Scientific Committee on Consumer Products

SCCP

OPINION ON

3-Methylamino-4-nitrophenoxyethanol

COLIPA n° B58

The SCCP adopted this opinion at its 13th plenary meeting on 2 October 2007

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 Products (SCCP), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

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

SCCP

Questions concerning the safety of consumer products (non-food products intended for the consumer).
In particular, the Committee addresses questions related to the safety and allergenic properties of cosmetic products and ingredients with respect to their impact on consumer health, toys, textiles, clothing, personal care products, domestic products such as detergents and consumer services such as tattooing.

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http://ec.europa.eu/health/ph_risk/risk_en.htm

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Opinion to be cited as: Opinion of the SCCP on 3-methylamino-4-nitrophenoxyethanol

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1. BACKGROUND

Submission I for 3-methylamino-4-nitrophenoxyethanol was submitted in July 1995 by COLIPA[1, 2].

The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) adopted at its plenary meeting on 20 May 1998 the opinion (XXIV/1290/97) with the opinion:

"*1-(2-hydroxyethyloxy)-3-methylamino-4-nitrobenzene has an acute oral toxicity of 3046 mg/kg bw in the rat. The substance can be classified as irritating to mucous membranes, not irritating to the skin and as a non sensitizer. Percutaneous absorption of a formulation was 0.71% in the presence of hair and 1.13% in the absence of hair for a concentration of the test formulation of 0.15%. In the 28 day study with rats 100 mg/kg/day is considered to be the NOAEL. In the teratogenicity study, no signs of maternal or foetal toxicity were observed after administration of 100 mg/kg bw.*"

The substance is currently regulated by the Cosmetics Directive (76/768/EC), Annex III, Part 2 under entry 28 on the List of substances provisionally allowed, which cosmetic products must not contain except subject to restrictions and conditions laid down.

Submission II for 3-methylamino-4-nitrophenoxyethanol was submitted by COLIPA in July 2005. According to this submission 3-methylamino-4-nitrophenoxyethanol is used in semi-permanent hair dye formulations at a maximum concentration of 0.15%.

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

2. TERMS OF REFERENCE

1. *Does the Scientific Committee on Consumer Products (SCCP) consider 3-methylamino-4-nitrophenoxyethanol safe for use as a non-oxidative hair dye with a concentration of maximum 0.15 % taken into account the scientific data provided?*

2. *Does the SCCP recommend any further restrictions with regard to the use of 3-methylamino-4-nitrophenoxyethanol in any non-oxidative hair dye formulations?*

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association
[2] According to records of COLIPA

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

3-Methylamino-4-nitrophenoxyethanol (INCI)

3.1.1.2. Chemical names

1-(2-Hydroxyethyloxy)-3-methylamino-4-nitrobenzene
2-[3-(methylamino)-4-nitrophenoxy]-ethanol (CAS name)
4-nitro-3-methylaminophenoxyethanol
(3-methylamino-4-nitro)phenyl-β-hydroxyethylether

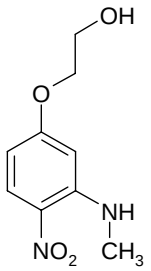
3.1.1.3. Trade names and abbreviations

Imexine FR (Chimex)
COLIPA B58

3.1.1.4. CAS / EINECS number

CAS: 59820-63-2
EINECS: 261-940-7

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_9H_{12}N_2O_4$

3.1.2. Physical form

Odourless, yellow crystalline powder

3.1.3. Molecular weight

Molecular weight: 212

3.1.4. Purity, composition and substance codes

Deduced specifications for the Material used in the market

Purity by UV-Vis: > 98.5 g/100g (Determination by spectrophotometry)
Purity (rel.) by HPLC: > 98.5%
Total impurities content: < 1.5 g/100g
Ash content: < 0.1 g/100g
Heavy Metals (*batch 0509824)*: < 20 mg/kg
- *As, Sb, Hg:* *< 5 mg/kg Each*
- *Cd:* *< 10 mg/kg*
- *Pb:* *< 20 mg/kg*

Batches used

The following table summarizes the results of characterization of five batches used in all the toxicological tests. In addition, Batch SEL/1438 of [^{14}C] 3-Methylamino-4-nitrophenoxy-ethanol (radiochemical purity ≥ 99%) was used in a skin absorption study realized in 2005 [ref. 10].

