Scientific Committee on Consumer Safety

SCCS

OPINION ON

2-Methoxy-methyl-p-phenylenediamine and its sulfate salt

COLIPA n° A160

The SCCS adopted this opinion at its 18th plenary meeting

of 26 February 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.

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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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1. BACKGROUND

The hair dyeing ingredient 2-Methoxy-methyl-p-phenylenediamine CAS n. 337906-36-2 (A160) is an ingredient, which is newly introduced on the EU market. The responsible company is submitting a safety dossier for review by the Scientific Committee on Consumer Safety.

Industry requests use of this ingredient in oxidative colouring products with a concentration on head of maximum of 1.8%.

Submission I, which has been received in March 2012, presents the scientific data on the above mentioned substance.

2. TERMS OF REFERENCE

1. *Does the SCCS consider 2-Methoxy-methyl-p-phenylenediamine (A160) safe for use as an oxidative hair dye with a concentration on-head of maximum 1.8% taken into account the scientific data provided?*

2. *And/or does the SCCS recommend any further restrictions with regard to the use of 2-Methoxy-methyl-p-phenylenediamine (A160) in any hair dye formulations?*

3. OPINION

3.1 Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1 Primary name and/or INCI name

2-methoxy-methyl-p-phenylenediamine (INCI name)
2-methoxy-methyl-p-phenylenediamine sulfate (INCI name)

3.1.1.2. Chemical names

Free base
1,4-Benzenediamine, 2-(methoxymethyl) (CA INDEX NAME, 9CI
2-(Methoxymethyl)benzene-1,4-diamine (IUPAC)
1,4-Diamino-2-methoxymethyl-benzene

Sulfate salt
1,4-Benzenediamine, 2-(methoxymethyl)-, sulfate (CA INDEX NAME, 9CI
2-(Methoxymethyl)benzene-1,4-diamine sulfate (IUPAC)
1,4-Diamino-2-methoxymethyl-benzene sulfate

3.1.1.3. Trade names and abbreviations

Free base
MBB (Dragon)
COLIPA A160

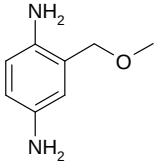
Sulfate salt
1,4-Diamino-2-methoxymethyl-benzene sulfate (DMMBS)
COLIPA A160

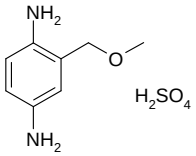
3.1.1.4. CAS / EC number

	Free base	*Sulfate*
CAS:	337906-36-2	337906-37-3
EC:	pending	/

3.1.1.5. Structural formula

Free base *Sulfate salt*





3.1.1.6. Empirical formula

Free base *Sulfate salt*

Formula: $C_8H_{12}N_2O$ $C_8H_{12}N_2O \cdot H_2SO_4$

3.1.2. Physical form

White to beige off-white / tan to light rose light pink powder.

3.1.3. Molecular weight

	Free base	*Sulfate salt*
Molecular weight:	152.20 g/mol	250.28 g/mol

3.1.4. Purity, composition and substance codes

Free base

Two batches were characterised and are reported in this dossier:

20080201 (Dragon), identification was based on NMR and elemental analysis
093-09/178-01 (Internal batch)

These batches meet the following limits:

Purity

HPLC qualitative (254 nm):	≥ 99.0 area%
NMR quantitative:	≥ 98.4%, w/w
Solvent content (water):	≤ 0.1%, w/w
Residue on ignition:	≤ 0.1%, w/w

Potential impurities

2-(Hydroxymethyl)benzene-1,4-diamine:	130 ppm
4-Amino-2-(methoxymethyl)-phenol:	< 34 ppm (LOD = 34 ppm)
1,4-Diamino-2-methylbenzene:	50 ppm
Toluene:	≤ 2100 ppm

Solvent residues
Toluene and ethyl acetate were used in the synthesis of this material. Ethyl acetate was not detected in the two batches. Toluene was detected with a maximum level of 2100 ppm.

Sulfate salt

Two batches were characterised and are reported in this dossier:

GST070-05/24-01
RD-CRU093-09/02-07

These batches meet the following limits:

Purity

HPLC qualitative (254 nm)	≥ 96.0 area%
NMR quantitative (salt):	≥ 95.0%, w/w
Solvent content (water):	≤ 0.9%, w/w
Residue on ignition:	≤ 0.1%, w/w

Potential impurities

2-(Hydroxymethyl)benzene-1,4-diamine:	≤ 5000 ppm
4-Amino-2-(methoxymethyl)-phenol:	≤ 35 ppm (LOD = 30 ppm)
1,4-Diamino-2-methylbenzene:	< 0.38 – 1.15 %, w/w

3.1.5. Impurities / accompanying contaminants

See above

3.1.6. Solubility

	Free base	*Sulfate salt*
Water*:	284 g/l at 20.15 °C, pH 8.87	119 g/l at 20 °C, pH 1.82
Water (pH adjusted)*:	84.9 g/l[a] at 20 °C, pH 7.35	116 g/l at 20 °C, pH 7.60
DMSO:	≥ 20% (w/w) at 20 °C	≥ 15% (w/w) at 20 °C

* Water solubility was determined by EU Method A.6

[a] In the dermal absorption study, the solubility of 2-methoxy-methyl-p-phenylenediamine, batch no. 20080201 (Dragon), at pH7.3 is described as 307.85 mg/ml (307.85 g/L)

3.1.7. Partition coefficient (Log Pow)

	Free base	*Sulfate salt*	
Log P_{ow}:	-0.649 (pH 7)	-0.450 (pH 8)	(EU Method A.8)

3.1.8. Additional physical and chemical specifications

	Free base	*Sulfate salt*
Melting point:	80.7-81.1 °C	200 °C (decomposition)
Boiling point:	/	/
Flammability:	not highly flammable	not highly flammable
Self-ignition temperature:	> 404 °C	376 °C
Explosive properties:	not explosive	not explosive
Oxidising properties:	not oxidising	not oxidising
Vapour pressure (20 °C, ex.-pol):	5.0 x 10^{-8} hPa	8.18 x 10^{-11} hPa
Density:	1.2028 g/ml	1.4769 g/ml
Surface tension:	70.86 mN/m	55.24 mN/m
pH:	8.87 (28.4%, w/w aq, 20 °C)	1.82 (11.9%, w/w aq, 20 °C)
pK_B:	5.86 ($[HB^+]*[OH^-]/[B]$)	not measured
particle size distribution:	166.6 µm (> 32 ≤ 250)	106.8 µm (> 32 ≤ 250)
UV_Vis spectrum (200-800 nm):	/	

3.1.9. Homogeneity and Stability

Free base
The substance was considered to be stable for more than 5 years, if stored dry and protected from light.

2-Methoxy-methyl-p-phenylenediamine
In DMSO (10% solution, w/v)
Recovery at t=0: 99.2%; t=6h: 100.3%; t=2d: 99.8%; t=3d: 100.2%
In water (10% solution, w/v)

Recovery at t=0: 98.7%; t=6h: 99.4%; t=2d: 99.7%; t=3d: 100.2%

Ref.: 29

Stability in solution
The stability of the test substance methoxymethyl-PPD sulfate in water (10% solution, w/v, pH 5.1) in the presence of L(+)-ascorbic acid acting as antioxidant substance was monitored over a total time period of 7 days using HPLC-chromatography at the 246 nm detection wavelength. During the test procedure, all stock solutions saved under nitrogen atmosphere were stored at ambient temperature in the absence of light. While the recovery rates point to an excellent stability of the PPD-derivative within the first two days (98.5%; 100.6%) the recovery rate is 94.6% after seven days. Overall, the recovery rates of the test substance show the good stability of methoxymethyl-PPD-sulfate under the conditions applied (Study no G2001/0099).

Stability testing in DMSO, water, acetone/water 1:1
The stability of the test substance (A2005/165) in 3 different solvents was monitored over a total time period of seven days using HPLC/DAD at a detection wavelength of 245 nm. During the test procedure, the test solutions were stored at ambient temperature in the absence of light. The indicated values were standardised on the initial value (t = 0).

Stability in DMSO (approx. 10% solution, w/v)
During the course of storage no decomposition of the substance could be observed.
Recovery at t=0: 100.0%: t=6h: 98.8%; t=2d: 98.1%; t=7d: 94.7%.

Stability in water (approx. 10% solution, w/v. with the correction of the pH to 5.9)
During the course of storage no decomposition of the substance could be observed.
Recovery at t=0: 100.0%; t=6h: 98.7%; t=2d: 96.1%; t=7d: 95.0%.

Stability in acetone/water 1:1 (approx. 1.5% solution, w/v)
During the course of storage no decomposition of the substance could be observed.
Recovery at t=0: 100.0%; t=6h: 96.7%; t=2d: 94.8%; t=7d: 90.8%

Summary
The solutions of the test substance in DMSO, water/acetone 1:1 and water can be regarded as stable during the test period of 7 days under the conditions described above. During the course of storage no decomposition of the test item could be observed.

Ref.: 43

General Comments on physico-chemical characterisation

- Two different values for the solubility of 2-methoxy-methyl-p-phenylenediamine in water at pH 7.3 were reported.
- Stability of 2-methoxy-methyl-p-phenylenediamine in typical hair dye formulations was not demonstrated.

