Scientific Committee on Consumer Safety

SCCS

OPINION ON

2-Chloro-p-phenylenediamine

COLIPA n° A8

The SCCS adopted this opinion at its 3rd plenary meeting

on 19 September 2013

About the Scientific Committees

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

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

SCCS

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

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http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

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1. BACKGROUND

Submission I for 2-chloro-p-phenylenediamine was submitted in August 1980 by COLIPA[1] according to COLIPA.

The Scientific Committee on Cosmetology adopted an opinion for this substance at its 48th plenary meeting of 4 October 1991 with the conclusion: "'The SCC requires a percutaneous absorption study, a 90 days repeated oral administration study and a study to determine the induction of UDS or DNA damage in the liver of rats treated in vivo. Classification: C'"

The current submission II for 2-chloro-p-phenylenediamine was submitted in November 2006.
According to this submission 2-chloro-p-phenylenediamine and its sulphate and hydrochloride salts is used in oxidative hair dye formulations for eyebrows and eyelashes in a maximum concentration of 4.6%. Prior to use in is mixed with a 3% hydrogen peroxide solution in a ratio 1:1.

2. TERMS OF REFERENCE

1. *Does SCCS consider 2-chloro-p-phenylenediamine safe for use as oxidative hair dye for eyebrows and eyelashes in a concentration of maximum 4.6 %, taking into account the scientific data provided?*

2. *And/or does the SCCS recommend any further restrictions with regard to the use of 2-chloro-p-phenylenediamine in any hair dye formulations for eyebrows and eyelashes?*

[1] 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

2-Chloro-p-phenylenediamine

3.1.1.2. Chemical names

1,4-diamino-2-chlorbenzene
o-Chloro-p-phenylenediamine
2-Chloro-1,4-benzenediamine
3-Chloro-4-aminoaniline

3.1.1.3. Trade names and abbreviations

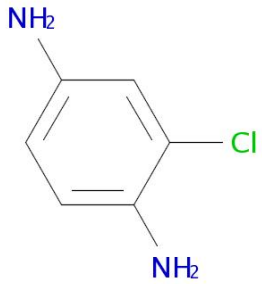
Ursol Brown O
CI 76065

3.1.1.4. CAS / EC number

CAS: 615-66-7 (free base)
EC: 210-441-2

CAS: 61702-44-1 (sulfate)
CAS: 615-46-3 (dihydrochloride)

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

$C_6H_7N_2Cl$

3.1.2. Physical form

Described as solid material in the dossier

3.1.3. Molecular weight

Free base: 140.6
Sulfate: 240.7
Dihydrochloride: 215.5

3.1.4. Purity, composition and substance codes

Free base
99%
Sulfate
>95%

Ash: 5% max

SCCS Comment
Analytical data on chemical characterisation and purity determination was not provided

3.1.5. Impurities / accompanying contaminants

Iron 100 ppm max

SCCS Comment
No information is provided on organic impurities in 2-Chloro-p-phenylenediamine

3.1.6. Solubility

Soluble in water and ethanol

SCCS Comment
It is not stated whether the solubility refers to free base or its salt. Water solubility has not been determined by EC method A.6

3.1.7. Partition coefficient (Log P_{ow})

/

3.1.8. Additional physical and chemical specifications

Melting point: 64°C (free base)
Boiling point: /
Flash point: /
Vapour pressure: /
Density: /
Viscosity: /
pKa: /
Refractive index: /
pH: /
UV_Vis spectrum : λmax 245 nm, 307 nm

3.1.9. Homogeneity and Stability

/

General Comments to physico-chemical characterisation

- No information was provided on qualitative chemical characterisation of 2-chloro-p-phenylenediamine and organic impurities in it. Analytical data on purity determination is not provided
- Solubility information on 2-chloro-p-phenylenediamine was insufficient. Water solubility should be determined by EC Method A.6
- Data on Log P_{ow} of 2-chloro-p-phenylenediamine was not submitted.
- Stability and homogeneity of 2-chloro-p-phenylenediamine in test solutions was not provided
- Stability of 2-chloro-p-phenylenediamine in typical hair dye formulation was not provided

3.2. Function and uses

2-Chloro-p-phenylenediamine is used at concentration of up to 4.6% (w/w) in oxidative hair dyes formulations; for eyebrows and eyelashes. Prior to use it is mixed with 3% hydrogen peroxide solution in a 1:1 ratio.

