EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

Scientific Committee on Consumer Products

SCCP

OPINION ON

4-AMINO-3-NITROPHENOL

COLIPA n° B51

Scientific Committees
on consumer products
on emerging and newly identified health risks
on health and environmental risk

The SCCP adopted this opinion at its 11th plenary on 21 March 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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Opinion to be cited as: Opinion of the SCCP on 4-amino-3-nitrophenol

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1. BACKGROUND

Submission I for 4-Amino-3-nitrophenol was submitted in July 1993 by COLIPA[1, 2].

Submission II for 4-Amino-3-nitrophenol was submitted in July 1996 by COLIPA[2].

The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) adopted in its 11th plenary meeting on 17 February 2000 the opinion (SCCNFP/0234/99) with the conclusion that:

"*The SCCNFP is of the opinion that 4-Amino-3-nitrophenol can be used safely in permanent hair dye formulations at a maximum concentration of 3.0%. However, as permanent hair dyes are mixed with hydrogen peroxide before application, the maximum in-use concentration should not exceed 1.5 %. In semi-permanent hair dye formulations, the maximum concentration is 3.0 %. The animal sensitisation data in the dossier was generated with a method not conforming to OECD n° 406.*
Cosmetic products containing this substance shall carry a label warning of a risk of sensitisation."

The substance is currently regulated by the Cosmetics directive (76/768/EC), Annex III, Part 2 under entry 3 on the List of substances, provisionally allowed, which cosmetic products must not contain except subject to restrictions and conditions laid down.

Submission III for 4-Amino-3-nitrophenol was submitted by COLIPA in July 2005. According to this submission the substance is used in oxidative hair dye formulations at a maximum concentration of 3.0%, which after mixing typically in 1:1 proportions ratios with hydrogen peroxide prior to use, corresponds to a concentration of 1.5% upon application (final, on-head concentration). It is also used in non-oxidative hair dye formulations at a maximum concentration of 1.0%.

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 4-Amino-3-nitrophenol safe for use as non-oxidative hair dye with an on-head concentration of maximum 1.0% taken into account the scientific data provided?*

2. *Does the SCCP consider 4-Amino-3-nitrophenol safe for use as an oxidative hair dye with an on-head concentration of maximum 1.5% taken into account the scientific data provided?*

3. *Does the SCCP recommend any further restrictions with regard to the use of 4-Amino-3-nitrophenol in any non-oxidative or 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

4-Amino-3-nitrophenol

3.1.1.2. Chemical names

Phenol, 4-amino-3-nitro- (CAS)
1-Hydroxy-3-nitro-4-aminobenzene

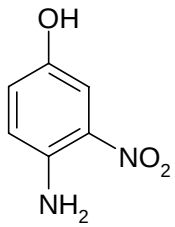
3.1.1.3. Trade names and abbreviations

IMEXINE® FN
COLIPA n° B051

3.1.1.4. CAS / EINECS number

CAS: 610-81-1
EINECS: 210-236-8

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_6H_6N_2O_3$

3.1.2. Physical form

Dark red powder

3.1.3. Molecular weight

Molecular weight: 154.12

3.1.4. Purity, composition and substance codes

Purity and impurities in 4-Amino-3-nitrophenol

Description	Batch number				
	0508916	**Op.253**	**Op. T268**	**Op. T283**	**Op. 238**
Identification/ characterisation	IR, NMR, MS, UV_Vis, HPLC, Elemental analysis	NMR, MS, HPLC, HPTLC	IR, HPTLC, UV_Vis	UV_Vis, HPTLC	UV_Vis, HPLC
Titre[1] (g/100 g)	97.5	>98	99.5	99.3	99.4
HPLC content (% peak area)	99.5				
Impurities (g/100 g) A B C D	 <0.01 D <0.01 ND <0.01 ND 0.34	 <0.01 D <0.01 ND <0.01 ND			
Water content (g/100 g)	0.3	<0.2			
Ash content (g/100 g)		<0.1			
Solvent residues (ppm) Methanol Isopropanol	 <100 ND <100 ND				

[1]Neutralisation of amine function by perchloric acid in an acetic acid medium
D: Detected, ND: Not detected
Impurity A: p-aminophenol
Impurity B: acetic acid, 4-acetylamino-phenyl ester
Impurity C: acetic acid, 4-acetylamino-3-nitrophenyl ester
Impurity D: N-(4-hydroxy-2-nitrophenyl)acetamide

3.1.5. Impurities / accompanying contaminants

See 3.1.4
Metal content (Batch No. 0508916):
Al: 51 ppm
Fe: 9 ppm
Ag, As, Ba, Bi, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Pd, Pt, Sb, Se, Sn, Ti, V, Zn: <1 ppm each
Hg: <0.1 ppm

3.1.6. Solubility

Water: 1.79 ± 0.08 g/l at 20°C ± 0.5 °C
Ethanol: >1 g/100 ml, <10 g/100 ml
DMSO: >20 g/100ml

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: 0.41 at 23°C ± 2°C, pH 7.45

3.1.8. Additional physical and chemical specifications

Melting point: 149.4 – 152.9 °C
Boiling point: /
Flash point: /
Vapour pressure: /
Density: /
Viscosity: /
pKa: /
Refractive index: /
UV_Vis maxima: 231.6 nm and 453 nm

3.1.9. Stability and Homogeneity

1 mg/ml and 100 mg/ml solutions of 4-Amino-3-nitrophenol in 0.5% carboxymethylcellulose (CMC), up to 6 hours at room temperature and up to 9 days at 4°C, were stable (maximum deviation from initial concentration = 6%), when stored protected from light and under inert gas atmosphere.

0.1 mg/ml and 500 mg/ml solutions of 4-Amino-3-nitrophenol in DMSO were stable at room temperature up to 4 hours study period (maximum deviation from initial concentration = 7%) when stored protected from light and under inert gas atmosphere.

0.5 mg/ml, 10 mg/ml and 250 mg/ml solutions of 4-Amino-3-nitrophenol in acetone/olive oil (4:1) were stable at room temperature up to 4 hours study period (maximum deviation from initial concentration = 7%) when stored protected from light and under inert gas atmosphere.