3.2 Function and uses

2-Methoxy-methyl-p-phenylenediamine is used as an oxidative hair colouring agent (precursor). The intended maximum on-head concentration is 1.82% in oxidative hair dye formulations, after mixing with the developer (1:3).

3.3 Toxicological Evaluation

3.3.1 Acute toxicity

No study on acute toxicity was provided for 2-Methoxy-methyl-p-phenylenediamine. The following details were taken from *in vivo* genotoxicity studies performed with 2-Methoxy-methyl-p-phenylenediamine sulfate.

Before an *in vivo* micronucleus study in mice (ref 51), a pre-test was performed. Two female mice treated i.p. with 2000 mg/kg bw died after 10 min. Two other female treated i.p. at the dose of 250 mg/kg bw died after 6 hours. In a third experiment, 3 male and 3 female mice were treated i.p. at the dose of 100 mg/kg. No deaths were reported but signs of toxicity were observed in all animals. The mice showed palpebral closure and lethargy at least the first 6 h after treatment.

Before an *in vivo* Comet assay in rats (ref 53), a pre-test was performed. Three male rats treated by oral gavage with 2000 mg/kg bw died shortly after administration. Three other male rats were treated by oral route at the dose of 200 mg/kg bw. Two rats died within 19 hours after treatment. Three other male rats were then treated at the dose of 100 mg/kg. No deaths were reported but signs of toxicity were observed in all animals. The rats showed reduced motility, roughened fur and discoloured urine.

Before an *in vivo* UDS assay in rats (ref 54), a pre-test was performed. Two male and 2 female rats treated orally with 100 mg/kg bw showed reduced motility, roughened fur and discoloured urine but no animal died. In a second experiment, 2 male and 2 female rats treated orally with 500 mg/kg bw showed clinical signs such as reduced motility, roughened fur, ventrolateral recumbence and discoloured urine and 1 male and 1 female died within 24 hours. In a third experiment, 2 male and 2 female rats treated orally with 200 mg/kg bw showed clinical signs such as reduced motility, roughened fur and discoloured urine and 1 rat died within 24 hours. In the last experiment, 2 male and 2 female rats treated orally with 150 mg/kg bw showed clinical signs such as reduced motility, roughened fur and discoloured urine but no rat died.

Conclusion
No acute oral toxicity study was performed. Based on the results from in vivo genotoxicity studies, the LD50 value of 2-Methoxy-methyl-p-phenylenediamine sulphate is between 100 and 250 mg/kg bw in mice after i.p. administration and between 150 and 200 mg/kg bw in rats after gavage.

The LD50 value of 2-Methoxy-methyl-p-phenylenediamine corrected for molecular weight is between 61 and 152 mg/kg bw in mice after i.p. administration and between 91 and 122 mg/kg bw in rats after gavage.

Comment
The SCCS noticed that the acute toxicity of 2-Methoxy-methyl-p-phenylenediamine sulphate appeared at very low doses and at levels similar to sub-chronic exposure. No explanation was given

3.3.1.1 Acute oral toxicity

Sulfate salt

LD50 rat (oral gavage):	150 – 200 mg/kg bw	(free base: 91.5 – 122.0 mg/kg bw)
LD50 mice (intraperitoneal):	100 - 250 mg/kg bw	

Ref.: 51, 53, 54

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

***In vitro* skin corrosion: Transcutaneous Electrical Resistance (TER) Test Method**

Guideline:	OECD 430 (2004)
Species/strain:	rat, HsdRccHan®™:WIST®™
Group size:	1 male
Test system:	*ex vivo* dorsal skin discs
Test substance:	2-methoxymethyl-p-phenylenediamine (WR 804025)
Batch:	20080201 (Dragon)
Purity:	100.0 area% at 254 nm (HPLC)
Vehicle:	distilled water
Test concentration:	neat
Positive control:	hydrochloric acid (approximately 36%)
Negative control:	sterile distilled water
GLP:	in compliance
Study period:	10 – 11 December 2008

2-Methoxymethyl-p-phenylenediamine was applied neat to the epidermal surface of three skin discs for a contact period of 24 hours. Sufficient test material was applied evenly to the skin discs to ensure that the whole surface of the epidermis was covered. 150 µl distilled water was applied to ensure good contact with the skin. At the end of the exposure period, the test material was removed by washing the skin disc with a jet of warm tap water until no further test material could be removed. Three positive (hydrochloric acid 10 M (~36%)) and negative control (sterile distilled water) discs were also assayed.

The TER was measured using a Wheatstone Bridge with a "low voltage alternating current", value in Ω/kΩ per skin disc was determined. The mean TER for the skin discs was calculated.

Results
Results are accepted if the mean positive and negative control results for the assay fall within the accepted ranges of 0.5 to 1.0kΩ for the positive control (hydrochloric acid 10 M (~36%)) and 10 to 25kΩ for the negative control (sterile distilled water). The test substance is classified as 'Non-Corrosive' if the mean TER value recorded for the 24 hour contact period is greater than 5kΩ. The test substance will be classified as 'Corrosive' if the mean TER value recorded for the 24 hour contact period is 5kΩ or lower.

The mean TER after a contact period of 24 hours with the test material 2-methoxymethyl-p-phenylenediamine was 14.0 kΩ (± 5.0 SD), after a contact period of 24 hours.

Conclusion
2-Methoxymethyl-p-phenylenediamine as neat substance is classified as "non-corrosive".

Ref.: 29

***In vitro* skin irritation: Episkin™ Reconstructed Human Epidermis (RHE) Test Method**

Guideline:	predates OECD 439 (2010)
Test system:	Episkin™ Reconstructed Human Epidermal model
Test substance:	2-methoxymethyl-p-phenylenediamine (WR 804025)
Batch:	20080201 (Dragon)
Purity:	100.0 area% at 254 nm (HPLC)
Vehicle:	sterile water
Test concentration:	neat; 1.83% w/v; 6.1% w/v in sterile water
Test volume:	10 mg (neat); 10 µl (1.83 and 6.1% w/v)
Positive control:	Sodium dodecyl sulphate (SDS), 5% w/v
Negative control:	sterile water
GLP:	in compliance
Study period:	10 December 2008 – 9 February 2009

The treatment period was 15 minutes followed by a rinsing step and a 42 ± 1 hour post-treatment incubation period. Cell viability was determined by the enzymatic reduction of the yellow MTT tetrazolium salt (3-[4,5-dimethylthiazol-2-y1]-2,5-diphenyl-tetrazolium bromide) to a blue formazan salt (within the mitochondria of viable cells) in the test material treated tissues relative to the negative control).
2-Methoxymethyl-p-phenylenediamine was applied as neat test item and at a concentration of 1.83 and 6.1% (w/v) in sterile water to triplicate tissues.

Results
The test material is classified based on tissue viability analysis according to the prediction model: non-irritant to skin if tissue viability is > 50% (Not Classified) and irritant to skin if tissue viability is ≤ 50% (Category 2). Histological evaluation of the tissues at the end of the treatment was performed as an additional measure to correlate with cytotoxicity to evaluate irritation for test items which may interfere with MTT. A decrease in MTT reduction capacity and changes in tissue morphology were used as indicators of potential irritancy.

2-Methoxymethyl-p-phenylenediamine, did not induce significant decrease in cell viability in the MTT assay for any of the concentrations tested with viability measurements of 97.9 ± 3.2% (neat), 109.7 ± 7.2% (1.83% (w/v) in sterile water) and 107.8 ± 0.9% (6.1% (w/v) in sterile water).

Histological evaluation of the treated tissues showed no marked epidermal effects in the treated cultures in comparison to the negative control cultures for 2-methoxymethyl-p-phenylenediamine tested neat, and at 1.83 and 6.1% (w/v) in sterile water.

Conclusion
In conclusion, 2-methoxymethyl-p-phenylenediamine is classified as non-irritant (MTT viability > 50%), when applied as neat test item, at 1.83 and 6.1% (w/v) in sterile water. The histological examinations confirmed the absence of cytotoxicity for 2-methoxymethyl-p-phenylenediamine tested neat, and at 1.83 and 6.1% (w/v) in sterile water.

Assessment of validity
Although conducted before adoption in July 2010 of OECD Test Guideline 439 (*In Vitro* Skin Irritation: Reconstituted Human Epidermis Test Method), the study described above was performed according to OECD Test Guideline 439.

Ref.: 30

Conclusion skin irritation
These studies showed that 2-methoxymethyl-p-phenylenediamine is classified as non-corrosive (neat) and non-irritant (neat and in dilution).

Therefore, the maximum on-head concentration of 1.82% does not have the potential to cause skin corrosion or skin irritation.

Comment
The available histological data suggests that 2-methoxymethyl-p-phenylenediamine is not irritant to skin at anticipated use exposure.

