

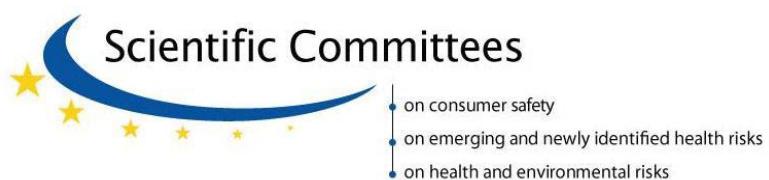


Scientific Committee on Consumer Safety

SCCS

**OPINION ON
o-Aminophenol**

COLIPA n° A14



The SCCS adopted this opinion at its 7th plenary meeting
of 22 June 2010

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat. They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

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).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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TABLE OF CONTENTS

ACKNOWLEDGMENTS	3
1. BACKGROUND	5
2. TERMS OF REFERENCE.....	5
3. OPINION.....	6
4. CONCLUSION	32
5. MINORITY OPINION.....	32
6. REFERENCES	32

1. BACKGROUND

According to COLIPA, submission I for o-Aminophenol was submitted in May 1983 by COLIPA¹, submission II in June 1985 and submission III was submitted in March 1987.

The Scientific Committee on Cosmetology (SCC) in 1993 issued an opinion (SPC/1396/93) based on submission IV and with summaries of submissions I-III and assigned the classification "A". The same conclusion was expressed in the reports of the Scientific Committee on Cosmetology by its 62nd plenary meeting of 18 January 1996.

o-Aminophenol and its salts are currently regulated in Annex III, part 2, under entry 34, on the list of substances provisionally allowed which cosmetic products must not contain except subject to restrictions and conditions laid down. For its use as an oxidative hair dye, a maximum concentration of 2% in the finished cosmetic products was established.

o-Aminophenol is classified as a mutagen, category 3.

Both the substance o-aminophenol (CAS 95-55-6) and its sulfate (CAS 67845-79-8) are listed in the inventory.

According to the current submission o-aminophenol is used in oxidative hair colouring products at a maximum of 0.6% in the finished cosmetic products.

2. TERMS OF REFERENCE

1. *Does the SCCS consider o-Aminophenol safe for use as an oxidative hair dyes with an on-head concentration of maximum 0.6% taken into account the scientific data provided?*
2. *And/or does the SCCS have any further scientific concern with regard to the use of o-Aminophenol in oxidative hair dye formulations?*

¹ COLIPA – the European Cosmetics Association

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

o-Aminophenol (INCI name)

3.1.1.2. Chemical names

2-Aminophenol
o-Aminohydroxybenzene
1-Hydroxy-2-aminobenzene
2-Hydroxybenzenamine
o-Hydroxyaniline
2-Hydroxyaniline
o-Hydroxyphenylamine

3.1.1.3. Trade names and abbreviations

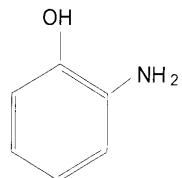
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BASF Ursol 3 GA
Benzofur GG
Fouramine OP
Nako yellow 3 GA
Paradone Olive Green B
Pelagol 3 GA
Pelagol Grey GG

3.1.1.4. CAS / EC number

CAS: 95-55-6
EC: 202-431-1

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: C₆H₇NO

3.1.2. Physical form

A light-beige powder, almost odourless

3.1.3. Molecular weight

Molecular weight: 109 g/mol

3.1.4. Purity, composition and substance codes

For batch 7120258 of 1-hydroxy-2-aminobenzene, a purity of 99.7% has been reported in submission IV without any further specification.

Chemical identity

Identification of the chemical structure of o-aminophenol was performed for batch 919151. IR, mass, ¹H and ¹³C NMR spectra are in accordance with the proposed structure.

The chemical identity of batch 445834 has been checked using UV spectroscopy.

High performance thin layer chromatography (HPTLC), an antiquated technique, was used for a qualitative test of o-aminophenol.

Ref.: summary (Subm. IV)

Impurities (batch 919151)

Possible impurities that may originate from reagents and intermediate products of reaction:

- m-aminophenol and p-aminophenol
- phenol
- o-nitrophenol
- aminophenozone

batch	m-amino-phenol	p-amino-phenol	phenol	o-nitro-phenol	amino-phenozone	heavy metals
919151	< 100 ppm	<100 ppm	< 100 ppm	< 100 ppm	< 25 ppm	< 10 ppm

m-, p-Aminophenol:

Impurities of m- and p-aminophenol were determined by HPLC-DAD at 281 nm (m-aminophenol) and 297 nm (p-aminophenol). Standard solutions of 100 ppm m- and p-aminophenol were used for calibration.