SCCS comment
SCCS was not informed whether it is free base or it is salt(s) used in hair dye formulations

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

35 male and 35 female rats of the CFY strain were administered by oral intubation with a 10% suspension in aqueous gum tragacanth (0.5%) at dosage levels 400, 640, 800. 1000, 1260 and 1600 mg/kg bw.

Results
LD_{50} (rats): 1,190 (1,070 - 1,320 95% confidence limits) mg/kg bw

Subm. I
Ref.: 1

LD_{50} (rats): 729 mg/kg bw
No study details are available

Ref.: 4

3.3.1.2. Acute dermal toxicity

3.3.1.3. Acute inhalation toxicity

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline: /
Species/strain: Albino rabbits
Group size: 3

Test substance:	1,4-diamino-2-chlorobenzene
Batch:	/
Purity:	/
Vehicle:	water containing 0.05% sodium sulphite; pH 7.0
Dose level:	2.5% w/v
Dose volume:	Not stated in original summary report
Observation:	24 hours and 72 hours
GLP:	/
Study period:	1976

Sample was applied to one intact and one abraded site on the clipped dorsum of each rabbit.

Results
None of the animals showed any observable response to treatment throughout the 72 hours observation period.

Conclusion
1,4-diamino-2-chlorobenzene is not considered to be an irritant to rabbit skin.

Subm. I
Ref.: 3

SCCS comment
Only a report summary was provided. The CTFA document gives details not included in the original report summary. In particular, the dose is said to have been 0.5 mL and the application 24 hours. 1,4-diamino-2-chlorobenzene 2.5% appears not to have been irritating to the skin under the conditions of this experiment.

3.3.2.2. Mucous membrane irritation

Guideline:	/
Species/strain:	Rabbit
Group size:	3
Test substance:	1,4-diamino-2-chlorobenzene
Batch:	/
Purity:	/
Vehicle:	water buffered to pH 7 with 0.05% sodium sulfite
Dose level:	2.5 % (w/v)
Dose volume:	Not stated in original summary report
Observation:	/
GLP:	/
Study period:	1976

2.5% (w/v) 1,4-diamino-2-chlorobenzene in water pH 7 was instilled into one eye of each of the three rabbits. Ten seconds after instillation the eyes were rinsed with 20 mL of distilled water.

Results
In one animal mild conjunctival inflammation was observed. Two animals did not show any observable response to treatment throughout the 7 days observation period.

Conclusion
1,4-diamino-2-chlorobenzene 2.5% solution gave a negative test for eye irritation.

Ref.: 3

Subm. I
Ref.: 2

SCCS comment
Only a report summary was provided. The CTFA document gives details not included in the original report summary. In particular, the dose is said to have been 0.5 mL.

The result provided in the summary does indicate that 1,4-diamino-2-chlorobenzene 2.5% aq. does have irritant potential to the rabbits eyes under the conditions of the described experiment.

As 1,4-diamino-2-chlorobenzene is intended for use on eyelashes, data from a guideline study should be provided to further assess the irritant potential of the substance on the eye.

3.3.3. Skin sensitisation

Guideline:	"Magnusson-Kligman protocol"
Species/strain:	Pirbright white guinea pigs; female
Group size:	15 with 10 controls
Test substance:	1,4-diamino-2-chlorobenzol (C.I.76 065)
Batch:	/
Purity:	/
Vehicle:	water
Concentration:	3% (0.1 mL) for intracutaneous injections.
Positive control:	/
GLP:	/
Study period:	1977

Fifteen female Pirbright white guinea pigs were given 3 x 0.1 mL intracutaneous injections of 3% 1,4-diamino-2-chlorobenzol in water on 5 consecutive days. 10 further animals, which received no injections, served as the negative control.
The above induction period was followed by a 4 week no-treatment period. The animals were then patch tested with 0.003, 0.006, 0.03 and 0.3% 1,4-diamino-2-chlorobenzol and their skin reactions scored after 24 hours and 48 hours.

Results
A Student's t-test was used to compare the skin reaction scores of the test versus control animals. Nine of 15 animals showed a reaction within 24 hours.

Conclusion
Due to the outcome of the test, 1,4-diamino-2-chlorobenzol was classified as strongly sensitising.

Ref.: 3
Subm. I
Ref.: 4

SCCS comment
The original report was provided in German.

