EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

Scientific Committee on Consumer Products

SCCP

Opinion on

3-Amino-2,4-dichlorophenol HCl

COLIPA n° A43

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 3-amino-2,4-dichlorophenol HCl

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1. BACKGROUND

Submission I for 3-amino-2,4-dichlorophenol hydrochloride was submitted in March 1992 by COLIPA[1,2].

The Scientific Committee on Cosmetology (SCC) expressed an opinion at the meeting the 53rd plenary meeting of 25 June 1993 with the conclusion, *"Industry should provide data to skin sensitization potential from in-use data in the context of the volume used, together with any available information on the toxicological profile of the compound, e.g. from animal studies and/or, from experience in use in either the consumer or occupational context."*

The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) expressed its opinion at the plenary meeting on 23 June 1999 with the conclusion, *"classification 1 under the conditions in use : in oxidative hair dyes at a maximum concentration of 2.0 %, in combination with hydrogen peroxide the maximum use concentration upon application is 1.0 %"*

Submission II for 3-amino-2,4-dichlorophenol hydrochloride was submitted in March 2001 by COLIPA[2].

The substance and its salts are currently regulated by the Cosmetics Directive (76/768/EC), Annex III, Part 2 under entry 19 on the List of provisionally allowed substances, which cosmetic products must not contain except subject to restrictions and conditions laid down.

According to current submission III, submitted by COLIPA in July 2005, 3-amino-2,4-dichlorophenol hydrochloride is used as a precursor for hair colours. It reacts with primary intermediates to form the final dye. The reaction can be accelerated by addition of an oxidizing agent. So the substance is used as an ingredient in hair dye formulations which may or may not contain a hydrogen peroxide based developer mix up to a final concentration of 1.5% (calculated for the hydrochloride) on scalp. Under the intended conditions of use the exposure is terminated 30 minutes after application of the mixture to the hair by shampooing and thoroughly rinsing with water.

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-amino-2,4-dichlorophenol hydrochloride safe for use as an ingredient in hair dye formulations in a concentration on-head of maximum 1.5% taking into account the scientific data provided?*

2. *Does the SCCP recommend any restrictions with regard to the use of 3-amino-2,4-dichlorophenol hydrochloride in hair dye formulations?*

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

3. OPINION

The substance, as synthesized, is present as the hydrochloride (3-Amino-2,4-dichlorophenol HCl). In previous submissions and Opinions it is not clear if it is referred to the free base or hydrochloride.

According to the INCI dictionary, CAS n° 61693-43-4 is for the hydrochloride

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

3-Amino-2,4-dichlorophenol HCl (INCI name)

Remark
The INCI Dictionary contains both, the hydrochloride and the free base.
The previous submission evaluated by SCC (opinion adopted on 25 June 1993, 53rd plenary) and the SCCNFP (opinion adopted on 23 June 1999, 6th plenary) referred both to the free base (*principal name*) connected CAS registry number 61693-43-4, which according to present data, corresponds to the hydrochloride.

3.1.1.2. Chemical names

Phenol, 3-Amino-2,4-dichloro-, hydrochloride (9CI)
1-Hydroxy-3-amino-2,4-dichlorobenzene hydrochloride
2,4-Dichloro-3-aminophenol hydrochloride
3-Amino-2,4-dichlorophenol hydrochloride

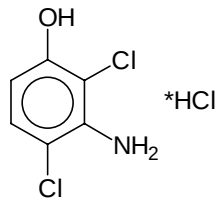
3.1.1.3. Trade names and abbreviations

Ro 151
COLIPA n° A43

3.1.1.4. CAS / EINECS number

Free base:	CAS:	61693-42-3
	EINECS:	262-909-0
Hydrochloride:	CAS:	61693-43-4
	EINECS:	applied for

3.1.1.5. Structural formula



3.1.1.6.	Empirical formula

Formula: $C_6H_6N_1O_1Cl_3$

3.1.2.	Physical form

Free base: Grey-white powder

Hydrochloride: Fine white powder (slightly pinkish – greyish)

3.1.3.	Molecular weight

Free base: Molecular weight: 178.02

Hydrochloride: Molecular weight: 214.49

3.1.4.	Purity, composition and substance codes

Purity by NMR assay: > 97% (w/w)
Purity by HPLC assay: > 99% (area)
Solvent content (water): < 1.0% (w/w)
Sulphated ash < 0.5% (w/w)

Ref.: 1

Specification of Batch 030120 = 03-01-20 = SAT 030629 = SAT 040233

Identity: 3-Amino-2,4-dichlorophenol hydrochloride, verified by ^{1}H-, ^{13}C- and DEPT-NMR-spectroscopy, IR-spectrometry and UV-spectrometry

Purity by NMR assay: 97.7% (w/w)
Purity by HPLC assay: 99.9% (area)
Chloride content: 16.9% (w/w)
Solvent content (water): < 0.1% (w/w)
Sulphated ash: < 0.1% (w/w)