Phenol:

Impurities of phenol were determined by HPLC-DAD at 270 nm.

o-Nitrophenol, aminophenozone:

Impurities of o-nitrophenol and aminophenozone were determined by HPLC-DAD at 277 nm (o-nitrophenol) and 437 nm (aminophenozone). Standard solutions of 100 ppm o-nitrophenol and 25 ppm aminophenozone were used as reference.

3.1.5. Impurities / accompanying contaminants

See section 3.1.4. Purity, composition and substance codes

3.1.6. Solubility

The substance is insoluble in water, but soluble in ethanol (96%) and in dimethylformamide (DMF)

Ref.: summary (Subm. IV)

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: no data supplied

3.1.8. Additional physical and chemical specifications

Melting point:	174-177 °C (determined by capillary tube method)
Boiling point:	/
Flash point:	/
Vapour pressure:	/
Density:	/
Viscosity:	/
pKa:	/
Refractive index:	/
UV_Vis spectrum (200-800 nm):	/

3.1.9. Homogeneity and Stability

A preparation of 0.5% o-aminophenol in aqueous carboxymethylcellulose was analyzed for homogeneity and stability. Homogeneity was determined in three samples, one of them at the top, middle and bottom of the high dosage level formulation.

Stability tests were performed on two samples of the high dose level at the beginning of the animal experiments and afterwards. After preparation the samples were stored at -18°C until analysis.

Ref.: 37 (Subm. IV)

Comment

Documentation of the results of homogeneity and stability tests was not provided. Because of these missing data, no conclusions can be drawn as to the homogeneity or stability of the formulations used for toxicological testing or of the products on the market.

General Comments to physico-chemical characterisation

Nearly all physicochemical information originates from submission IV. The documentation of these data in this submission is inadequate. Many of the data cannot be linked to certain batches. This leads to many shortcomings:

- only one batch (919151) was fully characterized for identity and impurities
- all other batches were not sufficiently characterized
- for all batches, the data on purity are insufficient
- data on the stability and homogeneity of o-aminophenol or its formulations have not been supplied.

Conclusion

On the basis of the data provided no conclusions can be drawn neither with respect to the purity of o-aminophenol batches nor to the homogeneity or stability of o-aminophenol or its formulations.

Possible impurities that may originate from reagents and intermediate products of synthesis have been reported to be below limit of detection. This might be a hint that the state of the

art syntheses of o-aminophenol lead to products with purities of about 99% or even better. In this case differing purities of o-aminophenol batches would have no influence on the toxicological evaluation. However, since descriptions of the synthesis are missing, this assumption cannot be ascertained.

3.2. Function and uses

o-Aminophenol is used in oxidative hair dye formulations at a maximum concentration of 1.2% which, after mixing 1:1 with H₂O₂ just prior usage, corresponds to an on-head concentration of 0.6% upon application.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

The oral LD₅₀ of 1-Hydroxy-2-aminobenzene following administration of 500, 1000, 1250, 2000 and 2500 mg/kg bw in 1% carboxymethylcellulose to 10 OFA rats per group (5 males, 5 females) was calculated to be 1,052 mg/kg bw.

Ref.: 1 (Subm. I)

Acute toxicity in the rat has been examined by oral administration of the substance as a 5% suspension in 3% starch solution. Methaemoglobin concentration was less than 10% of all haemoglobin. Death occurred within 1 to 48 hours of dosing and calculation gave a median lethal dose of 1,300 mg/kg bw.

Ref.: 1-5 (Subm. V)

In the mouse a similar value of 1,259 mg/kg bw was obtained

Ref.: 2, 4, 6 (Subm. V)

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

Six male albino New Zealand white rabbits were used. 1-Hydroxy-2-aminobenzene dissolved at 1% in propylene glycol was applied to the clipped skin and kept under occlusion for 23 h. The test substance was non-irritant to rabbit skin.

Ref.: 1 (Subm. I)

0.5 g powdered o-aminophenol was applied to the clipped skin of 6 male albino New Zealand white rabbits using three different protocols. The substance was found to be non-irritant.