There was no positive control.

1,4-diamino-2-chlorobenzene should be categorised as being at least a strong sensitiser when tested at 3% in this experiment.

3.3.4. Dermal / percutaneous absorption

No data available

SCCS comment
There is no information on percutaneous absorption of 2-chloro-p-phenylenediamine. A study conforming to current guidelines and using the maximum intended use concentration of 2-chloro-p-phenylenediamine (with appropriate adjustments for salts) should be provided. Otherwise 100% absorption may be considered.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity

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

A subchronic toxicity study with 2-chloro-p-phenylenediamine sulfate was mentioned in a review paper. Fischer 344 rats and B6C3F1 mice were used in the study. Concentrations of 0.03, 0.1, 0.3, 1.0 and 3.0% were incorporated into the feed and administered to the animals (5 males and 5 females per group) for 8 weeks. Results for the 3% group were not reported. At 1% 5 male rats and 1 female rat died. At 0.3% no deaths occurred but the mean body weight was depressed in both males and females. In mice, there was one death in the 1% male group and none in the female group. Mean body weights were depressed in males and in females. A concentration of 0.3% resulted in no deaths in either sex and no mean weight depression in males; a 16.1 % mean weight depression was reported in females.

Ref.: 3

SCCS comment
The study is not available. No conclusion can be derived with regard to subchronic toxicity.

3.3.5.3. Chronic (> 12 months) toxicity

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial gene mutation assay

Guideline:	/
Species/strain:	*E. coli 343/113*
Replicates:	duplicate cultures per "mutant" type in one experiment
Test substance:	1,4 –diamino-2-chlorbenzol
Batch:	/
Purity:	/
Solvent:	DMSO
Concentrations:	1, 10 and 100 µg/ml
Treatment:	2 h incubation and an expression period of 20 h for standard medium, of 40 h for gal^+ mutants, and of 72 h arg^+ , nad^+ and MTR mutants
GLP:	/

Study period: January 1976

1,4 –Diamino-2-chlorbenzol was investigated for the induction of gene mutations in *E. coli* strain 343/113 according to Mohn *et al.* (Mutation Research 25, 187-196, 1974). By using different selection mediums, the assay enables the simultaneous determination of both forward and reverse mutations in different genes: reverse mutations of *arg*$^-$ and *nad*$^-$ to prototrophy, forward mutations of 5-methyl-DL-tryptohan sensitivity (MTS) to MT resistance and forward (and reverse) mutations from R^s_{18} to *gal*$^+$. Test concentrations were based on the basis of survival in a toxicity test with 3 h incubation with 1,4–diamino-2-chlorbenzol in the dark. In the main test, bacteria were treated for 2 h with 1, 10 and 100 µg/ml 1,4–diamino-2-chlorbenzol followed with by an expression period of 20 h for standard medium, of 40 h for *gal*$^+$ mutants, and of 72 h *arg*$^+$, *nad*$^+$ and MTR mutants.

Results
In the main test, a decrease in survival compared to the untreated controls was not found. For none of the mutation endpoints, a biologically relevant increase in the number of revertants has been observed.

Conclusion
Under the experimental conditions used 1,4–diamino-2-chlorbenzol was not mutagenic in this gene mutation tests.

Subm. I
Ref.: 6
AR 3

SCCS comment
The experiment was performed before the implementation of the OECD guidelines. The test with *E. coli* 343/113 was not validated and does not belong to the standard OECD tests. Batch number and purity are lacking. Only the average findings are given, raw data are lacking. The performance of the test does not comply with the present standard requirements.
The test has only limited value and can only be used for conformation purposes.

Bacterial gene mutation assay

Guideline:	/
Species/strain:	*S. typhimurium*, TA98, TA100, TA1535, TA1537, TA1538
Replicates:	single cultures in one experiment
Test substance:	2-chloro-p-phenylenediamine
Batch:	/
Purity:	/
Solvent:	distilled water
Concentrations:	1, 10, 100, 1000 and 10000 µg/plate
Treatment:	/ (no details given)
GLP:	/
Study period:	6 April 1978

2-chloro-p-phenylenediamine was investigated for the induction of gene mutations in *Salmonella typhimurium* strains (Ames test) without and with metabolic activation. Test concentrations were based on the results of a bacteriostatic test with 4 concentrations of 2-chloro-p-phenylenediamine of up to 10000 µg/well. Toxicity was evaluated on the basis of a reduction in the number of spontaneous revertant colonies and/or completeness of the bacterial background lawn. Negative and relevant positive controls were included.