	Batch				
	0509824	**Op. 94**	**Op. T105**	**Op. T99**	**Op. 67**
Appearance	Yellow crystalline powder				
Titre (g/100g) UV Spectrometry	98.6	99.7	> 99	> 99	> 99 (1)
Water content (g/100g)	0.16	0.1			
Melting point (°C)	129.7 (2)	125-130 (3)	127.2 (3)	126.2 (3)	129.4 (3)
H.P.L.C. Profile (Purity %) (3)	> 98.5	In accordance with the specifications			> 99.5
Impurities H.P.L.C (g/100g)					
Impurities A and B	ND < 0.01	ND < 0.01			
Impurity C	D < 0.1	0.16			
Impurity D	1.0				
Residual solvent GC (µg/g)					
Methanol	D < 1000	D < 500			
Toluene	D < 900	500			
Vis. spectrum	The Vis. spectra are comparable (1)				
IR spectrum	In conformance with the proposed structure				
Mass spectrum	Compatible with the proposed structure				
^{1}H and ^{13}C NMR spectra	In accordance with the proposed structure				

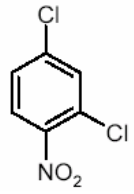
D: detected
ND: Not detected
- *Impurity A*: *2,4-dichloro-1-nitrobenzene*
- *Impurity B*: *5-chloro-2-nitrophenylmethylamine*
- *Impurity C*: *5-hydroxy-2-nitrophenylmethylamine*
- *Impurity D: 1,2-Di-(3-methylamino-4-nitrophenoxy)-ethane*

(1) *The characteristic wavelength in visible light (λ max) of batch Op.67 is 416.6 nm.*
(2) *DSC (differential scanning calorimetry)*
(3) *Thermo-microscopic method*

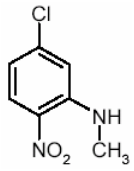
3.1.5. Impurities / accompanying contaminants

Some impurities are detected: total impurities content < 1.5% by UV. Possible impurities which may originate from reagents and intermediate reaction products were checked in batches 0509824 and Op. 94:

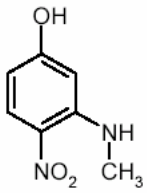
- Impurity A: 2,4-dichloro-1-nitrobenzene (t_R : 19.7 min)



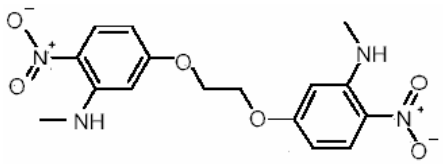
- Impurity B: 5-chloro-2-nitrophenylmethylamine (t_R : 19.1 min)



- Impurity C: 5-hydroxy-2-nitrophenylmethylamine (t_R : 19.7 min)



- Impurity D: Methyl-{2-nitro-5-[2-(3-methylamino-4-nitrophenoxy)-ethoxy]-phenyl}-amine (or 1,2-Di-(3-methylamino-4-nitro-phenoxy)-ethane, or 1,2- Di-(3-methylamino-4-nitro-phenoxy)-ethane (t_R : 5.6 min).



- Impurity A: Not detected < 0.01g/100g for the two batches
- Impurity B: Not detected < 0.01g/100g for the two batches
- Impurity C: 0.16 g/100 g in batch Op. 94 and detected < 0.1g/100g in batch 0509824
- Impurity D: 1 g/100 g in batch 0509824

3.1.6. Solubility

in water : 263 ± 9 mg/L at 20°C ± 0.5°C according to EEC method A6
in ethanol : ≤ 1 g/100 ml at 22°C after 24 hours
in DMSO : ≤ 20 g/100 ml at 22°C after 24 hours

3.1.7. Partition coefficient (Log P_{ow})

Log $P_{O/W}$: 1.43 at 25°C ± 1°C at pH 6.19 According to EEC method A8 (HPLC Method)

3.1.8. Additional physical and chemical specifications

Melting point: 125 - 130 °C
Boiling point: /

Flash point:	/
Vapour pressure:	/
Density:	/
Viscosity:	/
pKa:	/
Refractive index:	/
pH:	/
UV_Vis spectrum	The ultra-violet light absorption, in the range 220 to 350 nm of a 0.01 g/l solution in ethanol (95%), exhibits two maxima, at 233 nm (ε=0.885) and 312 nm (ε=0.388) and a less well defined maximum (shoulder) at 254 nm (ε=0.321). The visible light absorption, in the range 350 to 600 nm of a 0.02 g/l solution in ethanol (95 %), exhibits a maximum at 414 nm (ε=0.744).

3.1.9. Stability and Homogeneity

The stability of the test item in dosage forms at 10 and 200 mg/ml in 0.5% methyl-cellulose (MC), at 0.1 and 250 mg/ml in DMSO and at 1 and 250 mg/ml in DMF was satisfactory over a 4-hour period at room temperature, protected from light and under inert gas atmosphere (average recovery 2% and 3% respectively for the two concentrations). The homogeneity of the test item at 10 and 200 mg/ml in 0.5% MC on the day of preparation was satisfactory (average variability 5% and 4% respectively for the two concentrations).

