SCCS/1426/11
Revision of 18 September 2012

Scientific Committee on Consumer Safety

SCCS

OPINION ON

Tetrabromophenol Blue

COLIPA n° C183

The SCCS adopted this opinion at its 15th plenary meeting

of 26-27 June 2012

About the Scientific Committees

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

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

SCCS

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

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

TABLE OF CONTENTS

1. BACKGROUND

Submission I for Tetrabromophenol Blue, 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), was submitted by September 2003 by COLIPA[1].

The Scientific Committee on Cosmetic Products and Non Food Products intended for Consumers (SCCNFP) adopted at the 28th plenary meeting of 25 May 2004 the opinion (SCCNFP/0794/04) with the conclusion, "that the information submitted is inadequate to assess the safe use of the substance. Before any further consideration, the following information is required:

* percutaneous absorption study in accordance with the SCCNFP Notes of Guidance."

According to current update of submission I, submitted by COLIPA in July 2005, Tetrabromophenol Blue is used as a direct dye in oxidative and non-oxidative hair colouring products. The final concentration on the scalp can be up to 0.2%.

The current submission presents updated scientific data on the above mentioned substance in line with the second step of the strategy for the evaluation of hair dyes (http://europa.eu.int/comm/enterprise/cosmetics/doc/hairdyestrategyinternet.pdf) within the framework of the Cosmetics Directive 76/768/EEC.

2. TERMS OF REFERENCE

1. *Does the Scientific Committee on Consumer Safety (SCCS) consider Tetrabromophenol Blue safe for consumers, when used as a direct dye in oxidative and non-oxidative hair dye formulations at a maximum concentration on the scalp of 0.2% taking into account the scientific data provided?*

2. *Does the SCCS recommend any restrictions with regard to the use of Tetrabromophenol Blue in any hair dye formulations?*

[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 (INCI name)

Comment
This hair dye is a mixture of octa-, hepta- and hexa-bromo phenolsulfonphthaleins (see section 3.1.4. below). The name Tetrabromophenol Blue is used as synonym of Octabromosulfonphthalein, although it does not contain any Tetrabromo-homologue. See section 3.1.4. and the General Comments below.

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 names below correspond to the octabromo-derivative only, while the chemical structure of the other homologues is unknown.

- 3,3-Bis(3,5-dibromo-4-hydroxyphenyl)-4,5,6,7-tetrabromo-2,1[3H]-benzoxathiol-1,1dioxide
- Phenol, 4,4'-(4,5,6,7-tetrabromo-1,1-dioxido-3H-2,1-benzoxathiol-3-ylidene)bis[2,6-dibromo- (CA Index name, 6CI)
- 3',3'',4,5,5',5'',6,7-Octabromophenolsulfonphthalein
- Tetrabromophenol Blue (CA Index name, 9CI)*

* The labelling of the last name as "CA Index name, 9CI" probably indicates that the name Tetrabromophenol Blue has been accepted by the Chemical Abstracts Services, either for the octabromo-homologue alone, or for the mixture of all homologues; a copy of the respective entry (CAS 4430-25-5) was requested by the SCCP to be submitted in 2004 It was not been submitted yet.

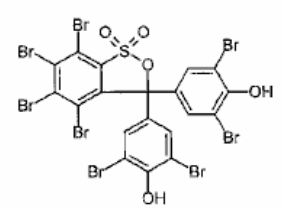
3.1.1.3. Trade names and abbreviations

Gardex Royal Blue (Wella)
Royal Blue (Wella)

3.1.1.4. CAS / EC number

CAS: 4430-25-5
EC: 224-622-9

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: 985.59 g/mol

3.1.4. Purity, composition and substance codes

Chemical characterization was performed using NMR, IR, LC-MS, and UV-Vis spectroscopy. This hair dye is a mixture of octa-, hepta- and hexa-bromo phenolsulfonphthaleins of the following relative composition (HPLC-peak area method at 210nm, 254nm and 615nm).

(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	38.7%	34.8%	40.0%
Heptabromo-minor homologue	7.1%	6.8%	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.
** The applicant declares: "*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 major impurities of them 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 1.1area%. 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 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%

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

Heavy metals

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.

Comment
There is no information on whether other batches of Tetrabromophenol Blue, used in hair dye formulations, meet the same mixture specifications as the one used for the toxicity testing described in this Opinion.

