EUROPEAN COMMISSION

Directorate-General for Health & Consumers

Scientific Committee on Consumer Safety

SCCS

OPINION ON

Acid Black 1

COLIPA n° B15

Scientific Committees
on consumer safety
on emerging and newly identified health risks
on health and environmental risks

The SCCS adopted this opinion at its 6th plenary meeting
of 23 March 2010

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 Evaluation Agency (EMEA), 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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http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

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This opinion has been subject to a commenting period of four weeks after its initial publication. All 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.

Keywords: SCCS, scientific opinion, hair dye, B15, Acid Black 1, 1064-48-8, EC 213-903-1, directive 76/768/EEC

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on Acid Black 1, 23 March 2010

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1. BACKGROUND

A dossier for Acid Black 1 (1-naphthol-3,6-disulfonic acid, 8-amino-7-(p-nitrophenylazo)-2-phenylazo disodium salt) (B015) was delivered to the Scientific Committee on Cosmetology (SCC) in March 1984 by COLIPA [1]. No opinion could be given at that time due to lack of data.

Acid Black 1 is identical with CI 20470 also used as a colouring agent allowed for use in cosmetic products intended to come into contact only briefly with the skin.

Submission II of Acid Black 1 was submitted by COLIPA in July 2005. Submission II 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://ec.europa.eu/enterprise/sectors/cosmetics/files/doc/hairdyestrategyinternet_en.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 Acid Black 1 safe for use in non-oxidative hair dye formulations with a concentration of maximum 0.5% in the finished product taken into account the scientific data provided?*

2. *Does the SCCS recommend any restrictions with regard to the use of Acid Black 1 in non-oxidative hair dye formulations e.g. max concentration in the finish cosmetic product, dilution ratio with hydrogen peroxide, warning?*

[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

Acid Black 1 (INCI name)

3.1.1.2. Chemical names

1-Naphthol-3,6-disulfonic acid, 8-amino-7-(p-nitrophenylazo)-2-phenylazo- disodium salt
2,7-Naphthalenedisulfonic acid, 4-amino-5-hydroxy-3-[(4-nitrophenyl)azo]-6-(phenylazo)-, disodium salt

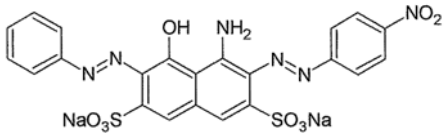
3.1.1.3. Trade names and abbreviations

Black n° 401
Naphthalene Black 10B
Amido Black 10B
CI 20 470
COLIPA n° B15

3.1.1.4. CAS / EC number

CAS: 1064-48-8 (disodium salt)
EC: 213-903-1 (disodium salt)

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_{22}H_{14}N_6Na_2O_9S_2$

3.1.2. Physical form

Dark red to black powder

3.1.3. Molecular weight

Molecular weight: 616.49 g/mol

3.1.4. Purity, composition and substance codes

Chemical characterisation by NMR, IR and LC-MS. UV spectrum 200-800 nm and a LC chromatogram was provided.

Ref.: 12

General description

Overall purity (NMR): > 85%
Water content: < 10%
Ash Content: < 5%
4-Aminoazobenzene: < 130 ppb
p-Nitroaniline: < 8 ppm
Aniline: < 100 ppm
Heavy metal content: < 5 ppm

Batch	9405	M90114	J 00927
Purity	94.8%	97.1%	94.2 %
Benzene	360 ppm	400 ppm	60 ppm
p-nitroaniline	14.74 ppm	45.89 ppm	7.98 ppm
4-Aminoazobenzene	8.18 ppm	0.88 ppm	0.13 ppm
4-Aminobiphenyl	0.31 ppm	0.068 ppm	0.062 ppm
Aniline	9.39 ppm	10.6 ppm	3.0 ppm

Comments

1. CMR classification of the impurities in EU:
   Benzene: carcinogenic, category 1 (GHS: Carc. 1A); mutagenic, category 2 (GHS: Muta. 1B)
   4-Aminoazobenzene: carcinogenic, category 2 (GHS: Carc. 1B)
   4-Aminobiphenyl: carcinogenic, category 1 (GHS: Carc. 1A)
   Aniline: carcinogenic, category 3 (GHS: Carc. 2); mutagenic, category 3 (GHS: Muta. 2)
   (GHS: Globally Harmonised System)
   p-nitroaniline: MAK commission; Carcinogenic, category 3A (Germany)
   These CMR substances will not pose any relevant cancer risk at the levels given in the table above.
2. The method used for the determination of the purity of the three batches of Acid Black 1 was not described.
3. Approximately 3-6% of the impurity(ies) in the various batches of Acid Black 1 have not been characterised.
4. 4-aminobiphenyl, found up to 310 ppb in the three batches of Acid Black 1, is not listed in the general description. In one batch, the amount of 4-aminobiphenyl is relatively high (0.31 ppm)
5. The amount of 4-Aminoazobenzene in two of the batches of Acid Black 1 is higher than that given in the general description of Acid Black 1.
6. The amount of aniline given in the general description is much higher than in the three batches of Acid Black 1.
7. The amount of p-nitroaniline in the three batches of Acid Black 1 is higher than that given in the general description.

3.1.5. Impurities / accompanying contaminants

See point 3.1.4. Purity, composition and substance codes

3.1.6. Solubility

Water: > 3%
DMSO: > 10%
Ethanol: < 0.2%

Comment
The water solubility was not determined by the EC method.

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: *- 4.53 (dianionic form) (calculated)*
Log P_{ow}: 1.2 (determined by EEC method) Ref. 10

Comment
The calculated Log P_{ow} value is significantly different from the value experimentally determined.

3.1.8. Additional physical and chemical specifications

Melting point:	> 350 °C
Boiling point:	/
Flash point:	/
Vapour pressure:	/
Density:	/
Viscosity:	/
pKa:	/
Refractive index:	/
UV_Vis spectrum (200-800 nm)	Absorption at 323 nm and 620 nm (λ_{max}) – similar in acidic, basic and neutral solution

3.1.9. Homogeneity and Stability

Acid Black 1 is stable under normal laboratory conditions.
0.5 mg/ml, 3.0 mg/ml and 18.0 mg/ml solutions of Acid Black 1 in distilled water containing 1% carboxymethylcellulose (used in 13 week oral toxicity study), stored at 2 – 8 °C for 7 days were stable (variation up to ± 12% of the nominal concentration). These solutions were also homogeneous.