Ref.: 2

3.1.5.	Impurities / accompanying contaminants

Heavy metals
- Hg: < 1 ppm
- Pb: < 20 ppm
- Sb, Ni: < 10 ppm
- Cd, As: < 5 ppm

Ref.: 1

3.1.6.	Solubility

in water: 1 – 10 g/l room temperature
in ethanol: > 100 g/l room temperature
in DMSO: > 100 g/l room temperature

3.1.7.	Partition coefficient (Log P_{ow})

Log P_{ow}: 2.6 (calc. Syracuse Vers. 1.66)

3.1.8.	Additional physical and chemical specifications

Melting point:	171 – 186°C
Boiling point:	/
Flash point:	/
Vapour pressure:	/
Density:	/
Viscosity:	/
pKa:	/
Refractive index:	/
pH:	/
UV_Vis spectrum	λ_{max} at 209 nm and 293 nm

3.1.9.	Homogeneity and Stability

/

General Comments to physico-chemical characterisation

- Data on solubility, stability and additional physico-chemical specifications were not adequately reported.
- Data on the stability of the test solutions and of the marketed product were not reported.
- Log P_{ow}: calculated values cannot be accepted as estimates of the true physical constant without justification, indicating that the reported values are realistic.

3.2. Function and uses

3-Amino-2,4-dichlorophenol HCl is used in oxidation 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.

Comment
According to the present submission, the 3-amino-2,4-dichlorophenol HCl content in currently marketed oxidative hair dye formulations is 3.0 %, which is higher than the content of 2 % as laid down in Directive 76/768/EC, Annex III, part 2 entry 19.
In the background, it is stated that the substance is used as an ingredient in hair dye formulations which may or may not contain a hydrogen peroxide based developer mixed up to a final concentration of 1.5% (calculated for the hydrochloride) on scalp.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline: OECD 423 (2001), acute toxic class method
Species/strain: Wistar rats
Group size: 6 females and 3 males
Test substance: A043 (3-amino-2,4-dichlorophenol hydrochloride) in corn oil
Batch: SAT 040233 - 030120
Purity: 99.7%
Doses: 300, 2000 mg/kg bw by gavage
Observation: 2 weeks
GLP: in compliance

The acute oral toxicity of 3-amino-2,4-dichlorophenol hydrochloride was investigated in Wistar rats, 3 male and 6 female rats were used. A group of 3 female rats was administered a single dose of 300 mg /kg bw. During a two weeks observation period, mortalities and clinical-toxicological observations were recorded daily. Since no mortality was observed, additional 3 male rats were given a single dose of 300 mg/kg bw. As no mortality was observed at the dose level of 300 mg/kg bw in both female and male rats, a group of 3 female rats was tested at the dose level of 2000 mg/kg bw.

Results
No mortality was observed at the dose level of 300 mg/kg bw. All animals treated with 2000 mg/kg bw were found dead within 30 minutes post dosing. The visceral examination of the animals found dead revealed lesions in lungs (mild to moderate congestion) and stomach (moderate to severe ulceration). In addition to these effects one animal showed liver lesions. These lesions in different organs were correlated with test substance treatment.

Conclusion
The median acute lethal dose is 500 mg/kg bw (300 – 2000 mg/kg bw).

Ref.: 3

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: Rabbit, New Zealand White
Group: 3 male
Substance: A043 / SAT 030629 (3-amino-2,4-dichlorophenol HCl)
Batch: 03-01-20
Purity: 99.7%

Dose:	0.5 g of the test substance moistened with 1 ml water on a semi-occlusive patch
GLP:	in compliance

Each animal was treated by dermal application of 0.5 grams of the test substance. The test substance was moistened with 1 ml water and applied to the clipped back skin of one flank, using a semi-occlusive patch mounted on Micropore tape wrapped around the abdomen and secured by elastic bandage. The patch was removed 4 hours after application and the skin was cleaned using water. The skin reactions were assessed at approximately 1, 24, 48 and 72 hours after removal of the dressings and test substance. Adjacent areas of untreated skin served as control in each animal.

Results
4 hours exposure resulted in well-defined erythema and slight oedema in the treated skin-areas of the 3 rabbits. Scaling was observed on the treated skin area of all animals 7 days after exposure. The skin irritation had resolved within 14 days after exposure in all animals.

Conclusions
Under the conditions of the study, A043 caused irritation to rabbit skin.

Ref.: 4

3.3.2.2. Mucous membrane irritation

Guideline:	OECD 405
Species:	Rabbit, New Zealand White
Group:	1 male
Substance:	A043 / SAT 030629 (3-amino-2,4-dichlorophenol HCl)
Batch:	03-01-20
Purity:	99.7%
Dose:	44.3 mg (approximately 0.1 ml)
GLP:	in compliance

The equivalent of 0.1 ml test substance was instilled into the conjunctival sac of one eye of the test animal. The eye lids were held together for about one second to avoid loss of test substance. The other eye served as control. After the 24-hour observation, a solution of 2% fluorescein in water was instilled into both eyes to quantitatively determine corneal epithelial damage. Based on the severity of the ocular lesions observed during the study, the animal was sacrificed for ethical reasons after the 24 hours observation and the two further rabbits assigned to the study were not treated.