3.1.5. Impurities / accompanying contaminants

See 3.1.4

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 %

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: 3.71 (pH 4.0, room temperature) by EC Method A.8

Log P_{ow}: 5.98 ± 0.20 (calculated for pure Tetrabromophenol Blue-most acidic)

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).

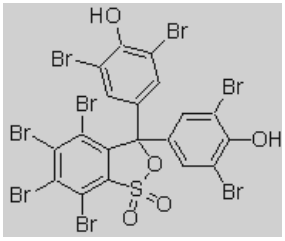
A long-term stability of the dye stuff in a common market formulation (90% recovery) is also reported on the basis of a single determination of the dye content after storage for 10

months at 25°C and comparison with the "theoretical content". This is not an acceptable stability test.

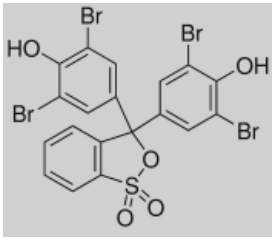
The stability of Tetrabromophenol Blue has not been tested under the conditions of use of an oxidative hair dye formulation.

General Comments to physico-chemical characterisation

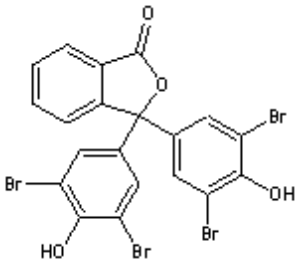
* The name ***Tetrabromophenol Blue*** of the test material is misleading, since the main component is an ***octabromo***-derivative (42%, quantitative HPLC analysis using standard reference material) and no tetrabromo-homologue is present. This material is a mixture of 3 substances: an ***octabromo***-derivative (42%), two heptabromo-homologues (< 46% HPLC peak area) and a hexabromo-homologue (< 13% HPLC peak area). The tetrabromo-homologue is a well known pH indicator named ***Bromophenol Blue*** while the respective non-sulfonated derivative is also a well known compound which is named ***Tetrabromophenolphthalein***. Using the same terminology, the correct name for the octabromo-homologue is ***Tetrabromo Bromphenol Blue*** (instead of Tetrabromophenol Blue).



Tetrabromophenol Blue



bromophenol Blue



tetrabromophenolphthalein

* 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.
* There is no information on how other batches of Tetrabromophenol Blue, used in hair dye formulations, meet the same mixture specifications as the one used for the toxicity testing described in this Opinion.
* The stability of Tetrabromophenol Blue is not tested under the conditions of use of an oxidative hair dye formulation.
* The stability of the test material in typical hair dye formulations has not been performed adequately (only one measurement).

3.2. Function and uses

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.

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

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Taken from SCCNFP/0797/04

Guideline:	OECD 404 (1992)
Species/strain:	Albino Rabbit, New Zealand White, (SPF-Quality)
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

Taken from SCCNFP/0797/04

Study 1, neat substance

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 hours 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

Study 2, diluted substance

Guideline:	OECD 405 (1998)
Species/strain:	Albino Rabbit, New Zealand White, (SPF-Quality)
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

Local Lymph Node Assay

Guideline:	OECD 429 (2000)
Species/strain:	Mouse: CBA/J
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 pinnae. Each concentration was tested on one animal group, which consisted of 5 animals.
Morbidity/mortality checks were performed generally 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 sensitizer under the defined experimental conditions.

Ref.: 16

3.3.4. Dermal / percutaneous absorption

New study, updated submission I, 2005

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 rinsings (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

Comment
Only 5 chambers were used and the dose of dye was too high; therefore, the mean +2SD should be used in calculating the MOS. This equates to 2.71+ (2 x 0.89) = 4.49 µg/cm² of Tetrabromophenol Blue (1.62% of the applied dose).

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

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 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 to have 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

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

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral toxicity

Taken from SCCNFP/0797/04

Guideline:	OECD 407 (1995)
Species/strain:	SPF-bred Wistar rats
Group size:	5 males and 5 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

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 daily with the test substance by gavage for 28 days. Once daily, clinical observations were made. During week 4, functional observations including a motor activity test were performed. Body weights and food consumption were measured weekly. At the end of the study, clinical biochemistry, macroscopic and microscopic examinations were performed, organ weights were determined and histopathology was carried out. Organs and tissues were analysed from all animals of the highest dose group and controls.