Results

In the initial bacteriostatic test, the highest concentration of 2-chloro-p-phenylenediamine occasionally demonstrated toxicity resulting in an incomplete bacterial lawn. Therefore, this high concentration was also chosen as the top concentration in the main test.
In the presence of metabolic activation only, a concentration dependent increase in the number of revertants was found in TA98, TA100 and TA1538. In the absence of metabolic activation and for TA1535 and TA 1537 in the presence of metabolic activation, a biologically relevant increase in revertants was not observed.

Conclusion
Under the experimental conditions used 2-chloro-p-phenylenediamine was mutagenic in this gene mutation tests in bacteria.

Subm. I
Ref.: 7

SCCS comment
The experiment was performed before the implementation of the OECD guidelines. Batch number, purity and information on treatment are lacking. However, information may be available in the HRC Protocol MCB/101A. Only the average findings are given, raw data are lacking. In the report it is stated that "Full mutation data are available on request". The performance of the test does not comply with the present standard requirements.
The test has only limited value and can only be used for conformation purposes.

Bacterial gene mutation assay

Guideline:	/
Species/strain:	*S. typhimurium*, TA98, TA100, TA1535, TA1537, TA1538
Replicates:	single cultures in one experiment
Test substance:	2-chloro-p-phenylenediamine mixed 1:1 with hydrogen peroxide
Batch:	/
Purity:	/
Solvent:	distilled water
Concentrations:	1, 10, 100 and 1000 µg/plate
Treatment:	/ (no details given)
GLP:	/
Study period:	6 April 1978

2-chloro-p-phenylenediamine mixed 1:1 with hydrogen peroxide was investigated for the induction of gene mutations in *Salmonella typhimurium* strains (Ames test) with and without metabolic activation. Test concentrations were based on the results of a bacteriostatic test with 4 concentrations of 2-chloro-p-phenylenediamine mixed 1:1 with hydrogen peroxide up to 10000 µg/well. Toxicity was evaluated on the basis of a reduction in the number of spontaneous revertant colonies and/or completeness of the bacterial background lawn. Negative and relevant positive controls were included.

Results
Although toxic effects in the initial bacteriostatic test were not reported, 1000 µg 2-chloro-p-phenylenediamine mixed 1:1 with hydrogen peroxide per plate was chosen as the highest concentration in the main test.
In the presence of metabolic activation only, a concentration dependent increase in the number of revertants was found in TA98, TA100 and TA1538 (highest concentration only). In the absence of metabolic activation and for TA1535 and TA 1537 in the presence of metabolic activation, a biologically relevant increase in revertants was not observed.

Conclusion

Under the experimental conditions used 2-chloro-p-phenylenediamine mixed 1:1 with hydrogen peroxide was mutagenic in this gene mutation tests in bacteria.

Subm. I
Ref.: 7

SCCS comment
The experiment was performed before the implementation of the OECD guidelines. Batch number, purity and information on treatment are lacking. However, information may be available in the HRC Protocol MCB/101A. Only the average findings are given, raw data are lacking. In the report it is stated that "Full mutation data are available on request". The performance of the test does not comply with the present standard requirements.
Since 2-chloro-p-phenylenediamine was mixed 1:1 with hydrogen peroxide the effective concentrations are a factor 2 lower than mentioned in the method part of the report and in the tables showing the summary of the mutation data.
The test has only limited value and can only be used for conformation purposes.

***Salmonella umu* test**

In a publication from 2006, Yasunaga *et al.* reported on a *Salmonella umu* test with 2-chloro-p-pheynelenediamine. The test performed without and with metabolic activation according to the method of Yasunaga et al. (2004), used *Salmonella typhimurium* strain TA1535/pSK1002. Liver S9 fraction from phenobarbital/5,6-benzoflavone-induced rats was used as exogenous metabolic activation system. The S9-mix contained 10% S9 fraction. The maximum concentration used was 5 mg/ml (final concentration) in DMSO. A positive reaction was defined for a minimum of a twofold increase of the ratio maximum activity of treatment/ maximum activity of negative control. Under identical experimental condition for comparison, a gene mutation test (Ames test) using *Salmonella typhimurium* strain TA98 was performed. For both the *umu* test (DNA damage) as the gene mutation test, in the presence of metabolic activation, an increase in DNA damage and in the number of revertants, respectively, was observed following treatment with 2-chloro-p-phenylenediamine. In the absence of metabolic activation, a biological relevant increase was not found. It was concluded that under the experimental conditions used, 2-chloro-p-phenylenediamine was mutagenic in this *Salmonella umu* test. However, it should be noted that these conclusions are based on the data from a publication in open literature. Very little detail on the performance of the test was reported and raw data were not available. The test was not conducted in compliance with GLP or OECD guidelines. Therefore, the test has only limited value and can only be used for confirmation purposes.