The solutions of 4-Amino-3-nitrophenol in CMC were found to be homogeneous during the 9 days storage period (CV maximum 10), when stored at 4°C, protected from light and under inert gas atmosphere

0.5 mg/ml, 10 mg/ml and 250 mg/ml solutions of 4-Amino-3-nitrophenol in acetone/olive oil (4:1) are shown to be homogeneous (CV maximum 4%), just after preparation, at room temperature.

General comments to physico-chemical characterisation

- The stability of 4-Amino-3-nitrophenol in marketed products is not reported.

3.2. Function and uses

4-Amino-3-nitrophenol is used as an ingredient in oxidative hair dye formulations at a maximum concentration of 3.0%, which after mixing typically in 1:1 ratio with hydrogen peroxide prior to use, corresponds to a concentration of 1.5% upon application. It is also used in semi-permanent hair dye formulations at a maximum concentration of 1.0%.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline:	OECD 401
Species/strain:	Sprague-Dawley
Group size:	15 male and 15 female
Test substance:	4-amino-3-nitrophenol (ANP)
Batch:	op 238
Purity:	99.4%
Dose:	500, 1000, 1500 mg/kg bw
Route:	oral in 1,2-propanediol

Exposure: single administration and a 14 days observation period
GLP: not in compliance

On the day of exposure, the animals had a mean weight of 173 ± 10 g for the males and 139 ± 5 g for the females. The day before treatment, the animals were fasted for about 18 hours before administration of ANP. ANP was suspended in 1,2-propanediol and aliquots of 10 ml/kg bw were administered once. The doses were 500, 1000, 1500 mg/kg body weight.

During two weeks of observation, mortalities (500: 1/10, 1000: 7/10, 1500: 7/10) and clinical observations were recorded on a daily basis. The main observations were sedation, dyspnoea, tonic clonic convulsions and orange colouration of urogenital area in all groups

LD_{50} was determined to be > 500 mg/kg bw.

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: New Zealand white rabbit
Group size: 3 males
Test substance: IMEXINE FN
Batch: Op238
Purity: 99.4%
Concentration: 6%
Vehicle: 1,2-propanediol (99%)
GLP: in compliance

The flanks of each animal were shaved with an electrical clipper. A dose of the fine grounded (dust) test substance at a concentration of 6% in 1,2-propanediol in a 6 cm^2 dry hydrophilic gauze patch was applied to the right flank of each animal. The test substance and the gauze patch were held in contact with the skin by means of an adhesive aerated semi-occlusive dressing attached to a restraining bandage. After 4 hours, the dressings were removed and residual test substance was removed with water. The cutaneous reactions were observed 1 h, 24 h, 48 h and 72 h after removal of the dressing. The left flank did not receive any substance and served as control.

Results
No oedema was observed 1 h, 24 h, 48 h and 72 h after removal of the test material. The macroscopic examination of erythema was obscured by coloration of the skin, but a microscopic examination of the skin revealed no lesions related to the application of the test substance.

Conclusion
Under the experimental conditions of the study, IMEXINE FN at 6% was considered as non irritant to rabbit skin.

Ref.: 2

3.3.2.2. Mucous membrane irritation

Guideline:	OECD 405
Species/strain:	New Zealand albino rabbit
Group size:	3
Test substance:	IMEXINE FN
Batch:	T 283
Purity:	99.3%
Dose:	0.1 g
Vehicle:	/
GLP:	not in compliance

100 mg of test substance was instilled into the conjunctival sac of the right eye after gently pulling the lower lid away from eye ball. The lids were then gently held for about one second in order to prevent loss of the material. The untreated eye served as control. The reactions in the eyes were checked from 1 hour to one week after application of the test material.

Results
One hour after instillation, signs of moderate irritation, slight chemosis of conjunctivae and discharge, were observed.
Redness of conjunctivae and iris congestion were not evaluated due to orange discoloration induced by the test material. 24 h later, redness of conjunctivae, discharge and chemosis were observed in all animals. An iris congestion and a partial corneal opacity were also noticed. Iris congestion and corneal opacity were reversible in less than 72 h. Conjunctival reactions disappeared within a week.

Conclusion
IMEXINE FN is an irritant for the rabbit eye, when tested undiluted.

Ref.: 3

Guideline:	OECD 405
Species/strain:	New Zealand albino rabbit
Group size:	3
Test substance:	IMEXINE FN
Batch:	Op 238
Purity:	99.4%
Concentration:	6%
Vehicle:	1,2-Propanediol
GLP:	in compliance

0.1ml aliquot of IMEXINE FN at 6% in 1,2-propanediol was instilled into the left conjunctival sac of test animals. The eyes were not rinsed following application of the test item. The non-treated eye served as control. The ocular reactions were assessed 1, 24, 48 and 72 h after instillation.

Results
There were no reactions following instillation of IMEXINE FN at 6% in rabbit eye.

Conclusion
Under the conditions of this study, IMEXINE FN at 6% was not irritating to rabbit eye.

Ref.: 4

3.3.3. Skin sensitisation

Local Lymph Node Assay

Guideline:	OECD 429
Species:	CBA/J mice
Group:	2 independent experiments, 28 animals in each experiment involving 7 groups of 4 animals each. 5 groups were treated with the test substance, one group was treated with the reference positive control substance and a group treated with the vehicle served as (negative) control
Test Substance:	4-Amino-3-nitrophenol (B051)
Batch:	0508916
Purity:	97.5%
Concentration:	0, 1, 2.5, 5, 10 and 25% (w/v)
Vehicle:	Acetone/olive oil (4:1)
Positive control:	25% (v/v) α-hexylcinnamic aldehyde in acetone/olive oil (4:1)
GLP:	in compliance

In a preliminary study, 25% (w/v) 4-Amino-3-nitrophenol in acetone/olive oil (AOO) was found to be non-irritant, and that was the maximal practicable concentration, based on solubility studies. [15].
In the first experiment, animals were separated in 7 groups (4 mice/group) consisting of:

