µm)
Test item:	5 g of formulation with 5.0 % of Tetrabromophenol Blue
Diffusion cells:	flow-through system, 6 replicates
Batch:	TBFB3/02/30 (formulated in batch 6746 11.06.2002)
Dose:	400 mg of test item (oxidative formulation) containing 1.67 % of Tetrabromophenol Blue on 4 cm²; i.e. 1.67 mg Tetrabromophenol Blue / cm²
Assay:	HPLC
GLP:	in compliance

The cutaneous absorption of Tetrabromophenol Blue was determined in a representative hair dye formulation containing 1.67% of the test substance using pig skins *in vitro*. A dose of 400 mg formulation was applied on skin samples (1670 µg Tetrabromophenol Blue/cm² pig skin) for 30 minutes and subsequently rinsed off with water and shampoo. After 72 hours, the amount of the test substance was determined in the receptor fluid, in the skin extracts (epidermis and upper dermis separated) and in the rinsing solution using HPLC analysis.

Results

The content of Tetrabromophenol Blue in all fractions in the receptor fluid was below the limit of quantification of 56 ng/cm² per fraction or 339 ng/cm² adding up all 6 fractions. Considering the limit of quantification as the upper limit, the amount of Tetrabromophenol Blue in the receptor fluid was < 0.339 µg/cm² (or < 0.02% of the applied dose).
Correspondingly, the amount of <0.339 µg/cm² was regarded as having passed the skin barrier during the experimental period of 72 hours. The concentrations of Tetrabromophenol Blue detected in the separated skin layers were 0.901 ± 0.116 µg/cm² (or 0.054 ± 0.007%) in the epidermis, and 0.04 ± 0.013 µg/cm² (or 0.002 ± 0.001%) in the upper dermis. A total recovery of 95.1% was calculated, including the amount of test substance in the rinsing solution (1584 µg/cm² or 95%).

Conclusion

According to the study authors, under the described test conditions that correspond to realistic in-use conditions, a dermal penetration rate of <0.339 µg/cm²/72h was obtained. For the worst case assumption, the amount of the test item found in the upper dermis was added, resulting in a maximum dermal penetration rate of 0.379 µg/cm²/72h for the final risk assessment.

Comments

- The exact composition of the oxidative formulation is unknown.
- The use of full thickness skin is not justified.
- An "Infinite dose" of formulation was applied (100 mg/cm²) instead of a finite dose (1-5 mg/cm²). Therefore, the results expressed in percentage are of no value for any calculation.
- The absorption should take into account the amount of material recovered in the epidermis (stratum corneum and epidermis were not separated at the end of the test) for the calculation of the total absorption. In this case, the amount of material would be about 1.280 µg/cm² instead of 0.379 µg/cm².

Ref.: 20

SCCS comment

This dermal absorption study with pig skin under oxidative conditions was not considered acceptable due to methodological shortcomings.

Overall SCCS conclusion on dermal absorption

New *in vitro* dermal absorption studies using human skin show that the bioavailable amount of C183 is 0.05 ± 0.02 µg/cm^2 and 0.02 ± 0.02 µg/cm^2 under non-oxidative and oxidative conditions, respectively. In accordance with the SCCS Notes of Guidance, the mean + 1 SD will be used for the MoS calculation i.e. 0.07 µg/cm^2 for non-oxidative conditions and 0.04 µg/cm^2 for oxidative conditions.

3.3.5 Repeated dose toxicity

3.3.5.1 Repeated Dose (14 days) oral toxicity

No data submitted

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

Taken from SCCNFP/0797/04, re-evaluated

Guideline:	OECD 408 (1998)
Species/strain:	SPF-bred Wistar rats
Group size:	10 males and 10 females per dose group
Test substance:	Tetrabromophenol Blue dissolved in water containing 5.3% polyglycol 600 and 4.2% of a 50% aqueous decyl glucoside solution
Batch:	TBFB3/02/30
Purity:	96.7-98.8%
Dose levels:	0, 3, 10 and 100 mg/kg bw/day by oral gavage
Route:	oral gavage
GLP:	in compliance
Study period:	November 2002 – February 2003

The test substance was added to the vehicle and heated to 80 °C under stirring. The formulation was cooled down to room temperature and homogenised. The stability of the test substance in the vehicle was analysed. The animals were treated with the test substance by gavage, 7 days per week, for 91 (males) or 92 (females) days. Clinical observations were made once daily. During week 12-13, a motor activity test was performed. Body weights and food consumption were measured weekly. Ophthalmoscopy was done at pre-test and week 13. At pre-test and at the end of the study, clinical biochemistry, macroscopic and microscopic examination was performed, organ weights were determined and histopathology on organs was examined. Lungs, livers and kidney of all dose groups were examined and the other organs and tissues were analysed from the highest dose group and controls.

Results

No treatment-related mortality occurred. Motor activity, body weight gain and food consumption revealed no treatment-related effects.

