OPINION OF THE SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD PRODUCTS INTENDED FOR CONSUMERS

CONCERNING

TETRABROMOPHENOL BLUE

COLIPA n° : /

Adopted by the SCCNFP during the 28th plenary meeting
of 25 May 2004

1. Terms of Reference

1.1 Context of the question

The adaptation to technical progress of the Annexes to Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products.

1.2 Request to the SCCNFP

The SCCNFP is requested to answer the following questions :

* Is Tetrabromophenol blue safe for use as a hair dye ingredient?

* Does the SCCNFP propose any restrictions or conditions for its use in cosmetic products?

1.3 Statement on the toxicological evaluation

The SCCNFP is the scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food products intended for consumers.

The Commission's general policy regarding research on animals supports the development of alternative methods to replace or to reduce animal testing when possible. In this context, the SCCNFP has a specific working group on alternatives to animal testing which, in co-operation with other Commission services such as ECVAM (European Centre for Validation of Alternative Methods), evaluates these methods.
The extent to which these validated methods are applicable to cosmetic products and its ingredients is a matter of the SCCNFP.

SCCNFP opinions include evaluations of experiments using laboratory animals; such tests are conducted in accordance with all legal provisions and preferably under chemical law regulations. Only in cases where no alternative method is available will such tests be evaluated and the resulting data accepted, in order to meet the fundamental requirements of the protection of consumer health.

2. Toxicological Evaluation and Characterisation

2.1. General

2.1.1. Primary name

Tetrabromophenol Blue*

* The name Tetrabromophenol Blue is used as synonym of Octabromosulfonphthalein although it does not contain any Tetrabromo-homologue. See section 2.1.7. and General Comments below.

2.1.2. Chemical names

This hair dye is a mixture of octa-, hepta- and hexa-bromo phenolsulfonphthaleins (see section 2.1.7. 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)

Note
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) should be submitted for clarification.

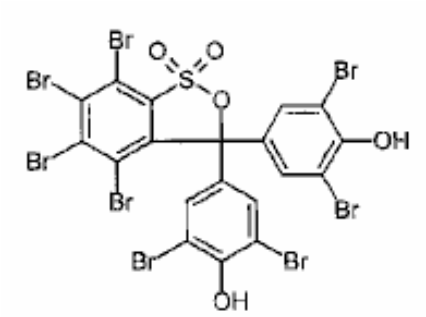
2.1.3. Trade names and abbreviations

COLIPA n° : /
Trade name : Gardex Royal Blue (Wella), Royal Blue (Wella)

2.1.4. CAS / EINECS / COLOUR INDEX number

CAS : 4430-25-5
EINECS : 224-622-9
Colour Index : /

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : $C_{19}H_6Br_8O_5S$
Mol weight : 985.59

2.1.7. Purity, composition and substance codes

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 %	6.5 %	4.6 %
Hexabromo-homologue	12.9 %	10.7 %	6.8 %
Sum of octa-, hepta- and hexa-bromo (corrected values)*.....	**96.6 % (96.7 %)***	**97.2 % (97.5 %)***	**98.7 % (98.8 %)***
UV-absorbing impurities (number) " " " (%)	(13) 3.4%	(8) 2.8%	(7) 1.3%

* Corrected values are reported, but without any information about the correction method.

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). Therefore, the purity 96.7-98.8 % mentioned in all toxicological studies carried out with the batch TBFB3/02/30 should be corrected accordingly, and the general analytical data for this hair dye should be expressed as follows:

Purity (as sum of Octa-, Hepta, and Hexabromo phenolsulfonephthaleins) :
< 95 % (96.6 %for the batch TBFB3/02/30)

Loss on drying	:	< 1%
Water content	:	< 1%
Ash content	:	< 2% (sulfated)

Potential impurities
7-13 UV-absorbing materials of unknown identity : 2-4 %

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.