It is described in the dossier that solutions of Acid Black 1 in DMSO were stable for 48 hours under laboratory conditions; the dye was stable under oxidative conditions.

Comment
No documentation is provided for the stability of Acid Black 1 in DMSO solutions used for the LLNA assay.

General Comments to physico-chemical characterisation

- The calculated Log P_{ow} is significantly different from the measured value.
- The P_{ow} strongly depends on the pH, especially for ionisable molecules, zwitterions etc. Therefore, a single calculated value of Log P_{ow}, usually without any reference to the respective pH, cannot be correlated to physiological conditions and to the pH conditions of the percutaneous absorption studies.
- All batches Acid Black 1 contain 3-6% unidentified impurities.
- Acid Black 1 contains several carcinogenic and/or mutagenic impurities. The concentration of these impurities should be kept at a minimum.
- The stability of Acid Black 1 in DMSO is not documented.
- The stability of Acid Black 1 in a typical formulation is not reported.

3.2. Function and uses

Acid Black 1 is used as a direct hair colouring agent up to an on-head concentration of 0.5% in non-oxidative hair dye formulations.

Acid Black 1 is listed in Annex IV – List of colouring agents allowed for use in cosmetic products – to Directive 76/768/EEC on cosmetic products, column 4: colouring agents allowed exclusively in cosmetic products intended to come into contact only briefly with the skin.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline: /
Species/strain: rat, Wistar II
Group size: 50 females, 10 per group
Test substance: Acid Black 10B
Batch: /
Purity: /
Vehicle: water
Dose levels: 3.1, 10, 15, 16 and 18 g/kg bw, 30% concentration
Route: stomach tube
GLP statement: /
Study period: September 1974

This study was provided as a 2004 certified translation with the comment that 'the raw data documentation did not correspond to current standards.
The doses started at higher concentration than the current guideline. Clinical signs noted 1 h after dosing, were piloerection, diarrhoea, prone/lateral position.

Ref.: 1

Comment
The results as presented are valueless as the table provided has transcriptional errors in it.

Guideline: /
Species/strain: mouse, CF1 (Winkelmann)
Group size: 10 males
Test substance: Acid Black n° 1
Batch: /
Purity: /
Vehicle: bi-distilled water
Dose levels: 5000 mg/kg bw
Dose volume: 20 ml/kg bw
Route: stomach tube
Administration: single application
GLP statement: /
Study period: February 1983

This study was provided as a 2005 certified translation. The lethal dose (LD_{50}) was > 5,000 mg/kg body weight. There were no symptoms of intoxication.

Ref.: 2

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

Guideline: /
Species/strain: albino rabbit, New Zealand
Group size: 6 males
Test substance: Acid Black 1
Purity: /
Batch: /
Vehicle: tap water
Dose level: 10% (w/v) Acid Black 1 in tap water
Dose volume: 0.5 ml
GLP: /
Study period: July 1982

The rabbits were clipped on the back 24 hours prior to the test. 10% aqueous solution/suspension (pH adjusted with ammonia to pH 8-10) of the dye was applied on the skin and covered with occlusive patches, which were secured to the skin. At the end of a 2 hours exposure, the patches were removed and skin reactions were examined immediately and after 24, 48 and 72 hours of patch removal.

Results
None of the 6 animals showed signs of irritation, neither after immediate reading nor after 72 hours.

Conclusion
Under the conditions in this experiment, the test substance (10% Acid Black 1) is not irritant to rabbit skin.

Ref.: 4

Comments
It is not reported if any staining of the skin affected the evaluation. No explanation was given for the pH adjustment. The batch and the purity of the test material were not identified, but it was mentioned that the sponsor of the study supplied the test material as 100% pure.

3.3.2.2. Mucous membrane irritation

Guideline: /
Species/strain: albino rabbit, New Zealand
Group size: 6 males
Test substance: Acid Black 1
Purity: /
Batch: /
Vehicle: tap water
Dose level: 5% (w/v) Acid Black 1 in tap water
Dosing volume: 0.1 ml

GLP: /
Study period: July 1982

On test day 1, an aliquot of 0.1ml of 5% aqueous solution/suspension (pH adjusted with ammonia to pH 8 - 10) was applied in the conjunctival sac of the right eye of each animal. The left eye remained untreated and served as reference control. Rinsing of the eyes was not performed.

Scoring of irritation based on the Draize method was performed 1, 24, 48 and 72 hours after single application.

Results
No irritation reactions of cornea, iris and conjunctiva were observed.

Conclusion
Acid Black 1 at 5% (w/v) concentration is not irritating to eye.

Ref.: 3

Comment
No explanation was given for the pH adjustment. The batch and the purity of the test material were not identified, but it was mentioned that the sponsor of the study supplied the test material as 100% pure.

3.3.3. Skin sensitisation

Local Lymph Node Assay (LLNA)

Guideline: OECD 429 (2002)
Species/strain: mouse, CBA/CaOlaHsd (nulliparous and non-pregnant)
Group size: 20 females (4 animals per group)
Test substance: Acid Black 1
Batch: J00927
Purity: 94.2%
Vehicle: DMSO
Concentration: 1, 5, 10 and 20% (w/v) in DMSO
Positive control: α-hexylcinnamaldehyde in acetone:olive oil, 4:1 (v/v) (October 2004)
GLP: in compliance
Study period: 19 – 25 January 2005

In a non-GLP pre-test in two mice, 2.5, 5.0, 10.0 and 20.0% (w/v) suspension of Acid Black 1 in DMSO were tested on one ear each. Due to the intense black colour of the test material, local irritation reactions could not be detected. No swelling of the ears was observed. The results of the pre-test indicated that the final test can be performed at the highest test concentration (20%).

In the final test, four groups each of four female mice were treated by 25 µl topical application of 1% (w/v), 5% (w/v), 10% (w/v) and 20% (w/v) Acid Black 1 in DMSO at the dorsum of each ear lobe (both left and right) on three consecutive days. In addition a control group of four female mice was similarly treated with 25 µl vehicle. Five days after the first application, ^{3}H-methyl thymidine was intravenously injected into a tail vein. 5 hours later mice were sacrified by intraperitoneal injection of Na-thiopental and the draining auricular lymph nodes taken and pooled for each experimental group. Single cell suspensions of pooled lymph nodes were prepared. Cells were washed with PBS and precipitated with 5% trichloro-acetic acid (TCA). 18 hours later the pellets were resuspended in TCA and transferred into the scintillation cocktail. The proliferation capacity of lymph node cells was determined by the incorporation of ^{3}H-methyl thymidine.