Clinical signs included blue discolouration of the fur and faeces in all dose groups. Alopecia, chromodacryorrhoea and other skin problems such as scabbing were also common in all dose groups but the study authors considered that these were within the normal range. However, chromodacryorrhoea increased in a dose-related manner in females. By the end of the dosing period, these effects were more pronounced, both in numbers affected (control: 3; 3 mg/kg bw d: 7/10; 10 mg/kg bw d: 4/10 and 100 mg/kg bw d: 7/10 respectively) and with increasing severity of the response in the mid- and high-dose groups. Three females that had chromodacryorrhoea (1 mid and 2 high dose) also exhibited behavioural effects (hunching, piloerection and clonic spasms).

During ophthalmoscopy, multifocal corneal opacities were observed in 1/10 males at 10 mg/kg bw/day (bilateral) and in 4/10 males at 100 mg/kg bw/day (two bilateral and two unilateral). The incidence of this finding was considered by the study report authors to be higher than normally encountered in these types of studies. Since the test substance has corrosive properties based on the rabbit eye irritation test, these changes may have resulted from direct contact of the formulation present on e.g. the fur with the eye, causing local irritation.

However, microscopic examination of the eye of control and high dose animals did not reveal any treatment-related lesions. Therefore, these findings were considered by the study report authors to be of no primary toxicological significance.
Statistically significant but not dose-related differences in haemoglobin and haematocrit values between the dose groups were observed at pre-test and at the end of the study and not considered as toxicologically relevant, but changes in platelet values (males) at 100 mg/kg bw/day and changes in erythrocytes counts observed in males which were statistically significant at 10 and 100 mg/kg bw/day point to a haematotoxic potential of the test substance. Following the dose of 100 mg/kg bw/day changes in urea (males) and cholesterol (females) values were found. Discolouration of the gastro-intestinal tract was observed, related to the staining properties. No treatment-related changes were observed in organ weights or in the histopathological examination of organs and tissues.
The study report authors established a NOAEL of 100 mg/kg bw/day. Due to the ophthalmological and haematological findings at this dose level, the SCCNFP set the NOAEL to 3 mg/kg bw/day.

Ref.: 12

Comment

The SCCNFP remarked that according to Ref. 15 (Ref. 5 subm. I), a 2% solution of Tetrabromophenol Blue has not been classified as eye irritating and no corneal opacity was observed at this concentration. However, for the highest dose in this 90-day study, 100 mg per kg bw was administered in 5 ml volume per kg, which corresponds to a 2% solution and the observed ophthalmological effects were attributed to direct eye contact.

Reassessment by the SCCS

In 2004, only a draft study report was submitted. The final report has now been provided, but it does not change the previous Opinion.
The SCCNFP commented on the discrepancy in interpretation by the study authors between the eye irritation test and the 90-day study. A 2% solution of Tetrabromophenol Blue was not classified as an eye irritant, but in the 90-day study, 100 mg per kg bw/d; (equivalent to a 2% solution) the corneal opacities in males (1 mid and 4 high dose) were attributed to direct eye contact, causing local irritation, as microscopic eye examination did not reveal any other treatment-related lesions.
Chromodacryorrhoea was not considered toxicologically significant. However, there was a dose-related increase in the occurrence and severity of chromodacryorrhoea in females by the end of the dosing period. This suggests that these could be cholinergic effects, since overproduction of porphyrin from the Harderian gland is indicative of a non-specific response to stress. The three females (1 mid and 2 high dose) that exhibited behavioural changes also had chromodacryorrhoea, which supports this. This, in conjunction with the higher incidence of corneal opacities in males, suggests that the ophthalmic effects were systemic rather than due to direct contact.
The statistically significant reduced platelet and urea values (high-dose males), and increased cholesterol values (high-dose females) were considered to be not toxicologically significant as they were within the normal variation for rats of this age and strain.

Comments submitted under the Public Consultation to the SCCS Opinion on *Tetrabromophenol Blue*, Colipa n° C183 (SCCS/1479/11, adopted 26-27 June 2012)

The Applicant would like to comment that the findings on corneal opacity in the sub-chronic study are not inconsistent with the negative findings in the eye irritation study. A comparison of both study results is not possible because a single dose of the test material was used in the irritation study vs. repeated potential eye exposure in the sub-chronic oral toxicity study. Repeated exposure to the eye as a result of grooming behaviour, and microlesions on the

