Scientific Committee on Consumer Products

SCCP

OPINION ON

3-NITRO-P-HYDROXYETHYLAMINOPHENOL

COLIPA N° B54

The SCCP adopted this opinion during its 10th plenary of 19 December 2006

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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1. BACKGROUND

Submission I for **3-NITRO-P-HYDROXYETHYLAMINOPHENOL WAS** submitted in March 1992 by COLIPA[1].

The Scientific Committee on Cosmetology (SCC) expressed its opinion SPC/1075/93 at the 53rd plenary meeting of 25 June 1993 with the conclusion that the substance is safe (Classification A).
The substance is currently regulated by the Cosmetics Directive (76/768/EC), Annex III, Part 2 under entry 48 on the Preliminary list of substances, which cosmetic products must not contain except subject to restrictions and conditions laid down.

According to current submission II, submitted by COLIPA in July 2005, **3-NITRO-P-HYDROXYETHYLAMINOPHENOL** is used in oxidative hair colouring products at a maximum on-head concentration of 3%, after mixing the hair dye formulation with hydrogen peroxide typically in 1:1 proportions. **3-NITRO-P-HYDROXYETHYLAMINOPHENOL IS ALSO USED IN SEMI-PERMANENT** hair colouring products at a maximum concentration of 1.85%.

Submission III 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-nitro-p-hydroxyethylaminophenol safe for use as an ingredient in both oxidative hair dyes and non-oxidative hair dye formulations with a concentration on-head of maximum 3.0% respectively 1.85% taking into account the scientific data provided?*

2. *Does the SCCP recommend any restrictions with regard to the use of 3-nitro-p-hydroxyethylaminophenol in both oxidative hair dyes and non-oxidative 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

3-Nitro-p-hydroxyethylaminophenol (INCI)

3.1.1.2. Chemical names

Phenol, 4-[(2-hydroxyethyl)amino)]-3-nitro (CAS)
1-hydroxy-3-nitro-4-(β-hydroxyethyl)-aminobenzene

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association

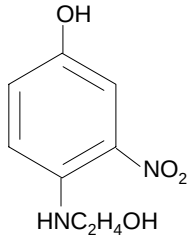
3.1.1.3. Trade names and abbreviations

IMEXINE FH
COLIPA n° B054

3.1.1.4. CAS / EINECS number

CAS: 65235-31-6
EINECS: 265-648-0

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_8H_{10}N_2O_4$

3.1.2. Physical form

Greenish-brown powder

3.1.3. Molecular weight

Molecular weight: 198.18

3.1.4. Purity, composition and substance codes

Purity and impurities in various batches of 3-Nitro-p-hydroxyethylaminophenol

Property	**Batch**	
	0509151	**Op.108***
Identification/characterisation	UV-Vis, IR, NMR, MS, elemental analysis	Visible spectrum is comparable
Melting point	133°C	139°C
Titre (g/100g)	98.3[1]	98.7[2]
HPLC purity (% peak area)	98.5	
Impurities[3] (g/100 g) A B C	 0.14 0.52 0.11	
Water content (g/100g)	0.3	
Solvent residues (ppm) Isopropanol Methanol	 <100, detected <100, detected	

* Only certificate of analysis (without signature), no supporting data
[1] Neutralisation of amine function with perchloric acid in acetic acid medium determination
[2] Spectrophotometric determination at 475 nm
[3] Impurities:

A: 4-Amino-3-nitrophenol
B: 2-{[4-(2-hydroxyethoxy)-2-nitrophenyl]amino}-ethanol
C: 4-{[2-(2-hydroxyethoxy)ethyl]amino}-3-nitrophenol

3.1.5.	Impurities / accompanying contaminants

See 3.1.4

3.1.6.	Solubility

Water: 1.06 ± 0.07 g/l, at 20 °C ± 0.5 °C
Ethanol: >1 g/100 ml, < 10 g/100 ml
DMSO: >20g/100 ml

3.1.7.	Partition coefficient (Log P_{ow})

Log P_{ow} : 0.60 (at 23°C±2°C, pH 7.45)

3.1.8.	Additional physical and chemical specifications

Organoleptic properties: /
Melting point: 133°C -139°C
Boiling point: /
Flash point: /
Vapour pressure: /
Density: /
Viscosity: /
pKa: /
Refractive index: /
UV-Vis spectrum: Absorption maxima at 237.2 nm and 477 nm

3.1.9.	Stability and Homogeneity

1 mg/ml and 200 mg/ml solutions of 3-Nitro-p-hydroxyethylaminophenol in 0.5% carboxymethylcellulose (CMC), at room temperature up to 6 hours and up to 9 days at 4°C, were stable (maximum deviation from initial concentration = 4%) when stored protected from light and under inert atmosphere.

0.1 mg/ml and 500 mg/ml solutions of 3-Nitro-p-hydroxyethylaminophenol in DMSO were stable at room temperature up to 4 hours study period (maximum deviation from initial concentration = 1%) when stored protected from light and under inert gas atmosphere.

1.0 mg/ml, 10 mg/ml and 500 mg/ml solutions of 3-Nitro-p-hydroxyethylaminophenol in DMF were stable at room temperature up to 4 hours study period (maximum deviation from initial concentration = 3%) when stored protected from light and under inert gas atmosphere

The solutions of 3-Nitro-p-hydroxyethylaminophenol in 0.5% CMC were found to be homogeneous during the 9 days storage period (CV maximum 5%) at when stored at 4°C, protected from light and under inert gas atmosphere.