Data were provided from a positive control study, performed in October 2004, with α-hexylcinnamaldehyde in acetone:olive oil 4:1 (v/v) using CBA/CaOlaHsd mice.

Results
No signs of local toxicity at the ears of animals and no systemic toxicity findings were observed during the study period. Due to the intense black colour of the test material, local irritation reactions such as ear redness could not be detected. However, no swelling of the ears was observed.

The stimulation indices of 2.4, 4.6, 5.9 and 7.4 were determined with the Acid Black 1 concentrations of 1, 5, 10 and 20% (w/v) in DMSO, respectively.

Concentration	Stimulation Index
Test item	
1%	2.4
5%	4.6
10%	5.9
20%	7.4
α-hexylcinnamaldehyde	
5%	2.0
10%	3.0
25%	4.9

Conclusion
Acid Black 1 was found to be a moderate skin sensitiser An EC3 value of 2.1% (w/v) was derived.

Ref.: 19

Comment
According to the grading scheme used by the SCCS (SCCP/0919/05), Acid Black 1 should be considered as a moderate sensitiser.

3.3.4. Dermal / percutaneous absorption

Guideline:	OECD 428 (draft, 2000)
Tissue:	split-thickness pig skin, 200 µm
Group size:	7 membranes from 3 donors
Diffusion cells:	flow-through diffusion cell, 0.64 cm², 31 ± 1 °C
Skin integrity:	permeability coefficient (Kp) < 2.5 x 10^{-3} cm/h, using tritium water
Test substance:	Acid Black 1 [Nitroaniline-ring-^{14}C] Acid Black 1; 2646 kBq/mg (71.5 µCi//mg)
Batch:	9405 (Acid Black 1) KL/141 (radio-labelled Acid Black 1)
Purity:	94.8% (Acid Black 1) 98.74% (radio-labelled Acid Black 1)
Test item:	Commercial product Elumen Hair Color Clear formulation containing 0.5% (w/w) [^{14}C] Acid Black 1
Doses:	95 µl (0.70 mg Acid Black 1/cm²)
Receptor fluid:	physiological saline, flow rate 3 ml/h
Solubility receptor fluid:	solubility in water > 3%
Stability:	stable in aqueous solution containing 1% carboxymethylcellulose
Method of Analysis:	Liquid scintillation counting
GLP:	in compliance
Study period:	20 - 27 October 2003

Split thickness skin membranes (thickness 200 µm) originating from frozen pig ear skin were used. The integrity of the skin membranes was determined by the permeability testing

using tritiated water. A 95 µl aliquot of the formulation Elumen Hair Color containing 0.5% (w/w) ^{14}C-Acid Black was applied manually to each skin membrane. Thus a dose level of approximately 0.70 mg Acid Black 1/cm^2 was applied to each skin membrane. The formulation was applied to the skin membranes for 30 minutes and then removed from skin with shampoo solution (three times and once with tap water). Following the washing procedure the donor chambers were filled with 1ml of saline. The perfusates were collected at ambient temperature in time intervals as follows:

0-4 hours: 0.5 hour interval (8 intervals)
4-8 hours: 1 hour interval (4 intervals)
8-48 hours: 2 hours interval (20 intervals)

48 hours after application, the perfusate sampling was terminated. The epidermis was separated from the dermis by tape strip method (18 tapes), the amount of dye found in the upper skin is considered not to have passed the skin. The consecutive strippings were combined to 3 fractions, 6 tape strips each. The remaining skin membranes after stripping were digested in tissue solubilizer reactivity was determined by LSC and considered as penetrated.

Results
93.8 ± 3.3% of the applied dose could be washed off from the skin membranes. Only 4% of the dose remained on the skin membrane after the washing procedure. A significant part of this radioactivity was found in physiological saline (2.3 ± 0.5%), which was applied to the skin after washing. The rest of the radioactivity (1.8%) was found in the stratum corneum of the skin membrane: tape strip I = 1.53 ± 0.56%, tape strip II = 0.28 ± 0.29%, tape strip III = 0.04 ± 0.04%. In the lower levels of the skin, a maximum absorption of 0.03% (range <0.01- 0.03% = 0.081-0.243 µg/cm²) of the dose was found. Most of the determined values in the lower skin were below the limit of determination (LQ), i.e. 0.01µg/cm². Thus, the study authors considered the LQ as the amount present in the lower skin. The amount of Acid Black 1 penetrated through the skin as measured in the receptor fluid was 0.173 ± 0.117 µg/cm^2**.** The amounts of Acid Black 1 measured in the receptor fluid and in the lower skin were considered to be absorbed by the skin. This amount was considered bioavailable.

Conclusion
According to the study authors, the worst case consideration of penetrated test item was 0.173 µg/cm² (receptor fluid) + 0.081 µg/cm² (lower skin) = 0.254 µg/cm². The mean recovery of the test item was 98.12 %.

Cell number	**1**	**2**	**4**	**6**	**10**	**11**	**12**	**mean**	**SD**
Penetrated (µg/cm^2)	0.390	0.288	0.110	0.108	0.105	0.105	0.105	**0.173**	0.117
Penetrated (%)	0.048	0.032	0.010	0.009	0.006	0.006	0.006	**0.017**	0.017
Amount present in lower skin (%)	<0.01	0.03	<0.01	0.01	<0.01	<0.01	0.01	**0.03 (max) = 0.24** µg/cm^2	/
Recovery (%)	105	99	95	97	99	95	97	98	3

Ref.: 11

Comments
Too few chambers were used for the study. The volume of the test material applied on the skin, 95 µl/cm² (approximately 95 mg/cm²) is too high compared to the recommended dose of 20 mg/cm².
Due to these shortcomings, an absorption of mean +2SD will be used in the calculation of MoS. For the amount present in lower skin, a SD could not be determined, since in 4/7

samples the amount of Acid Black 1 was below the LQ. For this compartment, the A_{max} (0.03%, corresponding to 0.24 µg/cm²) will be used in the calculation. The worst case estimation for dermal absorption of Acid Black 1 in non-oxidative hair dye formulation is therefore 0.17 + 2 x 0.12 (mean + 2SD, receptor fluid) + 0.24 µg/cm² (A_{max} lower skin) = 0.65 µg/cm².