Results
Instillation of the test substance into an eye of one rabbit resulted in effects on the cornea, iris and conjunctivae. The corneal injury consisted of opacity (maximum grade 4) and epithelial damage (maximum 90% of the corneal area). Iridial irritation grade 1 was observed up to termination, as well as the irritation of the conjunctivae (redness, chemosis and discharge) and grey-white discolouration (a sign of necrosis) on the eyelids and nictating membrane. In addition, irritation on the outside of the eyelids was noted at the 24-hour observation.

Conclusions
Under the conditions of the study, it was concluded that A043 was severely irritating to the rabbit eye.

Ref.: 5

3.3.3. Skin sensitisation

Guideline:	OECD 429
Species:	mice, CBA strain, inbred
Group:	females, 4 groups of 5 animals in each group (20 animals)
Substance:	A043 / SAT 030629 (3-Amino-2,4-dichlorophenol HCl)
Batch:	03-01-20
Purity:	99.7%
Dose:	25µl of A043 at 1, 10 and 25% in ethanol/water (7:3 v/v)
GLP:	in compliance

A Local Lymph Node Assay was performed to investigate the sensitisation potential of 3-Amino-2,4-dichlorophenol. A preliminary irritation study was performed. In the main study, 3 groups of 5 animals were treated with 3 test substance concentrations respectively. 1 group of 5 animals was treated with vehicle. During the induction phase, the test item was applied over the dorsal surface of each ear (25 µl per ear) for 3 consecutive days (days 1, 2, 3). On day 6, each animal was administered with ^{3}H-methyl thymidine by intravenous injection. After 5 hours, the animals were euthanized and the draining lymph nodes were excised and pooled to prepare single cell suspension for each animal. ^{3}H-methyl thymidine incorporation was measured by scintillation counting. The proliferative response of lymph node cells was expressed as the ratio of ^{3}H-methyl thymidine incorporation into lymph node cells of treated animals relative to that recorded in control animals.

In a separate experiment, reliability check with alpha-hexylcinnamic aldehyde as positive control was performed.

Results
The stimulation index values (SI) calculated for the substance concentrations 1, 10 and 25% were 1.5, 2.1 and 4.1 respectively. The data showed a dose-response and an EC3 value of 16.8% was calculated.

Conclusion
The results indicate that 3-Amino-2,4-dichlorophenol HCl is a moderate skin sensitiser.

Ref.: 6

3.3.4. Dermal / percutaneous absorption

Guideline	OECD 428
Species	Pig; dermatomed skin, 0.75mm
Group	2 pigs, 4 x 1cm^2 samples from each pig per experiment
Substance	A043 (SAT030629)
Radiolabel	[^{14}C]-A043 (SAT990410)
Batch	030120
Radiolabel Batch	3381-030
Purity	99.7%
Radiolabel Purity	99.0%
Samples/Dosing	Experiment A: Final concentration A043 1.65% in non-oxidative base Experiment B: Final concentration A043 1.65% in oxidative base Experiment C: Final concentration A043 1.5% in 25% aqueous ethanol
GLP	in compliance

The dermal absorption/percutaneous penetration of A043 from a standard hair dyeing cream mixed with a developer with and without hydrogen peroxide was studied on the clipped excised skin of two young pigs (both sexes), both with an approximate body weight of 30 kg. The dermatomed pig skin had a mean thickness of 0.75 mm.

The skin integrity of skin discs was checked by measuring the trans-dermal electrical resistance.

The intact, clipped excised pig skin of the flanks area was exposed for 30 minutes to the test substance in the different hair dyeing formulations without occlusion. Three different preparations were tested:

Experiment A: a standard basic cream mixed 1:1 (w/v) shortly before topical application to skin with a developer without hydrogen peroxide, resulting in a final A043 concentration of 1.65%.
Experiment B: a standard basic cream mixed 1:1 (w/v) shortly before topical application to skin with a developer containing hydrogen peroxide, resulting in a final A043 concentration of 1.65%.
Experiment C: A043 dissolved in 25% ethanol at a concentration of 1.5%.

The composition of the basic cream and the developer mix with and without hydrogen peroxide was:

Ingredients of basic cream	**Concentration in %**
A043	3.0
A005 (Toluene-2,5-diamine, 25%)	1:1 mol with A 043
Hydrenol D	9.35
Texapon NSO-UP	15.00
Dehyton K	12.50
Lorol techn.	2.20
Eumulgin B2	0.75
Ascorbic acid	0.20
Sodium sulphite	0.20
Ammonium sulphate	0.40
Ammonia	for pH adjustment
Citric acid (20%)	for pH adjustment
Water	ad 100
	pH 9.5

This formulation was traced with [^{14}C] radio-labelled material shortly before application resulting in a final concentration of 1.65% of A043.