2.1.8. Physical properties

Subst. Code	:	A 015828
Batches used	:	TBFB3/02/30
Appearance	:	yellowish grey powder
Melting point	:	204°C (decomposition)
Boiling point	:	/
Density	:	/
Rel. vap. dens.	:	/
Vapour Press.	:	/
Log P_{OW}	:	5.98 +/- 0.20 (calculated ACD for pure Tetrabromophenol blue-most acidic)
pKa	:	/

2.1.9. Solubility

In water (pH 3.6)	:	0.7 weight %
In acetone / water 1:1 (pH 2.6)	:	0.9 weight %
In DMSO	:	> 10 weight %

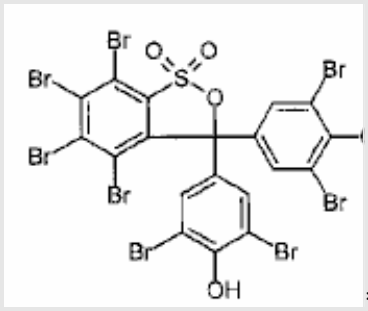
2.1.10 Stability

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

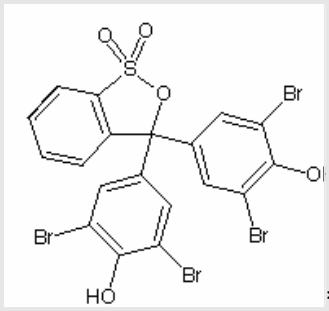
The good long-term stability of the dye stuff in a common market formulation (90% recovery) is also reported on the basis of a single determination of the dye content after storage for 10 months at 25°C and comparison with the "theoretical content". This is not an acceptable stability test.

General comments on analytical and physico-chemical characterisation

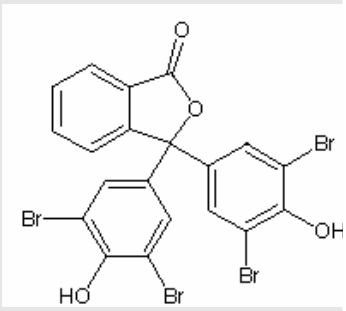
* This name ***Tetrabromophenol Blue*** is quite controversial and confusing, since the main component is an ***octabromo***-derivative and no tetrabromo-homologue occurs. The tetrabromo-homologue is a well known pH indicator named ***Bromphenol Blue***, while the respective non-sulfonated derivative, 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 TetrabromophenolBlue).



*** Tetrabromophenol Blue**



*** Bromphenol Blue**



*** Tetrabromophenolphthalein**

* The information provided on the compound is incomplete concerning the chemical identity of the 7-13 organic impurities identifiable by HPLC.
* The pKa values of the ionisable groups are not reported.
* Calculated values can not be accepted as estimates of the true physical constants without justification, indicating that the reported values are realistic. Since log P_{ow} is known to strongly depend on the pH, the reported value 5.98 +/- 0.20, followed by the ambiguous specification "for pure Tetrabromophenol blue-most acidic" corresponding to a strongly acidic pH, is not related to physiological conditions and to the pH conditions of the percutaneous absorption studies.

2.2. Function and uses

Tetrabromophenol Blue is intended for use in oxidative hair dye formulations as a direct dye at a maximum concentration of 0.2%.

Note
It is mentioned that the formulation is mixed with 1.5 volumes of oxidizing solution, but it is not clear if 0.2 % is final concentration or before mixing with the oxidant.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

No data

2.3.2. Acute dermal toxicity

No data

2.3.3. Acute inhalation toxicity

No data

2.3.4. Repeated dose oral toxicity

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 number	:	TBFB3/02/30
Purity	:	96.7-98.8 %
Dose levels	:	0, 3, 10 and 100 mg/kg bw/day by 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 examination 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

2.3.5 Repeated dose dermal toxicity

No data

2.3.6. Repeated dose inhalation toxicity

No data

2.3.7. Subchronic oral toxicity

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 number	:	TBFB3/02/30
Purity	:	96.7-98.8 %
Dose levels	:	0, 3, 10 and 100 mg/kg bw/day by 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. Once daily, clinical observations were made. 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. Blue discolouration of the faeces and of the fur was seen in all dose groups. No relevant substance-related clinical findings were noted, but alopecia in all dose groups. Motor activity, body weight gain and food consumption revealed no treatment-related effects. 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.