* 5 treated groups receiving 4-Amino-3-nitrophenol at 1, 2.5, 5, 10 and 25% (w/v) in a mixture acetone/olive oil (4/1, v/v, AOO).
* A negative control group receiving the vehicle (AOO) alone
* A positive control group receiving alpha-hexylcinnamaldehyde (HCA) at 25% (v/v) in AOO

As positive results were observed in the first experiment, a similar second experiment was conducted at 0.05, 0.1, 0.5, 1 and 2.5% (w/v) 4-Amino-3-nitrophenol to better evaluate the skin sensitising potency of the test material.
In each of these experiments, the test substance, AOO or HCA was applied on the ears (25 µl per ear) of respective animals for three consecutive days designated as days 1, 2 and 3. After 2 days of resting (day 6), mice received a single intravenous injection of tritiated methyl thymidine (^{3}H-TdR). Lymph nodes draining the application sites (auricular nodes) were sampled, pooled per group, and the proliferation of lymphocytes was evaluated by measuring the incorporation of ^{3}H-TdR. The values obtained were used to calculate stimulation indices (SI), and the EC_3 was calculated in the second experiment. The irritant potential of the test item was assessed by measuring ear thickness on days 1, 2, 3 and 6.

Results
SI values of 10 and 8 were obtained with the positive control α-hexylcinnamic aldehyde in the first and second experiments, respectively Thus, the validity criteria were fulfilled in both experiments and these were considered to be valid.
In the first experiment, lymphoproliferative responses were observed at all concentrations tested, which were attributed to delayed contact hypersensitivity in the absence of local irritation. In the second experiment, performed at concentrations ranging from 0.05–2.5%, a dose dependent increase in the stimulation index (SI) was noted, and the positive value of 3 was exceeded at the concentrations ≥0.5% as described below. The EC_3 value was calculated to be 0.2%.

4-Amino-3-nitrophenol concentration	*SI*
0.05%	1.65
0.1%	1.82
0.5%	6.91
1.0%	8.88
2.5%	27.67
25% α-hexylcinnamic aldehyde	8.8

Conclusion
Under the conditions of this study, 4-Amino-3-nitrophenol induced delayed contact hypersensitivity. According to the EC_3 value calculated (0.2%), 4-Amino-3-nitrophenol was considered to have an extreme sensitising potency.

Ref.: 5

3.3.4. Dermal / percutaneous absorption

***In Vitro* Percutaneous Absorption Study using Human Dermatomed Skin**

Guideline:	OECD 428
Species/strain:	Four human breast skin samples and 3 abdominal skin samples were obtained from seven different female donors (27-53 years) subjected to plastic surgery. The skin was transferred stored on ice and kept frozen at –20°C for less than one month until use.
Test substance:	4-Amino-3-nitrophenol (4-A-3-NP) and 4-amino-3-nitro[U-^{14}C]phenol
Batch:	0508916 (unlabelled) and CFQ13913 Batch 1 (^{14}C-labelled)
Purity:	97.5% (unlabelled) and 99.3% radiochemical purity of the labelled material, defined as HPLC peak area
Vehicle:	A homogeneous cream formulation for dermal absorption under oxidative conditions (2.73% 4-A-3-NP and 0.13% ^{14}C-4-A-3-NP), a homogeneous cream formulation for dermal absorption under semi-permanent conditions (0.87% 4-A-3-NP and 0.13% ^{14}C-4-A-3-NP), and a developer containing 6% H_2O_2.

Doses and experimental set-up:

	Approximately 20 mg/cm^2 on 0.64 cm^2 skin membranes of ca. 400 µm thickness mounted on a flow through diffusion cell with 0.25 ml receptor fluid. Cream formulation to be tested under oxidative conditions was mixed 1:1 with the H_2O_2 developer before application.
Group size:	10 skin membranes from 4 donors were used for test under oxidative conditions, and 12 skin membranes from 5 donors were used for the test under semi-permanent conditions
Receptor fluid:	Phosphate buffered saline (PBS), calcium and magnesium free
Stability:	4-amino-3-nitrophenol was found to be stable in the cream formulations during the 24 hours study period.
GLP:	in compliance

Skin samples were dermatomed (393-400 µm in thickness) and mounted on flow-through diffusion cells. Calcium and magnesium-free phosphate-buffered saline was used as the receptor fluid. The integrity of the skin was checked by determination of the permeability coefficient for tritiated water (<2.5 x 10^{-3} cm/h for all selected membranes). Twenty-two diffusion cells were used in two separate experiments at approximately 32°C.
In the first experiment (oxidative conditions), an oxidative hair dye formulation containing ca.3.0% 4-amino-3-nitrophenol was mixed with the developer (1:1, w/w) to yield a final target concentration of 1.5% 4-amino-3-nitrophenol. About 20 mg/cm² of this mixture (corresponding to exactly 329 µg/cm² of 4-amino-3-nitrophenol) was applied on to the skin

surface (0.64 cm^2) and left for 30 minutes. After this time period, the remaining formulation on the skin surface was removed using a standardized washing procedure, simulating use conditions. Twenty-four hours after application, the percutaneous absorption of [^{14}C]-4-amino-3-nitrophenol was estimated by measuring its concentration by liquid scintillation counting (following combustion for non-liquid samples) in the following compartments: skin washes, *stratum corneum* (isolated by tape strippings), epidermis/dermis, unexposed skin and receptor fluid.
In a separate experiment, a similar experimental procedure was applied to evaluate the percutaneous absorption of 4-amino-3-nitrophenol under semi-permanent conditions, using a semi-permanent hair colouring formulation containing 4-amino-3-nitrophenol at a target concentration of 1.0% (about 20 mg/cm^2 were applied, corresponding exactly to 211 µg/cm^2 of 4-amino-3-nitrophenol).