General comments to physico-chemical characterisation

- 3-Nitro-p-hydroxyethylaminophenol is a secondary amine, and thus, it is prone to nitrosation. Nitrosamine content in 3-nitro-p-hydroxyethylaminophenol is not reported.
- No supporting data is provided for the chemical characterisation of 3-Nitro-p-hydroxyethyl-aminophenol, Batch Op. 108.
- Stability of 3-Nitro-p-hydroxyethylaminophenol in marketed products is not reported

3.2. Function and uses

The ingredient 3-nitro-p-hydroxethylaminophenol is used in oxidative hair colouring products at a maximum on-head concentration of 3%, after mixing the hair dye formulation with hydrogen peroxide typically in 1:1 proportions. 3-Nitro-p-hydroxethylaminophenol is also used in semi-permanent hair colouring products at a maximum concentration of 1.85%.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline:	Partly in compliance with OECD 420
Species/strain:	Rat, Sprague-Dawley
Test substance:	3-nitro-p-hydroxethylaminophenol
Batch:	0509151
Purity:	98.5%
Vehicle:	0.5% suspension of carboxymethylcellulose in purified water
GLP:	In compliance

In an acute oral toxicity study, the single oral administration (by gavage) of 3-nitro-p-hydroxethylaminophenol to female Sprague-Dawley rats at the dose of 2000 mg/kg resulted in death in 2/5 animals within 4 hours following the treatment. Hypoactivity, piloerection, lateral recumbency and dyspnoea were observed prior to death of these animals and in surviving animals on day 1. No mortality occurred at the dose of 1000 mg/kg and piloerection was noted on the day of dosing in all animals. No effects on body weight and no macroscopic abnormalities were observed at either dose level. In summary, a non-lethal dose of 3-nitro-p-hydroxethylaminophenol was 1000 mg/kg and a lethal dose was 2000 mg/kg in rats.

Ref.: 1

Comment
According to Annex 3 of OECD guideline 420, the second dose level should have been 300 mg/kg instead of 1000 mg/kg. Despite this deficiency, this study is useful for evaluation.

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: New Zealand White rabbits
Group: 3 males
Substance: 3-nitro-p-hydroxyethylaminophenol
Batch: 0509151
Purity: 98.3%
Dose: 0.5ml of 6 % 3-nitro-p-hydroxyethylaminophenol in a 0.5% suspension of carboxymethylcellulose in purified water
GLP: in compliance

0.5 ml of 6 % 3-nitro-p-hydroxyethylaminophenol in 0.5% carboxymethylcellulose was applied for periods of 3 minutes, 1 hour and 4 hours to a single male New Zealand White rabbit. The substance was held in contact with the skin by means of a semi-occlusive dressing. Cutaneous reactions were observed approximately 1 hour, 24, 48 and 72 hours after removal of the dressing and then daily until the end of the observation period.
Since persistent red coloration of the skin was noted in this animal, the study was then extended to include two additional animals by applying the test substance for 4 hours to the clipped flank. Observations for any cutaneous reaction were made at approximately 1 hour, 24, 48 and 72 hours after removal of the dressing.
Because of the discoloration, the animals were killed and skin samples were taken from the flanks of the animals (treated and untreated sites) for histological examination.

Results
The red discoloration may have masked any erythema. No associated cutaneous reactions (dryness of the skin, crusts or oedema) were observed.
The microscopic examination of the last two treated animals did not show any changes which could be attributable to the treatment with the test item.

Conclusion
Under the conditions of the study 6 % 3-nitro-p-hydroxyethylaminophenol, was not irritant to rabbit skin.

Ref.: 2

3.3.2.2.	Mucous membrane irritation

Guideline: OECD 405
Species: New Zealand White rabbits
Group: 3 males
Substance: 3-nitro-p-hydroxyethylaminophenol
Batch: 0509151
Purity: 98.3%
Dose: 0.1ml of 6 % 3-nitro-p-hydroxyethylaminophenol in a 0.5% suspension of carboxymethylcellulose in purified water
GLP: in compliance

0.1 ml of 6 % 3-nitro-p-hydroxyethylaminophenol in 0.5% carboxymethylcellulose was instilled into the left conjunctival sac; the right eyes served as the controls.
Observations were made at 1, 24, 48 and 72 hours and the daily until day 7.

Results

Slight chemosis and very slight conjunctival redness in all animals from day 1 up to day 4 after instillation. No other ocular lesions were observed during the study.

Conclusion
Under the conditions of the test 6 % 3-nitro-p-hydroxyethylaminophenol is irritant to rabbit eyes.

Ref.: 3

3.3.3.	Skin sensitisation

Local Lymph node assay

Guideline:	OECD 429
Species:	female mice, CBA/J
Group:	7 groups of 4 mice (28 animals) for each of 2 experiments
Substance:	3-nitro-p-hydroxyethylaminophenol
Batch:	0509151
Purity:	98.3%
Dose:	25 of 3-nitro-p-hydroxyethylaminophenol 2.5, 5, 10, 25 and 50% in dimethylformamide (first experiment) 25 µl 3-nitro-p-hydroxyethylaminophenol 0.03, 0.09, 0.28, 0.83 and 2.5% in dimethylformamide (second experiment)
GLP:	in compliance

A Local Lymph Node Assay was conducted in female CBA/J mice to investigate the sensitisation potential of 3-nitro-p-hydroxyethylaminophenol. The dose volume of 25 µL 3-nitro-p-hydroxyethylaminophenol in dimethylformamide was applied to the dorsal surface of both ears at the following concentrations: 2.5%, 5%, 10%, 25% and 50%. As positive lymphoproliferative responses were obtained at all these concentrations, a second experiment using the concentrations of 0.03%, 0.09%, 0.28%, 0.83% and 2.5% 3-nitro-p-hydroxyethylaminophenol was performed.
A positive control group received 25% alpha-hexylcinnamaldehyde (HCA) in dimethylformamide and the negative control the vehicle alone.

In each experiment, during the induction phase, the test item, vehicle or positive control was applied over the ears (25 µl per ear) for 3 consecutive days (days 1, 2 and 3). After 2 days of resting, the proliferation of lymphocytes in the lymph node draining the application site was measured by incorporation of tritiated methyl thymidine (day 6). The obtained values were used to calculate stimulation indices (SI).

Results
No mortality, clinical signs or compound-related changes in body weight were observed in either experiment. Red colouration of ear skin that could have masked discrete to severe erythema was observed in both experiments, but because no increase in ear thickness was noted, it was considered that 3-nitro-p-hydroxyethylaminophenol produced no excessive local irritation.

In the first experiment, positive lymphoproliferative responses occurred with no clear relationship to dose.

Treatment	Concentration (%)	Signs of local irritation	Stimulation Index (SI)
Test item	2.5	no	14.62
Test item	5	no	23.88
Test item	10	no	14.01
Test item	25	no	22.02
Test item	50	no	4.62
HCA	25	-	6.76

In the second experiment, a dose-related increase in stimulation index was observed, and the threshold positive value of 3 was exceeded at concentrations ≥ 0.09%.