Comment
According to Ref. 5 a 2 % solution of Tetrabromophenol Blue has not be classified as eye irritating. For the highest dose in this study 100 mg per kg bw was administered in 5 ml volume per kg. This may be approximately a 2 % solution.

Ref.: 2

2.3.8. Sub-chronic dermal toxicity

No data

2.3.9. Sub-chronic inhalation toxicity

No data

2.3.10. Chronic toxicity

No data

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

Guideline	:	OECD 404 (1992)
Species/strain	:	Albino Rabbit, New Zealand White, (SPF-Quality)
Size	:	3 (same sex/male)
Test item	:	Tetrabromophenol Blue
Batch no.	:	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.: 3

2.4.2. Irritation (mucous membranes)

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 no.	:	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.: 4

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 no.	:	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 and 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.: 5

2.5. Sensitisation

Local Lymph Node Assay

Guideline	:	OECD 429 (2000)
Species/strain	:	Mouse: CBA/J
Size	:	5 females per concentration
Test item	:	Tetrabromophenol Blue
Batch no.	:	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 ^{3}H-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. The experimental conditions used in this study have been stricter than use conditions. It is therefore concluded that Tetrabromophenol Blue does not pose a sensitizing risk to consumers when used as intended.

Ref.: 6

2.6. Reproductive Toxicity / Teratogenicity

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 number	:	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.: 7

2.7. Toxicokinetics (incl. Percutaneous Absorption)

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 no.	:	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 cm^2; i.e. 1.67 mg Tetrabromophenol Blue /cm^2
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/cm^2 per fraction or 339 ng/cm^2 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" 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.: 8

2.8. Mutagenicity/Genotoxicity

2.8.1 Mutagenicity/Genotoxicity *in vitro*

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 number	:	TBFB 3/02/30
Lot number	:	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 any 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.: 9

***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 number	:	TBFB3/02/30
Lot number	:	/
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.
Because of the toxicity observed in some doses, the results of this assay must be confirmed or rejected by a second test.
The study is inadequate for the evaluation. It may indicate some possible mutagenic/clastogenic activity of the compound.

Ref.: 10

2.8.2 Mutagenicity/Genotoxicity *in vivo*

Mammalian Erythrocyte Micronucleus Test

Guideline	:	OECD 474 (July 1997)
Species/strain	:	NMRI mice
Test substance	:	Royal Blue WR 802175
Batch number	:	TBFB3/02/30
Lot number	:	/
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.: 11

A published paper reports a series of negative results obtained in other experiments conducted with this substance by another testing laboratory: the enclosed results cannot be evaluated.

Ref.: 12

2.9. Carcinogenicity

No data

2.10. Special investigations

No data

2.11. Safety evaluation

Not applicable

2.12. Conclusions

This hair dye is a mixture of octa-, hepta- and hexa-bromo phenolsulphonphthaleins.

No data on acute toxicity was submitted.
However, no treatment-related mortality occurred in a repeated dose and a sub-chronic toxicity study. Missing values in male control organ and body weights make it difficult to evaluate possible substance-related changes in absolute and relative body weights (repeated dose study). Blue discolouration of the faeces and other body parts was seen in the dose groups 10 and 100 mg/kg bw/day (repeated dose) and in all doses (sub-chronic). No relevant substance-related clinical findings were noted. The NOAEL in the subchronic toxicity study was set at 3 mg/kg bw/day. The NOAEL for maternal toxicity was set at 50 mg/kg bw/day, the NOAEL for teratogenicity was 5 mg/kg bw/day, for embryotoxicity a NOAEL could not be established.