Results
All diffusion cells yielded data that could be analysed. Most of the 4-amino-3-nitrophenol applied on the skin surface was removed with the skin wash (about 97% and 96% of the applied dose under oxidative and semi-permanent conditions respectively). Mass balance of 4-amino-3-nitrophenol in oxidative conditions was 97-104% and in semi-permanent conditions was 94-104%.
In oxidative conditions, the mean amounts of 4-amino-3-nitrophenol absorbed (sum of amounts measured in epidermis/dermis and receptor fluid) were as follows: 3.00 ± 1.75 µg equiv/cm^2 (0.92 ± 0.54% of the applied dose, range 0.53-5.19 µg equiv/cm^2)
In non-oxidative conditions, the amounts absorbed were: 0.59 ± 0.58 µg equiv/cm^2 (0.28 ± 0.27% of the applied dose, range 0.06-0.83 µg equiv/cm^2) in semi-permanent conditions.

Conclusion
The maximum dermal absorption of 4-amino-3-nitrophenol at 1.5% under oxidative conditions was 5.19 µg/cm^2 (or 1.6%)
The maximum dermal absorption of 4-amino-3-nitrophenol at 1.0% under non-oxidative conditions was 0.83 µg/cm^2 (or 0.4%).

Ref.: 14

Comment
Too few chambers were used. Because of the high variability of skin penetration, the study is considered inadequate.

3.3.5. Repeated dose toxicity

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

Guideline:	OECD 407
Species/strain:	Crl:CD-(SD)Br
Group size:	80 animals
Test substance:	4-amino-3-nitrophenol (ANP)
Batch:	/
Purity:	>98%
Dose:	0, 100, 250 and 600 mg/kg day
Route:	Oral, in carboxymethylcellulose
Exposure:	28 days
GLP:	in compliance

ANP was administered, by gavage, once daily to 4 groups of Crl:CD-(SD)BR rats (10/sex) for 28 days. The test substance was administered at dosage levels of 100, 250 or 600 mg/kg bw. The control group received the vehicle (carboxymethylcellulose). All animals were sacrificed at the end of the study. All animals were observed daily for mortality and clinical signs. Water consumption was recorded before treatment and during week 3. Body weights and food consumption were recorded individually in weekly intervals.

Ophthalmoscopic examination was performed. Blood samples were withdrawn from all survived animals for haematological and clinical chemistry investigations, during week 4. Organ weights (c. 15) were measured and macroscopy and histopathology (c. 40 organs/tissues) were performed on all control and high dose animals.

Results
Two animals (high dose group) died during the study (laboured respiration). All treated groups showed orange fur-staining, from day one. In the high dose group the following effects were observed: scabbing, perinasal staining, mild convulsions, significantly decreased body weight in the males. The dose level without adverse effects was 250 mg/kg bw.

Conclusion
The NOAEL was defined at 250 mg/kg bw per day.

Ref.: 5 of submission I

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

Guideline:	OECD 408
Species/strain:	Sprague-Dawley, SD
Group size:	40 per sex
Test substance:	4-amino-3-nitrophenol (ANP)
Batch:	Op T268
Purity:	>95.5%
Dose:	0, 10, 50 and 250 mg/kg day
Route:	Oral in 1.0% aqueous carboxymethylcellulose and 0.5% Tween 80
Exposure:	91 days
GLP:	in compliance

The subchronic toxicity of ANP was investigated in a 91 days oral (gavage) toxicity study in SD rats where ANP was given at 0, 10, 50 or 250 mg/kg/day. The dose levels were selected on the basis of the preliminary 4-week study.

Animals were observed daily, body weight and food consumption was recorded weekly. Ophthalmoscopy was performed before and after the exposure on control and high exposure groups. Haematology and blood chemistry investigations and urine analysis were performed at the end of the experiment. At the termination all animals were subjected to necroscopy.

Results
There were no treatment related unscheduled deaths in the study.
Food intake was not affected and the body weight development was similar in controls and the exposed groups.

There were no treatment related ocular findings.
Orange coloured urine was reported in all exposed groups demonstrating renal elimination of ANP and can therefore be considered a sign of systemic exposure.
No findings in haematology and blood chemistry are reported. Due to the strong discolouration of the urine of animals given 250 mg/kg day, most urinary parameters could not be evaluated at this particular dose level.
An increase in liver weights (+15%, relative weight) relative to control group was reported at the highest exposure group (males, 250 mg/bw/day). No abnormal histopathological findings in any tissues studied related to ANP exposure was reported.

Conclusion
The NOAEL was set at 250 mg/kg bw per day. Based on liver weight increase, the NOEL was set at 50 mg/kg bw per day.

Ref.: 6

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, TA1537, TA1538
Replicates:	Three independent experiments
Test substance:	4-amino-3-nitrophenol (B051)
Batch:	0508916
Solvent:	DMSO
Purity:	97.5%
Concentrations:	312.5, 625, 1250, 2500 and 5000 µg/plate, for all the strains in the 1st and 2nd experiments, with and without S9-mix; 1000, 2000, 3333, 4000 and 5000 µg/plate, for strain TA98 in the 3rd experiment, with and without S9-mix.
GLP:	in compliance

The objective of this study was to evaluate the potential of 4-amino-3-nitrophenol (B051) to induce reverse mutation in *Salmonella typhimurium*.

A preliminary toxicity test was performed to define the dose-levels of 4-amino-3-nitrophenol to be used for the mutagenicity study. The test item was then tested in three independent experiments, with and without S9-mix prepared from liver S9 fraction of rats induced with Aroclor 1254. The preliminary test, all experiments without S9-mix and the first as well as the third experiment with S9-mix were performed according to the direct plate incorporation method. The second experiment with S9-mix was performed according to the preincubation method (60 minutes, 37°C). Five strains of *Salmonella typhimurium* - TA98, TA100, TA102, TA1535, and TA1537 - were used. Each strain was exposed to five dose-levels of the test item (three plates/dose-level). After 48 to 72 h of incubation at 37°C, revertant colonies were scored. Toxicity evaluation was based on decrease in the number of revertant colonies or a thinning of the bacterial lawn. The test item was dissolved in dimethylsulfoxide (DMSO). The selected treatment-levels were: 312.5, 625, 1250, 2500 and 5000 µg/plate, for all the strains in the first and second mutagenicity experiments, with and without S9-mix, and 1000, 2000, 3333, 4000 and 5000 µg/plate, for the TA98 strain in the third experiment, with and without S9-mix. The following treatments were used as the positive controls without S9-mix: 1 µg/plate of sodium azide for TA1535 and TA100, 50 µg/plate of 9-aminoacridine for TA1537, 0.5 µg/plate of 2-nitrofluorene for TA98, and 0.5 µg/plate of mitomycin C for TA102. The following treatments were used as the positive controls with S9-mix: 2 µg/plate of 2-anthramine for TA1535, TA1537, TA98 and TA100, and 10 µg/plate of 2-anthramine for TA102 strain.