Treatment	Concentration (%)	Signs of local irritation	Stimulation Index (SI)
Test item	0.03	no	2.18
Test item	0.09	no	3.54
Test item	0.28	no	6.36
Test item	0.83	no	7.61
Test item	2.5	no	11.22
HCA	25	no	7.34

The EC_3 value (0.07%) indicated 3-nitro-p-hydroxyethylaminophenol should be considered as an extreme sensitiser in mice.

Conclusion
3-nitro-p-hydroxyethylaminophenol is an extreme sensitiser in the murine Local Lymph Node Assay.

Ref.: 4

3.3.4. Dermal / percutaneous absorption

***In vitro* Percutaneous Absorption**

Guideline: OECD draft 428
Species: Human
Group: females, (2 abdominal skin and 4 breast skin)
Substance: 3-nitro-p-hydroxyethylaminophenol (NPHEAP)
Batch: 0509151
Purity: 98.7%
Radiolabelled: 3-Nitro-4-(2-hydroxyethylamino)[ring-U-14C]phenol
Batch: CFQ1309
Purity: 99.5%
Dose: 20 mg/cm^2 of test formulations containing a) 3% w/w test substance in oxidative conditions or b) 1.85% w/w as a semi-permanent dye.
GLP: in compliance

The *in vitro* percutaneous absorption of 3-nitro-p-hydroxyethylaminophenol from two typical hair colouring products was determined under "in-use" conditions, in human dermatomed skin mounted in flow-through diffusion cells, using phosphate buffered saline containing serum bovine albumin at 5% (w/w).
The integrity of skin samples was assessed by the tritiated water permeability coefficient. From these, samples were chosen which had a Kp of < 2.5 x 10-3 cm.h-1.

Under oxidative conditions, 3-nitro-p-hydroxyethylaminophenol was incorporated into a typical oxidative hair dye formulation at 6% (w/w) before mixing with developer (hydrogen peroxide, 1:1, w/w) to give a final concentration of 3%.

Under semi-permanent conditions, 3-nitro-p-hydroxyethylaminophenol was incorporated into a typical semi-permanent hair dye formulation at 1.85% (w/w).

Approximately 20 mg/cm^2 of each test formulation was applied to the skin surface. After 30 minutes of exposure, the hair dye remaining on the skin surface was removed by washing. Twenty-four (24) hours after application, the cutaneous distribution of 3-nitro-p-hydroxyethylaminophenol was assessed by Liquid Scintillation Counting in the skin washes (dislodgeable dose), *stratum corneum* (isolated by tape strippings), skin (living epidermis plus dermis) and receptor fluid.

Results
A total of 12 samples of human skin obtained from 4 different donors were dosed topically with [14C]-NPHEAP in the oxidative test preparation of a hair dye formulation (3%, w/w). Cell 1 was rejected from the mean and SD as the mass balance was too low.

	Cell Number and Donor Number													
	Cell 1	Cell 3	Cell 5	Cell 10	Cell 11	Cell 12	Cell 14	Cell 15	Cell 17	Cell 18	Cell 20	Cell 21		
	0083	0083	0085	0085	0105	0105	0103	0083	0085	0105	0103	0103	Mean	SD
Dislodgeable Dose	*524.78*	656.76	688.11	596.22	563.61	630.40	599.46	542.55	598.90	599.94	633.81	535.10	604.08	46.61
Stratum Corneum	*1.01*	2.11	4.30	2.44	4.72	4.31	16.30	0.67	3.44	4.84	7.87	6.17	5.20	4.17
Total Unabsorbed	*525.79*	658.92	692.53	598.73	568.53	634.71	616.71	543.23	602.39	604.79	641.74	541.32	609.42	46.87
Total Absorbed	*0.08*	0.16	1.50	0.78	1.83	1.33	0.47	0.04	0.94	0.57	0.13	0.24	0.73	0.61
Dermal Delivery	*0.14*	0.32	2.14	1.79	3.24	2.91	8.21	0.07	1.88	2.83	2.33	1.81	2.50	2.14
Total Recovery	*525.93*	659.24	694.67	600.52	571.77	637.63	624.92	543.30	604.28	607.62	644.07	543.14	611.92	47.13

Cell 1 was rejected from mean and SD due to a low mass balance (outwith 100 ± 10%)

A total of 12 samples of human skin obtained from 5 different donors were dosed topically with of [14C]-NPHEAP in the semi-permanent test preparation (1.85% w/w).
Cell 33 was rejected as this has an absorbed dose value out with mean plus 2SD.

	Cell Number and Donor Number													
	Cell 24	Cell 26	Cell 27	Cell 28	Cell 30	*Cell 33*	Cell 35	Cell 37	Cell 38	Cell 39	Cell 41	Cell 42		
	0105	0085	0088	0088	0103	*0083*	0103	0105	0085	0083	0088	0103	Mean	SD
Dislodgeable Dose	373.09	348.07	361.27	357.38	356.52	*338.84*	350.04	352.53	366.13	367.50	361.71	345.38	358.15	8.73
Stratum Corneum	5.28	0.91	1.26	1.04	1.47	*5.13*	0.59	0.47	1.75	0.49	0.55	1.65	1.41	1.37
Total Unabsorbed	378.44	349.09	362.54	358.44	358.01	*345.62*	350.64	353.02	367.89	368.00	362.28	347.03	359.58	9.52
Total Absorbed	0.12	0.20	0.19	0.19	0.04	*15.08*	0.04	0.14	0.15	0.03	0.09	0.02	0.11	0.07
Dermal Delivery	0.57	1.10	0.45	0.31	0.71	*16.09*	0.17	0.41	0.79	0.07	0.17	0.26	0.45	0.31
Total Recovery	379.01	350.19	362.99	358.74	358.72	*361.70*	350.81	353.43	368.68	368.06	362.45	347.29	360.03	9.52

Cell 33 rejected due to absorbed dose outwith mean + 2SD

The dermal absorption (sum of the amounts measured in the living epidermis, dermis and receptor fluid) was 2.50 ± 2.14 µgeq/cm² (0.40%) and 0.45 ± 0.31 µgeq/cm² (0.12%) in oxidative and semi-permanent conditions, respectively

Conclusion
The maximum absorption values of 8.21 µgeq/cm² in oxidative and of 1.10 µgeq/cm² (excluding cell 33) in semi-permanent conditions will be used for the calculation of the MoS.
Ref.: 12