3.3.2.2 Mucous membrane irritation

Isolated Chicken Eye Test, study 1

Guideline:	OECD 438 (2009)
Test system:	isolated chicken eyes (ROSS, spring chickens)
Group size:	3 test eyes, 1 negative control eye, 3 positive control eyes
Test substance:	2-methoxymethyl-p-phenylenediamine (WR 804025)
Batch:	20080201 (Dragon)
Purity:	100.0 area% at 254 nm (HPLC)
Test concentration:	neat
Dose volume:	30 mg (test item and positive control) 30 µl (negative control)
Positive control:	sodium hydroxide
Negative control:	physiological saline, 0.9%
GLP:	in compliance
Study period:	17 June – 22 August 2011

Approximately 7 weeks-old chickens were used as eye-donors. Eyes with a corneal thickness deviating more than 10% of the average corneal thickness of the eyes, eyes that showed opacity (score higher than 0.5) or were unacceptably stained with fluorescein (score higher than 0.5) were rejected and were replaced. A total of 7 eyes were selected for testing: 3 for the test item, 3 for the positive control (sodium hydroxide (neat NaOH ground to a powder)) and 1 for the negative control (physiological saline). After an equilibration period of 45-60 minutes, the corneal thickness of the eyes was measured once more to determine the zero reference value for corneal swelling calculations.

Three corneas were treated with 30 mg of neat 2-methoxymethyl-p-phenylenediamine. After an exposure period of 10 seconds, the corneal surface was rinsed thoroughly with 20 ml of isotonic saline. After rinsing, each eye in the holder was returned to its chamber. The eyes were examined with a slit-lamp microscope at 0, 30, 75, 120, 180 and 240 minutes after treatment. Fluorescein retention was scored only at 30 minutes after treatment. After the final examination the test and control eyes were preserved in a neutral aqueous phosphate-buffered solution of 4% formaldehyde and then subjected to histopathological examination. Ocular effects were evaluated using the endpoints of corneal thickness (swelling), corneal opacity and fluorescein retention.

Results
Defined scoring scales are used for each endpoint to define the severity of effects into four categories (I-IV). Four classes of eye irritancy (not irritating; slightly irritating; moderately irritating; severely irritating) can be identified in the ICE test by combination of the categories defined for each of the evaluation parameters.

2-Methoxymethyl-p-phenylenediamine tested neat caused moderate corneal effects in that there was slight swelling (15%), moderate to severe opacity (2.3) and moderate to severe fluorescein retention (2.7). The Irritation Index calculated was 115. Microscopic examination of the treated corneas identified very slight or slight erosion of the epithelium with no abnormalities of the stroma or endothelium being observed.

The results of the positive and negative controls confirmed the validity of the study.

Conclusion
On the basis of the results obtained in the ICE study, 2-methoxymethyl-p-phenylenediamine tested neat is identified as irritating to eyes (classification Category 2 according to the EU-CLP classification scheme).

Ref.: 35

Isolated Chicken Eye Test, study 2

Guideline:	/
Test system:	isolated chicken eyes (ROSS, spring chickens)
Group size:	3 eyes per test group
Test substance:	2-methoxymethyl-p-phenylenediamine (WR 804025)
Batch:	20080201 (Dragon)
Purity:	100.0 area% at 254 nm (HPLC)
Test concentration:	1.83, 6.1% (w/w) aqueous solution
Dose volume:	30 µl
Positive control:	benzalkonium chloride, 5% w/w
Negative control:	physiological saline, 0.9%
GLP:	in compliance
Study period:	4 November 2008

Approximately 7 weeks old chickens were used as eye-donors. Eyes with a corneal thickness deviating more than 10% of the average corneal thickness of the eyes, eyes that showed opacity (score higher than 0.5) or were unacceptably stained with fluorescein (score higher than 0.5) were rejected and were replaced. A total of 12 eyes were selected for testing: 3 for each concentration of the test item, 3 for the positive control benzalkonium chloride (BAC) 5% (w/w)) and 3 for the negative control (physiological saline). After an equilibration period of 45-60 minutes, the corneal thickness of the eyes was measured once more to determine the zero reference value for corneal swelling calculations.

Three corneas were treated with 30 µl of 2-methoxymethyl-p-phenylenediamine at a concentration of 1.83% (w/w) aqueous. After an exposure period of 10 seconds, the corneal surface was rinsed thoroughly with 20 ml of isotonic saline of ambient temperature. After rinsing, each eye in the holder was returned to its chamber. This procedure was repeated for the three eyes exposed to 2-methoxymethyl-p-phenylenediamine at a concentration of 6.1% (w/w) aqueous.

All examinations were performed with a slit-lamp microscope. Fluorescein retention was scored only at 30 minutes after treatment. After the final examination the test and control eyes were preserved in a neutral aqueous phosphate-buffered solution of 4% formaldehyde and then subjected to histopathological examination. Ocular effects were evaluated using the endpoints of corneal thickness (swelling), corneal opacity and fluorescein retention.

Results
2-Methoxymethyl-p-phenylenediamine tested at 1.83% (w/w) aqueous caused almost no corneal effects with no swelling (0%), no or very slight opacity (0.3) and no or very slight fluorescein retention (0.2). The Irritation Index calculated was 10. Microscopic examination of the treated corneas confirmed the absence of meaningful corneal effects.

2-Methoxymethyl-p-phenylenediamine tested at 6.1% (w/w) aqueous also caused almost no corneal effects with almost no swelling (1%), very slight or slight opacity (0.7) and no or very slight fluorescein retention (0.2). The Irritation Index calculated was 19. Microscopic examination of the treated corneas confirmed the absence of meaningful corneal effects.

Conclusion

On the basis of the results obtained in the ICE study, 2-methoxymethyl-p-phenylenediamine tested at 1.83 and 6.1% (w/w) aqueous is identified as not irritating to eyes (Not Classified according to the EU-CLP classification scheme).

Ref.: 36

Isolated Chicken Eye Test, study 3

Guideline:	/
Test system:	isolated chicken eyes (ROSS, spring chickens)
Group size:	3 eyes per test group
Test substance:	2-methoxymethyl-p-phenylenediamine sulfate (WR 801337)
Batch:	RD-CRU093-09/140-06
Purity:	100.0 area% at 254 nm (HPLC)
Test concentration:	3, 10% (w/w) aqueous dilution
Dose volume:	30 µl
Positive control:	benzalkonium chloride, 5% (w/w) aqueous
Negative control:	physiological saline, 0.9%
GLP:	in compliance
Study period:	14 November 2008

Approximately 7 weeks old chickens were used as eye-donors. Eyes with a corneal thickness deviating more than 10% of the average corneal thickness of the eyes, eyes that showed opacity (score higher than 0.5) or were unacceptably stained with fluorescein (score higher than 0.5) were rejected and were replaced. A total of 12 eyes were selected for testing: 3 for each concentration of the test item, 3 for the positive control benzalkonium chloride (BAC) 5% (w/w)) and 3 for the negative control (physiological saline). After an equilibration period of 45-60 minutes, the corneal thickness of the eyes was measured once more to determine the zero reference value for corneal swelling calculations.
Three corneas were treated with 30 µl of 2-methoxymethyl-p-phenylenediamine sulfate at a concentration of 3% (w/w) aqueous. After an exposure period of 10 seconds, the corneal surface was rinsed thoroughly with 20 ml of isotonic saline of ambient temperature. After rinsing, each eye in the holder was returned to its chamber. This procedure was repeated for the three eyes exposed to 2-methoxymethyl-p-phenylenediamine sulfate at a concentration of 10% (w/w) aqueous.
All examinations were performed with a slit-lamp microscope. Fluorescein retention was scored only at 30 minutes after treatment. After the final examination the test and control eyes were preserved in a neutral aqueous phosphate-buffered solution of 4% formaldehyde and then subjected to histopathological examination. Ocular effects were evaluated using the endpoints of corneal thickness (swelling), corneal opacity and fluorescein retention.

Results
2-Methoxymethyl-p-phenylenediamine sulfate tested at 3% (w/w) aqueous caused almost no corneal effects with almost no swelling (2%), no or very slight opacity (0.3) and no or very slight fluorescein retention (0.3). The Irritation Index calculated was 14. Microscopic examination of the treated corneas confirmed the absence of meaningful corneal effects.

2-Methoxymethyl-p-phenylenediamine sulfate tested at 10% (w/w) aqueous also caused almost no corneal effects with almost no swelling (1%), no or very slight opacity (0.3) and no or very slight fluorescein retention (0.3). The Irritation Index calculated was 13. Microscopic examination of the treated corneas confirmed the absence of meaningful corneal effects.

Conclusion
On the basis of the results obtained in the ICE study, 2-methoxymethyl-p-phenylenediamine sulfate tested at 3 and 10% (w/w) aqueous is identified as not irritating to eyes (Not Classified according to the EU-CLP classification scheme).

Ref.: 37

Comment
The available data suggests that 2-methoxymethyl-p-phenylenediamine is not expected to be irritant to the eye at anticipated use exposures.

The CEET (ICE) is a screening method for hazard identification and not for risk assessment. The method has now been adopted as OECD guideline 438 (2009) for eye corrosivity and severe irritancy. No fully validated alternative methods for eye irritation exist.