Ingredient of developer mix	**without H_2O_2 in %**	**with H_2O_2 in %**
Dipicolinic acid	0.10	0.10
sodium diphosphate, acidic	0.03	0.03
Turpinal SL	1.50	1.50
Texapon NSO-UP	2.00	2.00
Ammonia, 25%	pH adjustment	pH adjustment
Tartaric acid	pH adjustment	pH adjustment
Aculyn 33 A	15.00	15.00
Hyprox 500 (= 50% H_2O_2 solution)	-	12.00
Water	ad 100	ad 100

The dermal absorption/percutaneous penetration of the test substance was investigated for the open application of about 20 mg formulation per cm² pig skin. Therefore the resulting dose of the test substance was approx. 0.33 mg/cm² skin. Skin discs of 1.0 cm² were exposed to the formulations for 30 minutes, terminated by gently rinsing with a 0.01% Tween 80 solution.
Each of the two formulations and the solution were analysed with eight replicates for adsorbed, absorbed and penetrated amount of the test substance. The receptor fluid used was Dulbecco's phosphate buffered saline (pH 7.35). In the static system, samples of the receptor fluid were drawn before the application of the test substance formulation and 0.5,

1, 2, 4, 6, 24, 29 and 48 hours after application. The removed volume was replaced by fresh receptor fluid.

Results
The mean quantities that had penetrated during the 30 minute exposure to A043 containing formulations and within the 48 hours after application are below:

ANALYSED SAMPLE	Formulation A without H_2O_2 [% of dose]	[µg/cm²]	Formulation B with H_2O_2 [% of dose]	[µg/cm²]	Solution C in ethanol:water (1:1) [% of dose]	[µg/cm²]
Skin rinsings	70.5	-	89.3	-	57.6	-
Adsorption (stratum corneum)	0.90	2.90	1.12	4.24	3.04	9.38
Not Bioavailable	71.4	-	90.4	-	60.6	-
Absorption (epidermis/dermis)	2.86	9.52	1.22	4.34	4.07	12.57
Penetration (receptor fluid)	12.42	41.04	5.91	20.95	28.28	87.32
Considered Bioavailable	15.28	50.56	7.13	25.29	32.35	99.89
Total recovery / mass balance	87.4	-	97.9	-	95.1	-

In this *in vitro* dermal penetration study the amount of A043 systemically available from a standard cream formulation mixed with a developer with or without hydrogen peroxide was found to be 25.29 ± 5.22 µg/cm² (7.13 ± 1.16%) and 50.56 ± 8.48 µg/cm² (15.28 ± 1.03%), respectively.

The maximum values were:

A_{max} (oxidative conditions) 35.28 µg/cm² (8.92%)
A_{max} (non-oxidative conditions) 63.68 µg/cm² (17.04%)

Ref.: 13

Comment
Although too few chambers (8) were used in this experiment, the variability of the data is low. Under these circumstances, the maximum absorption values may be used for the calculation of the Margin of Safety.

3.3.5. Repeated dose toxicity

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

No data submitted

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

Guideline: OECD 408 (1998)
Species/strain: Wistar strain (HsdBrlHan:WIST)
Group size: 10 per sex per dose, high dose (480 mg/kg bw/d) 12 per sex, separate 10 per sex control and 12 per sex (480 mg/kg bw/d) for recovery
Test substance: A043 (3-amino-2,4-dichlorophenol hydrochloride) in corn oil
Batch: SAT 040233 - 030120
Purity: 99.7%
Doses: 0, 80, 160 and 480 mg/kg bw/d
GLP: in compliance

The test substance was given as a suspension in corn oil for 91 consecutive days in daily doses of 80, 160 and 480 mg/kg bw. 20 rats (10 per sex) of the Wistar strain

(HsdBrlHan:WIST) were used per dose and control group, except for the high dose group which comprised 24 rats (12 per sex). Additionally, 20 rats (10 per sex) in a separate control group and 24 rats (12 per sex) in a separate high dose group were assessed for recovery of treatment-related effects, four weeks after the last administration. Mortality, signs of intoxication, body weight and food consumption were recorded. The animals of the recovery groups were additionally examined during the 4-week treatment-free period. At the end of the study, the animals were sacrificed and subjected to pathological investigations.