cornea occurring as a result of grooming, could very easily have produced an irritant or corrosive effect on the eye. Blue discoloration of the fur is suggestive of such exposure to the test material occurring as a result of grooming behaviour. Bacterial infections of microlesions of the cornea are known to directly induce degenerative processes, which will cause corneal opacity. Furthermore, no degenerative processes (protein denaturation and accompanying light reflection disturbance) were observed in the lens and the vitreous body during histopathological examination. Therefore, a systemic effect is considered unlikely.
The applicant would also like to comment on the changes in the erythrocyte count in males observed at 10 and 100 mg/ kg bw. Although statistically significant, in our opinion these observations can be concluded to be normal variations within the physiological range for that strain and age. The lack of histopathological evidence for a disturbance of haematopoiesis in the spleen, the bone marrow or the liver supports that interpretation.
Finally, the applicant would like to comment on the observed chromodacryorrhea in the treated animals. Although there was a higher incidence in treated females, chromodacryorrhea was also observed in controls. Chromodacryorrhea can occur as a non-specific response to stress, especially to environmental stress. Treatment with a test material could induce a higher level of stress and lead to a higher incidence of chromodacryorrhea in a treatment-related manner (discomfort after gavage, bad taste, etc.). If a direct cholinergic effect was involved, a clear dose response relationship would be expected, i.e., a ten-fold difference in dose between the mid and high dose would be expected to lead to a dramatic increase in chromodacryorrhea, This was not observed. Therefore, the applicant considers that a direct cholinergic effect of the test material is unlikely.
Based on the arguments above, the applicant concludes that a NOAEL of 100 mg/kg bw/day (expressed as administered dose) is justified for the 90-day oral toxicity study. The applicant acknowledges that this difference in interpretation regarding the NOAEL from this study does not impact the MoS calculation because the applicant has used the NOAEL from the developmental toxicity study (3 mg/kg bw/day) for the calculation.

Reassessment by the SCCS in 2016
The current SCCS agrees with the previous evaluation of the SCCNFP in 2004, as well as with the reassessment of the previous SCCS, i.e. the NOAEL is 3 mg/kg bw/day based on the ophthalmological and haematological findings at the higher dose levels in this study. The NOAEL of 3 mg/kg bw/day is taken forward to the MoS calculation.

3.3.5.3 Chronic (> 12 months) toxicity

3.3.6 Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Taken from SCCNFP/0797/04, SCCS/1426/11

Bacterial Reverse Mutation Assay

Guideline: OECD 471 (July 1997)
Species/strain: *S. typhimurium* TA 98; TA 100; TA102; TA1537; TA1535
Test substance: Tetrabromophenol Blue

Batch:	TBFB 3/02/30
Lot:	802175
Purity:	HPLC: 98.6%
Concentrations:	1–5000 µg/plate (5 doses): 1st experiment
	30–3000 µg/plate (5 doses): 2nd experiment
Replicate:	3 plates/dose
Positive controls:	according to the guideline
Metabolic activ.:	Aroclor 1254 induced rat liver homogenate (purchased)
GLP:	in compliance

Results

Toxicity: not stated

Mutagenicity: there was no increase over the control of the number of revertant colonies in the plates containing the test material.

Conclusion

Tetrabromophenol Blue is not mutagenic on bacterial cells.

Ref.: 22

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

Guideline:	OECD 476 (July 1997)
Species/strain:	Mouse Lymphoma L5178Y (Thymidine kinase locus)
Test substance:	Royal Blue WR 802175
Batch:	TBFB3/02/30
Lot:	/
Purity:	98.6 area % (HPLC)
Concentrations:	9-144 µg/ml 1st experiment (-S9); 18-288 µg/ml 1st experiment (+S9)
	18-288 µg/ml 2nd experiment (-S9)
Replicate:	2 cultures per experiment
Treatment time:	1st experiment = 4 hours; 2nd experiment = 24 hours
Metabolic acti.:	Phenobarbital/ß-Naphthoflavone induced rat liver homogenate
Positive controls:	MMS: -S9; 3MC: +S9
GLP:	in compliance

Results

Toxicity: concentrations of 18–2300 µg/ml were used to investigate the toxicity of the test item.

Toxicity was observed from a concentration of 144 µg/ml (-S9) and 288 µg/ml (+S9).

Mutagenicity: at 4 hours of treatment, MMS induced small and large mutant colonies, thus indicating a mutagenic/clastogenic activity; 3MC induced significant increase of small and large colony mutants only in one culture.

At 24 hours treatment, MMS induced a significant increase of small and large colony mutants.

After 4 hours treatment, the test item induced a dose-related significant increase of small colony mutants in the absence of the metabolic activation; this effect was not repeated in the 24 hours treatment. In the presence of a metabolic activation system, an increase of the induction of small colony mutants was also observed at the highest dose.

Ref.: 23

SCCS comment

After 4 h treatment without S9-mix, the increase in small colonies mutants was considered minor and of no biological relevance. No increase in mutant frequency was observed after 24 h treatment without S9-mix. No relevant increase in mutant frequency was observed with S9-mix. Therefore the SCCS considers the study to be negative.

Taken from SCCS/1426/11

***In vitro* Micronucleus Test**

Guideline:	OECD 487 (draft 2004)
Species/strain:	cultured human peripheral blood lymphocytes pooled from 3 male donors
Replicates:	two cultures per concentration and positive control (4 for negative control), three concentrations analysed
Test item:	Tetrabromophenol Blue
Batch:	9801090301
Purity:	98.8 area % (HPLC, at 254 nm)
Vehicle:	DMSO
Concentrations:	Exp. I: with S9-mix: 1000, 1200 and 1400 µg/ml without S9-mix: 225.3, 400.4 and 711.9 µg/ml Exp. II: with S9-mix: 1266, 1688 and 2250 µg/ml without S9-mix: 225.3, 400.4 and 711.9 µg/ml
Performance:	Exp. I: with S9-mix: 3 h treatment, 24 h after mitogen stimulation. Recovery period 45 h without S9-mix: 20 h treatment 24 h after mitogen stimulation. Recovery period 28 h Exp. II: with S9-mix: 3 h treatment, 48 h after mitogen stimulation. Recovery period: 45 h without S9-mix: 20 h treatment, 48 h after mitogen stimulation. Recovery period 28 h
Positive controls:	NQO and vinblastine in the absence of S9-mix, cyclophosphamide in the presence of S9-mix
GLP:	in compliance
Study date:	September 2005 – November 2005

The test agent was investigated for its clastogenic and aneugenic potential in the *in vitro* micronucleus assay. In a preliminary toxicity test, the highest concentration used (3000 µg/ml) was based on solubility in DMSO. The concentrations used in the main tests were limited by the toxicity of the test substance.