General Comments to physico-chemical characterisation

- The stability of the test substance in marketed products is not reported.
- The test substance is a secondary amine and thus is prone to nitrosation. It should not be used in combination with nitrosating substances. The nitrosamine content should be < 50 ppb. Data on nitrosamine content is not provided

3.2. Function and uses

3-Methylamino-4-nitrophenoxy-ethanol is used in non-oxidative (semi-permanent) hair colouring products at a maximum concentration of 0.15%.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline:	OECD 420 (2001)
Species/strain:	Rat, Female, Sprague-Dawley Rj: SD (IOPS Han)
Group size:	1 (sighting test) and 4 top dose, 5 at lower dose
Test substance:	3-methylamino-4-nitrophenoxy-ethanol
Batch:	0509824
Purity:	98.6%
Dose:	2000 and 1000 mg/kg
Route:	Gavage, 10 ml/kg.
Observation:	14 days
GLP:	in compliance

An initial test was performed at 2000 mg/kg in one animal. It survived, was hypoactive and had piloerection and dyspnoea on day 1 and 2.
Thus 4 female rats at 2000 mg/kg were used for the main study. Since these died a further five additional animals were treated at 1000 mg/kg.
Animals were observed at least once daily for mortality/morbidity and daily for clinical signs over a period of 14 days following a single administration of the test substance. Body weights were recorded on day 1 prior to treatment, and on days 8 and 15 thereafter. Individual weights of animals found dead were measured at necropsy when survival exceeded 24 hours and if no signs of "cannibalism" were present. All study animals were subjected to a macroscopic examination as soon as possible after death.

Results
In the main experiment, 3/4 animals treated at 2000 mg/kg died on day 2. Hypoactivity or sedation, piloerection, dyspnoea and lateral recumbency were observed prior to death. No clinical signs were observed in the surviving animal.
At 1000 mg/kg, 1/5 animals was found dead on day 2; hypoactivity or sedation, piloerection and dyspnoea were observed prior to death. In all surviving animals, hypoactivity or sedation and dyspnoea were noted on days 1 and 2. Piloerection was noted on day 1 only. Yellow colouration of the extremities was noted in almost all animals during the study.
No effect on body weight gain and no gross abnormalities were observed at either dose level.

Conclusion
The LD_{50} for 3-methylamino-4-nitrophenoxy-ethanol after single oral gavage to fasted female Sprague-Dawley rats was between 1000 mg/kg and 2000 mg/kg.

Ref.: 1

3.3.1.2. Acute dermal toxicity

No data submitted

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline:	OECD 404
Species/strain:	male New Zealand White rabbits
Group size:	3
Test substance:	3-Methylamino-4-nitrophenoxy-ethanol
Batch:	0509824
Purity:	99.0%
Vehicle:	2% of 3-Methylamino-4-nitrophenoxy-ethanol in 0.5% suspension of methylcellulose in purified water
GLP:	in compliance

Test material 0.5% was applied on the skin using a patch (semi-occlusive dressing), to shaved intact skin on one flank of each rabbit for 4 hours. Patch was removed and the site washed with water. The animals were examined 1, 24, 48 and 72 hours after patch removal. Adjacent areas of the treated skin of each animal served as controls.

Results

None of the treated animals showed any response to treatment, no skin irritation was observed after 4 hours exposure to 3-Methylamino-4-nitrophenoxy-ethanol. A slight yellow coloration of the skin was noted in all animals at all exposures between days 1 and 4.

Conclusion
Under the conditions of the experiment, 3-Methylamino-4-nitrophenoxy-ethanol was not-irritant to the rabbit skin.

Ref.: 2

3.3.2.2. Mucous membrane irritation

Guideline:	OECD 405
Species/strain:	male New Zealand White rabbits
Group size:	3
Test substance:	3-Methylamino-4-nitrophenoxy-ethanol
Batch:	0509824
Purity:	99.0%
Vehicle:	2% of 3-Methylamino-4-nitrophenoxy-ethanol in 0.5% suspension of methylcellulose in purified water
GLP:	in compliance

Test preparation (0.5% suspension of methylcellulose in purified water) was instilled into one eye of one of the rabbits. Observations were made 1, 24, 48 and 72 hours after instillation and then daily until reversibility of the ocular reactions.

Results
Instillation of the test substance resulted in very light or slight conjunctival reactions (very slight chemosis and/or very slight or light redness of the conjunctiva), observed in all animals on days 1 and 2; minimal conjunctival reactions (minimal chemosis and/or minimal redness of the conjunctiva) persisted until day 3, 4 or 6.

Conclusion
Under the conditions of the experiment, 3-Methylamino-4-nitrophenoxy-ethanol showed minimal irritancy to rabbit eyes.