Results
On day 25 one male animal in the high dose recovery group died approximately 30 minutes post gavage. Clinical signs (salivation, lacrimation, nasal discharge, tremor and lethargy) were transient and lasted approximately 30 minutes post-gavage. In the recovery period all animals appeared normal.
At the 480 mg/kg bw dose level (high dose and high dose recovery group animals) tremors were observed in some treated rats of these groups. In the mid dose group no other treatment related clinical signs were noticed. Ophthalmological examinations performed prior to commencement of treatment and sacrifice did not reveal ocular abnormalities.
Mean body weights of high dose recovery group males were significantly decreased from week one to week 15 as compared to controls. Mean body weight of high dose group males was (not significantly) decreased as compared to the control group males and maximum decrease was 4.0% during week 1. Mean food consumption of treatment group animals of both sexes was comparable to control group animals.
Neurobehavioral observations/detailed clinical observations (motor activity, hind limb and forelimb grip strength and hind limb foot splay as well as sensory reactivity tests) performed at weekly intervals did not reveal any treatment related changes.
At the 480 mg/kg bw dose level of the haematological parameters evaluated at the end of the 90 day exposure significant reduction in mean values of RBC, Hb (males) and HCT (males and females) was observed. Furthermore, significantly increased MCHC was observed in both sexes. Post 28 days of recovery period mean values of RBC, Hb and HCT of high dose recovery group animals were found to be comparable with the control.
Results of clinical chemistry analysis revealed significant increased phosphorus, sodium and chloride in males of mid and high dose groups and cholesterol in high dose group males as compared to control group males. In females, increases in phosphorus were not dose-related. After 28 days of recovery period, the mean value of chloride of high dose recovery group males was significantly increased and mean value of cholesterol was significantly decreased. A significantly decreased pH of urine in high dose group males was observed in males.
The absolute weight of liver in the high dose group males was significantly increased as compared to control group males. Relative weights of liver and kidneys in high dose group males and females were significantly increased as compared to respective control group animals. Relative weights of adrenal and kidneys of high dose recovery group males were significantly increased as compared to control recovery group males. The weight changes observed in liver and kidneys were correlated with concurrent histopathological changes observed in respective organs.
The microscopic examination of organs in some mid and high dose group animals revealed treatment related lesions suggestive of hepatotoxicity and nephrotoxicity. Histopathologically, the changes in liver of treatment group animals were characterized by degeneration and necrosis of hepatocytes with foci of mononuclear cell (MNC) infiltration. No treatment related changes were noticed in the liver of animals at 80 mg/kg bw/d. The microscopic examination of kidneys revealed degeneration and necrosis of tubules and hypertrophy of tubular epithelial cells specially affecting cortex and medulla in the mid and high dose group. In some cases these changes were also associated with foci of MNC infiltration. The degenerative changes were more pronounced in males as compared to females. The incidences and severity of degenerative changes observed in liver and kidneys of animals of the high dose recovery group animals were less as compared to the changes observed in high dose group animals at the end of treatment period.

Conclusion
Administration of 3-amino-2,4-dichlorophenol hydrochloride to Wistar rats at the dose levels of 160 and 480 mg/kg bw/day resulted in transient symptoms like salivation, lacrimation and lethargy. At the highest dose level (480 mg/kg bw/d) significant changes in absolute liver weight (males) and relative weights of liver and kidneys (males and females) were found. At 480 mg/kg bw/d some changes in haematological parameters were observed. In clinical chemistry significantly increased phosphorus, sodium and chloride in males also of the mid dose were observed. The histopathological examination viz. kidney and liver revealed degenerative changes suggestive of nephrotoxicity and hepatotoxicity at 160 and 480 mg/kg bw/d.
The No Observed Adverse Effect Level (NOAEL) of 3-amino-2,4-dichlorophenol hydrochloride in Wistar rats exposed over a period of 90 days was concluded to be 80 mg/kg bw/d.

Ref.: 11

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.2 Mutagenicity/Genotoxicity *in vitro*

Bacterial gene mutation assay

Guideline:	OECD 471	
Species/strain:	*Salmonella typhimurium* TA98, TA100, TA102, TA1535, and TA1537	
Replicates:	triplicates in 2 individual experiments both in the presence and absence of S9-mix	
Test substance:	A 043 (3-amino-2,4-dichlorophenol HCl)	
Solvent:	DMSO	
Batch:	03-01-20	
Purity:	99.7%	
Concentrations:	Experiment I:	33 - 5000 µg/plate without and with S9-mix
	Experiment II:	10 - 5000 µg/plate without and with S9-mix
Treatment:	Experiment I:	direct plate incorporation with at least 48 h incubation without and with S9-mix
	Experiment II:	pre-incubation method was used with 60 minutes pre-incubation and at least 48 h incubation without and with S9-mix.
GLP:	In compliance	

3-Amino-2,4-dichlorophenol HCl was investigated for the induction of gene mutations in *Salmonella typhimurium* (Ames test). Liver S9-fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the level of toxicity in a preliminary toxicity test with strains TA98 and TA100 both without and with S9-mix. Toxicity was evaluated for 8 concentrations up to the prescribed maximum concentration of 5000 µg/plate on the basis of a reduction in the number of revertant colonies and/or thinning of the bacterial background lawn. Since in this pre-experiment evaluable plates were obtained for five concentrations or more in all strains used the pre-experiment is reported as experiment I.
Experiment I was performed with the direct plate incorporation method, experiment II with the pre-incubation method. Negative and positive controls were in accordance with the OECD guideline.