Tetrabromophenol Blue is not a skin irritant. Although the neat substance poses a risk of serious damage to the eyes, no effect was noted at the proposed use concentration of 0.2%. Tetrabromophenol Blue is not a sensitiser when used as intended.

The percutaneous absorption study is inadequate.

Tetrabromophenol has been tested for the induction of gene mutations in bacterial and in mammalian cells. The test item is non mutagenic on bacterial cells. The *in vitro* mammalian cell test is inadequate. Tetrabromophenol has been found negative for the induction of MN in mice.

2.13. References

1. Identity, purity and stability test of Gardex Royal Blue (Tetrabromophenol Blue); Wella AG, D-64274 Darmstadt, Germany; Study No.: G 2002/005; September 23, 2002
2. 90-day oral toxicity study with Royal Blue/802175/Tetrabromophenol Blue by daily gavage in the rat; Notox B.V., NL-5231-DD's-Hertogenbosch, The Netherlands; Project 356219; Draft of April 4, 2003

2b. 28-day oral dose range finding; Notox B.V., NL-5231-DD's-Hertogenbosch, The Netherlands; Project 356208

3. Primary skin irritiation/corrosion study with Royal Blue/802175/Tetrabromophenol Blue in the rabbit (4-hour semi-occlusive application); Notox B.V., NL-5231-DD's-Hertogenbosch, The Netherlands; Project 356592; November 21, 2003

4. Acute eye irritation/corrosion study with Royal Blue/802175/Tetrabromophenol Blue in the rabbit; Notox B.V., NL-5231-DD's-Hertogenbosch, The Netherlands; Project 356603; November 21, 2003
5. Acute eye irritation/corrosion study with Royal Blue/802175/Tetrabromophenol Blue in the rabbit; Notox B.V., NL-5231-DD's-Hertogenbosch, The Netherlands; Project 370878; Draft of April 17, 2003
6. 802175 (Royal Blue) – Local lymph node assay; MDS Pharma Services, F-69210 Saint Germain sur l'Arbresle, France; Study number 762/018 (2002)
7. Embryotoxicity and teratogenicity study with Royal Blue/802175/Tetrabromophenol-blue by oral gavage in female Wistar rats; Notox B.V., NL-5231-DD's-Hertogenbosch, The Netherlands; Project 35623 ; Draft of May 12, 2003
8. Cutaneous absorption of WR802175 in formulation through pig skin in vitro; Cosmital SA, CH-1723 Marly 1, Switzerland; Study number KP 075; August 19, 2002
9. Assessment of the potential mutagenicity of WR802175 in the Ames reversion assay with Salmonella typhimurium; Cosmital SA, CH-1723 Marly 1, Switzerland; Study number AT 779; August 26, 2002
10. Cell mutation assay at the thymidine kinase locus ($TK^{+/-}$) in mouse lymphoma L5178Y cells with Royal Blue WR 802175; RCC – CCR GmbH, D-64380 Rossdorf, Germany; Study number 749802; December 2, 2002
11. Micronucleus assay in bone marrow cells of the mouse with Royal Blue WR 802175; RCC – CCR GmbH, D-64380 Rossdorf, Germany; Study number 749801; January 8, 2003
12. George H.Y. Lin and David J. Brusick, Mutagenicity studies on two triphenylmethane dyes, bromophenol blue and tetrabromophenol blue; Journal of applied toxicology, Vol. 12(4), 267-274 (1992).
13. Stability in formulation of Tetrabromphenol Blue; September 22, 2003. Wella AG; D-64274 Darmstadt

3. Opinion of the SCCNFP

The SCCNFP is of the opinion 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.

4. Other considerations

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5. Minority opinions

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