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: /
Species/strain: Rat, Sprague-Dawley
Group size: 10 animals per sex and dose
Test substance: Imexine FH
Batch: OP.108
Purity: 100%
Dose levels: 0, 40, 200 and 1000 mg/kg bw/day

Vehicle:	Hydrogel of 0.5 g polysorbate 80 and 0.5 g sodium carboxymethylcellulose in 100 ml sterile water containing 0.01% activated dimethicone
Route:	Oral, by oesophageal gavage
Dosing schedule:	7days/week for 96 days (males) or 97 days (females)
GLP:	In compliance

In a 3 month toxicity study the hair dye Imexine FH was administered to the rat by the oral route (oesophageal gavage) as a suspension in a volume of 5 ml/kg at doses of 40, 200 and 1000 mg/kg/day, seven days a week for three months (10 animals of each sex per dose). A control group of similar composition received the vehicle. Ophthalmologic, haematologic, blood biochemistry and urine evaluations were performed after 4 and 13 weeks of treatment. The animals were killed and autopsied; macroscopic and microscopic evaluations of principal organs were performed.

No mortality was observed. The fur and urine of the animals were stained red as of the first treatment and the intensity of the discolouration was a function of the dose administered. Ptyalism appeared at the dose of 1000 mg/kg/day, immediately after product administration, as of the seventh week of treatment. Body weight gain and food consumption were slightly decreased (7%) in males at the low dose; no change was observed at other doses. The ophthalmologic examinations performed in high dose animals revealed a slight yellow-orange discolouration of the choroid attributable to that of the sclera, without alteration of the appearance of the choroid vessels. The slight modifications observed in the haematology, blood biochemistry and urinalysis evaluations were without toxicological significance. Organ weights in both sexes revealed a slight increase in liver and kidney weights (less than 20% in relation to 100 g body weight) without pathological significance. Dark discolouration of the thyroids in 7/10 males at 1000 mg/kg/day observed at necropsy was identified microscopically as finely granular lipofuscin or melanin pigments in the thyroid follicles.

Conclusion
The oral administration of the product Imexine FH to the rat for three months at doses of 40, 200 and 1000 mg/kg/day caused no modifications in organs with the exception of pigment deposition in thyroid follicles of most of the males treated at the high dose. According to the authors, this abnormality does not constitute a toxicological alteration and the dose of 1000 mg/kg/day administered under the conditions described can thus be considered as a dose without toxic effect. Accordingly, the NOAEL was set at 1000 mg/kg/day (5a). Additional work on this study included an expert report which reviewed the urinalysis, organ weight, macroscopic and microscopic findings, and confirmed the conclusion of the initial study report (5b).

Ref.: 5a and 5b

Comment
Despite the deficiencies of this study (not according to a guideline) this study is useful for evaluation. Based on organ weight increase in the highest dose group (19% increase in relative liver weight (females, males 12%) and a 19% increase in relative kidney weight (males, females 15%), the NOAEL in this study was set at 200 mg/kg bw/day.

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6.	Mutagenicity / Genotoxicity

3.3.6.1	Mutagenicity / Genotoxicity *in vitro*

Bacterial gene mutation assay

Guideline:	OECD 471 (1997)
Species/strain:	*Salmonella typhimurium*, TA 1535, TA 1537, TA 98, TA 100 and TA 102
Replicates:	Three plates per concentration in two independent experiments both in the presence and absence of Aroclor 1254 induced rat liver S9.
Assay conditions:	Direct plate incorporation method, apart from the second test with S9 mix, which was performed according to the pre-incubation method (1 hour at 37°C).
Test substance:	3-nitro-p-hydroxethylaminophenol (B054)
Batch:	0509151
Purity:	98.3%
Concentrations:	At least five concentrations in both experiments
Solvent:	DMSO
GLP:	in compliance

B054 was tested in a preliminary toxicity test (TA98, TA 100 and TA102) and in two independent experiments in the absence and presence of metabolic activation (S9 mix prepared from the livers of rats given Aroclor 1254). The experiments were conducted according to the direct plating incorporation method, apart from the second test with S9 mix, which was performed according to the pre-incubation method with a preincubation period of 60 minutes at 37°C). Based on the preliminary toxicity test the following concentrations were tested:

Experiments without S9

- 312.5, 625, 1250, 2500 and 5000 µg/plate, for the TA 1535, TA 1537, TA 98 strains in the first experiment and for the TA 100 strain in both experiments,
- 78.13, 156.3, 312.5, 625, 1250 and 2500 µg/plate, for the TA 102 strain in the first experiment,
- 156.3, 312.5, 625, 1250 and 2500 µg/plate, for the TA 1537 strain in the second experiment,
- 312.5, 625, 1250, 2500 and 3750 µg/plate, for the TA 98 and TA 1535 strains in the second experiment,
- 19.53, 39.06, 78.13, 156.3 and 312.5 µg/plate, for the TA 102 strain in the second experiment.

Experiments with S9

- 312.5, 625, 1250, 2500 and 5000 µg/plate, for the TA 1535, TA 1537, TA 98 strains in the first experiment and for the TA 100 strain in both experiments,
- 78.13, 156.3, 312.5, 625, 1250 and 2500 µg/plate, for the TA 102 strain in the first experiment,
- 156.3, 312.5, 625, 1250 and 2500 µg/plate, for the TA 1537 strain in the second experiment,
- 312.5, 625, 1250, 2500 and 3750 µg/plate, for the TA 98 and TA 1535 strains in the second experiment,
- 19.53, 39.06, 78.13, 156.3 and 312.5 µg/plate, for the TA 102 strain in the second experiment.

Results

The test item was freely soluble in DMSO up to 50mg/mL and no precipitation was observed in the petri plates at any dose level. A moderate to marked toxicity, measured as a decrease in revertant colonies, was observed at dose-levels ≥ 312 µg/plate in TA102, ≥ 1250 µg/plate in TA1537 and at 5000 µg/plate in TA 1535 and TA98 without S9 and at dose-levels ≥ 312 µg/plate in TA102, ≥ 2500 µg/plate in TA1537, ≥ 3750 µg/plate in TA 1535 and TA98 and at 5000 µg/plate in TA100 with S9. B054 did not induce any noteworthy increase in the number of revertants in any of the five tester strains either with or without S9 mix.