Ref.: 8
AR: 1

In Submission II on 2-chloro-p-phenylenediamine form 2008 both an in *vitro* sister-chromatid exchange test and a chromosome aberration test were mentioned. Both tests were positive. It was reported that: "Much further details for these tests are not available, because the results are summarized without any details in NTP data base for testing status (Ref. 7)".

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Bone marrow micronucleus test in rats

Guideline: /

Species/strain:	Rat, CFY SPF
Group size:	5 rats/sex/group
Test substance:	1,4-diamino-2-chlorobenzene
Batch:	/
Purity:	/
Vehicle:	0.5% gum tragacanth
Dose levels:	0 and 900 mg/kg bw/day
Route:	orally, twice at 24h interval
Sacrifice times:	6 h after the last treatment
GLP:	/
Study period:	16 March 1977

1,4-Diamino-2-chlorobenzene has been investigated for induction of micronuclei in bone marrow cells of rats. Test doses were based on the results of a preliminary toxicity study with doses between 800 and 900 mg/kg bw/day on a group of 5 male and 5 female mice recording clinical signs and mortality for a period of 30 h performed under identical conditions as in the main study. In the main experiment male and female mice were exposed orally twice at 24 h intervals to 0 and 900 mg/kg bw/day. As in the preliminary toxicity study, the rats were starved the night before dosing. The mice were examined for acute toxic symptoms and/or mortality. Bone marrow cells were collected 6 h after the last treatment. For each mouse the percentage of polychromatic erythrocytes with a micronucleus was counted in 2000 polychromatic erythrocytes. Negative and positive controls were included.

Results

Both in the preliminary toxicity, and in the main study, all rats of all dose groups showed lethargy following dosing. In the highest dose group of the preliminary toxicity study, one male and one female rat died between 24 and 30 h after dosing. All rats excreted orange pigmented urine, indicating systemic distribution of 1,4-diamino-2-chlorobenzene and thus its bioavailability.
Biologically relevant increases in the number of polychromatic erythrocytes with micronuclei compared to the concurrent vehicle controls were not found in either males or females.

Conclusions

Under the experimental conditions used 1,4-diamino-2-chlorobenzene did not induce an increase in the number of bone marrow cells with micronuclei and, consequently, 1,4-diamino-2-chlorobenzene is not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of rats.

Subm. I
Ref.: 8

SCCS comment

The experiment was performed before the implementation of the OECD guidelines. Batch number and purity are lacking. The performance of the test does not comply with the present standard requirements.

3.3.7. Carcinogenicity

Oral administration

Rats

Guideline:	/
Species/strain:	F344 rats.

Group size:	50 animals per sex and dose. 20 animals per sex in control groups.
Test substance:	2-Chloro-*p*-phenylenediamine sulphate.
Batch:	Produced by Aldrich Chemical Company, Milwaukee, Wisconsin.
Purity:	98%
Dose level:	Dietary concentrations 0, 0.15, and 0.3%.
Route:	Oral, in feed.
Exposure period:	105 – 107 weeks.
GLP:	In compliance.
Study period:	Before 1978

The study was carried out by the US National Toxicology Program.

F344 rats, groups of 50 males and 50 females (about 7 weeks old at start of the study), were exposed to dietary concentrations 0.15, and 0.3% of 2-chloro-*p*-phenylenediamine sulphate in the feed for up to 105 – 107 weeks. The control group consisted of 20 female and 20 male rats.

Mean body weight depression was only slight for both male and female treated groups, indicating an observable effect of compound administration. There were no significant positive associations between the administered dietary concentrations of 2-chloro-p-phenylenediamine sulfate and mortality for rats of either sex.