3.3.3 Skin sensitisation

Local Lymph Node Assay (LLNA)

Guideline:	OECD 429 (2002)
Species/strain:	mouse, CBA/J
Group size:	5 females per test group
Test substance:	1,4-diamino-2-methoxymethyl-benzene sulfate (1:1) WR801337
Batch:	GST070-05/24-01
Purity:	99.5%
Vehicle:	DMSO
Concentration:	0.5, 1.5, 5.0 and 15% (w/v)
Positive control:	alpha-hexylcinnamaldehyde, 25.0% (v/v)
GLP:	in compliance
Study period:	29 June – 7 November 2005

25 µl of 0 (vehicle only), 0.50, 1.50, 5.0 and 15.0% 1,4-diamino-2-methoxymethyl-benzene sulphate in DMSO were applied to the surface of the ear of five female mice per group for three consecutive days. As a positive control, alpha-hexylcinnamaldehyde (HCA) at a concentration of 25.0% in DMSO was investigated in parallel under identical test conditions. At day 5, the mice received an intravenous injection of 250 µl solution containing 21.2 µCi of [^{3}H] methyl thymidine. Approximately five hours later, the mice were sacrificed, and the draining auricular lymph nodes were removed and collected in PBS. After preparing a single cell suspension for each mouse, cells were precipitated by 5% trichloro-acetic acid (TCA), and the radioactivity was determined (incorporation of [^{3}H] methyl thymidine in the pellets) by means of liquid scintillation counting as disintegration per minute (dpm).

Results
The positive control alpha-hexylcinnamaldehyde (HCA) at a concentration of 25.0% in DMSO induced a 7.2 fold increase in isotope incorporation in the draining auricular lymph nodes relative to the vehicle.

1,4-Diamino-2-methoxymethyl-benzene sulfate in DMSO was positive in the local lymph node assay, as there was more than a 3-fold increase in isotope incorporation in the draining auricular lymph nodes relative to the vehicle. The mean stimulation indices were 1.1, 1.2, 2.2 and 6.0 at the concentrations of 0.5, 1.5, 5.0 and 15.0%, respectively. The EC3 value was 7.11%.

Conclusion
1,4-Diamino-2-methoxymethyl-benzene sulfate is a moderate skin sensitizer under the defined experimental conditions, with a calculated EC3 value of 7.11%.

Ref.: 38

Comment
For the free base of 1,4-diamino-2-methoxymethyl-benzene:

The EC3 value of the free base may be calculated from the sulfate salt by using the conversion factor to account for the different molecular weight.

- EC3 value SO_4-salt: 7.11%
- 7.11% = 7.11 g/100 ml x 0.61 (conversion factor) = 4.3 g/100 ml = 4.3%
- EC3 value free base: 4.3%

Therefore, the calculated EC3 value for the free base of 1,4-diamino-2-methoxymethyl-benzene is 4.3%, which is a moderate skin sensitiser.

3.3.4 Dermal / percutaneous absorption

Guideline:	OECD 428
Tissue:	split thickness pig skin from back and flank (thickness: 1000 µm); 3 donors
Skin integrity:	tritiated water
Method:	flow through Teflon chambers with 9.2 cm² surface
Group size:	6 chambers in 2 independent experiments
Test substance:	2-methoxymethyl-p-phenylenediamine [ring-U-^{14}C]MEOME PPD
Batch:	20080201 (Dragon) FQ40384 Batch B1
Purity:	98.4 weight% (NMR) 96.7% (radiochemical purity)
Receptor fluid:	physiological receptor fluid
Solubility receptor fluid:	307.85 mg/ml (pH 7.3)
Test item:	Color Cream formulation with 1.824% 2-methoxymethyl-p-phenylenediamine
Dose volume:	100 mg/cm²
Method of Analysis:	Liquid scintillation counting
GLP:	in compliance
Study period:	29 September – 27 October 2008

Two independent experiments were performed with 6 diffusion cells per experiment. For calculations, the mean value of all valid skin samples (n=11, three donors) in contact with 1.824% 2-methoxymethyl-p-phenylenediamine in a typical hair dye formulation in the presence of hydrogen peroxide and a reaction partner was used.
Eleven of the twelve skin samples were within the limit of acceptance.

After checking the skin integrity, 400 mg of the formulation (= 100 mg/cm²), containing radiolabelled 1.824% of 2-methoxymethyl-p-phenylenediamine, was applied to the skin samples (= 1.824 mg of test item/cm²) for 30 minutes and subsequently washed off with water and shampoo. The determination of the amount of 2-methoxymethyl-p-phenylenediamine in the washings (= amount dislodgeable from the skin surface) was performed by measuring the radioactivity by liquid scintillation counting. At 16, 24, 40, 48, 64 and 72 hours, the content 2-methoxymethyl-p-phenylenediamine was determined in the receptor fluid by the same method. At termination of the experiment, the skin was heat-treated and the "upper skin" (stratum corneum and upper stratum germinativum) was mechanically separated from the "lower skin" (lower stratum germinativum and upper dermis). Both skin compartments were extracted separately and the radioactivity was quantified by liquid scintillation counting.

Results
72 hours cutaneous absorption of 1.824% 2-methoxymethyl-p-phenylenediamine in a typical hair dye formulation in the presence of hydrogen peroxide and a reaction partner 4-amino-2-hydroxytoluene:

	Skin	Integrity-Test	1)		2)		3)		4)		1) + 2) + 3) + 4)	
	No (series)	^3H_2O Permeation (4 hours cumulative)	Receptor fluid (72 hours cumulative)		Lower skin (after 72 hours)		Upper skin (after 72 hours)		Rinsing solution (after 30 minutes)		Total***	
		[% Dose]	[µg/cm²]	[% Dose]**	[µg/cm²]	[% Dose]**	[µg/cm²]	[% Dose]**	[µg/cm²]	[% Dose]**	[µg/cm²]	[% Dose]
Application of 100 mg 1.824 % 2-Methoxymethyl-p-phenylenediamine in a typical hair dye formulation* per 1 cm² skin	2 (1)	1.0	1.320	0.078	0.371	0.022	8.258	0.488	1546.15	91.34	1687.41	99.69
	4 (1)	1.5	2.290	0.133	0.412	0.024	7.992	0.464	1605.96	91.16	1716.85	99.60
	6 (1)	1.6	1.188	0.070	0.080	0.005	3.911	0.231	1565.94	92.65	1704.95	100.88
	8 (1)	0.9	1.218	0.069	0.057	0.003	4.082	0.233	1660.51	94.57	1734.08	98.76
	10 (1)	0.9	1.259	0.073	0.111	0.006	4.456	0.257	1595.98	92.10	1693.00	97.70
	12 (1)	0.9	1.172	0.067	0.106	0.006	5.009	0.288	1665.45	95.78	1756.90	101.04
	2 (2)	1.7	1.126	0.063	0.478	0.027	14.284	0.802	1676.74	94.13	1735.36	97.42
	4 (2)	2.5****	*1.999*	*0.114*	*0.545*	*0.031*	*16.086*	*0.919*	*1652.69*	*94.39*	*1744.43*	*99.63*
	6 (2)	0.8	1.207	0.068	0.245	0.014	6.675	0.378	1646.69	93.23	1712.46	96.95
	8 (2)	1.0	1.164	0.066	0.219	0.012	11.248	0.637	1643.12	93.05	1713.86	97.05
	10 (2)	0.7	1.423	0.081	0.176	0.010	7.098	0.406	1581.05	90.41	1665.02	95.21
	12 (2)	0.9	1.385	0.080	0.157	0.009	5.932	0.342	1615.09	93.22	1713.96	98.92
Mean		**1.2**	**1.341**	**0.077**	**0.219**	**0.013**	**7.177**	**0.411**	**1618.43**	**92.88**	**1712.17**	**98.47**
± S.D		0.5	0.328	0.019	0.143	0.008	3.211	0.179	43.40	1.59	25.10	1.79
(n)		(12)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)

*vehicle: (typical hair dye formulation as detailed in Annex III); **Corrected for individual applied dose; *** Total is corrected for losses on tips; ****outlier: not considered for the calculation of the mean values.

The majority of the test substance was found in the rinsing solutions (1618.43 ± 43.40 µg/cm²). Small amounts of 2-methoxymethyl-p-phenylenediamine were found in the upper skin (7.177 ± 3.211 µg/cm²), in the lower skin (0.219 ± 0.143 µg/cm²) and in the fractions of the receptor fluid collected within 72 hours (1.341 ± 0.328 µg/cm²).

The mass balance of the test substance resulted in values of 95.2 to 101.0% recovery for all (11) skin samples taken into consideration for the calculation of the mean values.

Amount of 2-methoxymethyl-p-phenylenediamine in:	µg/cm² (mean ± S.D, n=11)			%* (mean ± S.D, n=11)		
Receptor fluid (72 hours)	1.341	±	0.328	0.077	±	0.019
Lower skin (72 hours)	0.219	±	0.143	0.013	±	0.008
Upper skin (72 hours)	7.177	±	3.211	0.411	±	0.179
Rinsing solution (after 60 min.)	1618.43	±	43.40	92.88	±	1.59
Total balance (recovery)**	1712.17	±	25.10	98.47	±	1.79

Under the assumption that a depot effect is absent, the mean amount of 1.56 µg/cm² (1 SD 0.387 µg/cm²) of 2-methoxymethyl-p-phenylenediamine in a typical oxidative hair dye formulation with 1.824% dye and in the presence of reaction partners was detected as the mean bioavailable fraction (n=11, three donors; receptor fluid + lower skin; 1.341 µg/cm² + 0.219 µg/cm²). This is equivalent to 0.0896 ± 0.023% of the applied dose.