Results
The number of revertants for the vehicle and positive controls was as specified in the acceptance criteria. The study was therefore considered valid. Since the test item was freely soluble and non-severely toxic in the preliminary test, the highest dose-level was 5000 µg/plate, according to the criteria specified in the international guidelines. No precipitate was observed in the Petri plates when scoring the revertants at any dose-level. No toxicity was noted towards any of the strains used, with and without S9-mix. A reproducible

increase in the number of revertant colonies was observed in the TA98 strain, with and without S9-mix, using the direct plate incorporation method.

Conclusion
Under the experimental conditions, 4-amino-3-nitrophenol was mutagenic in the bacterial reverse mutation test with *Salmonella typhimurium* TA98 strain.

Ref.: 7

In vitro chromosome aberration test

Guideline:	OECD 473
Cells:	Human lymphocytes
Replicates:	Two independent experiments
Test substance:	4-amino-3-nitrophenol (B051)
Batch:	0508916
Purity:	97.5%
Solvent:	DMSO
Concentrations:	0.31, 0.63 and 1.25 mM (toxicity tested for: 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5 and 10 mM) in the 1st experiment, with and without S9-mix; 0.512, 1.02 and 1.28 mM (0.128, 0.256, 0.512, 1.02, 1.28, 1.6, 2 and 2.5 mM tested for toxicity) in the 2nd experiment, with and without S9-mix. Treatment volume was 27.5 µl/5.5 ml
GLP:	in compliance

The objective of this study was to evaluate the potential of 4-amino-3-nitrophenol to induce chromosome aberrations in cultured human lymphocytes.

Method
The tests were performed in two independent experiments, both with and without liver S9-mix obtained from rats treated with Aroclor 1254. The highest dose-level for treatment in the first experiment was selected on the basis of pH, osmolality and solubility. For selection of the dose-levels for the second experiment, any toxicity indicated by the reduction of mitotic index in the first experiment was also taken into account. For each culture, heparinized whole blood was added to culture medium containing phytohemagglutinin and incubated at 37°C, for 48 hours. In both experiments, lymphocyte cultures were exposed to the test or control items (with or without S9-mix) for 3 h, followed by rinsing. The cells were harvested 20 h after the beginning of the treatment, corresponding to approximately 1.5 normal cell cycles. 1.5 h before harvest, each culture was treated with colcemid to block cells at the metaphase-stage. After hypotonic treatment, the cells were fixed in a methanol/acetic acid mixture, spread on glass slides and stained with Giemsa. All the slides were coded for scoring. 4-Amino-3-nitrophenol was dissolved in dimethylsulfoxide (DMSO). The positive controls treatments were mitomycin C without S9-mix (3 µg/ml) and cyclophosphamide with S9-mix (12.5 or 25 µg/ml).

Results
In the culture medium, the dose-level of 10 mM (corresponding to 1540 µg/ml) showed a slight to moderate precipitate. At this dose-level, pH and the osmolality were equivalent to those of the vehicle control culture. In the first experiment, a slight to marked decrease in mitotic index was noted at dose-levels ≥ 0.31 mM (41- 95% decrease) without S9-mix. In the second experiment, a moderate to marked decrease in mitotic index was noted at dose-levels ≥ 1.28 mM (54-89% decrease). The highest dose-level selected for metaphase analysis (1.25 mM) for the first experiment induced a 42% decrease in mitotic index, and higher dose-levels inducing more than 75% decrease. In the second experiment, the highest dose scored for chromosome aberrations (1.28 mM) induced a 54% decrease in mitotic index. A significant increase in the frequency of cells with structural chromosomal

aberrations was noted after the 3-h treatment without S9-mix in the first experiment. Since this slight increase was not reproduced in the second experiment at a dose-level inducing more than 50% decrease in mitotic index, it was considered as non-biologically relevant.

In the first experiment, a marked decrease in mitotic index was noted at dose-levels ≥ 2.5 mM (78-99% decrease) with S9-mix. In the second experiment, a slight to marked decrease in mitotic index was noted at dose-levels ≥ 1.02 mM (39-94% decrease). In the first experiment, the highest dose scored for chromosome aberrations (1.25 mM) induced a 23% decrease in mitotic index, while higher dose-levels inducing more than a 75% decrease. In the second experiment, the highest dose examined for chromosome aberrations (1.28 mM) induced a 55% decrease in mitotic index. A significant and dose-related increase in the frequency of cells with structural chromosomal aberrations was noted in both experiments. The frequency of cells with structural chromosome aberration of the vehicle and positive controls were as specified in acceptance criteria. The study was therefore considered valid.

Conclusion
Under the experimental conditions, 4-amino-3-nitrophenol induced chromosome aberrations in cultured human lymphocytes with S9-mix.