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (14 days) oral / dermal / inhalation toxicity

Guideline:	/
Species/strain:	rat, Wistar Hanlbm: WIST (SPF)
Group size:	40 (5 per sex and per dose)
Test substance:	Acid Black 1
Batch:	9405
Purity:	94.8%
Vehicle:	bi-distilled water containing 1% carboxymethylcellulose
Dose levels:	0, 100, 300 and 1000 mg/kg bw
Dose volume:	10 ml/kg bw
Route:	oral gavage
Administration:	daily for 14 days
GLP:	/
Study period:	10 – 30 December 1999

This was a range finding study for the 90 day study. Doses were prepared daily. Homogeneity and stability of Acid Black 1 in bi-distilled water containing 1% CMC was performed.
The animals were observed daily for clinical signs or death. Food consumption and bodyweight recorded once prior to treatment then weekly.
Haematology, clinical chemistry, and urinalysis were carried out prior to the animals being killed. On Day 15, animals were anesthetized, weighed, and killed. Macroscopic observations were recorded, selected organs weighed, and selected tissues collected and preserved.

Results
At the high dose, two males (Days 5 and 9) and four females (Days 2, 5 12 and 15) were found dead. Clinical observations included dark faeces at all doses; orange urine and sedation at the high and mid doses; and piloerection, salivation, half closed lids and emaciation at the high dose. Body weight, body weight gain and food consumption decreased only in the high dose group.
The spleen in animals from both the mid dose and high dose groups showed a dose related increase in weight. In the high dose males there was a significant increase of the relative organ/body weight ratio of the liver. In addition animals in the high dose groups showed black discolouration of the intestine, liver, kidney, spleen, stomach, urinary bladder and uterus.

Conclusion
Based on these results, the highest dose for the 90 day study was proposed to be 180 mg/kg/bw.

Ref.: 5

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

Guideline:	OECD 408 (1998)
Species/strain:	Wistar rat, Hanlbm:WIST (SPF)
Group size:	80 (10 per sex and dose)

Test substance:	Acid Black 1
Batch:	9405
Purity:	94.8%
Stability:	> 72h (in water)
Vehicle:	bi-distilled water containing 1% carboxymethylcellulose
Dose levels:	0, 5, 30 and 180 mg/kg bw
Dose volume:	10 ml/kg bw
Route:	oral gavage
Administration:	daily for at least 91 days
GLP:	in compliance
Study period:	7 February – 17 November 2000

The doses were prepared weekly. Concentration, homogeneity and stability (after 2 hours and 7 days) of doses were determined.
The following parameters were evaluated: clinical signs daily; functional observation tests in week 12/13; body weight and food consumption weekly; ophthalmoscopy at pre-test and in week 13; clinical pathology and macroscopy at termination; organ weights and histopathology on a selection of tissues. There were no recovery groups.

Results
There were no deaths. Body weight and food consumption, locomotor activity grip strength and opthalmoscopy were comparable with the controls.
Clinical signs were dark faeces, green urine and bedding stained orange in the mid and high dose groups.
Significant haematological changes (decreased red blood cell count, decreased haemoglobin, MCV, MCH) indicated haemolytic anaemia with compensatory reticulocytosis at all dose levels in males and in the high dose females. Increased methemoglobin levels were noted at all doses and were statistically significant in the mid and high dose groups. There was a statistically significant increase in Heinz bodies in the high dose groups.
Increased spleen weight and increased kidney organ/body weight ratio were observed in the high dose group. These correlated with the histology (extramedullary erythropoiesis in the spleen at all doses; increased lipofuscin in kidneys of mid and high dose females and high dose males). In addition, in the kidneys, a brownish pigment was recorded in tubular cells of all treated groups and minor morphological alterations like lymphoic foci, tubular mineralization in high dose males and pelvic mineralization in high dose females. In the liver, increased incidence and/or severity of and haemopoietic cell foci and haemosiderin deposits mainly in macrophages were seen in high dose females. These were considered to be adaptive in nature.

Conclusion
Due to the changes in haematological parameters and the correlated histological findings in the spleen, the NOEL/NOAEL of Acid Black 1 could not be established.

Ref.: 6

Guideline:	OECD 408 (1998)
Species/strain:	rat, HanBrl:WIST (SPF)
Group size:	100 (group 1 and 4: 15 per sex; group 2 and 3: 10 per sex)
Recovery group:	5 per sex and per dose (control and high dose group)
Test substance:	Acid Black 1
Batch:	9405
Purity:	94.8%
Stability:	> 72h (in vehicle)
Vehicle:	bi-distilled water containing 1% carboxymethylcellulose sodium salt
Dose levels:	0, 0.3, 1.5 and 5 mg/kg bw
Dose volume:	10 ml/kg bw
Route:	oral gavage

Administration: daily for 91 days
GLP: in compliance
Study period: 11 December 2003 – June 2004

The doses were prepared weekly. Concentration, homogeneity and stability (after 2 hours and 7 days) of doses were determined.
The following parameters were evaluated: clinical signs daily; functional observation tests in week 12/13; body weight and food consumption weekly; ophthalmoscopy at pre-test and in week 13; clinical pathology and macroscopy at termination; organ weights and histopathology of selected tissues. The recovery groups were kept for a further treatment-free 28 days.

Results
There were no adverse effects on mortality, absolute and relative food consumption, mean body weight and mean body weight gain, ophthalmology. Dark faeces were seen from week 2 up to the first week of recovery in the high dose group.
Haematological changes were thought to be adaptive by the authors as they were reversed in the recovery period and were within the historical control range. However, significantly decreased mean haemoglobin levels were seen at the mid and high dose in males, mean corpuscular haemoglobin was seen at all doses in males and in high dose females. Red cell distribution width was significantly reduced in mid and high dose males and high dose females. The reticulocyte maturity indices in the high dose males were shifted significantly to the high fluorescence reticulocytes by the end of the treatment phase but were within the historical control range. The study controls were considered by the authors to have abnormally low values skewing the clinical picture. Mid and high dose females had significantly increased eosinophil counts during the treatment period but returned to control levels during the recovery period. These were considered to be indicative of metabolic reactions rather than adverse changes.
Biochemical and urine parameters changes were seen in high dose females, but were minor and within the historical control values except triglycerides. The mean triglycerides were significantly lower and outside the lower historic control range. The levels rose during the recovery phase but not to levels comparable to the controls. The authors suggested that this was due to metabolic changes in the liver. Alpha-1-globulin levels were also significantly reduced at the high dose.
Absolute or relative organ weights were comparable with the controls. No substance related macroscopic or adverse microscopic findings were noted.