Conclusion
Under the experimental conditions used in this study, 3-nitro-p-hydroxethylaminophenol (B054) did not show mutagenic activity in the bacterial reverse mutation assay.

Ref.: 6

Mammalian Cell Gene Mutation Test in Mouse Lymphoma Cells (tk locus)

Guideline:	OECD 476
Species/strain:	Mouse lymphoma cell line L5178Y (*tk* (tymidine kinase) locus)
Replicates:	Duplicate cultures in two independent experiments
Metabolic act.:	Aroclor 1254 rat liver S9
Test substance:	3-nitro-p-hydroxethylaminophenol (B054)
Batch:	0509151
Purity:	98.3%
Concentrations:	1. experiment: 0.63, 1.25, 2.5, 5 and 7.5 mM (-S9) 0.63, 1.25, 2.5, 5, 7.5 and 10 mM (+S9) 2. experiment: 1.75, 3.5, 5.25, 7, 8 and 9 mM (-S9) 1.75, 3.5, 5.25, 7, 8, 9 and 10 mM (+S9)
Treatment:	Pulse (3h) treatment both in the absence and presence of S9 and 2 days expression period. Viability and mutant frequency was scored 7 and 11-12 days after the expression period, respectively.
Solvent:	DMSO
GLP:	In compliance

B054 was evaluated for its ability to induce point mutations and chromosomal aberrations in the *tk* locus in the mouse lymphoma cell line L5178Y. Two independent experiments using duplicate cultures each (single cultures for positive controls) was performed. Approximately 0.5×10^6 cells were exposed to the test substance in the presence or absence of S9 for 3 hours at 37°C. The selected concentrations for the main experiments were based on a preliminary toxicity test. Since the test item was not severely toxic in the preliminary test and freely soluble the highest concentration-level was 10 mM (corresponding to 1980 µg/ml)
At the end of the expression period (2 days), acceptable cultures were then plated for viability (2 plates per concentration level) and Trifluorothymidine resistance (4 plates per concentration level). Cell viability and number of mutant clones (differentiating small and large colonies) were determined after 7 and 11-12 days respectively. Six to 7 concentrations were selected for evaluation:

Without S9
0.63, 1.25, 2.5, 5 and 7.5 mM for the first experiment (where cytotoxicity measured by the adjusted relative total growth (RTG) was 22% for the top concentration);
1.75, 3.5, 5.25, 7, 8 and 9 mM for the second experiment (adjusted RTG: 18% for the top concentration);

With S9

0.63, 1.25, 2.5, 5, 7.5 and 10 mM for the first experiment (adjusted RTG: 12% for the top concentration);
1.75, 3.5, 5.25, 7, 8, 9 and 10 mM for the second experiment (adjusted RTG: 21% for the top concentration).
Cyclophosphamide (CPA) in the presence of S9 mix and methylmethanesulfonate (MMS) in the absence of S9 mix were used as positive controls. Negative controls consisted of cultures treated with the solvent alone (DMSO).

Results
In both main experiments a moderate to marked toxicity was observed at all tested concentrations. At the concentrations selected for evaluation the adjusted RTG were between 12 and 22%.
In the absence of S9, slight dose-related increases of MF were observed in the first experiment from 2.5 mM (reaching a 3.6-fold increase at 7.5 mM compared to vehicle controls); however this increase did not reach the threshold of + 126 x 10^{-6} requested by IWTG recommendations, though appropriate cytotoxicity was observed (adj RTG = 22%). In the second experiment, a significant increase of MF (addition of +169 x 10^{-6} mutants, or 3.8-fold increase, compared to controls) was observed only at the concentration of 1.75 mM which was slightly cytotoxic (adjusted RTG: 44%); however, higher B054 concentrations (3.5 mM and higher) produced weaker and not dose-related increases in MF (i.e. 2-fold to 2.3 increases), with cytotoxicity reaching appropriate level (adjusted RTG: 18%). Overall, the slight increases observed in both experiments were of week magnitude and were not reproducible and therefore not considered biological significant.
In the presence of S9, both experiments yielded significant increases in MF at relatively high concentrations (e.g. at 10 mM in the first experiment (addition of +135 x 10^{-6} mutants, or 2.5-fold increase, compared to controls), and at 8 mM in the second experiment (addition of +128 x 10^{-6} mutants, or 3.4-fold increase, compared to controls), where four other concentration levels (from 5.25 to 10 mM) produced higher than 2.2-fold increases of MF compared to controls.
A tendency toward increases in small colonies was also observed in both experiments, with and without metabolic activation. This was considered as possible evidence of clastogenic potential.

All negative solvent controls mutation frequencies (MF) fell within normal range defined in protocol (50-250 x 10^{-6}) and within historical range of the laboratory (72-159 x 10^{-6}), except in the first experiment, in the absence of S9, where MF were slightly lower (44 x 10^{-6}). All positive controls yielded significant increases in MF.

Conclusion
Under the experimental conditions of this study B054 showed genotoxic activity in the mouse lymphoma assay (*tk locus*) with S9 mix which can be considered as a clastogenic potential.

Ref.: 7

Mammalian Cell Gene Mutation Test in Mouse Lymphoma Cells (hprt locus)

Guideline:	OECD 476
Species/strain:	Mouse lymphoma cell line L5178Y (*hprt* locus for 6-thioguanine (6-TG) resistance)
Replicates:	Duplicate cultures in two independent experiments
Metabolic act.:	Aroclor 1254 rat liver S9
Test substance:	3-nitro-p-hydroxethylaminophenol (B054)
Batch:	0509151
Purity:	98.3%
Concentrations:	1. experiment: 8 concentrations from 50 to 1000 µg/ml – S9 and 6 concentrations from 100 to 1200 µg/ml + S9

	2. experiment: 7 concentrations from 400 to 1200 µg/ml – S9 and 8 concentrations from 400 to 1600 µg/ml + S9
Treatment:	Pulse (3h) treatment both in the absence and presence of S9 and 7 days expression period. Viability and mutant frequency was scored 7 and 11-12 days after the expression period, respectively.
Solvent:	DMSO
GLP:	In compliance