Ref.: 8

In Vitro HPRT Gene Mutation Test

Guideline:	OECD 476
Cells:	L5178Y mouse lymphoma cells
Replicates:	Two independent experiments
Test substance:	4-amino-3-nitrophenol (B051)
Batch:	0508916
Purity:	97.5%
Solvent:	DMSO
Concentrations:	Experiment 1: nine doses (25-400 µg/ml) without S9-mix, ten doses (2.5-50 µg/ml) with S9-mix Experiment 2: ten doses were tested, ranging 25-500 µg/ml without S9-mix and 1.25 to 30 µg/ml with S9-mix.
GLP:	in compliance

4-Amino-3-nitrophenol was assayed for mutation at the *hprt* locus (6-thioguanine resistance) in mouse lymphoma cells using a fluctuation protocol. The study consisted of a cytotoxicity range-finding experiment followed by two independent experiments, each conducted in the absence and presence of metabolic activation by an Aroclor 1254 -induced rat liver post-mitochondrial fraction (S-9). A 3-h incubation period was employed for all treatments in the absence and presence of S9-mix. The test article was dissolved in sterile anhydrous analytical grade dimethyl sulphoxide (DMSO). In experiment 1, nine doses of 4-amino-3-nitrophenol, ranging from 25 to 400 µg/ml, were tested in the absence of S9-mix, and ten doses, ranging from 2.5 to 50 µg/ml, were tested in the presence of S9-mix. These doses were selected on the basis of the results of an cytotoxicity range-finding experiment. Seven days after the treatment, the highest doses analysed to determine viability and 6-TG resistance were 400 µg/ml in the absence of S-9 and 10 µg/ml in the presence of S-9, which yielded 18% and 8% relative survival, respectively.

In experiment 2, ten doses, ranging from 25 to 500 µg/ml in the absence of S9-mix and from 1.25 to 30 µg/ml in the presence of S9-mix, were tested. Seven days after treatment, the highest doses analysed to determine viability and 6-TG resistance were 500 µg/ml in the absence of S9-mix and 15 µg/ml in the presence of S9-mix, which yielded 17% and 8% relative survival, respectively. Negative (solvent) control and positive control treatments were included in each experiment in the absence and presence of S9-mix.

Results
Mutant frequencies in the negative control cultures fell within normal ranges, and clear increases in mutation were induced by the positive control chemicals 4-nitroquinoline 1-oxide (without S9-mix) and benzo(a)pyrene (with S9-mix). Therefore, the study was accepted as valid. When tested up to toxic doses in the absence of S9-mix, no statistically significant increases in mutant frequency were observed following treatment with 4-amino-3-nitrophenol at any dose level tested in Experiment 1 or 2. When tested up to toxic doses in the presence of S9-mix, no statistically significant increases in mutant frequency were observed following treatment at any dose level tested in experiment 1. In experiment 2, a statistically significant increase (2.2-fold) in mutant frequency was observed following treatment at 5 µg/ml, but no statistically significant increases were observed at two higher concentrations (6.25 and 7.5 µg/ml), where 10-20% relative survival was also observed. Furthermore, the effect was not reproduced at 5 µg/ml in experiment 1 and there was no evidence of a statistically significant linear trend in experiment 2. The increase observed at 5 µg/ml in experiment 2 was therefore considered as a chance event of no biological relevance.

Conclusion
It was concluded that, under the conditions employed in this study, 4-amino-3-nitrophenol (B051) is not mutagenic in this test system.

Ref.: 9

In vitro micronucleus test

Guideline:	OECD draft guideline
Cells:	Human lymphocytes
Replicates:	Two independent experiments, both with duplicate cultures (two female donors)
Test substance:	4-amino-3-nitrophenol (B051)
Batch:	0508916
Purity:	97.5%
Solvent:	DMSO
Concentrations:	Experiment 1(24h after PHA stimulation): 84.66, 132.3, 165.4, and 206.7 µg/ml without S9-mix (20 h + 28 h), 788.5, 985.6, and 1232 with S9-mix (3 h + 45 h) Experiment 2(48h after PHA stimulation): 250, 300, and 450 µg/ml without S9-mix (20 h + 24 h), 985.6, 1232, and 1540 µg/ml with S9-mix (3 h + 45 h).
GLP:	in compliance

4-Amino-3-nitrophenol was tested in an *in vitro* micronucleus assay using duplicate human lymphocyte cultures prepared from the pooled blood of two female donors in two independent experiments. Treatments covering a broad range of doses, separated by narrow intervals, were performed both in the absence and presence of metabolic activation (S9-mix). The test article was dissolved in sterile anhydrous analytical grade dimethyl sulphoxide (DMSO), and the highest dose level used, 1540 µg/ml was equivalent to 10 mM (an acceptable maximum concentration for *in vitro* cytogenetic studies according to current regulatory guidelines). In experiment 1, treatment of cells commenced approximately 24 h following mitogen stimulation. In the absence of S9-mix, this was for 20 h, followed by a 28-h recovery period prior to harvest (20+28 h). Treatment in the presence of S9-mix was for 3 h, followed by a 45-h recovery period prior to harvest (3+45 h). The S9-mix used was prepared from a rat liver post-mitochondrial fraction (S9-mix) from Aroclor 1254 induced animals. The test article dose levels for micronucleus analysis were selected by evaluating the effect of 4-amino-3-nitrophenol on the replication index (RI). Micronuclei were analysed

at three or four dose levels. The highest concentrations chosen for the analysis, 206.7 µg/ml in the absence of S9-mix, and 1232 µg/ml in the presence of S9-mix, induced approximately 69% and 62% reduction in replication index, respectively. Appropriate negative (solvent) control cultures were included in the test system under each treatment condition. 4-Nitroquinoline 1-oxide NQO, (5 µg/ml without S9-mix), vinblastine (0.08 µg/ml; without S9-mix), and cyclophosphamide (6.25 µg/ml; with S9-mix) were used as positive controls. The cells receiving these were sampled 48 h after the start of the treatment.