Ref.: 3

Comment
At a use concentration of 0.15%, no relevant irritancy is to be expected.

3.3.3. Skin sensitisation

Local Lymph Node Assay (LLNA)

Guideline:	OECD 429
Species/strain:	mouse – CBA/J
Group size:	5 females (10-11 weeks) per dose group
Test substance:	3-Methylamino-4-nitrophenoxy-ethanol in DMF
Batch:	0509824
Purity:	99.0 %
Doses:	1.0%, 2.5%, 5.0%, 10% and 25% (w/v)
Positive control	alpha-hexylcinnamaldehyde (25% in DMF)
Vehicle control	DMF
GLP:	in compliance

25 µl of test material or vehicle control and positive control was applied to the dorsal surface of both ear lobes once daily for 3 consecutive days. 5 days after the first application, animals were injected with 250µl 3H-methyl thymidine in the tail vein. Mice

were sacrificed 5 hours later. Draining lymph nodes were excised and pooled to prepare a single cell suspension for each group. Thymidine incorporation was measured by β-scintillation counting. The disintegrations per minute per lymph node (DPM/node) was measured and expressed as the ratio of the control group (stimulation index, S.I. = dpm of treated group / dpm of control group).

Results

Treatment	*Concentration (%)*	*Signs of local irritation*	*Stimulation Index (SI)*
Test item	1	no	1.69
Test item	2.5	no	2.70
Test item	5	no	2.13
Test item	10	no	2.59
Test item	25	no	1.85
HCA	25	-	14.64

The test substance was not irritant so the highest concentration retained for the test was the maximal practicable concentration. No dose-response of the SI was recorded according to the concentration.

Conclusion
3-Methylamino-4-nitrophenoxy-ethanol was not shown to be a skin sensitizer.

Ref.: 4

3.3.4. Dermal / percutaneous absorption

***In Vitro* Percutaneous Absorption**

Guideline:	OECD 428
Tissue:	human skin, 4 female donors, dermatomed skin thickness set at 500 µm
Tissue integrity:	tritiated water diffusion measurement
Method:	flow-through diffusion cells, exposed membrane area 0.64 cm²
Receptor phase:	phosphate buffer saline (pH not specified) with 0.01 % sodium azide
Test substance:	3-Methylamino-4-nitrophenoxy-ethanol [^{14}C]- 3-Methylamino-4-nitrophenoxy-ethanol
Batch:	non-radiolabelled: 0509824 [^{14}C] radiolabelled: SEL/1438
Purity:	non-radiolabelled: 99.9% [^{14}C] radiolabelled: 99.8%
Formulation:	semi-permanent (direct) hair dye under « in use » conditions
Concentration:	target 0.15% (experimental concentration 0.17%)
Dose applied:	20 mg / cm²
Contact:	30 minutes, then washing of the skin surface, and monitoring of the diffusion during 23.5 hours.
No. of replicates:	8 cells (2 cells from each different donor)
Assay:	liquid scintillation
GLP:	in compliance

After 30 minutes of exposure, the hair dye remaining on the skin surface was removed by washing. Twenty-four (24) hours after application, skin samples were removed and analysed by liquid scintillation counting to assess the cutaneous distribution of 3-Methylamino-4-nitrophenoxy-ethanol. The mean total dermal absorption (sum of the amounts measured in the living epidermis, dermis and receptor fluid) of B058 under the conditions of the experiment represented 0.14 ± 0.05 µgeq/cm² (0.45 ± 0.15% of the applied dose).

	% of dose applied	$\mu g_{eq}/cm^2$
Skin wash	94.6 ± 5.8	30.1 ± 2.3 (27.1 – 32.9)
Dislodgeable dose *	94.7 ± 5.8	30.1 ± 2.3 (27.1 – 32.9)
Stratum corneum	0.08 ± 0.02	0.03 ± 0.01 (0.01 – 0.04)
Skin (epidermis + dermis)	0.03 ± 0.02	0.01 ± 0.01 (0.00 – 0.03)
Receptor fluid	0.42 ± 0.14	0.13 ± 0.04 (0.05 – 0.18)
Unabsorbed dose **	94.8 ± 5.8	30.1 ± 2.3 (27.2 – 32.9)
Absorbed dose ***	0.45 ± 0.15	0.14 ± 0.05 (0.06 – 0.19)
Total recovery	95.2 ± 5.7	30.3 ± 2.2 (27.3 – 33.1)

* Dislodgeable dose is defined as the amount of test substance removable from the application site (skin wash, cotton swabs and donor compartment wash)
** Unabsorbed dose is defined as the dislodgeable dose including the amount recovered in the stratum corneum
*** Absorbed dose (dermal delivery) is defined as the amount in the receptor fluid, the receptor compartment wash and skin membrane, excluding tape strips

Conclusion
Under the described test conditions, a mean skin penetration of 0.14 ± 0.05µg/cm^2 (0.45 ± 0.15% of the applied dose) was obtained for 3-Methylamino-4-nitrophenoxy-ethanol, formulated in a semi-permanent (direct) hair dye. By summing up the amounts for receptor fluid, dermis and epidermis, the maximum skin absorption was 0.19µg/cm^2.