Conclusion
The study authors considered the NOAEL for Acid Black 1 to be 5 mg/kg/day on the basis of the minimal reduction of haemoglobin accompanied by left shifting of reticulocyte maturity indices without compensatory reticulocytosis.

Ref.: 7

Comment
Erythropoetic effects of Acid Black 1 suggesting the development of haemolytic anaemia were seen in both studies. Males seem to be more susceptible. The shift in the reticulocyte maturity indices combined with the alteration of the mean corpuscular haemoglobin at doses as low as 0.3 mg/kg bw/day in males and 5 mg/kg bw/day in females suggest compensatory reticulocytosis. Thus the SCCS considers that a NOEL could not be established and that the NOAEL is 0.3 mg/kg bw/day.

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial Reverse Mutation Assay, study 1

Guideline:	OECD 471 (1997)
Species/Strain:	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *Escherichia. coli* uvrA
Replicates:	triplicates in three independent experiments
Test substance:	Acid Black 1
Batch:	M90114
Purity:	> 85%
Vehicle:	de-ionised water
Concentration:	Experiment I and II: 42, 125, 417, 1250, 2500 and 5000 µg/plate, without and with S9-mix Experiment III: 666, 1000, 1666, 2500, 3333 and 5000 µg/plate, without S9-mix; TA98 and TA1537 only
Treatment:	pre-incubation method with 30 minutes pre-incubation and at least 48 h incubation, both without and with S9-mix
GLP:	in compliance
Study period:	31 May – 19 July 1999

Acid Black 1 was investigated for the induction of gene mutations in *Salmonella typhimurium* and *Escherichia coli* (Ames test). Liver S9 fraction from male Syrian golden hamsters was used as exogenous metabolic activation system. Test concentrations were based on the results of a pre-experiment with all strains; eight concentrations up to the prescribed maximum concentration of 5 mg/plate were tested for toxicity and mutation induction. The pre-experiment was reported as experiment I since criteria for a proper experiment were met. Toxicity was evaluated on the basis of a reduction in the number of spontaneous revertant colonies and/or a clearing of the bacterial background lawn. All experiments were performed with the pre-incubation method. Negative and positive controls were in accordance with the OECD guideline.

Results
In experiment II a slight toxic effect, evident as a reduction in the revertant colony numbers, was seen in strain TA1537 at 5000 µg/plate in the presence of S9-mix. A clearing of the background lawn was not observed in any of the experiments.
In experiment I without S9-mix a dose dependent increase in the number of revertants was observed in TA1537. However, this result could not be reproduced in experiments II and III. In all three experiments Acid Black 1 induced a dose-dependent increase in the number of revertants in strain TA98 without S9-mix.

Conclusion
The study authors concluded that under the experimental conditions used Acid Black 1 was mutagenic in the gene mutation tests in bacteria in TA98 in the absence of S9 metabolic activation.

Ref.: 13

Comment
The SCCS noted that, although the increases in TA98 were dose-dependent and more than two-fold, they were within the historical control range of the laboratory.

Bacterial Reverse Mutation Assay, study 2

Guideline:	OECD 471 (1997)
Species/Strain:	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2 uvrA

Replicates:	triplicates in two independent experiments
Test substance:	Acid Black 1
Batch:	9405
Purity:	94.8%
Vehicle:	de-ionised water
Concentration:	Experiment I and II: 33, 100, 333, 1000, 2500 and 5000 µg/plate, without and with S9-mix
Treatment:	pre-incubation method with 30 minutes pre-incubation and at least 48 h incubation, both without and with S9 mix
GLP:	in compliance
Study period:	12 - 21 January 2000

Acid Black 1 was investigated for the induction of gene mutations in *Salmonella typhimurium* and *Escherichia coli* (Ames test). Liver S9 fraction from male Syrian golden hamsters was used as exogenous metabolic activation system. Test concentrations were based on the results of a pre-experiment with all strains; eight concentrations up to the prescribed maximum concentration of 5 mg/plate were tested for toxicity and mutation induction. The pre-experiment was reported as experiment I since criteria for a proper experiment were met. Toxicity was evaluated on the basis of a reduction in the number of spontaneous revertant colonies and/or a clearing of the bacterial background lawn. All experiments were performed with the pre-incubation method. Negative and positive controls were in accordance with the OECD guideline.

Results
Neither relevant toxic effects nor a clearing of the background lawn were observed in any of the experiments. A biologically relevant increase in the number of revertants was not found in any of the tester strains following treatment with Acid Black 1 at any dose level neither in the presence or absence of metabolic activation.

Conclusion
Under the experimental conditions used Acid Black 1 was not mutagenic in this gene mutation tests in bacteria both in the absence and the presence of metabolic activation.

Ref.: 15

In vitro Mammalian Cell Gene Mutation Test, study 1

Guideline:	OECD 476 (1997)
Species/strain:	L5178Y $tk^{+/-}$ mouse lymphoma cells
Replicates:	two parallel cultures in two independent experiments
Test substance:	Acid Black 1
Batch:	M90114
Purity:	> 85%
Vehicle:	de-ionised water
Concentrations:	Experiment I: 75, 150, 300, 600 and 1200 µg/ml without and with S9-mix Experiment II: 156.3, 312.5, 625, 1250 and 2500 µg/ml without and with S9-mix
Treatment	Experiment I: 4 h both without and with S9 mix, expression period 72 h, selection growth 10-15 days. Experiment II: 24 h without S9 mix, expression period 72 h, selection growth 10-15 days
GLP:	in compliance
Study period:	28 June – 24 August 1999

Acid Black 1 was assayed for gene mutations at the *tk* locus of mouse lymphoma cells both in the absence and presence of S9 metabolic activation. Test concentrations were based on

the results of a pre-test on toxicity measuring relative survival growth. In the main test, cells were treated for 4 h (experiment I: both without and with S9-mix) or 24 h (experiment II: without S9-mix), followed by an expression period of 72 h to fix the DNA damage into a stable *tk* mutation and a selection growth 10-15 days. Liver S9-mix fraction from male Syrian golden hamsters was used as exogenous metabolic activation system. To discriminate between large (indicative for mutagenic effects) and small colonies (indicative for a clastogenic effect) colony sizing was performed. Toxicity was measured in the main experiments as percentage relative total growth of the treated cultures relative to the total growth of the solvent control cultures. Negative and positive controls were in accordance with the OECD guideline.