Results
The positive control compounds induced statistically significant increases in the proportion of cells with micronuclei. In experiment 1 (24 h PHA stimulation prior to treatment), 4-amino-3-nitrophenol did not significantly induce micronucleated binucleate (MNBN) cells in the presence of S9-mix. In the absence of S9-mix at 132.3 µg/ml, a small (1.9-fold concurrent control) but statistically significant increase ($p<0.01$) was, however, noted. The effect was of similar magnitude as observed with the concurrent aneugenic positive control treatment, vinblastine (1.6-fold). The MNBN cell frequency of both replicate cultures at this concentration exceeded the historical negative control (normal) range. However, this increase was not dose-related such that higher and lower concentrations of 4-amino-3-nitrophenol analysed (84.66 and to 206.7 µg/ml) exhibited normal (within historical range) frequencies of MNBN cells. This increase was therefore considered of questionable biological relevance. The MNBN cell frequency of all other 4-amino-3-nitrophenol-treated cultures (both treatment regimes) in experiment 1 fell within normal values.

In experiment 2 (48 h PHA stimulation prior to treatment), treatment of cells with 4-amino-3-nitrophenol (B051) in the absence and presence of S9-mix resulted in frequencies of MNBN cells, which were significantly elevated compared with those in concurrent vehicle controls for the majority of concentrations analysed. For treatment in the presence of S9-mix, significantly elevated frequencies of MNBN cells were observed for all three concentrations analysed. The MNBN cell frequency of both replicate cultures at the highest two concentrations analysed (1232 and 1540 µg/ml) and a single culture at the lowest (985.6 µg/ml) exceeded the normal range. These results were therefore considered of biological relevance. For treatment in the absence of S9-mix, statistically significant increases in MNBN cells were observed for the two highest concentrations tested (300 and 450 µg/ml; 2.4-fold and 1.8-fold concurrent control, respectively). MNBN cell frequencies that exceeded the historical negative control (normal) range were observed in single cultures at each of the three concentrations analysed. However, the increases observed were small so that group mean MNBN cells frequencies for the highest and lowest concentrations (250 and 450 µg/ml) fell within historical negative control values. No dose-response was apparent and for each concentration, the MNBN cell frequency fell within normal values in single replicate cultures. It was therefore considered that the increases observed without S9-mix were spurious and of no biological relevance.

Conclusion
It was concluded that 4-amino-3-nitrophenol induced micronuclei in cultured human peripheral blood lymphocytes following 3-h treatment in the presence of a rat liver S9-mix (3+45 h) where treatment commenced 48 h following PHA (mitogen) stimulation. No effect was seen in the presence of S9-mix when treatment commenced 24 h after PHA stimulation. Statistically significant increases seen in MNBN frequency at the two higher concentrations following treatment in the absence of S-9 (20+28 hour –S9-mix) where treatment commenced 48 h after PHA stimulation, were considered of no biological importance. An isolated increase in micronucleated cells was also observed following 20+28 h treatment in the absence of S9-mix where treatment commenced 24 h post mitogen stimulation. This increase was not observed at higher or lower concentrations analysed and was therefore, considered of questionable biological relevance.

Ref.: 10

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Rat bone marrow micronucleus test

Guideline:	OECD 474
Species/strain:	Rat, Crl:CD (SD)BR
Group size:	4 males and females
Test substance:	4-amino-3-nitrophenol (B51)
Batch No.:	0508916
Purity:	97.5%
Dose levels:	500, 1000, and 2000 mg/kg, once by oral gavage in 0.5% carboxymethylcellulose (24 h sacrifice), 2000 mg/kg (48 h sacrifice).
Sacrifice time:	24 h and 48 h (highest dose only) after the administration
GLP:	in compliance

The objective of the study was to evaluate the ability of 4-amino-3-nitrophenol to induce micronucleated polychromatic erythrocytes (PCEs) in rat bone marrow.

In a range-finding study, the test article was formulated in 0.5% aqueous carboxymethylcellulose and administered once by oral gavage to three males and three females per dose level. The animals were dosed at 500, 1000 or 2000 mg/kg and observed for up to 2 days after dosing for toxic signs and mortality. Based on the results, the top dose selected for the micronucleus assay was 2000 mg/kg, the limit of the assay.

In the micronucleus assay, the test article was formulated in 0.5% aqueous carboxymethylcellulose and administered once by oral gavage at 500, 1000, or 2000 mg/kg (at 10 ml/kg volume) to male and female rats. Cyclophosphamide (60 mg/kg) served as a positive control treatment. Bone marrow was extracted and at least 2000 PCEs per animal were analyzed for the frequency of micronuclei. Cytotoxicity was assessed by scoring the number of PCEs and normochromatic erythrocytes (NCEs) in at least 500 total erythrocytes for each animal.

Results
The test article induced mortality in one male treated at 1000 mg/kg and one female at 2000 mg/kg. Clinical observations included squinted eyes, slight hypoactivity/hypoactivity, hunched posture, tremors, orange genital discharge, red orange discharge, yellow ocular discharge, orange oral discharge, or red to orange discoloration of various parts of the body in animals treated with 1000 or 2000 mg/kg. Orange genital discharge was considered to be evidence of systemic exposure following administration of 1000 and 2000 mg/kg of the test article.

4-Amino-3-nitrophenyl did not induce statistically significant increases in micronucleated PCEs at any dose examined. However, the test agent was cytotoxic to the bone marrow, as evidenced by a statistically significant decrease in PCE:NCE ratio in males at 2000 mg/kg at the 48-h harvest time, indicating bone marrow exposure to the test compound. The vehicle control group had 0.0-0.4% micronucleated PCEs; the group mean was within the historical control. The positive control substance, cyclophosphamide, induced a statistically significant increase in micronucleated PCEs in comparison with the vehicle control.

Conclusion
4-Amino-3-nitrophenol was evaluated as negative in the rat bone marrow micronucleus assay under the conditions of this assay, when tested up to the testing limit (2000 mg/kg) for this assay which gave evidence of bone marrow toxicity.