B054 was evaluated for its ability to induce point mutations in the *hprt* locus in the mouse lymphoma cell line L5178Y. Two independent experiments using duplicate cultures each (single cultures for positive controls) was performed. At least 10^7 cells were exposed to the test substance in the presence or absence of S9 for 3 hours at 37°C. The selected concentrations for the main experiments were based on a preliminary toxicity test
In the first experiment, ten concentrations were tested, ranging from 50 to 1500 µg/ml in the absence of S9 and from 100 to 1980 µg/ml (10 mM) in the presence of S9. After 7 days expression period, the highest doses selected to determine viability and 6 thioguanine resistance were 1000 µg/ml in the absence of S9 and 1200 µg/ml in the presence of S9, which yielded 16% and 18% relative survival, respectively.
In the second experiment, ten doses were tested, ranging from 100 to 1400 µg/ml in the absence of S9 and from 100 to 1600 µg/ml in the presence of S9. The highest doses selected to determine viability and 6-thioguanine resistance were 1200 µg/ml in the absence of S9 and 1600 µg/ml in the presence of S9, which yielded 19% and 11% relative survival, respectively.
At the end of the expression period, acceptable cultures were then plated for viability (2 plates per culture, 7 days) or 6-TG resistance (4 plates per culture, 11-12 days). Six to eight different concentrations were evaluated for survival and mutagenicity.
Benzo[a]pyrene (BP) in the presence of S9 mix and 4-nitroquinoline 1-oxide (NQO) in the absence of S9 mix were used as positive controls. Negative controls consisted of cultures treated with the solvent alone (DMSO).

Results
When tested up to toxic doses, no statistically significant increases in mutant frequency were observed following treatment with B054 at any dose level tested in both experiments either with or without S9.
Mutant frequencies in negative control cultures fell within normal ranges, and the positive control chemicals induced clear increases in mutation frequencies.

Conclusion
Under the test conditions used B054 did not induce mutation at the *hprt locus* of L5178Y mouse lymphoma cells.

Ref.: 8

In Vitro Micronucleus Test in cultured Human Lymphocytes

Guideline:	OECD draft guideline 487 (2004)
Species/strain:	Human lymphocytes from two healthy, non-smoking female donors.
Metabolic act.:	Aroclor 1254 induced rat liver S9.
Test substance:	3-nitro-p-hydroxethylaminophenol (B054)
Batch:	0509151
Purity:	98.3%
Concentrations:	Experiment 1: 265.8, 332.2, 648.8 µg/ml (–S9); 519.0, 811.0, 1980 µg/ml (+S9) Experiment 2: 500.0, 600.0, 750.0; 900.0 µg/ml (–S9); 1700, 1800, 1980 µg/ml (+S9)

Treatment:	Experiment 1: 24 hours mitogen (PHA) stimulation before treatment Experiment 2: 48 hours mitogen (PHA) stimulation before treatment Both experiments: With S9: 3 h treatment followed by 45 h recovery period Without S9: 20 h treatment followed by 28 h recovery period The last 27-28 hours of incubation in the presence of cytochalasin B
Solvent:	DMSO
GLP:	In compliance

B054was evaluated for its ability to induce micronuclei (clastogenic and/or aneugenic potential) using duplicate cultures of human lymphocytes in two independent experiments in the absence and presence of metabolic activation. The highest concentration in each experiment and test condition was either 1980 µg/ml (equivalent to 10 mM) or was selected on the basis of cytotoxicity criteria (reduction in replication index, RI).
In experiment 1, cultures were incubated in the presence of the mitogen phytohaemagglutinin (PHA) for 24 hours and then received a 20 or 3-hour treatment in the absence or presence of S9 mix, respectively. Cells were harvested 72 hours after the beginning of incubation (the last 27-28 hours of incubation being in the presence of cytochalasin B). In experiment 2, a similar test procedure was used except that cultures were incubated in the presence of PHA for 48 hours prior to treatment (harvesting took place 96 hours after the beginning of incubation).
Lymphocytes were then harvested, fixed and placed on microscope slides for evaluation. Slides were examined for proportions of mononucleate, binucleate and multinucleate cells and the replication index (RI) calculated based on the analysis of 500 cells per replicate (1000 per dose) to determine the doses to be analysed for micronuclei. Based on RI, the following concentrations were selected for analysis:

Experiment 1:	265.8, 332.2, 648.8 µg/ml (–S9); 519.0, 811.0, 1980 µg/ml (+S9) (Reduction in RI: 59 and 37%)
Experiment 2:	500.0, 600.0, 750.0; 900.0 µg/ml (–S9); 1700, 1800, 1980 µg/ml (+S9) (Reduction in RI: 65 and 11%)

One thousand binucleate cells from each culture selected (2000 per dose level) were analysed for micronuclei.
4-nitroquinoline-1-oxide, NQO, and vinblastine, VIN were used as positive control clastogene and aneugene respectively without S9 and cyclophosphamide, CPA, as clastogen positive control in the presence of S9. Solvent-treated cultures (DMSO, four replicates) were used as negative controls.

Results
In the first experiment (24-hour PHA stimulation prior to treatment), no compound-related increases in micronucleus formation were observed in the presence (up to 10mM) or absence (up to limit of cytotoxicity) of metabolic activation.

In the second experiment (48-hour PHA stimulation prior to treatment), treatment of cells with B054 in the absence and presence of S9 resulted in frequencies of micronucleated binucleate (MNBN) cells, which were significantly elevated as compared to concurrent vehicle controls for all concentrations analysed. Dose dependent increases in numbers of MNBN cells were noted for both treatment regimes. For treatment in the absence of S9 frequencies of MNBN cells exceeded the historical negative control (normal) range for both replicate cultures at a concentration of 750 µg/ml and also in single cultures at concentrations of 600 and 900 µg/ml. For treatment in the presence of S9 the MNBN cell frequency of both replicate cultures exceeded normal values at concentrations of 1800 and 1980 µg/ml and also in a single culture at a concentration of 1700 µg/ml. These results were therefore considered to be biological significant.

The positive control substances induced statistically significant increases in the incidence of micronuclei.