There were no statistically significant positive associations between dietary exposure to 2-chloro-p-phenylenediamine sulfate and the incidences of any tumor. The increased incidence of transitional-cell hyperplasia of the renal pelvic epithelium in both male and female rats and the presence of transitional-cell tumors of the urinary bladder in three dosed rats is suggestive of, but not considered as sufficient evidence of carcinogenicity.

The study authors concluded that under the conditions of this bioassay there was insufficient evidence that dietary administration of 2-chloro-p-phenylenediamine sulfate was carcinogenic to Fischer 344 rats.

Ref.: 7

Mice

Guideline:	/
Species/strain:	B6C3F1mice.
Group size:	50 animals per sex and dose. 20 animals per sex in control group.
Test substance:	2-Chloro-*p*-phenylenediamine sulphate.
Batch:	Produced by Aldrich Chemical Company, Milwaukee, Wisconsin.
Purity:	98%.
Dose level:	Dietary concentrations 0, 0.3, and 0.6% 2-chloro-*p*-phenylenediamine sulphate.
Route:	Oral, in feed.
Exposure period:	104 – 105 weeks in low dose mice. High dose mice were supplied with dosed feed for a total of 87 weeks, followed by an 18-week untreated observation period.
GLP:	In compliance.
Study period:	Before 1978.

The study was carried out by the US National Toxicology Program.

B6C3F1 mice, groups of 50 males and 50 females (about 7 weeks old at start of the study), were exposed to dietary concentrations 0.3, and 0.6% of 2-chloro-*p*-phenylenediamine

sulphate in the feed. The low dose mice were exposed for up to 105 weeks. High dose mice were supplied with dosed feed for a total of 87 weeks, followed by an 18-week untreated observation period. The control group consisted of 20 female and 20 male mice.

Mean body weight depression was only slight for both male and female treated groups indicating an observable effect of compound administration. There were no significant positive associations between the administered dietary concentrations of 2-chloro-p-phenylenediamine sulfate and mortality for male mice. There was a significant positive association between dosage and mortality for female mice; however, adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumours.

A variety of proliferative hepatocellular lesions were observed in the mice in this bioassay. When those male mice having either hepatocellular carcinomas or hepatocellular adenomas were combined and the resulting tumor incidences were statistically analyzed, there was a significant positive association between the dietary concentration of the chemical and the incidences. The Fisher exact tests were not, however, supportive. There were no other tumors in mice for which a significant positive association could be established between dosage and incidence.

The study authors concluded that under the conditions of this bioassay there was insufficient evidence that dietary administration of 2-chloro-p-phenylenediamine sulfate was carcinogenic to B6C3F1 mice.

Ref.: 9
Ref.: 10

SCCS comment

The carcinogenicity of 2-chloro-p-phenylenediamine sulfate has been studied under the US National Toxicology Program in rats and mice after oral administration for 2 years. The study authors concluded that under the conditions of this bioassay there was insufficient evidence that dietary administration of 2-chloro-p-phenylenediamine sulfate was carcinogenic to B6C3F1 mice. Weisburger *et al* (Ref.: 10) points out that "substitution of a chlorine in the 2-position of p-phenylenediamine changed an inactive compound to one which tended to increase hepatocellular carcinomas in female mice and hyperplasia of the renal pelvis in rats". Today, NTP subdivide level of carcinogenicity as *Clear Evidence of Carcinogenic Activity. Some Evidence of Carcinogenic Activity, Equivocal Evidence of Carcinogenic Activity, Inadequate Study of Carcinogenic Activity* and *No Evidence of Carcinogenic Activity.* According to this subdivision SCCS considers that the study on 2-chloro-p-phenelendiamine may represent *Equivocal Evidence of Carcinogenic Activity* or *Inadequate Study of Carcinogenic Activity.* Based on the information available no conclusion can be drawn with regard to carcinogenicity.

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

o-Chloro-p-phenylenediamine was administered by gavage to Sprague-Dawley rats on days 6-15 of gestation at dose levels of 100, 200 and 400 mg o-chloro-p-phenylenediamine/kg bw/d dissolved in propylene glycol. Vitamin A was used as positive control. Each group consisted of 11 animals; in the control group of 20 animals only propylene glycol was applied.