Results

The highest concentrations used for analysis in the first experiment: 711 µg/ml in the absence of S9 and 1400 µg/ml in the presence of S9 induced approximately 62% and 76% reduction in replication index (RI) respectively. In the second experiment, the highest analysed concentrations: 711 µg/ml in the absence of S9 and 2250 µg/ml in the presence of S9 induced

approximately 58% and 35% reduction in RI respectively. In experiment 1, with 24 h growth stimulation with PHA prior to treatment, there was no significant increase in the frequencies of micronucleated binucleated (MNBN) cells at any concentration evaluated either with or without S9-mix. In experiment 2, with 48 h growth stimulation with PHA, there was no induction in MNBN without S9-mix. With S9-mix there was a slight, but statistically significant increase in MNBN cells at the intermediate concentration (1688 µg/ml). However, this increase was only observed in one culture and not concentration related, and therefore not considered biological relevant.

Conclusion

Under the test conditions used, Tetrabromophenol Blue did not induce structural or numerical chromosomal aberrations in human lymphocytes.

Ref.: 24

3.3.6.2 Mutagenicity / Genotoxicity *in vivo*

Taken from SCCNFP/0797/04, SCCS/1426/11

Mammalian Erythrocyte Micronucleus Test

Guideline:	OECD 474 (July 1997)
Species/strain:	NMRI mice
Test substance:	Royal Blue WR 802175
Batch:	TBFB3/02/30
Lot:	/
Purity:	98.6 area % (HPLC)
Dose levels:	75, 150, 300 mg/kg (24 hours of treatment); 300 mg/kg (48 hours of treatment) (5 females and 5 males)
Treatment:	i.p. (no justification is reported)
Positive control:	CPA, 40 mg/kg, i.p.
GLP:	in compliance

Results

Toxicity: toxicity preliminary experiments were performed on 4 animals (2F+2M) with a dose of 100, 200, 400 and 300 mg/kg by i.p. treatment: toxic effects were observed at 400 mg/kg. Therefore, the doses of 75, 150, 300 mg/kg were chosen.
Mutagenicity: CPA, the positive control, induced 1.45% and 1.15% of micronucleated cells in comparison of 0.4% of the negative control (water). The test item did not induce MN in the conditions of the assay; some reduction of the PE/NE ratio was observed in the treated animals.

Conclusion

Tetrabromophenol Blue does not induce clastogenic/aneugenic effects in mice, treated *in vivo*.

Ref.: 25

3.3.7 Carcinogenicity

No data submitted

3.3.8 Reproductive toxicity

3.3.8.1 Two generation reproduction toxicity

No data submitted

3.3.8.2 Other data on fertility and reproduction toxicity

No data submitted

3.3.8.3 Developmental Toxicity

Teratogenicity

Taken from SCCNFP/0797/04, SCCS/1426/11

Guideline:	OECD 414 (2001)
Species/strain:	SPF-bred Wistar rats
Group size:	24 females per dose group
Test substance:	Tetrabromophenol Blue dissolved in water containing 5.3% polyglycol 600 and 4.2% of a 50% aqueous decyl glucoside solution
Batch:	TBFB3/02/30
Purity:	96.7-98.8%
Dose levels:	0, 5, 50 and 500 mg/kg bw/day by oral gavage
GLP:	in compliance

110 females were mated, aiming at 96 pregnant females. From day 6-20 post coitum 24 females per dose group were treated by gavage with the test substance. Clinical signs were observed once daily. The body weights were determined on days 0, 3, 6, 9, 12, 15, 18 and 21 post coitum and food consumption was recorded for the respective intervals. On day 21, the study was terminated and all animals were subject to necropsy. The common reproduction parameters were recorded (corpora lutea, uterus weight, live and dead foetuses, foetal weight, implantations, resorptions, external abnormalities). Alternate foetuses of each litter were preserved and analysed for skeletal or visceral anomalies.