Ref.: 11

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

Old study

Guideline:	OECD 414
Species/strain:	Sprague-Dawley, SD
Group size:	96
Test substance:	4-amino-3-nitrophenol (ANP)
Batch:	Op T268
Purity:	>95.5 %
Dose:	0, 100, 250 and 600 mg/kg day
Route:	Oral in 1.0 % aqueous carboxymethylcellulose and 0.5% Tween 80
Exposure:	from day 6 to 15 of gestation
GLP:	in compliance

The test substance was administered, by gavage, to 4 groups of 24 pregnant Sprague-Dawley rats (OFA-SD). The test substance was daily administered at dosage levels of 100, 250 or 600 mg/kg bw. The control group received the vehicle (carboxymethylcellulose) only. All mated females were sacrificed at day 20 of gestation. The animals were observed daily for clinical signs. Individual body weights were recorded at days 0, 6-15 and 20. Food consumption was measured for the day-intervals 0-6, 6-11, 11-15 and 15-20. Immediately following sacrifice, the uterus was removed, weighed and the number of (non)viable foetuses, early and late resorptions and the number of total implantations and corpora lutea was recorded. A macroscopic examination of the organs was carried out. All foetuses were individually weighed and the sex of the foetuses was determined. Two third of the foetuses was examined for skeletal defects and variations of the ossification process by Alizarin Red staining and one third was evaluated for visceral imperfections (organic defects).

Results
Two females of the high dose group died during the study. Most females of all treated groups had yellow/orange fur staining and yellow/orange stained urine. The high dose females showed significantly reduced body weights. A dose related increase in the number of foetuses exhibiting the skeletal variant of uni- or bilateral vestigial (rudimentary) 14th rib; significant from 250 mg/kg bw onwards, was observed. No irreversible structural changes were observed.

Conclusion
The dose level without maternal toxicity was 250 mg/kg bw and the dose level without embryo/foetotoxicity was 100 mg/kg bw (NOEL).

Ref.: 12

New study

Guideline: OECD 414

Species/strain:	Sprague-Dawley, SD
Group size:	96
Test substance:	4-amino-3-nitrophenol (ANP)
Batch:	0508916
Purity:	>97.5%
Dose:	0, 5, 20 and 400 mg/kg day
Route:	Oral in 0.5 % aqueous carboxymethylcellulose
Exposure:	from day 6 to 19 of gestation
GLP:	in compliance

Pregnant SD rats were exposed to APN daily using oral gavage administration at 0, 5, 20, and 400 mg/kg/day from day 6 through day 19 of gestation. ANP was administered in 0.5% aqueous carboxymethylcellulose.

Maternal evaluations and measurements included daily clinical signs, food intake and body weight gain. The dams were sacrificed and subjected to macroscopic examinations, gravid uterus weights were measured and foetuses removed. Typical litter parameters were recorded and foetuses were sexed, weighed and examined. Half of the foetuses were examined for soft tissue anomalies and the other half of the foetuses were examined for skeletal anomalies.

Results
No deaths were reported and clinical signs were limited to orange coloured urine. This fact indicates a renal elimination of ANP or its coloured metabolites and thus systemic exposure following oral exposure to ANP. An increase of short supernumerary rib was reported at 400 mg/kg/day in foetuses of some of the litters which was however, not statistically significant. Because the incidence (4.1%) was within the historical control range (0.0 – 7.6%) and because no foetuses had full supernumerary rib or abnormal pre-sacral vertebrae, the observation was considered not to be adverse.

Conclusion
There was no maternal toxicity or effects on embryo-foetal development in any of the exposure levels used. The NOAEL for maternal and developmental toxicity was 400 mg/kg/day.

Ref.: 13

Comment
Although the effect seen at 400 mg/kg bw was considered as part of the normal variation, a conservative NOEL of 20 mg/kg bw could be used for the calculation of the margin of safety.

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

Not applicable

3.3.14. Discussion

Physico-chemical specifications
4-Amino-3-nitrophenol is used as an ingredient in oxidative hair dye formulations at a maximum concentration of 1.5%, after mixing with hydrogen peroxide. It is also used in semi-permanent hair dye formulations at a maximum concentration of 1.0%. The stability of 4-Amino-3-nitrophenol in marketed products is not reported.

General toxicity
LD_{50} was determined to be > 500 mg/kg bw.
In a 28 day oral study in rats, the NOAEL was defined at 250 mg/kg bw per day. In an oral 90 day study in rat, the NOAEL was set at 250 mg/kg bw per day.
The dose level without maternal toxicity was 250 mg/kg bw and the dose level without embryo/foetotoxicity was 100 mg/kg bw (NOEL). In a more recent study, the NOAEL for maternal and developmental toxicity was set at 400 mg/kg/day.

Irritation / sensitisation
A 6% solution of 4-amino-3-nitrophenol in 1,2-propanediol was not irritating to rabbit skin under semi-occlusive conditions. There were no reactions following instillation of 4-amino-3-nitrophenol at 6% in rabbit eye. It is an irritant for the rabbit eye, when tested undiluted. 4-Amino-3-nitrophenol is an extreme sensitiser.

Dermal absorption
Too few chambers were used. Because of the high variability of skin penetration, the study is considered inadequate.

Mutagenicity / Genotoxicity
With metabolic activation, 4-amino-3-phenol induced mutations in *Salmonella typhimurium* strain TA98 and chromosomal aberrations in cultured human lymphocytes. Micronuclei were induced in cultured human lymphocytes *in vitro* but the effect was equivocal and the biological significance was unclear. It did, however, not induce *hprt* mutations in mouse lymphoma cells *in vitro* or micronuclei in rat bone marrow after *in vivo* administration by

gavage. Relevant exposure of the bone marrow was indicated by bone marrow toxicity. Thus, 4-amino-3-phenol does not have a relevant clastogenic potential *in vivo*.

Carcinogenicity
No data submitted

4. CONCLUSION

The SCCP is of the opinion that the information submitted is insufficient to allow a final risk assessment to be carried out. Before any further consideration

- an *in vitro* percutaneous absorption study should be performed following the relevant SCCNFP/SCCP opinions and in accordance with its Notes of Guidance.

- an additional mutagenicity / genotoxicity test should be performed following the relevant SCCNFP/SCCP opinions and in accordance with its Notes of Guidance in order to exclude a gene mutation potential.

Moreover, 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.

4-Amino-3-nitrophenol is an extreme sensitiser.

5. MINORITY OPINION

Not applicable

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