Results
In experiment I precipitation of Acid Black 1 occurred at 1200 µg/ml and above and in experiment II at 1250 and 2500 µg/ml both without and with S9-mix. The appropriate level of toxicity (10-20% survival after the highest dose) was not reached in both experiments without or with S9-mix pointing to insufficient exposure of the cells. A rather weak but reproducible increase in mutant frequency was observed at the highest dose tested in experiment I. The ratio of small *versus* large colonies was shifted towards the small colonies at concentrations producing a mutagenic effect, indicating a clastogenic rather then a mutagenic potential of Acid Black 1.
The authors stated that under the experimental conditions reported a weak mutagenic potential of Acid Black 1 can not be entirely excluded both without and with S9-mix at concentrations at or above the limit of solubility. On the other hand, they stated that there was also no indication of a clear mutagenic response leading to an equivocal outcome of the study.

Conclusion
According to the study authors and under the experimental conditions used, the results obtained with Acid Black 1 in this mouse lymphoma assay at the *tk* locus have to be considered as inconclusive.

Ref.: 14

Comment
The appropriate level of toxicity (10-20% survival after the highest dose) was not reached in both experiments without and with S9-mix which may point to insufficient exposure of the cells.

In vitro Mammalian Cell Gene Mutation Test, study 2

Guideline:	OECD 476 (1997)	
Species/strain:	L5178Y $tk^{+/-}$ mouse lymphoma cells	
Replicates:	two parallel cultures in two independent experiments	
Test substance:	Acid Black 1	
Batch:	9405	
Purity:	94.8%	
Vehicle:	de-ionised water	
Concentrations:	Experiment I:	156.3, 312.5, 625, 1250 and 2000 µg/ml without S9-mix 156.3, 312.5, 625, 1250 and 2500 µg/ml with S9-mix
	Experiment II:	312.5, 625, 1250, 2500 and 5000 µg/ml without S9-mix 156.3, 312.5, 625, 1250 and 2500 µg/ml with S9-mix
Treatment	Experiment I:	4 h both without and with S9-mix, expression period 72 h, selection growth 10-15 days.
	Experiment II:	4 h without S9-mix, expression period 72 h, selection growth 10-15 days 24 h both without and with S9-mix, expression period 72 h, selection growth 10-15 days.

GLP: in compliance
Study period: 14 December 1999 – 21 February 2000

Acid Black 1 was assayed for gene mutations at the *tk* locus of mouse lymphoma cells both in the absence and presence of S9 metabolic activation. Test concentrations were based on the results of a pre-test on toxicity measuring relative survival growth. In the main test, cells were treated for 4 h (experiment I: both without and with S9-mix; experiment II with S9-mix) or 24 h (experiment II: without S9-mix only), followed by an expression period of 72 h to fix the DNA damage into a stable *tk* mutation and a selection growth 10-15 days. Liver S9-mix fraction from male Syrian golden hamsters was used as exogenous metabolic activation system. To discriminate between large (indicative for mutagenic effects) and small colonies (indicative for a clastogenic effect) colony sizing was performed. Toxicity was measured in the main experiments as percentage relative total growth of the treated cultures relative to the total growth of the solvent control cultures. Negative and positive controls were in accordance with the OECD guideline.

Results
Precipitation of Acid Black 1 was only reported in the pre-experiment; at 5000 µg/ml after 4 h treatment both without and with S9-mix and at 2500 µg/ml and above after 24 h treatment without S9-mix only. The appropriate level of toxicity (10-20% survival after the highest dose) was mostly reached in experiments without S9-mix but not with S9-mix, pointing to insufficient exposure of the cells in the latter case. An increase in mutant frequency was observed at 2000 µg/ml without S9-mix and at 2500 µg/ml with S9-mix in culture 1 of experiment I. Since these results were not reproducible, they were considered not biologically relevant. In the other cultures of both experiments I and II a biologically relevant and dose dependent increase in the number mutant colonies was not observed independent of the test concentration or the presence or absence of S9-mix.

Conclusion
Under the experimental conditions used, Acid Black 1 was not mutagenic in this mouse lymphoma assay using the *tk* locus as reporter gene.

Ref.: 16

Comment
The appropriate level of toxicity (10-20% survival after the highest dose) was not reached in cultures treated in the presence of S9-mix which may point to insufficient exposure of the cells.

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

In vivo Mammalian Erythrocytes Micronucleus Test

Guideline: OECD 474 (1997)
Species/strain: mouse, NMRI
Group size: 5 mice/sex/group
Test substance: Acid Black 1
Batch: M90114
Purity: > 85%
Vehicle: deionised water
Dose level: 500, 1000 and 2000 mg/kg bw
Route: oral
Sacrifice times: 24 h after treatment for all concentrations, 48 h for the highest dose only.
GLP: in compliance
Study period: 11 April – 24 May 2000

Acid Black 1 has been investigated for the induction of micronuclei in bone marrow cells of mice. Test concentrations were based in a pre-experiment on acute toxicity at 1, 6, 24 and 48 h after start of treatment. In the main experiment mice were exposed orally to 0, 500, 1000 and 2000 mg Acid Black 1/kg bw. Bone marrow cells were collected 24 h or 48 h (high dose only) after dosing. Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and total erythrocytes (PCE/NCE). Bone marrow preparations were stained with May-Grünwald/Giemsa and examined microscopically for the PCE/NCE ratio and micronuclei. Negative and positive controls were in accordance with the OECD guideline.

Results
In the a pre-experiment on acute toxicity with exposure up to 2000 mg Acid Black 1/kg bw, reduction of spontaneous activity and coloured urine was found up to 24 h after administration. On the basis of these data the oral application of 2000 mg/kg bw was estimated to be suitable. The mean number of NCEs was not relevantly increased after treatment with Acid Black 1 as compared to the mean value in untreated concurrent control animals indicating that Acid Black 1 was not cytotoxic for bone marrow cells. Biologically availability of Acid Black 1 is certain since coloured urine of treated animals confirms systemic distribution. A biologically relevant and/or statistically significant increase in the number of cells with micronuclei was not found following treatment with Acid Black 1 at any dose or time point.