Ref.: 11 (Subm. V)

3.3.2.2. Mucous membrane irritation

1-Hydroxy-2-aminobenzene was dissolved at 1% in propylene glycol and instilled into the eyes of 6 rabbits. The 1% solution was found slightly irritant to the rabbit eye one hour after instillation without rinsing. The effect was reversible within 24 hours.

Ref.: 1 (Subm. V)

3.3.3. Skin sensitisation

o-Aminophenol was tested in 20 albino Hartley guinea-pigs following a modification of the method of Magnusson and Kligman. The induction comprised 10 topical occlusive applications of 0.5 g of the test substance and 2 intradermal injections of Freund's complete adjuvant on day 1 and 10. On day 36 0.5 g of the test substance was applied to a skin area never treated (challenge reaction). o-Aminophenol did not produce any cutaneous sensitizing reaction.

Ref.: 2 (Subm. I)

In albino Hartley guinea-pigs two protocols were investigated to assess the allergic potential. The first protocol used open epidermal induction and challenge supported by injection of Freund' complete adjuvant into the food pad, in the second protocol both adjuvant and the test substance were injected for induction. In the first protocol, an induction concentration of 0.18 mM and a challenge concentration of 0.09 mM were used and no response to o-aminophenol was observed while with the second method (induction concentration 0.18 mM, challenge concentration 0.09 mM) a 20% percent response was induced. The number of tested animals was not given.

Ref.: 12 (Subm. V)

In a guinea-pig maximisation test with p-phenylenediamine-sensitized animals moderately strong cross reactions to o-aminophenol were observed in 4/10 animals.

Ref.: 13 (Subm. V)

In a study in humans on cross-sensitivity of hair dyes, 3 of 10 patients sensitized to p-phenylenediamine showed weak positive patch test reactions to o-aminophenol, in a similar degree as in the control panel

Ref.: 14 (Subm. V)

A sensitized man was subjected to patch tests with 27 aromatic amines. While after 48 and 72 h all tests were negative one week later the patient reported a focal flare of the test sites.

Ref.: 3 (Subm. I)

In a compilation of LLNA data, 2-aminophenol was found to be a sensitisier. No study details are available.

Add. Ref. 2

3.3.4. Dermal / percutaneous absorption

Percutaneous absorption study of a hair dye formulation in rats

Hair dye formulations containing ¹⁴C-labelled 0.8% o-aminophenol in the presence of m-aminophenol and absence of human hair (**group I**) or presence of m-aminophenol and human hair (**group II**) were mixed with 1.5 parts H₂O₂ and applied to the back of hairless rats in the presence of human hair, the final concentration on the scalp was 0.32%. In an additional group human hair but no m-aminophenol was added (**group III**). After 30 min the skin was shampooed and rinsed. The animals were sacrificed 96 h after treatment and radioactivity was measured in urine, faeces and body organs. Stratum corneum was

removed by 6 tape strippings. Absorption was determined by addition of radioactivity in the epidermis (without stratum corneum), dermis, carcass, urine and faeces. Excretion was mainly into the urine (ca. 85%). 2.91 ± 0.32 , 2.98 ± 0.16 and $4.24 \pm 0.31 \mu\text{g}/\text{cm}^2$ were absorbed in the groups III, II and I, respectively.

Ref.: 26 (Subm. II)

Comment of the SCCS

The study was not acceptable since it was performed in the presence of human hair and/or m-aminophenol. Furthermore, the concentration used (0.32%) was lower than the one applied for (0.6%).

In vitro study using human skin

Guideline:	/
Tissue:	human breast epidermis
Group size:	2 donors, 6-8 membranes per donor
Diffusion cells:	diffusion cell, 2 cm^2
Skin integrity:	visual control through magnifying binocular
Test substance:	1-hydroxy-2-aminobenzene
Batch:	/
Purity:	/
Test item:	<ul style="list-style-type: none"> - 2% (w/w) 1-hydroxy-2-aminobenzene in a formulation + oxidant <ul style="list-style-type: none"> formulation 20% H_2O_2, 1 + 1 (I) - 2% (w/w) 1-hydroxy-2-aminobenzene in a formulation with 1.98% 1,4-diaminobenzene + oxidant formulation 20% H_2O_2, 1 + 1 (II) - 2% (w/w) 1-hydroxy-2-aminobenzene in a formulation with 2% 1-hydroxy-3-aminobenzene + oxidant formulation 20% H_2O_2, 1 + 1 (III)
Doses:	20 $\mu\text{g}/\text{cm}^2$
Receptor fluid:	solution 0.9% sodium chloride and 10 $\mu\text{g}/\text{ml}$ sodium ascorbate
Solubility receptor fluid:	/
Stability:	/
Method of Analysis:	HPLC + detection UV 240 nm+ amperometric detection
GLP:	/
Study period:	May 1990

The penetration of 1-hydroxy-2-aminobenzene was measured *in vitro* through human breast epidermis from a formulation containing 2 % of the test substance, mixed 1:1 with 20 % H_2O_2 , the final concentration was 1 % (**I**).