Ref.: 10

Comment
The number of replicates (8, 2 from each donor) is not in accordance with the SCCP Notes of Guidance. However, the study was well conducted and therefore, the maximum absorption (A_{max}) observed in the experiment, 0.19 µg/cm^2 can be used for calculating the Margin of Safety.

3.3.5. Repeated dose toxicity

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

No data submitted

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

Guideline: OECD 408
Species/strain: Rat, Sprague-Dawley Crl CD (SD) BR
Group size: 10/sex/group
Test substance: Imexine FR
Batch: op T 105
Purity: > 99 %
Dose: 0, 25,100, 400 mg/kg
Vehicle: 0.5% methylcellulose
Route: Gavage, 5 ml/kg/day.
Exposure: 92/93 days
GLP: in compliance

In the preliminary study [ref. 13 (cited in ref 5, SCCNFP opinion n° XXIV/1290/97 of 20 May 1998)], Sprague-Dawley rats (10/sex/group) received 100, 300 or 1000 mg/kg/day B058 by oral gavage for 30 days. The controls received the vehicle (0.5% aqueous methylcellulose). There were no deaths, hypersalivation was observed in 1/10 females at 1000 mg/kg/day; orange coloured urine in all animals at 300 and 1000 mg/kg/day. Staining of fur and the mucosa of the digestive and urinary tracts were attributed to the compound. Slightly lower body weight gain was observed in males at 300 and 1000 mg/kg/day. A slight

increase in cholesterol levels in both sexes at 1000 mg/kg/day was considered toxicologically significant. Compound-related increases in liver weights were observed in both sexes at 1000 mg/kg/day. Slight to moderate diffuse or centrilobular hepatocellular hypertrophy was observed in 2/10 males at 100 mg/kg/day and in all males and 6/10 females at 1000 mg/kg/day; this was attributed to possible enzyme induction. The NOAEL for the study was 100 mg/kg/day.

In the 13 week study, animals were observed once daily for clinical signs and twice daily for mortality/morbidity. Body weight and food consumption were recorded weekly; efficiency of food utilization was calculated weekly using these values. Water consumption was recorded for 7 days during week 10 for control and 400 mg/kg/day dose group males and for 4 days in week 13 for all animals of the control and 400 mg/kg/day dose groups. Ophthalmologic evaluations on control and high-dose animals were performed before the treatment period and during week 13. Haematology, clinical chemistry and urinalysis evaluations were performed once during week 13.
At the end of the treatment period, all animals were killed and grossly examined. Selected organs were weighed. All animals were submitted to a complete macroscopic examination. All macroscopic lesions and required tissues from animals in the control and high-dose groups were evaluated microscopically; only macroscopic lesions and lungs, liver and kidneys were evaluated in the low and intermediate dose groups.

Results
No mortality occurred during the study. Ptyalism was seen in 3/10 males and 2/10 females at 100 and in all animals at 400 mg/kg/day. Soiled litter was observed in all males and 4/10 females from week 9 at 400 mg/kg/day. This was linked and became more marked for the remaining dosing period with higher water consumption, swollen abdomen, dehydration and signs of poor clinical condition. Yellow staining of urine, tail and body extremities at all dose levels occurred.
Body weight gain in males of the high dose group was lower overall during the study period (-40%) but was marked from week 5 (-82%). In females, lower body weight gain (-28%) was noted from week 5 but higher food consumption in both sexes from week 8 and week 10 (+63% and +38% in males and females, respectively) were observed at 400 mg/kg/day. Efficiency of food utilisation was comparable with controls in all animals up to week 11. In males dosed at 400 mg/kg/day only, it increased after this, linked with increased food consumption. Water consumption also increased (6.9- and 3.9-fold in males and females, respectively).
Treatment-related ophthalmalogical effects were seen at 400 mg/kg/day such as yellow staining of the fundus in all animals, subcapsular, anterior cortical and total opacification of the lens in both sexes (6/10 male. 2/10 female).

Statistically significant differences were seen between the controls and the high dose group in the following organ weights.

	Absolute	**Relative**
Kidney	+18% male, + 19% female	+69% male, + 36% female
Liver		+37% male,+ 22% female
Thymus	-46% male, -32% female	-30% male, -24 female
Spleen	-27% male, -21% female	
Heart	-26% male, -12% female	

The kidney, liver and thymus effects were considered to be treatment-related.