Ref.: 41

Comment

The dose volume was too high. 3 donors instead of 4 were used and recovery was over 72 hours.

In a typical oxidative hair dye formulation with 1.824% 2-methoxymethyl-p-phenylenediamine and in the presence of reaction partners, the bioavailable fraction used for the MOS is (mean + 2SD) 2.33 µg/cm², or 0.14% of the applied dose.

3.3.5 Repeated dose toxicity

3.3.5.1 Repeated Dose (28 days) oral toxicity

No data submitted

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

Guideline:	OECD 408 (1998)
Species/strain:	rat, HanRcc:WIST (SPF)
Group size:	15 males and 15 females (group 1 and 4); 10 males and 10 females (groups 2 and 3)
Test substance:	1,4-diamino-2-methoxymethyl-benzene sulphate (1:1) (WR801337)
Batch:	GST070-05/24-01
Purity:	99.5 area% (HPLC at 254 and 312 nm)
Vehicle:	0.4% aqueous solution of ascorbic acid, adjusted with NaOH (1N) to pH 5.7 - 6.1
Dose levels:	0, 10, 30 and 90 mg/kg bw/day
Dose volume:	10 mL/kg bw
Route:	oral, by gavage
Administration:	daily for 91/92 days
GLP:	in compliance
Study period:	13 March 2006 – 7 January 2010

In this sub-chronic study, Wistar rats were exposed daily during 91/92 days by gavage to 1,4-diamino-2-methoxymethyl-benzene sulphate at the dose of 0, 10, 30 and 90 mg/kg bw/day (n=10 males and 10 females per dose except for the control and maximum dose: n = 15). The rats were sacrificed after the last administration (group 2 and 3) or 28 days later (group 1 and 4). Clinical signs, food consumption, body weights were recorded periodically. Opthalmoscopic examinations and functional tests were performed. A peer review of the results and conclusion of the study was performed in 2010.

Results
All rats survived until sacrifice.

Corneal opacity reported in all groups including the control at a frequency greater than the historic control was not considered related to the treatment.

Orange discoloration of the urine was reported at the doses of 30 and 90 mg/kg bw/d.

At the dose of 90 mg/kg bw/d, minor modifications in biochemistry (marginal increase in AST, LDH and CK) values were reported. They were mostly in the range of historical control values. A small increase in absolute and relative liver weight was observed in male rats and was not considered toxicologically relevant but as an adaptive response. In addition, treatment-related hepatocellular hypertrophy was observed in one high dose male. However, there were no significant differences in these parameters after recovery.

At doses of 10 and 30 mg/kg bw/day, no test item related changes were observed in the animals compared to the controls. No significant histopathological lesions were observed.

Conclusion
A NOAEL of 90 mg/kg bw/d (highest dose tested) and a NOEL of 30 mg/kg bw/d may be derived from this study. The equivalent NOAEL for the free base is 55 mg/kg bw/d.

Ref.: 42, 43

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

Guideline:	OECD 471 (1997)
Species/Strain:	*Salmonella typhimurium* TA98, TA100, TA102, TA1535 and TA1537
Replicates:	triplicates in a single experiment
Test substance:	1,4-diamino-2-methoxymethyl-benzene sulphate (1:1) (WR801337)
Batch:	GST070-05/24-01
Purity:	99.5 area% (HPLC)
Solvent:	deionised water
Concentrations:	0, 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate without and with S9-mix
Treatment:	direct plate incorporation with at least 48 h incubation, without and with S9-mix
GLP:	in compliance
Study period:	19 December 2006 – 22 December 2006

1,4-diamino-2-methoxymethyl-benzene sulphate was investigated for the induction of gene mutations in *Salmonella typhimurium* (Ames test). Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the results of a pre-experiment for toxicity and mutation induction with all strains both without and with S9-mix. Toxicity was evaluated for 8 concentrations up to the prescribed maximum concentration of 5000 µg/plate on the basis of a reduction in the number of revertant colonies and/or clearing of the bacterial background lawn. Since in this pre-experiment evaluable plates were obtained for five concentrations or more in all strains used, the pre-experiment is reported as the main experiment. The experiment was performed with the direct plate incorporation method. Negative and positive controls were in accordance with the OECD guideline.

Results
No precipitation occurred up to the highest concentration investigated. Toxic effects evident as reduction in the number of revertants or clearing of the bacterial background lawn were not observed up to the highest concentrations without and with S9-mix in all strains.
A concentration dependent and statistically significant increase in the number of revertants was seen in TA98, TA100, TA1535 and TA1537 in the presence of metabolic activation. In the absence of metabolic activation, a biologically relevant increase in revertant colonies was not found in any tester strain.

Conclusion
Under the experimental conditions used 1,4-diamino-2-methoxymethyl-benzene sulphate was mutagenic in this gene mutation tests in bacteria in the presence of metabolic activation.

Ref.: 44

Comment
The results were not confirmed in an independent repeat experiment.

***In vitro* Mammalian Cell Gene Mutation Test (*tk*-locus)**

Guideline:	OECD 476 (1997)
Cells:	Mouse lymphoma L5178Y cells
Replicates:	duplicate cultures in a single experiment
Test substance:	WR 801337 (1,4-diamino-2-methoxymethyl-benzene sulphate (1:1))
Batch:	GST070-05/24-01
Purity:	99.5 area% (HPLC)
Solvent:	cell culture medium (RPMI + 3% HS)
Concentrations:	0, 10, 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 µg/ml without S9-mix 0, 400, 600, 800, 1000, 1250, 1500, 1750, 2000 and 2250 µg/ml with S9-mix
Treatment	4 h treatment without and with S9-mix; expression period 72 h and selection period of 11-14 days
GLP:	in compliance
Study period:	8 April 2002 – 29 July 2002

WR 801337 was assayed for gene mutations at the *tk* locus of mouse lymphoma cells in both the absence and presence of S9 metabolic activation. Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the results of a pre-test on toxicity measuring relative suspension growth and relative total growth. Six concentrations, up to 2636 µg/ml, were tested without metabolic activation. In the main tests, cells were treated for 4 h followed by an expression period of 72 h to fix the DNA damage into a stable *tk* mutation. Toxicity was measured in the main experiments as percentage total growth of the treated cultures relative to the total growth of the solvent control cultures. To discriminate between large (indicative for mutagenic effects) and small colonies (indicative for a clastogenic effect) colony sizing was performed. In combination with a positive effect in the MF, an increased occurrence of small colonies indicated by a low large/small colony ratio (< 4), is associated with clastogenic effects and/or chromosome aberrations. Negative and positive controls were in accordance with the OECD guideline.

Results
Without and with metabolic activation growth inhibition was observed. With metabolic activation, the highest biologically relevant concentration evaluated was 1750 µg/ml with a relative total growth of 9.14%; without metabolic activation, the highest biologically relevant concentration was 450 µg/ml with a relative total growth of 8.8%.
With metabolic activation, a more or less concentration dependent and biologically relevant increase in the mutant frequency was found; a slight decrease in the MF was seen at the mid concentration of 1500 µg/ml. Without metabolic activation, a biologically relevant increase in the mutant frequency was observed as well.
With metabolic activation the ratio large/small colonies was below 4 at some concentrations (1250 and 1750 µg/ml) without clear indications for a more clastogenic or mutagenic effect of WR 801337. Whereas without metabolic activation the number of large and small colonies increased, the ratio large/small colonies concentration dependently decreased with value below 4 at 350 µg/ml and above indicating to mutagenic and clastogenic effects of WR 801337.

Conclusion
Under the experimental conditions, used WR 801337 was mutagenic in this gene mutation test in mouse lymphoma cells. Since both the number of large and small colonies increased and the ratio large/small colonies decreased, the results indicate mutagenic and clastogenic effects of WR 801337.

Ref.: 46

Comment
The results were not confirmed in an independent repeat experiment.

***In vitro* Micronucleus Test in human lymphocytes**

Guideline:	In accordance with recommendations of IWTG workshop, draft OECD 487 (2004) and accepted scientific/regulatory principles described in current guidelines for clastogenicity testing *in vitro*.	
Test system:	cultured human peripheral blood lymphocytes	
Replicates:	duplicate cultures, 2 independent experiments	
Test item:	1,4-diamino-2-methoxymethyl-benzene sulphate (1:1) (WR801337)	
Batch:	GST070-05/24-01	
Purity:	99.5 area% (HPLC)	
Solvent:	DMSO	
Concentrations:	experiment 1:	200, 300, 450 and 575 µg/ml without S9-mix 1000, 1750 and 2560 µg/ml with S9-mix
	experiment 2	200, 300 and 350 µg/ml without S9-mix 2000, 2250 and 2560 µg/ml with S9-mix
Treatment	experiment 1:	24 h PHA, 20 h treatment and 28 h recovery without S9-mix 24 h PHA, 3 h treatment and 45 h recovery with S9-mix
	experiment 2:	48 h PHA, 20 h treatment and 28 h recovery without S9-mix 48 h PHA, 3 h treatment and 45 h recovery with S9-mix
GLP:	in compliance	
Study period:	3 August 2005 – 9 November 2005	

1,4-diamino-2-methoxymethyl-benzene sulphate has been investigated for the induction of micronuclei in cultured human lymphocytes in the absence and presence of metabolic activation. Blood from two healthy, non-smoking female volunteers was used in this study. The mitogen phytohaemagglutinin (PHA) was included in the culture medium in order to stimulate the lymphocytes to divide, and blood cultures were incubated at 37° C for 24 h or 48 h and rocked continuously. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system.
The concentration selection for the micronucleus test was based on the cytotoxicity data from a range-finder experiment with concentrations up to 1600 µg/ml measuring replication index (RI). The top concentration for micronucleus analysis was to be the one at which approximately 60% reduction in RI occurred.
Both in the range finder and in the main test, cells were treated either for 3 h in the presence of S9-mix or 20 h in the absence of S9-mix, and sampled 48 h after start of treatment. Cytochalasin B (final concentration 6 µg/ml) was added the final 28 h before harvest. Negative and positive controls were in accordance with the OECD draft guideline.