In other experiments a formulation containing 2 % of the test substance and 1.98 % of p-phenylenediamine (**II**) or a formulation containing 2 % of the test substance and 2 % of 1-hydroxy-3-aminobenzene mixed with 1:1 H_2O_2 were assessed (**III**).

After 30 minutes the upper part of the skin was washed and dried. The mean quantity of test compound in the receiving chamber 4.5 h after application was measured. Approximately 40 mg of the formulation were applied to the dermatomed membranes, which corresponds to $0.2 \mu\text{g}/\text{cm}^2$ of the test substances. In some experiments, hair was put in contact with the skin. The applications were rinsed off with distilled water and a 2% solution of sodium lauryl sulphate after a 30 min contact period, with the penetration of o-aminophenol through the membrane being assessed throughout 4 h exposure period. At the end of the exposure period, the amount of o-aminophenol in the receptor fluid was measured.

Samples collected during this study were analysed by HPLC followed by two extra analyses: detection UV 240 nm + amperometric detection.

Results

I: Formulation with 1% 1-hydroxy-2-aminobenzene

In two different experiments in the absence of hair, 500.77 ± 442.64 and 87.02 ± 53.41 ng/cm², which correspond to 0.24 and 0.04% respectively of the test compound applied on the skin, were measured in the receiving chamber.

In two different experiments in the presence of hair, 221.25 ± 292.23 ng/cm² and 99.32 ± 76.6 ng/cm², which correspond to 0.105% and 0.04% respectively of the test compound applied on the skin, were measured in the receiving chamber.

Skin without hair

Cell number	Amount recovered (ng/cm²)									
	1	4	5	6	7	8	9	10	mean	SD
Penetrated	1321.2 3	279.33	810.20	176.35	207.33	139.62	197.43	874.66	500.77	442.64
Penetration (%)	0.661	0.140	0.368	0.088	0.094	0.063	0.079	0.417	0.24	0.22

Cell number	Amount recovered (ng/cm²)							mean	SD
	7	8	9	10	11	12			
Penetrated	88.31	55.60	56.30	80.64	191.44	49.80	87.02	53.41	
Penetration (%)	0.042	0.024	0.026	0.032	0.096	0.023	0.04	0.03	

Skin in contact with hair

Cell number	Amount recovered (ng/cm²)								mean	SD
	1	2	3	4	5	6	7	8		
Penetrated	123.41	560.48	800.38	98.88	25.85	39.55	56.84	64.64	221.25	292.23
Penetration (%)	0.05	0.273	0.390	0.042	0.012	0.019	0.024	0.028	0.105	0.144

Cell number	Amount recovered (ng/cm²)						mean	SD
	1	2	3	4	5	6		
Penetrated	232.00	49.70	51.00	153.73	55.60	53.90	99.32	76.60
Penetration (%)	0.095	0.021	0.020	0.068	0.028	0.023	0.04	0.03

II: Formulation 1 % 1-hydroxy-2-aminobenzene + 0.99 % 1,4-diaminobenzene

Skin without hair

Cell number	Amount recovered (ng/cm²)									mean	SD
	1	2	3	4	5	6	8	9			
Penetrated	<48.9	75.6	<52.6	83.35	174.49	81.82	55.2	71.57	n.c.		
Penetration (%)	<0.025	0.04	<0.022	0.036	0.079	0.038	0.027	0.029	n.c.		