Histopathological changes seen at 400 mg/kg/day were
slight to moderate centrilobular hepatocyte hypertrophy, probably due to increased liver function,
slight to marked tubular nephrosis

slight to moderate lymphoid depletion in the thymus in 7 males and 3 females.

Similar but non significant lymphoid depletion was seen at 100 mg/kg/day in 1 male and 2 females, but liver and kidney effects seem to be similar to the high control values.
According to the study authors, no treatment-related findings were found in these organs in any animals given 25 or 100 mg/kg/day.
Males at 400 mg/kg/day had slightly higher haemoglobin concentration (+13%), packed cell volume (+11%) and fibrinogen levels (+30%); these were considered signs of haemoconcentration caused by the dehydratation observed in some animals.

Higher glucose, phosphorus, urea, bilirubin, cholesterol, triglyceride, alkaline phosphatase, ASAT and ALAT levels as well as lower blood sodium, potassium and chloride levels were observed in some animals of both sexes at 400 mg/kg/day. Higher urine volume and glucosuria were also observed at this dose level.
The study authors considered the NOAEL to be 100 mg/kg/day.

Ref.: 5

Comment
The SCCP considered the lymphoid depletion in the thymus at 100 and 400 mg/kg bw in males as adverse. Therefore, the NOAEL was set at 25 mg/kg bw per day.

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial gene mutation assay

Guideline:	OECD 471	
Species/strain:	*Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 *Escherichia coli* WP2uvrA	
Replicates:	triplicates in 2 individual experiments both in the presence and absence of metabolic activation.	
Test substance:	Imexine FR (3-methylamino-4-nitrophenoxy-ethanol)	
Solvent:	DMSO	
Batch:	op T 105	
Purity:	> 99%	
Concentrations:	Experiment 1:	312.5 - 5000 µg/plate without and with S9-mix
	Experiment 2:	312.5 - 5000 µg/plate without and with S9-mix
Treatment:	Experiment 1:	direct plate incorporation with 48 - 72 h incubation without and with S9-mix
	Experiment 2:	direct plate incorporation with 48 - 72 h incubation without S9-mix pre-incubation method with 60 minutes pre-incubation and 48 - 72 h incubation with S9-mix.
GLP:	In compliance	

Imexine FR was investigated for the induction of gene mutations in both *Salmonella typhimurium* and *Escherichia coli* (Ames test). Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the level of toxicity in a preliminary toxicity test with strain TA100 both without and with S9-mix. Toxicity was evaluated for 6 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 thinning of the bacterial background lawn. Experiment 1 and experiment 2 without metabolic activation was performed with the direct plate incorporation method, experiment 2 with metabolic activation with the pre-incubation method. Negative and positive controls were in accordance with the guideline.

Results
Toxic effects were only found in the presence of metabolic activation at 1250 µg/plate and above for TA100 (experiment 1) and TA1535 (experiment 2). Precipitation of Imexine FR was found exclusively in experiment 2 both in the presence and absence of metabolic activation for all strains at 5000 µg/plate. In both experiments in the presence or absence of metabolic activation Imexine FR did not induce an increase in the number of revertants in any of the strains at any concentration tested.

Conclusion
Under the experimental conditions used, the test substance was not mutagenic in this gene mutation tests in bacteria.

Ref. 6

In vitro chromosome aberration test

Guideline:	/
Cells:	CHO cells
Replicates:	2 slides per culture per concentration in 2 independent experiments
Test substance:	Imexine FR (3-methylamino-4-nitrophenoxy-ethanol)
Solvent:	DMSO
Batch:	Op T 99
Purity:	/
Concentrations:	25, 125 and 250 µg/ml without S9-mix 250, 1250 and 2500 µg/ml with S9-mix
Treatment:	both in absence and presence of S9 two harvest times were reported.
GLP:	In compliance

The test substance has been investigated in the absence and presence of metabolic activation for the induction of chromosomal aberrations in CHO cells. Test concentrations were based on the level of cytotoxicity in a range finder experiment, measuring suppression of mitotic index as endpoint, at 7 concentrations up to the prescribed maximum concentration of 5000 µg/ml without and with S9-mix. Details on treatment and harvest times were not reported. However, the tables describing the results mention 2 harvest times. Two hours before harvest, each culture was treated with colcemid solution (final concentration of 0.1 µg/ml) to block cells at metaphase of mitosis. Liver S9 fraction from sodium phenobarbitone/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. Toxicity was determined by measuring the reduction in mitotic index (MI). Chromosome (metaphase) preparations were stained with Giemsa and examined microscopically for MI and chromosomal aberrations. Negative and positive controls were included.