Results

No mortality or substance-related clinical signs were observed. Due to the staining properties 4/24 females of the 5 mg/kg bw/day group and all other test substance-dosed animals exhibited blue staining of body parts and/or faeces. Females of the 500 mg/kg bw/day group showed decreases in body weights, body weight gain and corrected body weight gain compared to controls accompanied by reduced food consumption in some periods. Foetal body weights were decreased at 50 and 500 mg/kg bw/day. Cranial bone ossification was reduced in nearly all high-dose group foetuses and in about one half of the 50 mg/kg dose. At the low dose of 5 mg/kg bw/day, a generalised reduction in ossification was seen. Incidental cases of malformations were seen in all dose groups including controls (e.g. polydactyly, exencephaly, spina bifida, abnormal shape of limb bones) but the effects were not dose-related. In the high-dose group, 18 of 166 analysed foetuses showed changes of the major arteries, which should

be attributed to treatment. Even in the medium dose, one foetus with persistent truncus arteriosus was found.

Conclusion

The NOAEL of maternal toxicity was 50 mg/kg bw/day, the NOAEL of teratogenicity was 5 mg/kg bw/day. For embryotoxicity, a NOAEL cannot be established.

Ref.: 17

Taken from SCCS/1426/11

Guideline:	OECD no. 414 (2001)
Species/strain:	Rat, strain Wistar rats HanBrl: WIST, outbred (SPF)
Group size:	22 mated females per dose group
Test item:	Tetrabromophenol Blue
Batch:	TBFB3/02/30
Purity:	98.8 area % (at 615 nm, HPLC)
Dose levels:	0, 3, 30 and 300 mg/kg bw/day
Vehicle:	5% w/w polyglycol 600, 4% w/w Plantaren 2000 UP (50% aqueous decyl glucoside), 90.5% milli-U water
Route:	oral, gavage
GLP:	in compliance
Study date	3 January – 20 July 2005

Eighty-eight successfully mated females were allocated to 4 groups of 22 animals per group. Animals were dosed from Gestation Day (GD) 6 through to GD 20, with a standard dose volume of 10 ml/kg bw with a daily adjustment to the actual body weight. Samples for determination of concentration, homogeneity and stability (7 days) of the dose formulations were taken during the first week of the administration period. Additionally, samples for determination of concentration and homogeneity were taken during the last week of the administration period. On each occasion, three samples of approximately 2 g were taken from the top, middle and bottom of each formulation and transferred into flat-bottomed flasks. Stability samples were taken from the middle only. The samples were frozen (-25°C to -15°C) pending analysis. The test item was used as analytical standard.

Dose selection was based on the previous study.

Animals were checked daily for clinical signs and twice daily for mortality. Body weights were recorded daily from GD 0 - 21. Food consumption was recorded on 3-day intervals: GD days 0-3, 3-6, 6-9, 9-12, 12-15, 15-18 and 18-21.

On GD 21, all were killed under $C0_2$-asphyxiation and a complete autopsy and a macroscopic examination of the organs was carried out.

The intact uterus (prepared by caesarean section) was removed and the presence of resorption sites (early, late) and foetuses (live or dead) as well as their uterine position were recorded. In addition, placental and uterine weights were determined.

The number of implantation sites and corpora lutea was also determined. Each viable foetus was weighed, sexed and examined for gross external malformations.

After fixation and staining, skeletal and visceral examinations of the foetuses were performed. At least one half of the foetuses from each litter were fixed in Bouin's fixative (one foetus per container). They were examined by a combination of serial sections of the head and microdissection of the thorax and abdomen. This included detailed examination of the major blood vessels and sectioning of the heart and kidneys. After examination, the tissues were preserved in a solution of glycerine/ethanol. Carcasses of the other half of the foetuses were processed through solutions of ethanol, glacial acetic acid with Alcian blue (for cartilage staining), potassium hydroxide with Alizarin red S (for clearing and staining ossified bone) and aqueous glycerin for preservation and storage. Examinations were conducted by means of a

dissecting microscope.

Results

Investigations of the homogeneity, stability and correctness of concentrations in the used formulations were within the required ranges.
No mortality occurred during this study.
No clinical signs or behavioural changes were noted in any dose group. In the mid- and high-dose groups, the faeces were bluish, discoloured from GD 7 until necropsy, due to the colouring property of the test item.
Food consumption was distinctly reduced in the high-dose group throughout the treatment period (GD 6 21). Consequently, body weight development was reduced in this group from GD 8 - 9 onwards, and the mean corrected body weight gain (corrected for uterus weight) was also distinctly reduced. These findings were considered to be related to treatment with Tetrabromophenol Blue.
There were no findings in the dams of low- and mid-dose groups (3 and 30 mg/kg bw), which were considered to be treatment-related.
The relevant reproduction data (incidence of post-implantation loss and number of foetuses per dam) were similar in all groups and not affected by treatment with the test item.

Mean foetal body weights were reduced in the high dose group when compared with the control group. Compared with the control group, increased incidences of the following findings occurred in the high- and mid-dose: cleft palates, (high - 2/22; mid - 1/22) and in addition increased incidences of left-sided umbilical arteries and cranially elongated thymuses at the high dose and anophthalmia in the mid-dose group. There was an increased incidence of fused zygomatic arches at the high dose (21 in 12 litters) when compared with the control group (12 in 9 litters). A statistically significant increase in supernumerary rudimentary ribs was observed in the mid and high doses.
No changes were noted in the foetuses of the low dose group (3 mg/kg bw).