Results
In experiment I toxic effects evident as were observed at 1000 µg/plate for TA102, at 2500 µg/plate for TA98 and TA100 without S9-mix and at 5000 µg/plate for TA98 (with S9-mix), TA100 (with S9-mix), TA1535 (with S9-mix) and TA1537; in experiment II at 2500 µg/plate for TA98 (without S9-mix), TA100 (without S9-mix), TA102 and TA1537, and at 5000 µg/plate for TA98 (with S9-mix), TA100 (with S9-mix) and TA1535. Reduction in background growth was reported in experiment I at 5000 µg/plate for TA98, TA 100 and TA102; in experiment II at 5000 µg/plate for TA98 without S9-mix and for all strains with S9-mix.
In experiment I in the presence of S9-mix the number of colonies did not reach the lower limit of the historical control data in the negative controls. Since these deviations are rather small these results are judged to be not detrimental for the outcome of the study.
In both experiments 3-amino-2,4-dichlorophenol HCl treatment did not result in a biologically relevant increase in revertant colonies in any of the five tester strains neither in the absence nor in the presence of S9-mix.

Conclusion
Under the experimental conditions used 3-amino-2,4-dichlorophenol HCl was not genotoxic (mutagenic) in this gene mutation tests in bacteria.

Ref.: 7

In Vitro Mouse Lymphoma assay (*tk* locus)

Guideline:	OECD 476	
Cells:	L5178Y Mouse lymphoma cells	
Replicates:	duplicates in 2 parallel cultures in 2 independent experiments	
Test substance:	A 043 (3-amino-2,4-dichlorophenol HCl)	
Solvent:	DMSO	
Batch:	03-01-20	
Purity:	99.7%	
Concentrations:	Experiment I:	100.0 - 500.0 µg/ml (without S9-mix) 5.0 - 25.0 µg/ml (with S9-mix)
	Experiment II:	25.0 - 300.0 µg/ml (without S9-mix) 2.5 - 20 µg/ml (with S9-mix)
Treatment	Experiment I:	4 h treatment without and with S9-mix; expression
period		72 h and selection period of 10-15 days
	Experiment II:	24 h treatment without S9-mix; expression period 48 h and selection period of 10-15 days 4 h treatment with S9-mix; expression period 72 h and selection period of 10-15 days.
GLP:	In compliance	

3-Amino-2,4-dichlorophenol HCl was assayed for gene mutations at the *tk* locus of mouse lymphoma cells both in the absence and presence of S9-mix metabolic activation. Test concentrations were based on the results of a pre-test on toxicity measuring relative suspension growth. In the main test, cells were treated for 4 h or 24 h (without S9-mix experiment II) followed by an expression period of 72 or 48 h (without S9-mix experiment II) to fix the DNA damage into a stable *tk* mutation. Liver S9-fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. Toxicity was measured in the main experiments as percentage relative total growth of the treated cultures relative to the total growth of the solvent control cultures. Negative and positive controls were in accordance with the OECD guideline.

Results
There was no relevant shift in pH values nor in osmolarity even at the maximal concentration of 3-amino-2,4-dichlorophenol HCl (2200 µg/ml ≈ 10 mM) measured in the pre-test without S9-mix.
Exclusively, in experiment I culture I the required toxicity was reached (10-20% survival compared to the concurrent negative controls). In experiment I in the presence of S9-mix the appropriate level of toxicity at the three highest doses was beyond the required toxicity and these data points were not evaluated. Consequently in experiment I with S9-mix only two data points remained.
An increase in the number of mutant colonies was never observed at any concentration tested in both experiments either with or without S9-mix.

Conclusion
Under the experimental conditions used, 3-amino-2,4-dichlorophenol HCl did not induce gene mutations in this gene mutation test in mammalian cells.

Ref.: 8

Comments
The required toxicity (10-20% survival at the highest concentration tested compared to the concurrent negative controls) was mostly not reached.
Historical control data were only reported for the total number of mutant colonies; historical data for "small" and "large" colonies were not available.

***In vitro* micronucleus test**

Guideline:	treatment conditions, time of exposure and dose selection were performed according the guideline for the "*in vitro* chromosome aberration test" (OECD 473)
Cells:	V79 cells
Replicates:	duplicates
Test substance:	A 043 (3-amino-2,4-dichlorophenol HCl)
Solvent:	DMSO
Batch:	030120
Purity:	99.7%
Concentrations:	125.0 – 500.0 µg/mL (without S9-mix) 3.1 – 12.5 µg/mL (with S9-mix)
Treatment	4 h treatment both without and with S9-mix, harvest time 24 h after the start of treatment
GLP:	In compliance

3-Amino-2,4-dichlorophenol HCl has been investigated in the absence and presence of metabolic activation for the induction of micronuclei in V79 cells. Liver S9-fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the results of a pre-test on cell growth inhibition (XTT assay) with 4 h treatment. Treatment period was 4 h: harvest time 24 h after the beginning of culture. In the main experiment toxicity was determined by measuring the reduction in cell count and cell growth inhibition. Micronucleus preparations were stained with May Grünwald and Giemsa and examined microscopically for micronuclei. Negative and positive controls were in accordance with the OECD 473.