Results
Although not reaching the prescribed 50 % decrease in mitotic index, a decrease in mitotic index was observed indicating to Imexine FR-induced toxicity and thus exposure of the cells.
A biologically significant and reproducible increase in the number of cells with chromosomal aberration was not found in the absence or presence of metabolic activation at any of the concentrations tested in both experiments.

Conclusion

Under the experimental conditions used, the test substance did not induce an increase in chromosomal aberrations and, consequently, is not genotoxic (clastogenic) in CHO cells.
Ref. 6

Comments
Although an OECD or EU guideline was not reported, the study protocol showed a strong resemblance with the guideline protocol. The purity of Imexine FR was not reported. Details about treatment and harvest times were not reported. However, the tables describing the results mention 2 harvest times. Historical data were not reported. Despite these shortcomings, this study can be used for evaluation.

In vitro micronucleus test

Guideline:	OECD 487 (draft 2004)
Cells:	human lymphocytes from 2 healthy, non-smoking male volunteers
Replicates:	duplicates in 2 independent experiments
Test substance:	3-methylamino-4-nitrophenoxy-ethanol
Solvent:	DMSO
Batch:	0509824
Purity:	98.6 %
Concentrations:	Experiment 1: 0, 100, 400 and 700 µg/ml (without S9-mix) 0, 750, 1000 and 1200 µg/ml (with S9-mix) Experiment 2: 0, 250, 800 and 1100 µg/ml (without S9-mix) 0, 1000, 1200 and 1400 µg/ml (with S9-mix)
Treatment	Experiment 1: 24 h PHA followed by 20 + 28 h treatment (without S9-mix) 24 h PHA followed by 3 + 45 h treatment (with S9-mix) Experiment 2: 48 h PHA followed by 20 + 28 h treatment (without S9-mix) 48 h PHA followed by 3 + 45 h treatment (with S9-mix)
GLP:	In compliance

3-Methylamino-4-nitrophenoxy-ethanol has been investigated in the absence and presence of metabolic activation for the induction of micronuclei in cultured human lymphocytes. The suitable top concentrations for experiments 1 and 2 were based on the results of a cytotoxicity range-finding experiment measuring replication index (RI). To determine the test concentrations for micronucleus analysis in each separate experiment the RI is measured in cultures treated with increasing concentrations of 3-methylamino-4-nitrophenoxy-ethanol. The top dose for micronucleus analysis was to be the one at which approximately 60% reduction in RI occurred or the highest dose tested. Two lower doses were selected so that a range of cytotoxicity from maximum (60%) to little or none is covered. Treatment periods were 20 h without and 3 h with S9-mix. Harvest times were 72 hours (experiment 1) or 96 hours (experiments 2) after the beginning of culture. The final 28 h of incubation was in the presence of cytochalasin B (at a final concentration of 6 µg/ml). Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Negative and positive controls were in accordance with the draft guideline.

Results
Measurements on post-treatment media in the absence or presence of S9-mix indicated that 3-methylamino-4-nitrophenoxy-ethanol had no effect on osmolarity or pH as compared to concurrent vehicle controls.
In both experiments, biologically relevant increases in the number of micronucleated binucleate cells compared to concurrent control values were not found at any concentration tested both in the absence or presence of S9-mix.

Conclusion
Under the experimental conditions used 3-methylamino-4-nitrophenoxy-ethanol did not induce micronuclei and, consequently, is not genotoxic (clastogenic and/or aneugenic) in cultured human peripheral lymphocytes *in vitro*.

Ref.: 8

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

No data submitted

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

3.3.8.1. One generation reproduction toxicity

No data submitted

3.3.8.2. Prenatal Developmental Toxicity

Guideline:	OECD 414
Species/strain:	Rat, Sprague-Dawley Crl CD (SD) BR
Group size:	25 mated female per dose (20 pregnant per dose)
Test substance:	Imexine FR
Batch:	op 67
Purity:	99.8%
Dose:	0, 100, 250 or 750 mg/kg/day
Vehicle:	0.5% aqueous carboxymethylcellulose
Route:	gavage, 5 ml/kg/day
Exposure:	Gestation day (GD) 6-15
GLP:	in compliance

Dose levels for this study were selected based on the results of the preliminary embryo-foetal developmental toxicity study in rats in which the NOEL was 300 mg/kg/day [14].
Animals were observed twice daily for mortality/morbidity. Clinical signs were checked once daily. Food consumption and body weight were recorded at designated intervals during gestation.
On day 20 of gestation, the animals were killed and examined macroscopically. Foetuses were removed by Caesarean section. The following litter parameters were recorded: number of corpora lutea, number of implantation sites, number and distribution of early and late resorptions, and number of dead and live foetuses. Foetuses were weighed, sexed and submitted to external, soft tissue and skeletal examinations. Statistical analysis was performed on the following data: body weight, food consumption, litter data, and the proportions of live foetuses, pre- and post-implantation loss and foetuses with abnormalities. The homogeneity and concentrations of test substance preparations were verified.