Conclusion
Under the conditions of the study, B054 was considered to be genotoxic (clastogenic and/or aneugenic) in cultured human lymphocytes in the absence and presence of metabolic activation

Ref.: 9

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Rat bone marrow micronucleus test

Guideline:	OECD 474 (1997)
Species/strain:	Sprague-Dawley (SD) rats
Group size:	Five males and 5 females
Test substance:	3-nitro-p-hydroxethylaminophenol (B054)
Batch:	0509151
Purity:	98.3%
Dose level:	0, 500, 1000 or 2000 mg/kg bw administrated as single dose
Route:	Oral gavage
Vehicle:	0.5% aqueous carboxymethylcellulose
Sacrifice times:	24 hours after dosing. Additional negative control (0.5% aqueous carboxymethylcellulose) and high dose (2000 mg/kg) PMP groups (5 rats/sex) were killed 48 hours after dosing.
GLP:	In compliance

The clastogenic/aneugenic potential of B054 was evaluated in this study. A preliminary toxicity assay was performed in four groups of 3 male and 3 female rats each to determine dose levels for the main study.
In the main study, five groups of 5 male and 5 female rats were used. Three of these groups received a single dose of 500, 1000 or 2000 mg/kg B054 by oral gavage. A fourth group received the vehicle (0.5% aqueous carboxymethylcellulose) under the same experimental conditions and served as a control. A fifth group received the positive control compound (cyclophosphamide) in a single oral dose of 60 mg/kg. An additional 3 males and 3 females received a single dose of 2000 mg/kg B054 and were reserved for pharmacokinetics analysis.
All animals were examined for mortality and/or clinical signs immediately after dosing, approximately one hour after dosing, and at least daily for the duration of the study.
Animals were killed either 24 hours (all groups) or 48 hours (vehicle control and 2000 mg/kg groups only) after the last treatment. For each animal, bone marrow smears were prepared and the micronucleated polychromatic erythrocytes were counted in 2000 polychromatic erythrocytes. The polychromatic (PCE) / normochromatic (NCE) erythrocyte ratio was established by scoring at least 500 erythrocytes per animal.

Results
No mortality occurred during the study. Red urine and red-stained tail were observed in all groups administered B054; no clinical signs of toxicity were observed.
No differences were observed between the micronucleated PCE frequencies for control and treated groups at any test article dose examined (500, 1000 and 2000 mg/kg).
B054 was cytotoxic to the bone marrow since the PCE/NCE ratio was significantly lower in 2000 mg/kg females killed 24 hours after compound administration.
The results of plasma analysis confirmed the systemic exposure of the test animals after oral administration of B054 at 2000 mg/kg (0.5 hour mean = 14.5 µg/ml for males and 4.02 µg/ml for females; 4 hour mean = 3.53 µg/ml for males and 2.48 µg/ml for females).
The positive control article yielded a significant increase in micronucleated PCE, which demonstrated sensitivity of the assay. The vehicle control group had less than

approximately 0.4% micronucleated PCEs and the group mean was within the historical control range (24 h: 0.085±0.007 (males) and 0.068±0.011 (females); 48 hours: 0.073±0.007 (males) and 0.040±0.010 (females).

Ref.: 10

Conclusion
B054 was evaluated as negative in the bone marrow micronucleus assay in Sprague-Dawley rats, when treated orally up to the testing limit for this assay of 2000 mg/kg. There were indications of bone marrow toxicity and systemic exposure.

Comments
Only a minor reduction in the PCE:NCE (from 0.85 ± 0.03 to 0.75 ± 0.02) was observed and only in the high dose group and in females at 24 hours harvest time only. No reduction was observed in males and in females at 48 hours harvest time.

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
Species/strain:	Rat, Sprague-Dawley
Group size:	20 mated females per dose group
Test substance:	Imexine FH
Batch:	TG CIACO89004 or lot. OP 108
Purity:	Not specified
Dose levels:	0, 100, and 1000 mg/kg bw/day
Exposure:	Gestation Days (GD) 6 through 15, once a day, 7 days/week
Recovery:	GD 16 -20
Route:	Oral, by oesophageal gavage
Vehicle:	0.5% aqueous hydrogel carboxymethylcellulose solution
GLP:	In compliance

The hair dye Imexine FH was administered by oral route to female rats from day 6 to day 15 of pregnancy in a 0.5% aqueous hydrogel carboxymethylcellulose solution at dose levels of 100 and 1000 mg/kg. Simultaneously, the control animals were given the vehicle alone. During pregnancy, females were observed for clinical signs, body weight and food consumption.
On day 20 of pregnancy, they were sacrificed and the foetuses were delivered by caesarean section. The litter parameters, i.e. corpora lutea, implantation sites, resorptions, dead and live foetuses were determined. The live foetuses were weighted and submitted to external and skeletal or visceral examinations.

In the treated groups, all females showed reddish coloured urine from day 7 to day 16 of pregnancy. No treatment-related effects on the body weight and food consumption were observed during the study. No mortalities and no abortions occurred. The numbers of corpora lutea and implantations, the rate of live foetuses, post-implantation loss, and the foetal body weight were similar in the control and the 100 mg/kg/day groups. In the 1000 mg/kg/day group, the numbers of corpora lutea and implantations and the foetal body

weight were comparable to the control group, while the rate of live foetuses was slightly decreased (95.4% vs. 98.7%, p <0.05) and the post-implantation loss slightly increased (4.6% vs. 1.3%: p<0.05).
No treatment-related foetal external anomalies or malformations, skeletal variation, anomalies or malformations, and soft tissue anomalies or malformation were observed in the 100 mg/kg/day group. In the 1000 mg/kg/day dose level, 2 foetuses presented with malformation: 1/292 showed an external astomia, which was associated with malformation of the face and brain, and 1/150 shoed a polydactyly.

Conclusion
According to the authors, the test substance Imexine FA was not materno-toxic, not embryotoxic nor was it teratogenic after oral administration of 100 mg/kg/day to mated female rats during the sensitive phase of organogenesis, under the experimental conditions of the study. The 1000 mg/kg/day dose level was not materno-toxic, slightly embryotoxic and 2 foetuses showed malformations (11a). According to an additional position paper in which the significance of the polydactyly was investigated, the presence of a polydactyly was not confirmed by the re-examination of the specimen by two reviewers. The changes noted in carpals were therefore considered an artefact and not to be test item related (11b).