Conclusion

Based on these results, the maternal NOAEL was considered to be 30 mg/kg bw/ day.
A NOAEL for embryo-foetal effects was derived at 3 mg/kg bw/day.

Ref: 18

3.3.9 Toxicokinetics

3.3.9.1 Toxicokinetics in laboratory animals

Taken from SCCS/1426/11

Guideline:	OECD 417 (1984) and OECD 427 (2004)
Species/strain:	Rat, Wistar CRL: WI BR (outbreed) (SPF)
Group size:	Females, mass balance groups (groups 1,2,3,4) 4 per dose; toxicokinetics groups (groups 5, 6, 7, 8) 6 per dose
Test substances:	Tetrabromophenol Blue-(Phenol-UL-^{14}C)
Batch:	064K9418
non-labelled	Tetrabromophenol Blue
Batch:	TBFB3/02/30
Purity:	Radiochemical purity: 88.8% by HPLC, specific activity 48.8 mCi/mmol

	Non-labelled: 97.5% (HPLC, 254 nm)
Stability	Not indicated
Vehicles:	
Oral	5.3% w/w polyglycol 600, 4.2% w/w Plantaren 2000 UP (50% aqueous decyl glucoside), 90.5% milli-U water
Intravenous	0.05 M phosphate buffer (pH 7.6)
Dermal	Water/acetone 1:1
Dose levels:	
Oral	10 and 100 mg/kg bw by gavage
Intravenous	5 ml/kg
Dermal	9 mg/kg bw (equivalent to 0.09 mg/cm² skin, 9 mg/ml)
Dosing schedule:	Single
GLP:	in compliance
Study date:	Oct 2004 - Sept 2005

In the mass-balance groups, animals were housed in metabolism cages in order to obtain a total ^{14}C-radioactivity material balance. After dosing, urine and faeces were collected over time intervals of 0-8 h, 8-24 h, 24-48 h, 48-72 h, 72-96 h. The animals were killed after 96 h and several tissues and organs were collected. Total radioactivity in urine, faeces, tissues, and organs was determined.
For metabolic studies, urine and faeces were pooled per group, and the metabolite profile of the pooled samples was obtained by HPLC and LC-MS/MS.
In the toxicokinetic groups, blood was sampled alternately from several rats per time point at 15 and 30 min, and 1, 2, 4, 8, 24, and 48 h. Total radioactivity Tetrabromophenol Blue equivalent concentrations were determined.

Results

Homogeneity and stability of test substance in the vehicle were demonstrated by HPLC. Accuracy of concentrations was sufficient to fulfil the study objectives.
Mortality and clinical signs: One animal (group 2; low oral dose group) died on day 2, probably due to misdosing.
No clinical signs were observed in the oral dose groups (groups 2, 3, 6 and 7) or in the intravenous dose groups (groups 1 and 5), except for blue/green discolouration of the faeces at day 2 and some blue discolouration of the tail in one animal.
After dermal dosing (groups 4 and 8), chromodacryorrhoea from nose and eye was observed. This was not a consequence of grooming, as the animals had neck collars.
Absorption and excretion: After oral dosing, the mean cumulative recovery of ^{14}C-Tetrabromophenol Blue radioactivity in the urine after 96 h was 0.031 ± 0.004 % (low dose) and 0.03 ± 0.001 % (high dose) and in faeces was 107.1 ± 5.06 % (low dose) and 119.5 ± 6.618 % (high dose). Mean residual radioactivity in the carcass, tissues and blood was 0.244 % (low dose) and 0.353 % (high dose). Less than 0.02 % of the total radioactivity was recovered in the cage wash. The mean mass balance was 107.40 ± 5.03 % (low dose) and 119.9.± 6.63 % (high dose). The percentage of oral absorption was calculated by comparison of the percentage of radioactivity recovered in urine after oral administration with the percentage of radioactivity recovered in urine after iv administration which yielded 29 and 30 %.
After intravenous administration, the mean percent recovery of radioactivity after 96 h was 0.102 ± 0.013 % in urine and 112.76 ± 14.30 % in faeces. Mean residual radioactivity in the carcass and tissues was 5.89 % of the dose. Less than 0.05 % of the total radioactivity was recovered in the cage wash. The mean mass balance was 113.49 ± 14.32 %.
After dermal application, the mean cumulative recovery of radioactivity was 0.013 ± 0.007 % of the dose for the urine and 0.838 ± 0.248 % of the applied dose for the faeces. Mean residual radioactivity in the carcass and tissues (without skin) was 0.314 %. The recovery from

the treated skin was 0.369 ± 0.151 %. Less than 0.05 % of the total radioactivity was recovered in the cage wash. The mean mass balance was 97.332 ± 2.521 %.

The chromatograms from the 3 treatments showed similar characteristics, although radioactivity in the dermal group was low and only a vague peak pattern observed. Hence, the results are based on the average of all groups. It was reported that no radioactivity peaks were detected in the urine samples. With both LC methods, two clusters of peaks were observed. In the first cluster, a peak with a retention time similar to ^{14}C-Tetrabromophenol Blue was detected, indicating unchanged compound in the faeces. The second cluster was thought to be metabolites.