Results
No mortality or abortion occurred during the study. Lemon-coloured urine was observed in all treated groups from day 7 to day 16. A transient decrease in body weight gain was observed at 250 and 750 mg/kg/day from GD 6 -9, and mean body weight gain was lower in 750 mg/kg/day females at the end of the treatment period compared with controls. Statistically decreased food consumption in the 750 mg/kg/day females from days 6 to 20

(mean decrease approximately 18 %) compared with controls. No other compound-related findings were observed in dams at any dose level.
At 750 mg/kg/day, higher pre-implantation loss was attributed to two outliers and was not considered treatment related. No other treatment-related changes in litter data or foetal examinations were noted. No anomalies or malformations of toxicological significance were observed.
The results show a slight maternotoxic effect at 250 mg/kg/day and a moderate maternotoxic effect at 750 mg/kg/day. Neither embryotoxic nor teratogenic effects were observed at any dose level.
The NOAEL for maternotoxicity was considered as 100 mg/kg/day and the NOAEL for developmental toxicity was 750 mg/kg/day in the present study for 3-methylamino-4-nitrophenoxy-ethanol.

Ref.: 9

3.3.9. Toxicokinetics

No data submitted

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

No data submitted

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

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

(3-methylamino-4-nitrophenoxy-ethanol**)**
(Non-oxidative / semi-permanent)

Maximum absorption through the skin	**A (µg/cm^2)**	**=**	**0.19 µg/cm²**
Skin Area surface	**SAS (cm^2)**	**=**	**700 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**0.133 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.002 mg/kg bw**
No observed adverse effect level (90day, rat, oral)	**NOAEL**	**=**	**25 mg/kg bw**
Margin of Safety	**NOAEL / SED**	**=**	**11280**

3.3.14. Discussion

This risk assessment relates to the use of 3-methylamino-4-nitrophenoxy-ethanol in non-oxidative hair dye formulations only.

Physico-chemical properties
The stability of the test substance in marketed products is not reported.
The test substance is a secondary amine and thus is prone to nitrosation. It should not be used in combination with nitrosating substances. The nitrosamine content should be < 50 ppb. Data on nitrosamine content is not provided

General toxicity
Based on lymphoid depletion in the thymus at 100 and 400 mg/kg bw in males, the NOAEL is set at 25 mg/kg bw per day (90-day study in rats). The NOAEL for maternal toxicity was considered as 100 mg/kg/day and the NOAEL for developmental toxicity was 750 mg/kg/day in the present study.

Irritation / sensitisation
Under the conditions of the experiments, 3-methylamino-4-nitrophenoxy-ethanol was not irritant to rabbit skin. It showed minimal irritancy to rabbit eyes. 3-Methylamino-4-nitrophenoxy-ethanol was not shown to be a skin sensitizer in a LLNA study.

Dermal absorption
The number of replicates (8, 2 from each donor) is not in accordance with the SCCP Notes of Guidance. However, the study was well conducted and therefore, the maximum absorption (A_{max}) observed in the experiment, 0.19 µg/cm^2 can be used for calculating the Margin of Safety.

Mutagenicity / genotoxicity
Overall, the genotoxicity of 3-methylamino-4-nitrophenoxy-ethanol was sufficiently investigated in valid genotoxicity tests for the 3 types of mutation: gene mutation, structural chromosome mutation and aneuploidy. 3-methylamino-4-nitrophenoxy-ethanol did not induce gene mutations in bacteria, chromosome aberrations in an *in vitro* chromosome aberration test nor micronuclei in an *in vitro* micronucleus test.
Consequently, 3-methylamino-4-nitrophenoxy-ethanol can be considered to have no genotoxic potential and additional tests are unnecessary.

Carcinogenicity
No data submitted

4. CONCLUSION

This risk assessment relates to the use of 3-methylamino-4-nitrophenoxy-ethanol in non-oxidative hair dye formulations only.

The SCCP is of the opinion that the use of 3-methylamino-4-nitrophenoxy-ethanol as a non-oxidative hair dye at a maximum concentration of 0.15% on the head does not pose a risk to the health of the consumer.

The test substance is a secondary amine and should not be used in combination with nitrosating substances. The nitrosamine content should be < 50 ppb.

5. MINORITY OPINION

Not applicable

6. REFERENCES

The references in italics (12-19) were not submitted by the applicant as full reports in the present dossier, since the studies reported therein were not considered to be adequate.

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