Results
Measurements on post-treatment media in the absence or presence of S9-mix indicated that 1,4-diamino-2-methoxymethyl-benzene sulphate had no marked effect on osmolarity or pH as compared to concurrent vehicle controls.
In both experiments without S9 metabolic activation 1,4-diamino-2-methoxymethyl-benzene sulphate did induce a statistically significant and more or less concentration dependent increase in the number of cells with micronuclei compared to the concurrent untreated controls. Increases were small and only single cultures showed frequencies of micronucleated lymphocytes outside the historical control range. The positive findings were considered of questionable biological importance.
In the presence of S9 metabolic activation, in experiment 1 (24 h PHA stimulation) a statistically significant and dose dependent increase in the number of cells with micronuclei compared to the concurrent untreated controls was observed. Again the increases were small and only one single culture showed a micronucleated lymphocytes frequency outside the historical control range and thus these positive findings were again considered of

questionable biological importance. In experiment 2 (48 h PHA stimulation) a biologically relevant increase in the number of cells with micronuclei compared to the concurrent untreated controls was not found.

Conclusion
Under the experimental conditions used, 1,4-diamino-2-methoxymethyl-benzene sulphate was genotoxic (clastogenic and/or aneugenic) in human lymphocytes *in vitro*. However, the positive findings were considered of questionable biological importance.

Ref.:47

Comment
Although 1,4-diamino-2-methoxymethyl-benzene sulphate did induce an increase in micronucleated human lymphocytes, the authors concluded the positive findings of questionable biological importance. SCCS agrees with this conclusion.

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 males and 5 females per test group
Test substance:	1,4-diamino-2-methoxymethyl-benzene sulphate (1:1)
Batch:	GST070-05/24-01
Purity:	99.5 area% (HPLC)
Vehicle:	aqua dest.
Dose level:	0, 10, 50 and 100 mg/kg bw
Route:	intraperitoneal injection
Sacrifice times:	24 h and 48 (highest dose only) h after injection
GLP:	in compliance
Study period:	3 July 2002 - 6 August 2002

1,4-diamino-2-methoxymethyl-benzene sulphate was investigated for the induction of micronuclei in bone marrow cells of mice. Test doses were based on the results of a pre-experiment for toxicity. Mice were treated ip with 100, 250 and 2000 mg/kg bw under identical conditions as in the main test and observed for acute toxic symptoms at intervals around 1, 6, 24, 48 and 72 h post application.
In the micronucleus test mice were treated by ip injection with 0, 10, 50 and 100 mg/kg bw. The mice of the highest dose group were examined for acute toxic symptoms at intervals of around 1, 6, 24 and 48 h after treatment. Bone marrow cells were collected 24 h and 48 h (highest dose only) after dosing. Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and total erythrocytes (PCE/TE). Bone marrow preparations were stained with May-Grünwald/Giemsa and examined microscopically for the PCE/TE ratio and micronuclei. Negative and positive controls were in accordance with the OECD guideline.

Results
In the pre-experiment for toxicity, mice (2 females/dose) treated with 2000 and 250 mg/kg bw showed palpebral closure, lethargy and convulsions (2000 mg/kg bw only) died 10 minutes and 6 h after treatment, respectively. Mice (3 mice/sex/dose) treated with 100 mg/kg bw showed palpebral closure and lethargy 1 h after treatment whereas no symptoms of toxicity were observed at 6, 24 and 48 h after treatment. For the main experiment 100 mg/kg bw was chosen as top dose.
In the micronucleus test, mice treated with the top dose (100 mg/kg bw) showed identical symptoms of toxicity 1 h after treatment which were gone at 6, 24 and 48 h after treatment.

In comparison to the concurrent negative controls, in the micronucleus test the PCE/TE ratio was slightly decreased in the higher dose groups. This effect is considered to be biologically relevant and indicates to exposure of bone marrow cells with 1,4-diamino-2-methoxymethyl-benzene sulphate.
Compared to the concurrent vehicle controls, a biologically relevant increase in erythrocytes with micronuclei at any preparation interval and dose level after treatment with 1,4-diamino-2-methoxymethyl-benzene sulphate was not observed.

Conclusion
Under the experimental conditions used 1,4-diamino-2-methoxymethyl-benzene sulphate is not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice.

Ref.: 51

In Vivo Unscheduled DNA Synthesis (UDS) Test

Guideline:	OECD 486 (1997)
Species/strain:	rat, Wistar Hanlbm: WIST (SPF)
Group size:	4 male rats per test group
Test substance:	1,4-diamino-2-methoxymethyl-benzene sulphate (1:1)
Batch:	GST070-05/24-01
Purity:	99.5 area% (HPLC)
Vehicle:	deionised water
Dose level:	0, 75 and 150 mg/kg bw
Route:	oral
Sacrifice times:	4 h and 16 h after dosing
GLP:	in compliance
Study period:	15 January 2007 – 23 February 2007

1,4-diamino-2-methoxymethyl-benzene sulphate was investigated for the induction of unscheduled DNA synthesis (UDS) in hepatocytes of rats. Test concentrations were based on a pre-experiment for toxicity measuring acute toxic symptoms at 1, 2-4, 6 and 24 h after oral administration of 100, 150, 200 and 500 mg/kg bw. In the main experiment rats were treated orally with 0, 75 and 150 mg/kg bw and were examined for toxic symptoms at 1, 2 and 4 h (4 h treatment) or at 1, 2-4 and 16 h (16 h treatment) after treatment.
To quantify the concentration of 1,4-diamino-2-methoxymethyl-benzene sulphate and putative metabolites, in plasma heparinised blood samples were taken by retroorbital puncture from rats treated with the highest dose and the controls.
Hepatocytes for UDS analysis were collected by perfusion with 0.05% w/v collagenase approximately 2 h (high dose only) and 16 h after administration of 1,4-diamino-2-methoxymethyl-benzene sulphate. The quality of the hepatocytes after perfusion was determined by the trypan blue dye exclusion method. Three cultures were established for each animal. At least 90 minutes after plating the cells were incubated for 4 h with 5 µCi/ml ^{3}H-thymidine (specific activity 20 Ci/mmol) followed by overnight incubation with unlabelled thymidine. Evaluation of autoradiography was done after 14 days.
The nuclear and cytoplasmic grain counts, the net grains counts (nuclear minus cytoplasmic grains) as well as the mean and percentage of cells in repair (cells with a net grain count >5) were reported. Increased net grain counts should be based on enhanced nuclear grain counts rather than on decreased cytoplasmic grain counts. Negative and positive controls were in accordance with the OECD guideline.

Results
In the pre-experiment for toxicity at 500 and 200 mg/kg bw 3 female rats per dose died. All rats showed reduction of spontaneous activity and ruffled fur; rats treated with 500 mg/kg bw showed additionally abdominal position and eyelid closure. For all mice red coloured urine was reported indicating systemic distribution and thus bioavailability of 1,4-diamino-2-

methoxymethyl-benzene sulphate. For the main experiment 150 mg/kg bw was chosen as top dose.
In the main test, the rats demonstrated the same toxic reactions, reduction of spontaneous activity and ruffled fur, and had red coloured urine, as well.
The viability of the hepatocytes determined by means of the trypan blue dye exclusion assay was not substantially effected by the treatment with 1,4-diamino-2-methoxymethyl-benzene sulphate at any of the treatment periods or dose groups.
A biological relevant increase in UDS induction as compared to the untreated control was not found in hepatocytes of any treated animal both for the 2 h and the 16 h treatment time. No substantial shift to higher values was obtained in the percentage of cells in repair.

Conclusions
Under the experimental conditions used, 1,4-diamino-2-methoxymethyl-benzene sulphate did not induce unscheduled DNA synthesis and, consequently, is not genotoxic in rats in this *in vivo* UDS test.

Ref.: 54

Comment
To quantify the concentration of 1,4-diamino-2-methoxymethyl-benzene sulphate and putative metabolites in plasma heparinised blood, samples were taken by retro-orbital puncture from rats treated with the highest dose and the controls. The actual blood concentrations were not mentioned in the present report.