^{14}C-Tetrabromophenol Blue has at least 5 components that differ in the number of bromine atoms (6-8). Each of these forms metabolites. The major metabolic reactions resulted in metabolites with longer retention times on the LC system and with m/z ratios 2 amu (atomic mass unit) higher than the corresponding ^{14}C-Tetrabromophenol Blue components. Mass Spectroscopic data on these metabolites did not yield sufficient information for proposal of a chemical structure because elimination of *Br and HBr were the main fragmentation reactions.

The most important route of excretion of Tetrabromophenol Blue and its metabolites was through the faeces, suggesting some biliary excretion. With oral dosing, 107-119 % of the administered dose was recovered in the faeces. After dermal administration, excretion via faeces was low, (0.8 %), reflecting the poor dermal absorption.

Excretion in urine was low, representing 0.03-0.1 % of the dose after oral and iv administration and 0.01 % after dermal application. Excretion of Tetrabromophenol Blue and its metabolites was much slower after dermal application, which was probably a sign of the slow dermal absorption and consequent slow systemic availability.

Toxicokinetics: Oral toxicokinetics, over the dose range investigated, was linear with C_{max} values of 0.431 mg/kg bw (low dose) and 7.32 mg/kg bw (high dose). $AUC_{0-\infty}$ values were 4.58 and 111.0 mg_{eq}hr/kg for the low and high dose groups respectively. The dose-normalised AUC values were in the same order of magnitude, i.e. 0.450 and 1.070, respectively. Apparent terminal half-lives of ^{14}C-Tetrabromophenol Blue were also similar in both oral administered groups with 19 and 15 hours, respectively. After intravenous administration, half-life was 23.04 hours. No toxicokinetic evaluation could be performed for the dermal group.

Toxicokinetic parameters of Tetrabromophenol Blue equivalents after iv and oral dosing

Parameters		**Intravenous**	**Oral**	
		5 mg/kg bw	**10 mg/kg bw**	**100 mg/kg bw**
Dose	mg/kg	4.360	10.182	103.67
T_{max}	hr	N/a	4	4
C_{max}	mg/kg	n/a	0.431	7.32
Dose-norm C_{max}	mg/kg/mg-*kg	n/a	0.042	0.071
AUC_{last}	hr*mg/kg	28.2	4.44	107
AUC_{∞}	hr*mg/kg	28.9	4.58	111
Dose-norm AUC_{∞}	mg/kg/mg-*kg	6.634	0.45	1.07
% extrapolated	%	2.4	3.0	3.33
λ_z	1/hr	0.0301	0.0366	0.0476
$t_{1/2}$	hr	23.04	18.93	14.56
No. points		3	3	5
Corr. coef.	r^2	0.974	0.99	0.991
F_{oral}	%	n/a	7	16

Conclusion

Absorption, distribution, metabolism and excretion have been investigated in the female Wistar rat. After oral administration, 14C-Tetrabromophenol Blue was moderately absorbed, readily distributed into all organs and excreted mainly via the faeces. The oral absorption of ^{14}C-Tetrabromophenol Blue was moderate, 29 % (100 mg/kg) and 30 % (10 mg/kg).

Dermal absorption of 0.9% of aqueous ^{14}C-Tetrabromophenol Blue was 1.2% of the applied dose.

When dermally absorbed, excretion took place mainly via the faeces and the rate of elimination was slower than after oral dosing.

Ref.: 21

SCCS comment

In the dermal part of the study, a 0.9% solution was applied while only 0.2% was requested by the applicant. Chromodacryorrhoea from the nose and eye were observed. Chromodacryorrhoea was seen in females in the 90-day study at the 10 mg/kg bw d and 100 mg/kg bw d doses.

3.3.9.2 Toxicokinetics in humans

No data submitted

3.3.10 Photo-induced toxicity

3.3.10.1 Phototoxicity / photo-irritation and photosensitisation

No data submitted

3.3.10.2 Photomutagenicity / photoclastogenicity

No data submitted

3.3.11 Human data

No data submitted

3.3.12 Special investigations

No data submitted

3.3.13 Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

(non-oxidative conditions)
(In formulation, on-head concentration 0.2%)

Absorption through the skin	**A**	**=**	**0.07 µg/cm²**
Skin Area surface	**SAS**	**=**	**580 cm²**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**0.0406 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/…**	**=**	**0.00068 mg/kg bw**
No observed adverse effect level (90-day, oral, rat)	**NOAEL**	**=**	**3 mg/kg bw/d**
Bioavailability 30%*		**=**	**0.9 mg/kg bw/d**
Margin of Safety	**adjusted NOAEL/SED**	**=**	**1300**

* based on the toxicokinetic study (ref. 21).

CALCULATION OF THE MARGIN OF SAFETY

(oxidative conditions)
(In formulation, on head concentration 0.2%)

Absorption through the skin	**A**	**=**	**0.04 µg/cm²**
Skin Area surface	**SAS**	**=**	**580 cm²**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**0.0232 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/…**	**=**	**0.00039 mg/kg bw**
No observed adverse effect level (90-day, oral, rat)	**NOAEL**	**=**	**3 mg/kg bw/d**
Bioavailability 30%*		**=**	**0.9 mg/kg bw/d**
Margin of Safety	**adjusted NOAEL/SED**	**=**	**2300**

* based on the toxicokinetic study (ref. 21).