Results
3-amino-2,4-dichlorophenol HCl had no effect on osmolarity. The pH of the two highest concentrations was adjusted with small amounts of NaOH.

Cytotoxicity, measured as cell count, reached appropriate levels in the range of tested doses. However, at the highest dose tested with S9-mix cell growth inhibition was still 96% of the value of the concurrent negative control.
A dose dependent and biologically relevant increase in micronucleated V79 cells was found both in the absence and the presence of S9-mix. However, at the highest dose in the experiment without S9-mix the number of micronucleated V79 cells decreased compared to second highest dose possibly due to an increase in cytotoxicity.

Conclusion
Under the experimental conditions used 3-amino-2,4-dichlorophenol HCl induced micronuclei and, consequently, is genotoxic (clastogenic and/or aneugenic) in V79 cells.
Ref.: 9

Comment
3-Amino-2,4-dichlorophenol HCl was considered clastogenic after 4 h treatment. Therefore, a second experiment with longer treatment was not performed.

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Mouse bone marrow micronucleus test

Guideline:	OECD 474
Species/strain:	NMRI
Group size:	5 mice/sex/group
Test substance:	A 043 (3-amino-2,4-dichlorophenol HCl)
Batch:	030120
Purity:	99.7%
Dose level:	37.5, 75.0 and 150.0 mg/kg bw
Route:	*i.p.*
Vehicle:	aqueous ethanol (20%)
Sacrifice times:	24 and 48 h after the treatment.
GLP:	In compliance

3-Amino-2,4-dichlorophenol HCl has been investigated for the induction of micronuclei in bone marrow cells of mice. Test concentrations were based on the acute toxicity in a pre-test with 2 animals per sex/group, measured at various intervals around 1 to 48 h after treatment. In the main experiment mice were exposed to single *i.p.* doses of 0, 37.5, 75.0 and 150.0 mg/kg bw. 24 h or 48 h (highest dose only) after dosing bone marrow cells were collected. The animals of the highest dose group were examined for acute toxic symptoms 1, 2-4, 6 and 24 h after start of treatment.
Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and total erythrocytes (PCE/TE). Satellite groups of 3 male mice per sampling time (1 h and 4 h after start of treatment) treated with 100 mg/kg bw were included for determination of blood concentrations of 3-amino-2,4-dichlorophenol HCl.
Bone marrow preparations were stained with May-Grünwald/Giemsa and examined microscopically for the PCE/TE ratio and micronuclei. 5 mice/sex/group were analysed; the remaining 6^{th} animals of each group were only evaluated in case a mouse died spontaneously. Negative and positive controls were in accordance with the OECD guideline.

Results
In the main experiment one animal died after the highest dose of 150 mg/kg bw. This animal was replaced.
Treatment with 3-amino-2,4-dichlorophenol HCl did not result in substantially decreased PCE/TE ratios compared to the untreated controls indicating that 3-amino-2,4-dichlorophenol HCl did not have cytotoxic properties in the bone marrow. In contrast, clinical signs like reduction in spontaneous activity, abdominal position, eyelid closure and

ruffled fur indicating to systemic toxicity were observed in almost all treated animals up to 24 h (highest doses) or 6 h (lowest doses) after start of the treatment.
Biological relevant increases in the number of micronucleated PCEs compared to the concurrent vehicle controls were not found for bone marrow collected at 24 h following treatment with 3-amino-2,4-dichlorophenol HCl. The increase in micronucleated PCEs after 48 h treatment is due to the results found in 2 individual animals out of 10. Since all other values are in a normal lower range these findings may be considered as outliers.

Conclusion
Under the experimental conditions used 3-amino-2,4-dichlorophenol HCl did not induce micronuclei in bone marrow cells of treated mice and, consequently, 3-amino-2,4-dichlorophenol HCl is not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice.

Ref.: 10

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

Guideline:	OECD 414 (2001)
Species/strain:	Wistar rats
Group size:	25 sperm positive females per dose group
Test substance:	A043 (3-amino-2,4-dichlorophenol hydrochloride) in corn oil
Batch:	SAT 040233 - 030120
Purity:	99.7%
Doses:	0, 115, 230 and 460 mg/kg bw/d, GD 5-19
GLP:	in compliance

The test substance was suspended in corn oil and, based on dose finding studies, the doses 0, 115, 230 and 460 mg/kg bw/d were administered by gavage from gestational day 5 to 19 to pregnant Wistar rats. Clinical signs were observed daily, body weights and feed intake were recorded on days 0, 3, 5, 8, 11, 14, 17 and 20 of gestation.
On day 20 of gestation, the animals were sacrificed by asphyxiation using carbon dioxide. Immediately, the maternal viscera including uteri were examined macroscopically and gross pathological observations if any, were recorded. The number of alive and dead foetuses, their distribution and site in the uterus, early and late resorptions, implantations and number of *corpora lutea* were determined. The weight of the foetuses, gravid uteri, uteri without foetuses, placentas and the sex of foetuses were recorded. Approximately one-half of the foetuses were selected at random and examined for visceral alterations. The remaining foetuses were examined for skeletal malformations, variations and retardation of the normal organogenesis after appropriate staining.