In vivo **alkaline single cell gel electrophoresis (comet) assay in rats**

Guideline:	/
Species/strain:	male rats, CRL:WI) BR Wistar
Organs studied:	liver, stomach and urinary bladder epithelium cells
Group size:	5 male rats per dose group
Test substance:	methoxymethyl-PPD-sulfat (WR 801337)
Batch:	GST070-05/24-01
Purity:	99.5 area % (HPLC)
Vehicle:	deionised water with 0.4% ascorbic acid, adjusted to pH 5.1 with NaOH
Dose level:	0, 25, 50, 100 mg/kg bw/treatment
Treatment:	orally by gavage, twice 20 h apart
Sacrifice times:	3 h after second treatment
GLP:	in compliance
Study period:	1 September 2003 – 10 October 2003

Methoxymethyl-PPD-sulfat has been investigated for the induction of DNA damage in the alkaline single cell gel electrophoresis (comet) assay in various tissues of rats. Test doses were based on the results of a pilot study in which 3 male rats were treated orally with 2000, 200 and 100 mg/kg bw/day. Rats were observed 1 h after each treatment and before the second treatment.
In the main experiment, male rats were treated twice orally 20 h apart with 0, 25, 50 and 100 mg/kg bw/treatment. Food was withdrawn from about 4-5 h before until 1 h after the 1st treatment. Immediately after each treatment and just before anaesthesia rats were observed for mortality and clinical signs of stress.
Tissues were collected 3 h after the 2nd treatment. Liver cells were obtained after perfusion with type CLS II collagenase, stomach cells and urinary bladder epithelial cells after incubation with 0.25% trypsine. Viability of liver, stomach and urinary bladder epithelial cells of the vehicle control animals should normally exceed 70%; below 50% preparations from control animals are considered unacceptable. No such limits were set for treated rats. Cytotoxicity was determined as viability relative to the vehicle control.

After lysis, alkaline treatment at pH ≥ 13 was done followed by electrophoresis, which was performed for 40 min (30 min for stomach cells) at 25 V and 300 mA.
Tail length defined as the distance between the middle of the head and the end of the tail, was used as assessment parameter. Fifty cells per slide and 2 slides per tissue were evaluated. Relevant positive controls were included in the experiments.

Results
In the pilot study, all rats died shortly after application of 2000 mg/kg bw. At 200 mg/kg bw 2 of the 3 rats died about 19 h after application. One h after the 1st application and just before the 2nd application of 100 mg/kg bw, the rats showed reduced motility, roughened fur and reddish discoloured urine; directly after the 2nd treatment roughened fur, pallor, hunched posture were seen; 20 h after the 2nd application red coloured urine was observed and 48 h after the 2nd treatment the rats showed increased water intake and micturition. Based on these results 100 mg/kg bw was chosen as the top-dose.
Treatment with methoxymethyl-PPD-sulfat did not result in a substantial decrease in viability of liver, stomach and urinary bladder epithelial cells.

A biologically relevant increase in tail length was not observed in cells of the stomach and urinary bladder. In livers, increases in mean tail length values per rat were observed for 2-3 rats per dose group. Since not all rats showed increased tail length values, the increases in tail length per dose group were relatively small, and a dose dependency (an inverted dose dependency was observed) of the result for the liver was not found, the results found for the liver were concluded to be equivocal.

Conclusion
Under the experimental conditions used, methoxymethyl-PPD-sulfat did not induce a biologically relevant increase in DNA damage in cells of stomach and urinary bladder of rats and, consequently, methoxymethyl-PPD-sulfat is not genotoxic in these tissues of rats. The results obtained for liver cells treated with methoxymethyl-PPD-sulfat were considered equivocal.

Ref.: 53

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

Guideline:	OECD 414 (2001)
Species/strain:	Sprague-Dawley rats Crl: OFA.SD
Group size:	25 females per dose group
Test substance:	1,4-diamino-2-methoxymethyl-benzene sulphate (1:1)
Batch:	GST070-05/24-01
Purity:	99.5 area% at 254 and 312 nm (HPLC)
Vehicle:	0.4% ascorbic acid in water for injection
Dose levels:	0, 10, 30 and 90 mg/kg bw/day
Dose volume:	5 ml/kg bw/day
Route:	oral (gavage)
Administration:	once daily from day 6 (G6) to day 19 (G19) of gestation inclusive
GLP statement:	in compliance

Study period: 4 May 2006 – 25 September 2007

In this developmental toxicity study, Sprague-Dawley female rats were exposed by gavage to 1,4-diamino-2-methoxymethyl-benzene sulphate (1:1) once daily from day 6 to day 19 of gestation at doses of 10, 30 and 90 mg/kg bw/day. A control group of 25 mated rats received the vehicle only. The day of mating was designated as day 0 of gestation. Animals were observed twice daily for morbidity/mortality. Clinical signs were checked daily. Food consumption and body weight gain were recorded. On day 20 of gestation, the animals were killed and examined macroscopically and subjected to necropsy to determine several ovary and uterine related parameters. Foetuses were weighed, sexed and examined for possible external abnormalities.

Results
No unscheduled deaths were reported in any group. At the dose of 30 and 90 mg/kg bw/day, orange discoloration of the urine was noted.

In the time period of gestation day 6 to day 9, food consumption and body weight gain showed statistically significant reductions in the high dose group. Thereafter mean weight gain was comparable between control and treated rats.

There were no treatment related macroscopic findings at necropsy of the adult females. There was a slightly higher incidence of late resorptions in the high dose group compared with the control group but this was related to a single female. This isolated finding was considered to be incidental. Another female in the high dose group showed one late resorption and no viable foetuses. This incidental finding was considered to be a normal physiological response due to the presence of the single implantation and of no toxicological relevance.

No external abnormalities and no visceral or skeletal malformation related to the treatment were seen in foetuses. The incidences of foetuses with delayed ossification of the cranium, paws or sternum were slightly higher in the 90 mg/kg bw/day groups. Similar but less marked increase was observed in the 30 and 10 mg/kg bw/day dose groups but none of these increases were statistically significant. These findings were indicative of a minor delay in foetal ossification which was considered too slight for being of physiological relevance. A slightly greater incidence of rudimentary 14^{th} rib in all the treated groups was observed. These findings were considered by the applicant of no toxicological relevance.

Conclusions
Based on the reduction of the body weight gain and food consumption in the high dose group, the NOAEL for maternal toxicity was 30 mg/kg bw/day, while the NOAEL for embryo-toxicity/teratogenicity was 90 mg/kg bw/ day.

Ref.: 58

Comments
The equivalent NOAEL for the free base is 18 mg/kg bw/day for the maternal toxicity and 55 mg/kg bw/day for the embryo-toxicity/teratogenicity.

3.3.9 Toxicokinetics

In vitro metabolism of methoxymethyl-PPD sulfate in cryopreserved human hepatocytes

Guideline:	/
Test system:	cryopreserved human hepatocytes
Test design:	one experiment with suspended and one with plated hepatocytes
Test substance:	2-methoxy-methyl-PPD-sulfate ^{14}C-2-(methoxymethyl)benzene-1,4-diamine

Batch:	radiolabelled: CFQ40317,B1 Non-radiolabelled: GST070-05/24-01
Purity:	99.5%
Test concentrations:	1.0 and 10 µg/ml (suspended hepatocytes) 1.08, 10.8 and 107.08 µg/ml (plated hepatocytes)
Incubation time:	180 min
GLP statement:	Not in compliance
Study period:	/

In this study, 2-(methoxymethyl)benzene-1,4-diamine sulfate was incubated with human suspended or plated hepatocytes to assess the metabolism. Two concentrations (1.0 and 10 µg/ml) were tested for suspended hepatocytes and 3 for plated hepatocytes (1.08, 10.8 and 107.8 µg/ml).

Results
In the suspended hepatocytes incubations, only a N-monoacetylated metabolite was observed. The amount of the metabolite was greater at the highest concentration but did not increase proportionally (0.16 µg/ml at the low dose and 0.49 µg/ml at the high dose).

In the plated hepatocytes study, two metabolites were observed: N-mono-acetylated and a cysteine conjugate. The amount of the monoacetylated metabolite increase in the dose range of 1.08 to 10.8 µg/ml but decrease in the dose range of 10.8 to 107.8 µg/ml. The cysteine conjugate was only found at the highest test concentration.

Two additional products were observed: one conjugate of 2-(methoxymethyl)benzene-1,4-diamine sulfate with glucose and a cyclisation product (not confirmed).

Ref.: 59

Comment
The cryo-conservation might impair some metabolising enzymes.

In vitro skin metabolism of 2-methoxymethyl-p-phenylene diamine sulfate following 24 hours incubation with HaCaT cells

Guideline:	/
Test system:	HaCaT cells (human keratinocytes)
Test substance:	^{14}C-2-(methoxymethyl)benzene-1,4-diamine
Batch:	/
Purity:	/
Vehicle:	stable cell number media (DMEM with 5% foetal bovine serum and 1% Pen-Strep diluted 1:10 in HBSS with 2.15 g glucose)
Dose levels:	0.625, 1.25, 2.5, 5 and 10 µg/ml
GLP statement:	not in compliance
Study period:	2010

In this study, 2-(methoxymethyl)benzene-1,4-diamine sulfate was incubated with human keratinocytes to assess the metabolism. Five concentrations (0.625, 1.25, 2.5, 5 and 10 µg/ml) were tested.