SCCS comment

The above MoS calculations only refer to batch TBFB3/02/30 that was used for the toxicity tests, not the current market quality batches.

3.3.14 Discussion

Physicochemical properties

Tetrabromophenol Blue is used in oxidative -as well as in non-oxidative hair dye formulations at a maximum concentration of 0.2% on the scalp.

The test material is not composed of a single substance, but of different homologues. Analysis of different batches shows a large variation in homologue mixture composition of the test material intended for commercial use versus the batch used for toxicity testing and considered in this Opinion.

For the batch used for the toxicity tests, the information provided on the compound is incomplete concerning the chemical identity of the 9 organic impurities identifiable by HPLC which may comprise up to 3.4% of the test material. SCCS notes that because of an optimised manufacturing process the impurity present at 11.86 min is no longer present in the current market quality batches of C183. With respect to the batches intended to be used in hair dye formulations, the information provided shows the presence of the Hexabromo-homologue (between 0.6 and 0.8%).
The analytical data provided by the Applicant suggests that the substance is sufficiently stable (>90%) during storage and also under oxidative conditions during use.

General toxicity

No data on acute toxicity were submitted.
The study authors established a NOAEL of 100 mg/kg bw/day for the subchronic study. However, the SCCNFP set the NOAEL as 3 mg/kg bw/day based on the ophthalmological (corneal opacity), clinical signs and haematological findings. The SCCS concurs with this decision. The ophthalmic effects were considered to be systemic cholinergic effects due to an underlying stressor effect rather than direct eye contact.
A NOAEL for embryo-foetal effects was derived at 3 mg/kg bw/day.
No data on reproductive toxicity were provided.

Irritation/sensitisation

Tetrabromophenol Blue is not a skin irritant. Based on the degree and persistence of the corneal injury, the pure substance poses a risk of serious damage to eyes. Tetrabromophenol Blue in a dilution of 2% is not irritant for the eyes.
Tetrabromophenol Blue does not pose a sensitising risk to consumers when used as intended.

Dermal absorption

Two new *in vitro* experiments using human skin, one under oxidative and one under non-oxidative conditions, were performed to measure the dermal absorption of Tetrabromophenol Blue. Under non-oxidative conditions, the dermal delivery of Tetrabromophenol Blue was considered to be 0.05 ± 0.02 µg/cm², whereas a dermal absorption of 0.02 ± 0.02 µg/cm² was considered under oxidative conditions. For the calculation of the MoS, a dermal absorption of the mean + 1SD is used: 0.07 µg/cm^2 for non-oxidative conditions and 0.04 µg/cm^2 for oxidative conditions. Because of large variation in chemical composition, this MoS calculation only refers to batch TBFB3/02/30 that was used for the toxicological testing, but not for the batches intended for commercial use as proposed by the Applicant in the current submission.

Mutagenicity

Tetrabromophenol Blue has been tested for the three genetic endpoints: gene mutations, structural and numerical chromosomal aberrations. The test agent did not induce gene mutations in bacteria and mammalian cells. In an *in vitro* micronucleus assay, the substance did not induce an increase in the number of cells with micronuclei and was also negative in an *in vivo* micronucleus assay. It can therefore be concluded that Tetrabromophenol Blue has no genotoxic potential.

Carcinogenicity

No data submitted

Toxicokinetics

In the toxicokinetics study in rats, ^{14}C-Tetrabromophenol Blue was moderately absorbed (~30%) after oral administration whereas dermal absorption was low (1.2%). The systemically available portion was readily distributed into all organs and excreted mainly via the faeces, as the parent compound and to a lesser extent, its metabolites. In the dermal part of the study, chromodacryorrhoea from the nose and eye were observed. Similar systemic effects on the eyes were seen in the 90-day study at the high and medium doses.

Human data

No data submitted

4. CONCLUSION

1. In light of the new data provided, does the SCCS consider Tetrabromophenol Blue (C183) safe when used as a direct dye in oxidative and non-oxidative hair colouring products with a final on-head concentration up to 0.2%?

The margin of safety calculated in this Opinion relates to the previously supplied batch quality of the material. However, because of the large discrepancies noted between the specifications provided for the representative market quality batch intended for commercial use and that used in toxicological testing, SCCS cannot conclude on the safety of Tetrabromophenol Blue (C183).

The test material is not composed of a single substance, but of different homologues. Analysis of different batches has shown a large variation in the homologue mixture composition of the test material intended for commercial use. The safety assessment of Tetrabromophenol Blue (C183) will require a clear well-defined set of specifications for the composition of the substance intended for use in cosmetic products. This will also require toxicological data on a representative batch, and/or a scientifically valid justification for showing toxicological similarities amongst the homologues.

2. Does the SCCS have any further scientific concerns with regard to the use of Tetrabromophenol Blue (C183) in other cosmetic products?

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5. MINORITY OPINION

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