Comments
Despite the deficiencies of this study (purity unknown) this study is useful for evaluation. In the submission the applicant concluded that the NOAEL for both maternal and developmental toxicity was 1000 mg/kg day. The applicant argued that the external astomia associated with the face and brain observed in a single foetus from the 1000 mg/kg/day group is unlikely to be related to administration of the test substance, given the isolated nature of this finding and because this malformation is known to occur spontaneously in rats of this strain at a low incidence. However, no historical control data on the occurrence of external astomia is provided. It can not be ruled out that the observed teratogenic effect (external astomia) was related to the administration of the test substance. In addition, embryotoxic effects (a statistically significant decrease in the number of live foetuses and a statistically significant increased post-implantation loss) were observed in the 1000 mg/kg/day group.

Therefore, the SCCP considers the NOAEL for developmental toxicity to be 100 mg/kg/day and the NOAEL for maternal toxicity 1000 mg/kg/day, which means that it can not be ruled out that teratogenic (external astomia) and embryotoxic (decreased number of live foetuses and increased post-implantation loss) effects occurred at dose levels which were not toxic to the pregnant dams.

Ref: 11a and 11b

3.3.9. Toxicokinetics

No data submitted

3.3.10. Photo-induced toxicity

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-nitro-p-hydroxyethylaminophenol)
(Oxidative/permanent)

Maximum absorption through the skin	**A (µg/cm²) µg/cm²**	**=**	**8.21**
Skin Area surface	**SAS (cm²)**	**=**	**700 cm²**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**5.75 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60 mg/kg**	**=**	**0.096**
No observed effect level (mg/kg) (developmental, oral, rat)	**NOAEL**	**=**	**100 mg/kg**
Margin of Safety	**NOAEL / SED**	**=**	**1042**

(3-nitro-p-hydroxyethylaminophenol)
(Non-oxidative/semi-permanent)

Maximum absorption through the skin	**A (µg/cm²) µg/cm²**	**=**	**1.10**
Skin Area surface	**SAS (cm²)**	**=**	**700 cm²**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**0.71 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60 mg/kg**	**=**	**0.0128**
No observed effect level (mg/kg) (developmental, oral, rat)	**NOAEL**	**=**	**100 mg/kg**
Margin of Safety	**NOAEL / SED**	**=**	**7792**

3.3.14. Discussion

Physico-chemical specifications
The ingredient 3-nitro-p-hydroxethylaminophenol is used in oxidative hair colouring products at a maximum on-head concentration of 3%, after mixing the hair dye formulation with hydrogen peroxide typically in 1:1 proportions. 3-Nitro-p-hydroxethylaminophenol is also used in semi-permanent hair colouring products at a maximum concentration of 1.85%. 3-Nitro-p-hydroxyethylaminophenol is a secondary amine, and thus, it is prone to nitrosation. It should not be used in combination with nitrosating agents. Nitrosamine content in 3-nitro-p-hydroxyethylaminophenol is not reported. Stability of 3-Nitro-p-hydroxyethylaminophenol in marketed products is not reported

General toxicity
A non-lethal dose of 3-nitro-p-hydroxethylaminophenol was 1000 mg/kg. A lethal dose was 2000 mg/kg in rats.
On the basis of significant increase in organ weights in the highest dose group, the NOAEL in the 90-day study was 200 mg/kg bw/day.

The NOAEL for developmental toxicity was set at 100 mg/kg/day and the NOAEL for maternal toxicity 1000 mg/kg/day.

Irritation / sensitisation
6% 3-nitro-p-hydroxyethylaminophenol was not irritant to rabbit skin. It was irritant to rabbit eyes.
3-nitro-p-hydroxyethylaminophenol was an extreme sensitiser in the murine Local Lymph Node Assay.

Dermal absorption
The maximum absorption values of 8.21 µgeq/cm² in oxidative and of 1.10 µgeq/cm² in semi-permanent conditions will be used for the calculation of the MoS.

Mutagenicity
3-nitro-p-hydroxethylaminophenol (B054) was not mutagenic in the Bacterial reverse mutation assay and did not induce mutations at the *hprt* locus of mammalian L5178Y cells ; it was positive in mammalian L5178Y cells (*tk* locus), with indication of clastogenic rather than gene mutagenic activity *in vitro*; B054 induced micronucleus formation *in vitro* (in cultured human peripheral blood lymphocytes); finally, an *in vivo* rat bone marrow micronucleus test showed that high doses did not affect chromosomes *in vivo*.
Overall, the genotoxicity program on B054 investigated the principal endpoints of modern genotoxicity testing: a) gene mutations (bacteria, mouse lymphoma cells, *tk* and *hprt* loci), b) clastogenicity (*in vitro* and *in vivo* micronucleus tests) and c) aneugenicity (*in vitro* and *in vivo* micronucleus tests). B054 induced no gene mutations in bacteria or in mammalian cells *(hprt)*. The possible clastogenic activity of B054 *in vitro* observed in L5178Y cells (*tk* locus) was consistent with the clastogenic/aneugenic activity induced in human lymphocytes in the *in vitro* micronucleus test. The *in vivo* micronucleus test was conducted at the limit dose to maximize target tissue exposure and was negative with evidences that tested animals were systemically exposed (urine discolouration, B054 plasma levels) and that bone marrow was exposed to the test compound. Given that this *in vivo* test was negative, B054 was considered to have no genotoxic potential *in vivo* and further testing with the substance itself was deemed unnecessary.

Carcinogenicity
No data submitted

4. CONCLUSION

Based on the information provided, the SCCP is of the opinion that the use of 3-nitro-p-hydroxyethylaminophenol itself as an oxidative hair dye substance at a maximum concentration of 3.0% in the finished cosmetic product (after mixing with hydrogen peroxide) or as an ingredient in semi-permanent hair colouring products at a maximum concentration of 1.85% does not pose a risk to the health of the consumer, apart from its sensitising potential.

3-Nitro-p-hydroxyethylaminophenol 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.

3-Nitro-p-hydroxyethylaminophenol itself is not mutagenic *in vivo*.

However, Studies on genotoxicity/mutagenicity in finished hair dye formulations should be undertaken following the relevant SCCNFP/SCCP opinions and in accordance with its Notes of Guidance.

5. MINORITY OPINION

Not applicable

6. REFERENCES

References in *italics* [14-23] were not submitted as full reports in the present dossier. They consist of reports for studies considered to be inadequate. These can be provided upon request.

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