Results
No treatment-related mortality occurred. Blue discolouration of the faeces and other body parts was seen in the dose groups 10 and 100 mg/kg bw/day. No relevant substance-related clinical findings were noted. Functional observations, body weight gain and food consumption revealed no treatment-related effects. High dose animals showed an increase in white blood cell counts (males) and increases in cholesterol and glucose (females) while at 10 mg/kg bw/day only one male showed a high glucose value. Discolouration of the caecum related to the staining properties of the test substance was noted in high dose animals. Missing values in male control organ and body weights make it difficult to evaluate possible substance-related changes in absolute and relative body weights. The authors established a NOAEL of 3 mg/kg bw/day.

Ref.: 2b, subm I

3.3.5.2. Sub-chronic (90 days) toxicity (oral, dermal)

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

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 pretest and week 13. At pretest 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, 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 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 and in 4/10 males at 100 mg/kg bw/day. According to the study authors this effect may be due to the corrosive properties of the substance and a direct eye contact with the fur.
Statistical significant, but not dose-related differences in haemoglobin and haematocrit values between the dose groups were observed at pretest 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 and related to the staining properties.
The study authors established a NOAEL of 10 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. However 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, support this. This, in conjunction, with the higher incidence of corneal opacities in males suggest that the ophthalmic effects were systemic rather than by 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.

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Taken from SCCNFP/0797/04

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 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

Comment of the SCCS
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.

New micronucleus study, updated submission I, 2005

***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 toxicity of the tests 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

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.

Conclusions
Tetrabromophenol 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. Teratogenicity

Taken from SCCNFP/0797/04

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.

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 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.

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

New study, submission 2005

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 tissue 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 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

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 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

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.

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

No data submitted

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

No data submitted

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

INCI Name

Non oxidative conditions

Absorption through the skin	**A**	**=**	**4.49 µg/cm^2**
Skin Area surface	**SAS**	**=**	**580 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**2.60 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose	**SAS x A x 0.001/60**	**=**	**0.0434 mg/kg bw/d**
No Observed Adverse Effect Level (rat, oral, teratogenicity and sub-chronic toxicity)	**NOAEL**	**=**	**3 mg/kg bw/d**
Corrected for 30% oral bio-availability (Ref. 21)		**=**	**0.9 mg/kg bw/d**
MOS		**=**	**21**

3.3.14. Discussion

Physico-chemical 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 name *Tetrabromophenol Blue* of the test material is misleading, since the main component is an *octabromo*-derivative (42%, quantitative HPLC analysis using standard reference material) and no tetrabromo-homologue is present. This material is a mixture of 3 substances: an *octabromo*-derivative (42%), two heptabromo-homologues (< 46% HPLC peak area) and a hexabromo-homologue (< 13% HPLC peak area). The tetrabromo-homologue is a well known pH indicator named *Bromophenol Blue* while the respective non-sulfonated derivative is also a well known compound which is named *Tetrabromophenolphthalein*. Using the same terminology, the correct name for the octabromo-homologue is *Tetrabromo Bromophenol Blue* (instead of Tetrabromophenol Blue).

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. In addition, there is no information on whether other batches of Tetrabromophenol Blue, used in hair dye formulations, meet the same mixture specifications as the one used for the toxicity testing described in this Opinion. The stability of Tetrabromophenol Blue has not tested under the conditions of use of an oxidative hair dye formulation. The stability test of Tetrabromophenol Blue in typical hair dye formulations has not been performed adequately.

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 sensitizing risk to consumers when used as intended.

Dermal absorption

In an *in vitro* dermal absorption study with a non-oxidative hair dye formulation, only 5 chambers were used and the dose of dye was too high. Therefore, the mean+2 SD were used in calculating the MOS. This equates to 2.71+ (2 x 0.89) = 4.49 µg/cm^2 of Tetrabromophenol Blue (1.62% of the applied dose). No adequate study is available to assess dermal absorption under oxidative conditions.

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.

General toxicity
No data on acute toxicity were submitted
The study authors established a NOAEL of 10 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.

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

4. CONCLUSION

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 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.

5. MINORITY OPINION

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