Maternal body weight gain was significantly reduced at the dose levels 200 and 400 mg/kg bw/d. Rats given the high dose revealed a significant reduction in male and female foetal body weights. Evaluation of foetuses for gross, visceral and skeletal anomalies revealed no statistically significant differences between dye-treated and vehicle control groups, but showed a significant increase (up to 25-91%) in the incidence of abnormal foetuses in the group exposed to Vitamin A; the positive control.
The NOAEL of maternal toxicity is 100 mg/kg bw/d, that of developmental toxicity is 200 mg/kg bw/d whereas the NOAEL teratogenicity from this study can be estimated to be 400 mg/kg bw/d.

Ref.: 11

SCCS comment
The evaluation was a published study. The study did not follow a guideline.

3.3.9. Toxicokinetics

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

No data available

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

3.3.11. Human data

A 29 year old woman developed an eczematous reaction after use of a cream dye on her lashes and brows. She had used the same product 4x a year for several years without any problems. She had developed an intensely itchy dermatitis on her brows but not at her lashes, despite these having been coloured at the same time.
Patch testing with 2-chloro-p-phenylenediame (CAS 615-66-7) 1% in petrolatum gave a +++ reaction and the cream dye 'as is' a ++ reaction. P-Phenylenediamine 1% in petrolatum, tested at the same time, gave a + reaction.

A 30 year old woman developed an eczematous reaction after using a cream dye on her lashes and brows. She used to regularly colour her lashes and brows but following the last two colourings she developed an itchy dermatitis on her eyelids.
Patch testing with 2-chloro-p-phenylenediame (CAS 615-66-7) 1% in petrolatum gave a +++ reaction and the cream dye 'as is' a +++ reaction, p-Phenylenediamine 1% in petrolatum, tested at the same time, also gave a +++ reaction.

Ref: 14

A 30 year old male presented with eye lid swelling and conjunctival chemosis a day after having had his eyelashes dyed for the first time and without any history of any earlier exposure to similar compounds. He was treated with oral prednisolone and complete resolution occurred over a week. The authors state that the patient was patch test positive to chloro-PPD (dilution and methodology not described).

AR 2:

SCCS comment
Interpretation of the results of this case report is difficult in the absence of information on patch test methodology. Hence, the conclusion of the authors that their report highlights the hypersensitivity reaction to hair dye cream should be considered as speculative.

3.3.12. Special investigations

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3.3.13. Safety evaluation (including calculation of the MoS)

Proposal of the applicant

In the carcinogenicity study in rats as published in Ref. 10 a transitional cell hyperplasia of the kidney and renal pelvis was observed also in the lowest dose investigated (0.15% in the diet corresponding to 67 mg/kg bw/d) at a rate of 35%. From this a benchmark dose was derived (lower level BMD_{10}, about 15 mg/kg bw/d). Using an uncertainty factor of 300 a reference dose of about 0.05 mg/kg was calculated. Exposure was corrected by the use frequency (12 out of 365 days per year). The worst case calculation assuming 100 % dermal absorption resulted in a MoS of 1181.

SCCS comment

According to the Notes of Guidance, it is not the average daily dose that should be used but the actual dose on the exposure days. Following this, assuming an exposure of 23 mg and 100% dermal absorption a systemic exposure dose of 0.38 mg/kg bw/d was calculated which is higher than the reference dose proposed by the applicant. Furthermore, the reference dose must be corrected for oral bioavailability.
The SCCS concludes that no sufficient margin of safety can be deduced.

3.3.14. Discussion

Physico-chemical properties

2-Chloro-p-phenylenediamine is used at concentration up to 4.6% (w/w) in oxidative hair dyes formulations, for eyebrows and eyelashes. Prior to use it is mixed with 3% hydrogen peroxide solution in 1:1 ratio. However, it was not informed whether it is free base or it is salt(s) used in hair dye formulations.
No information was provided on qualitative chemical characterisation of 2-chloro-p-phenylenediamine and organic impurities in it. Analytical data on purity determination is not provided.
Solubility information on 2-chloro-p-phenylenediamine was insufficient. Water solubility should be determined by EC Method A.6. Data on Log P_{ow} of 2-chloro-p-phenylenediamine was not submitted. Stability and homogeneity of 2-chloro-p-phenylenediamine in test solutions was not provided. Stability of 2-chloro-p-phenylenediamine in typical hair dye formulation was not provided

General toxicity

The LD_{50} in rats was reported to be 1190 mg/kg bw and 729 mg/kg bw in different studies No study details are available.