Results
At 460 mg/kg bw/d 2 of 25 animals died. Several clinical symptoms (e.g. salivation, lethargy, lacrimation, nasal discharge or irritation, bronchial rales, gasping) were observed at 230 and 430 mg/kg which were dose-dependent and transient. Significant decreases in maternal body weight as well as in maternal body weight changes were observed in the 230 and 460 mg/kg dose groups. This was accompanied by decreases in feed consumption.

No significant changes were observed in the number of corpora lutea, implantation sites, foetuses and resorptions as well in the sex ratio. Uterine weights and foetal body weight (males and females) were significantly decreased at 460 mg/kg bw/d. Whereas no treatment-related changes were recorded in the frequency of external anomalies, visceral observations and section findings, a significant increase in incompletely ossified *os parietale* and sternebral ossification centers was observed at 460 mg/kg. This was interpreted as related to maternal toxicity and may also be due to the reduced foetal weight.

Conclusion
For 3-amino-2,4-dichlorophenol hydrochloride the NOAEL of maternal toxicity is 115 mg/kg bw/d and the NOAEL of embryo-/foetal toxicity is 230 mg/kg bw/d.

Ref.: 12

3.3.9. Toxicokinetics

No data submitted

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

No data submitted

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

No data submitted

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

3-Amino-2,4-dichlorophenol HCl

Maximum absorption through the skin	**A (µg/cm^2) µg/cm²**	**=**	**63.68**
Skin Area surface	**SAS (cm^2)**	**=**	**700 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**44.58 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.74 mg/kg**
No observed adverse effect level (mg/kg) (90 day, oral, rat)	**mg/kg bw**	**NOAEL**	**= 80**
Margin of Safety	**NOAEL / SED**	**=**	**108**

3.3.14. Discussion

Physico-chemical specifications
3-Amino-2,4-dichlorophenol HCl is used in oxidation 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.
However, the physico-chemical data were not adequately reported.

General toxicity
The median acute lethal dose is 500 mg/kg bw (300 – 2000 mg/kg bw).
The No Observed Adverse Effect Level (NOAEL) of 3-amino-2,4-dichlorophenol hydrochloride in Wistar rats exposed over a period of 90 days was set at 80 mg/kg bw/d. The NOAEL for maternal toxicity was set at 115 mg/kg bw/d. For embryo-/foetal toxicity, it was set at 230 mg/kg bw/d.

Irritation / sensitisation
Under the conditions of the study, A043 caused irritation to rabbit skin. It was severely irritating to the rabbit eye. However, the instillation of the test substance into the eye was not considered as the most relevant experiment.
The results indicate that 3-Amino-2,4-dichlorophenol HCl is a moderate skin sensitiser.

Dermal absorption
Although too few chambers (8) were used in this experiment, the variability of the data is low. Under these circumstances, the maximum absorption values may be used for the calculation of the Margin of Safety.

Mutagenicity
Overall, the genotoxicity of 3-amino-2,4-dichlorophenol HCl is sufficiently investigated for the three endpoints of genotoxicity: gene mutations, structural chromosome aberrations and aneuploidy. 3-Amino-2,4-dichlorophenol HCl did not induce gene mutations in bacteria whereas in mammalian cells at the *tk* locus of mouse lymphoma cells slight indications were found for a clastogenic effect. 3-amino-2,4-dichlorophenol HCl induced an increase in the number of micronucleated V79 cells. The indications for a clastogenic effect of 3-amino-2,4-dichlorophenol HCl in vitro were not confirmed in an adequately performed bone marrow micronucleus test in mice.
Consequently, 3-amino-2,4-dichlorophenol HCl can be considered to have no *in vivo* genotoxic potential and additional tests are unnecessary.
To reach a definitive conclusion, appropriate tests with 3-amino-2,4-dichlorophenol HCl in combination with hydrogen peroxide have to be provided.

Carcinogenicity
No data submitted

4. CONCLUSION

The SCCP is of the opinion that the use of 3-amino-2,4-dichlorophenol HCl itself as an oxidative hair dye at a maximum concentration of 1.5% in the finished cosmetic product (after mixing with hydrogen peroxide) does not pose a risk to the health of the consumer, apart from its sensitising potential.

3-Amino-2,4-dichlorophenol HCl itself has no mutagenic potential *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

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