Conclusion
Under the experimental conditions used, Acid Black 1 is not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice.

Ref.: 17

In vivo unscheduled DNA synthesis (UDS) test

Guideline:	OECD 486 (1997)
Species/strain:	rat, Wistar Hanlbm: WIST (SPF)
Group size:	4 male rats/group
Test substance:	Acid Black 1
Batch:	M90114
Purity:	> 85%
Vehicle:	carboxymethylcellulose 1% w/v
Dose level:	500 and 2000 mg/kg bw
Route:	oral (gavage)
Sacrifice times:	2 h and 16 h after dosing
GLP:	in compliance
Study period:	3 May – 18 July 2000

Acid Black 1 was investigated for the induction of unscheduled DNA synthesis (UDS) in hepatocytes of rats. Test concentrations were based on results from a pre-experiment on acute toxicity evaluated at 1 and 48 h after start of treatment. In the main experiment mice were exposed orally to 0, 500 and 2000 mg Acid Black 1/kg bw. Hepatocytes for UDS analysis were collected at 2 h and 16 h after administration of Acid Black 1. All animals from each group were perfused with collagenase for the collection of hepatocytes and establishment of cultures. After attachment of the cultures they were labelled for 4 h with 5 µCi/ml ^{3}H-thymidine (specific activity 20 Ci/mmol). Evaluation of autoradiography was done after 12 days exposure.
UDS was measured by counting nuclear grains and subtracting the number of grains in a nuclear sized area adjacent to the nucleus; this value is referred to as net grain count.
Unscheduled synthesis was determined on 2 slides and 50 randomly selected hepatocytes per animal. Negative and positive controls were in accordance with the OECD guideline.

Results
In the a pre-experiment on acute toxicity with exposure up to 2000 mg Acid Black 1/kg bw, reduction of spontaneous activity and black coloured urine was found up to 24 h after administration. On the basis of these data the oral application of 2000 mg/kg bw was estimated to be suitable. The viability of the hepatocytes was not substantially affected due to the *in vivo* treatment with Acid Black 1; variations were within the historical control data. No dose level of Acid Black 1 induced UDS in the hepatocytes of treated animals as compared to concurrent control values.

Conclusion
Under the experimental conditions used Acid Black 1 did not induce unscheduled DNA synthesis and, consequently, is not genotoxic in rats in the *in vivo* UDS test.

Ref.: 18

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

Dose-range finding prenatal development toxicity study

Guideline:	/
Species/strain:	rat, Wist HanIbm: WIST (SPF-Quality)
Group size:	20 mated females, 5 per group
Test substance:	Acid Black 1
Batch:	9405
Purity:	94.8%
Vehicle:	bi-distilled water containing 1% carboxymethylcellulose sodium salt
Dose levels:	0, 80, 240 and 720 mg/kg bw
Dose volume:	10 ml/kg bw
Route:	oral, gavage
Administration:	once daily, 11 days (GD 6 – GD 17)
GLP statement:	/
Study period:	10 December 1999 - 13 January 2000

The doses were prepared daily. Concentration, homogeneity and stability (after preparation and 4 hours) of doses were determined.
Females were killed on GD 21 and the foetuses were removed by Caesarean section.
Maternal data recorded included deaths, clinical signs, food consumption, body weights, reproductive data and pathology findings and foetal data such as external examination, sex ratios and body weights.

Results
Maternal effects
There was one death in the high dose group on GD 17. Clinical signs in this high group started GD7 and were tremor, ruffled fur, dyspnea, swollen throat region, dark faeces and green urine. The dead animal felt cold to touch on GD 16. Dark faeces were noted in the low and mid dose group throughout the study.
Food consumption was decreased in the mid and high dose groups with concomitant

reduction in bodyweights (5% mid dose; 10% high dose). Dose related increases in spleen weight were recorded (low dose 33%, mid dose 64% and high dose 166%).
Reproductive effects
One low dose animal was not pregnant. Live foetuses were found in 4 mid dose and 2 high dose dams at the end of the study period. Pre-implantation losses were highest in the high dose group, since implantation occurs on GD6, this could be treatment related. Increased post-implantation losses were seen in the high (50%) and mid (11%) dose resulting in decreased foetal numbers and increased foetal deaths. Foetal resorptions were seen in one mid and 2 high dose dams.
Foetal findings
At the high dose, 13/14 live foetuses were male. The high post-implantation losses were possibly of female foetuses. Mean foetal weight significantly reduced (60%) compared with controls. 12/14 foetuses from the 2 litters had malformations such as hydrocephalus (11), micrognathia (9), oligodactylia (4) and syndactylia (3)
In the low and mid dose groups, no treatment related effects were seen. The foetal sex ratio was not affected.

Ref.: 8

Embryo-foetal development study

Guideline:	OECD 414 (1981)
Species/strain:	rat, Wist HanIbm: WIST (SPF-Quality)
Group size:	88 mated females, 22 per group
Test substance:	Acid Black 1
Batch:	9405
Purity:	94.8%
Vehicle:	bi-distilled water containing 1% carboxymethylcellulose sodium salt
Dose levels:	0, 5, 40 and 320 mg/kg bw
Dose volume:	10 ml/kg bw
Route:	oral, gavage
Administration:	once daily, from day 6 through day 17 *post coitum*
GLP statement:	in compliance
Study period:	7 February – 8 March 2000

Based on the previous range finding study, doses were lowered. The doses were prepared daily. Concentration, homogeneity and stability (after preparation and 4 hours) of doses were determined.
Females were killed on GD 21 and the foetuses were removed by Caesarean section.
Maternal data recorded included deaths, clinical signs, food consumption, body weights, reproductive data and pathology findings and foetal data such as external examination, sex ratios and body weights.

Results
Maternal effects
No deaths occurred during the study and only in the high dose group were all animals pregnant. One non-pregnant female was found in the other groups. At the high dose, clinical symptoms observed were dark faeces, discoloured urine and ruffled fur, combined with reduced food consumption and body weight gain. Compared with the controls, increased spleen weights (50%) were observed. In the low and mid dose groups, no treatment related effects were seen.
Reproductive effects
No treatment related effects were seen in any dose group.