Results
2-(methoxymethyl)benzene-1,4-diamine sulphate is acetylated at the non-hindered amine position to monoacetylated 2-(methoxymethyl)benzene-1,4-diamine sulphate. As a second transformation step, this metabolite likely undergoes O-demethylation under the conditions applied.
Two additional products were observed: one conjugate of 2-(methoxymethyl)benzene-1,4-diamine sulfate with glucose and a cyclisation product (not confirmed).

Ref.: 60

ADME of 1,4-diamino-2-methoxymethyl-benzene sulfate (WR801337) in the Wistar rat

Guideline:	OECD 417 (1984); OECD 427 (2004)	
Species/strain:	rat: Wistar Crl:WI	
Group size:	4 females/dose level (group 1 to 5)	
	6 females/dose level (group 6, 7, 8 and 10)	
	7 females/dose level (group 9)	
Test substance:	1,4-diamino-2-methoxymethyl-benzene sulphate (WR801337)	
	[ring-U-^{14}C]MEOME PPD sulfate (740 MBq/mmol)	
Batch:	GST070-05/24-01	
	CFQ14701 batch 1 (radiolabelled)	
Purity:	99.5 area% (HPLC at 254 nm)	
	98.5% (HPLC) (radiochemical purity)	
Vehicle:	0.9% NaCl:	intravenous groups 1 and 6
	Milli-Q water:	oral groups 3, 4 and 7
	Milli-Q and 819051088B:	dermal groups 4 and 9
	DMSO:	dermal groups 5 and 10
Dose levels:	25 mg/kg bw:	iv
	25, 100 mg/kg bw:	oral
	0.3, 1.25 mg/cm² (25, 120 mg/kg bw):	dermal
GLP statement:	in compliance	
Study period:	23 November – 14 December 2006	

In this ADME study, 5 groups of rats (n=4) were used for the mass balance and 5 groups (n=6) for the toxicokinetic study. Rats were exposed to 1,4-diamino-2-methoxymethyl-benzene sulphate by iv at the dose of 25 mg/kg bw, by gavage at the doses of 25 and 100 mg/kg bw and by dermal route at the doses of 25 mg/kg bw in water during 30 minutes and 120 mg/kg bw in DMSO during 24 hours. The dermal application at the low dose was considered representative of hair dye use conditions. The dermal application at the high dose was designed to favour skin penetration.

In the toxicokinetic groups, blood was sampled from two rats at 0.25, 0.5, 1, 4, 8, 24 hours post dosing. Total radioactivity and 1,4-diamino-2-methoxymethyl-benzene sulphate equivalent was determined.

In the mass balance groups, total radioactivity in urine, faeces, tissues and organs was determined.

Oral and dermal bioavailability was calculated by comparing blood AUC in the toxicokinetic groups and by comparing urine concentrations in the mass balance groups.

Results
Based on the mass balance studied, oral absorption was 99% in the low dose group and 84% in the high dose group: dermal absorption was 2% or 0.01 mg/cm^2 (2.7% when considering the residual amount in the skin) in the low dose – short exposure group and 21% or 0.3 mg/cm^2 (22% when considering the residual amount in the skin) in the high dose - 24h exposure group.

Based on the toxicokinetic study, oral absorption was 97% in the low dose group and 128% in the high dose group: dermal absorption was 2.5% in the low dose – short exposure group and 47% in the high dose - 24h exposure group.

T_{max} were 1 hour after dermal exposure to the low dose and 2 hours after the high dose. Terminal half lives ranged from 1.22 hours to 3.9 hours respectively.

When absorbed, excretion took mainly place in the urine. In urine, 3 metabolites were identified: 2 mono-acetylated and 1 di-acetylated metabolites of 1,4-diamino-2-methoxymethyl-benzene sulphate. Following low dermal exposure, only the di-acetylated metabolite could be detected in the urine. As a minor metabolic pathway, glutathione conjugation occurred resulting in mercapturic acid metabolite. No major qualitative differences in the metabolite profile between the oral and dermal routes were observed.

Ref.: 61

Comments
The dermal absorption percentages derived from the mass balance group were considered more reliable since the total recovery was within the required criteria of 100 +/- 10%. Due to the high oral bioavailability, for the calculation of the MoS, the NOAEL does not need to be corrected.

3.3.10 Photo-induced toxicity

3.3.10.1 Phototoxicity / photoirritation and photosensitisation

No data submitted

3.3.10.2 Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11 Human data

No data submitted

3.3.12 Special investigations

No data submitted

3.3.13 Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

2-Methoxy-methyl-p-phenylenediamine

Oxidative / Non oxidative conditions

Absorption through the skin	**A**	**=**	**2.33 µg/cm²**
Skin Area surface	**SAS**	**=**	**580 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**1.351 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.0225 mg/kg bw/d**
No Observed Adverse Effect Level (Maternal toxicity in developmental toxicity, oral, rat)	**NOAEL**	**=**	**18 mg/kg bw/d**

MOS	**NOAEL/SED**	**=**	**800**

3.3.14 Discussion

Physico-chemical properties
2-Methoxy-methyl-p-phenylenediamine is used as an oxidative hair colouring agent (precursor). The intended maximum on-head concentration is 1.8% in oxidative hair dye formulations, after mixing with developer (1:3).
Two different values for the solubility of 2-methoxy-methyl-p-phenylenediamine in water at pH 7.3 were reported. Stability of 2-methoxy-methyl-p-phenylenediamine in typical hair dye formulations was not demonstrated.

Irritation, sensitisation
The available data suggests that 2-methoxymethyl-p-phenylenediamine is not irritant to skin and not a strong irritant to the eye at anticipated use exposure. The CEET (ICE) is a screening method for hazard identification and not for risk assessment but used in a weight of evidence approach.
2-Methoxymethyl-p-phenylenediamine is a moderate skin sensitiser (EC3 = 4.3%).

Dermal absorption
In a typical oxidative hair dye formulation with 1.824% 2-methoxymethyl-p-phenylenediamine and in the presence of reaction partners, the bioavailable fraction used for the MOS is (mean + 2SD) 2.33 µg/cm², or 0.14% of the applied dose.

General toxicity
No study on acute toxicity was provided for 2-Methoxy-methyl-p-phenylenediamine. However information on short term exposure to the sulfate form may be taken from *in vivo* genotoxicity studies. Based on a micronucleus pretest in mice, an LD50 value between 100 and 250 mg/kg bw after i.p. administration may be considered and based on Comet assay pretest in rats, an LD50 value between 150 and 200 mg/kg bw after gavage.

From the results of a 90-day repeated dose oral toxicity study in rats with 1,4-diamino-2-methoxymethyl-benzene sulfate, a NOAEL of 55 mg/kg bw/d (highest dose tested) may be derived.

From the results of a developmental toxicity study in rats, the NOAEL for maternal toxicity was determined to be 18 mg/kg bw/d based on the reduction of the body weight gain and food consumption at 55 mg/kg bw/d. The NOAEL for developmental toxicity in fetuses was determined to be 55 mg/kg bw/d which corresponds to the highest dose tested.
No additional reproductive toxicity study was submitted.

An ADME and toxicokinetic study in rats was performed to compare iv, oral and dermal routes. The results show a very good oral biovalaibility of 1,4-diamino-2-methoxymethyl-benzene sulfate. Due to the high oral bioavailability, for the calculation of the MoS, the NOAEL do not need to be corrected.

Mutagenicity
Overall, the genotoxicity of 2-methoxy-methyl-p-phenylenediamine is sufficiently investigated in valid genotoxicity tests for the 3 endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy.
2-Methoxy-methyl-p-phenylenediamine was mutagenic in all in vitro tests performed. It did induce gene mutations both in a gene mutation test in bacteria as well as in a mouse lymphoma assay in mammalian cells and chromosome aberrations in an in vitro micronucleus test although these positive findings were considered of questionable biological importance. The finding in the mouse lymphoma assay that both the number of

large and small colonies increased and the ratio large/small colonies decreased indicated to a clastogenic effect of 2-methoxy-methyl-p-phenylenediamine next to a mutagenic one.
The positive findings from the *in vitro* tests could not be confirmed in *in vivo* tests. In an UDS test exposure to 2-methoxy-methyl-p-phenylenediamine did not result in unscheduled DNA synthesis in liver of rats. In an *in vivo* micronucleus test, an increase in bone marrow cells with micronuclei was not seen indicating that 2-methoxy-methyl-p-phenylenediamine is not clastogenic nor aneugenic in this test. The results of a comet assay, an indicator test covering gene mutations and chromosome aberrations, confirmed that 2-methoxy-methyl-p-phenylenediamine is not genotoxic (mutagenic and clastogenic) in stomach cells and bladder epithelial cells of rats.
Although the results obtained in the comet assay for liver cells were equivocal, the negative UDS test covers for DNA damage in these cells.
Consequently, based on these tests, 2-methoxy-methyl-p-phenylenediamine can be considered to have no genotoxic potential and additional tests are unnecessary.

Carcinogenicity
No data submitted

4. CONCLUSION

The SCCS is of the opinion that the use of 2-methoxy-methyl-p-phenylenediamine and its sulfate salt as oxidative hair dye with a concentration on head of maximum 1.8% does not pose a risk to the health of the consumer, apart from its sensitising potential.

5. MINORITY OPINION

Not applicable

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