A subchronic toxicity study with 2-chloro-p-phenylenediamine sulfate was mentioned in a review paper. The study is not available. No conclusion can be derived with regard to subchronic toxicity.

The results of teratogenicity study in rats were reported in a publication. The NOAEL of maternal toxicity is 100 mg/kg bw/d, that of developmental toxicity is 200 mg/kg bw/d whereas the NOAEL teratogenicity from this study can be estimated to be 400 mg/kg bw/d. However, the study did not follow a guideline.

Irritation / sensitisation

2-Chloro-p-phenylenediamine (not stated whether base or a salt) at 2.5% in water showed no irritant potential on rabbit skin but was irritating to the rabbit eye. As assessment for any potential for eye irritation is important when considering the use of 2-chloro-p-phenylenediamine on eyelashes and eyebrows, a guideline study to formally evaluate the eye irritating potential is required.

2-Chloro-p-phenylenediamine is categorised as at least a 'strong' sensitiser in the guinea pig when tested at 3% in a Magnusson Kligman test.

2-Chloro-p-phenylenediamine has been shown to be a sensitiser in man.

Dermal absorption

No information available. A guideline study using 2-chloro-p-phenylenediamine at intended use level (taking into account free base and salts) should be undertaken. Otherwise 100% absorption may be assumed.

Mutagenicity / genotoxicity

Overall, the genotoxicity of 2-chloro-p-phenylenediamine has been investigated in a limited number of genotoxicity tests; gene mutations were investigated exclusively in bacteria whereas chromosome aberrations and aneuploidy were studied under *in vivo* conditions only in a micronucleus test.
In tests using *S. typhimurium* strains 2-chloro-p-phenylenediamine induced gene mutations and DNA damage (*umu* test) whereas the gene mutation test with an *E. coli* strain was negative. The induction of gene mutations was not investigated in mammalian cells nor confirmed in a proper *in vivo* test for this endpoint. Chromosomal aberrations and aneuploidy were not investigated with (an) *in vitro* test(s). However, in rats exposure to 2-chloro-p-phenylenediamine did not result in an increase in erythrocytes with micronuclei.
Based on the available data and the lack of a proper *in vivo* test for gene mutation induction, it is not possible to give a conclusion on the genotoxic potential of 2-chloro-p-phenylenediamine. An *in vivo* test on the induction of gene mutations is essential. However, the SCCS appreciates that such a test is not permitted under current European cosmetics legislation.

Carcinogenicity

The carcinogenicity of 2-chloro-p-phenylenediamine sulfate has been studied under the US National Toxicology Program in rats and mice after oral administration for 2 years. The study authors concluded that under the conditions of this bioassay there was insufficient evidence that dietary administration of 2-chloro-p-phenylenediamine sulfate was carcinogenic to B6C3F1 mice. Weisburger *et al* (Ref.: 10) points out that "substitution of a chlorine in the 2-position of p-phenylenediamine changed an inactive compound to one which tended to increase hepatocellular carcinomas in female mice and hyperplasia of the renal pelvis in rats". Today, NTP subdivide level of carcinogenicity as *Clear Evidence of Carcinogenic Activity. Some Evidence of Carcinogenic Activity, Equivocal Evidence of Carcinogenic Activity, Inadequate Study of Carcinogenic Activity* and *No Evidence of Carcinogenic Activity.* According to this subdivision SCCS consider that the study on 2-chloro-p-phenelendiamine may represent *Equivocal Evidence of Carcinogenic Activity* or *Inadequate Study of Carcinogenic Activity.*

Based on the information available no conclusion can be drawn with regard to carcinogenicity.

4. CONCLUSION

The SCCS considers that no sufficient margin of safety could be deduced for the use of 2-chloro-p-phenylenediamine in oxidative hair dye formulations for eyebrows and eyelashes in a concentration of maximum 4.6 % taking into account the scientific data provided.

2-Chloro-p-phenylenediamine is categorised as at least a 'strong' sensitiser.

A complete chemical characterisation of 2-chloro-p-phenylenediamine is needed.

The SCCS is of the opinion, that based on the available data and the lack of a proper *in vivo* test for gene mutation induction, it is not possible to give a conclusion on the genotoxic potential of 2-chloro-p-phenylenediamine.

Therefore, the use of 2-chloro-p-phenylenediamine cannot be considered safe for the consumer.

5. MINORITY OPINION

6. REFERENCES

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