Foetal findings
At the high dose, reduced foetal body weight was noted. Consequently, though statistically significant differences in skeletal ossification were seen, these were considered to be due to

delayed maturation and not evidence of a teratogenic potential. The frequency of other foetal abnormalities noted during external examination, visceral examination or skeletal examination were comparable with the controls at all doses.

Conclusion
Based on this study, the maternal and foetal No-Observable-Effect-Level (NOEL) of Acid Black 1 was considered to be 40 mg/kg bw/day.

Ref.: 9

3.3.9. Toxicokinetics

No data submitted

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

40 hairdressers with a known relevant allergy to 4-phenylenediamine (PPD) and or 2,5-diaminotoluene sulphate (DTS) and/or 2-nitro-4-phenylenediamine (ONPPD) and a history of hand eczema were examined.

Study Phase 1
Patch test concentration range finding study was performed among 10 volunteers. Acid Black 1 (0.2%, 0.6%; 2%) and a representative formulation containing 0.5% Acid Black 1 (1%, 3%, 10%) were prepared in aqua or petrolatum.

Study Phase 2
40 hairdressers with known allergy (to PPD and/or DTS and/or ONPPD) were tested with the highest non-irritating concentration of Acid Black 1.
The patch tests were performed with Van der Bend square chambers. Readings were made 2 and 3 days after application and if necessary on day 4.
The grading was performed according to the guidelines of the European Society of Contact Dermatitis as follows: -, +, +, ++, +++, IR (IR = irritant reaction).

Results
Study 1:
In 10 volunteers no irritant reactions were observed at any of the concentrations. Based on the result the highest test concentration was used for study phase II.

Study 2:
No positive reaction was observed on Acid Black 1 neither at 48 hours nor at 72 hours.

Conclusion
Acid Black 1 is not an irritant under the reported test conditions. In addition, there is no cross sensitisation to PPD, DTS and ONPPD.

Ref.: 21

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Acid Black 1

Absorption through the skin (mean +2SD)	**A (µg/cm^2)**	**=**	**0.65 µg/cm²**
Skin Area surface	**SAS (cm^2)**	**=**	**580 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**0.377 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.0063 mg/kg**
No observed adverse effect level (90-day, oral, rat)	**NOAEL**	**=**	**0.3 mg/kg bw/d**
Margin of Safety	**NOAEL / SED**	**=**	**48**

3.3.14. Discussion

Acid Black 1 is used as a direct hair colouring agent up to an on-head concentration of 0.5% in non-oxidative hair dye formulations.

Acid Black 1 is also listed in Annex IV – List of colouring agents allowed for use in cosmetic products – to Directive 76/768/EEC on cosmetic products, column 4: colouring agents allowed exclusively in cosmetic products intended to come into contact only briefly with the skin. Exposure of Acid Black 1 from such products is not considered in this risk assessment.

Physico-chemical properties
Besides several carcinogenic and/or mutagenic impurities, all batches of Acid Black 1 contain 3-6% unidentified impurities. The concentration of carcinogenic/mutagenic impurities should be kept at a minimum. Stability of Acid Black 1 in DMSO is not documented. Also, the stability of Acid Black 1 in a typical hair dye formulation is not reported.

Toxicity
Erythropoetic effects of Acid Black 1 were seen in two 90-day studies. Adult males seem to be more susceptible to developing haemolytic anaemia. The shift in the reticulocyte maturity indices combined with the alteration of the mean corpuscular haemoglobin at doses as low as 0.3 mg/kg bw/day in males and 5 mg/kg bw/day in females suggest compensatory reticulocytosis. This is borne out by the effects on the spleen. Thus the SCCS considers the NOAEL of the 90 oral studies in rats should be 0.3 mg/kg bw/day. The maternal and foetal No Observable Effect Level (NOEL) of Acid Black 1 was considered to be 40 mg/kg bw/day in a developmental toxicity study in rats.
No two generation reproduction study was submitted.

Skin/eye irritation and sensitisation
10% (w/v) Acid Black 1 in tap water was shown to be non-irritant to rabbit skin. Acid Black 1 at 5% (w/v) concentration was not irritating to eye. In a LLNA study, Acid Black 1 was found to be a moderate skin sensitiser.

Percutaneous absorption
Too few chambers were used for the study. The volume of the test material applied on the skin, 95 µl/cm² (approximately 95 mg/cm²), is too high compared to recommended dose of 20 mg/cm². As a worst case estimation, dermal absorption of Acid Black 1 in a non-oxidative hair dye formulation was considered as 0.65 µg/cm².

Mutagenicity/genotoxicity
Overall, the genotoxicity of Acid Black 1 is sufficiently investigated for the three endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy. Acid Black 1 did induce gene mutations in the first gene mutation tests in bacteria (batch M90114, purity >85%) but not in the second (batch 9405, purity >94.8%), although both tests were performed identically. In the same way in the first mouse lymphoma assay the results obtained with Acid Black 1 were inconclusive (batch M90114, purity >85%) whereas in the second experiment (batch 9405, purity >94.8%) negative results were found. The positive *in vitro* results for gene mutations were not confirmed in an *in vivo* unscheduled DNA synthesis test in rats.
The induction of chromosome aberrations by Acid Black 1 was only studied in an *in vivo* micronucleus test with mice; Acid Black 1 exposure did not result in an increase of cells with micronuclei in bone marrow cells.
As the positive *in vitro* results were not confirmed in an *in vivo* test, Acid Black 1 can be considered to have no *in vivo* genotoxic potential and additional tests are unnecessary.

Carcinogenicity
No data submitted on carcinogenicity

4. CONCLUSION

Based on the low margin of safety for the use as a direct hair colouring agent in non-oxidative hair dye formulations, the SCCS is of the opinion that Acid Black 1 at a maximum on-head concentration of 0.5% poses a risk to the health of the consumer.

Acid Black 1 (CI 20470) is listed in Annex IV, part 1 – List of colouring agents allowed for use in cosmetic products, field of application: 4 – colouring agents allowed exclusively in cosmetic products intended to come into contact only briefly with the skin.

The use of Acid Black 1 (CI 20470) as a cosmetic colorant should be evaluated.

5. MINORITY OPINION

Not applicable

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