Skin in contact with hair

Cell number	Amount recovered (ng/cm²)								mean	SD
	2	3	4	5	6	7	8	9		
Penetrated	191.5 2	197.1 1	220.4 1	120.7 9	199.4 3	112.5 5	90.58	177.9 4	163.7 9	48.39
Penetration (%)	0.091	0.092	0.11	0.055	0.091	0.048	0.04	0.087	0.08	0.03

Cell number	Amount recovered (ng/cm²)							mean	SD
	3	4	5	6	7	8			
Penetrated	102	<51.3	<43.7	<52	<50	59.18	n.c.		
Penetration (%)	0.046	<0.022	<0.022	<0.019	<0.021	0.02	n.c.		

n.c. not calculated

III: Formulation with 1 % 1-hydroxy-2-aminobenzene + 1 % hydroxy-3-aminobenzene

Skin without hair

Cell number	Amount recovered (ng/cm²)								mean	SD
	3	4	5	6	7	8	9	10		
Penetrated	<50.3	<49.2	<49.5	<49.4	<43.3	<52.1	99.79	117.72	n.c.	
Penetration (%)	<0.025	<0.021	<0.024	<0.024	<0.02	<0.024	0.04	0.049	n.c.	

Skin in contact with hair

Cell number	Amount recovered (ng/cm²)							mean	SD
	4	5	6	7	8	9			
Penetrated	<50.5	<50.3	42.7	52.1	<55.3	<48.9	n.c.		
Penetration (%)	<0.022	<0.025	0.019	0.025	<0.031	<0.023	n.c.		

Ref.: 40, 41, 42 (Subm. IV)

Comments

The method did not follow a guideline and was not in compliance with GLP. Only epidermis was used, not the full skin. The control of the integrity was not done correctly. No data were supplied on the amount of test substance present in stratum corneum, epidermis and dermis. The variability between samples in one experiment and between different experiments under the same conditions is very high. Because of these many shortcomings, the studies cannot be used for the safety assessment.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (30 days) oral toxicity

30 days repeated dose oral toxicity, study 1

Guideline: OECD 407 (1981)
 Species/strain: rat, Sprague Dawley OFA
 Group size: 10 males and 10 females per group
 Test substance: orthoaminophenol
 Batch: 7-12-0 258
 Purity: /
 Vehicle: hydrogel of 0.2 g polysorbate 80 and 0.2 g sodium carboxymethylcellulose in 100 ml aqua pro injectione
 Dose levels: 0, 20, 80 and 320 mg/kg bw/d
 Route: oral gavage
 Administration: daily for 30 days
 GLP: in compliance
 Study period: 9/1988 to 2/1989

The test substance (suspended in a hydrogel of 0.2 g polysorbate 80 and 0.2 g sodium carboxymethylcellulose) was administered by gavage for 30 consecutive days to groups of 20 Sprague Dawley (OFA) rats (10/sex) at doses 0 (group I), 20 (group II), 80 (group III) and 320 (group IV) mg/kg bw/d. The age of the animals at the start of the study was approximately 6 weeks and the body weight range was 164 – 186 g for males and 134 – 169 g for females. Food and water was supplied *ad libitum*.

Clinical observation and mortality was checked daily. Ophthalmoscopic examination was performed before and at the end of the study. Body weights, food and water consumption were recorded twice weekly.

Haematology and blood biochemistry were determined at the end of the study. Urinalysis was performed at the end of the study. Necropsy, main organ weights, histopathological examination were undertaken at sacrifice time.

Results

Orange discolouration of the urines was observed throughout the study groups III and IV and orange discolouration of the fur in group IV females from day 7 on.

Signs of regenerative macrocytic anaemia in group IV males and females were seen. Increase of GOT activity in group IV (males and females) and group III (males) was registered. Increase of blood urea nitrogen was observed in group IV females as well as an increase of urinary proteins in group IV males and females. Renal cells were seen in the urine in males of group II.

Increased relative liver and kidney weights in group IV males and females were recorded. Kidneys were pale or mottled at macroscopic examination and showed renal tubular lesions at histopathological examination in males of group III and IV. Increased vacuolisation of the urothelium of the bladder in males and females in group II and III was present.

Conclusion

None of the three tested doses could be considered as NOAEL.

Ref.: 36 (Subm. IV)

30 days repeated dose oral toxicity, study 2

Guideline:	OECD 407 (1981)
Species/strain:	rat, Sprague Dawley Crl:CD(SD)BR (VAF plus)
Group size:	10 males and 10 females per group
Test substance:	orthoaminophenol
Batch:	TOES 189001
Purity:	/
Vehicle:	0.5% aqueous carboxymethylcellulose
Dose volume	10 ml/kg bw
Dose levels:	0, 2, 5 and 15 mg/kg bw/d
Route:	oral gavage
Administration:	daily for 28 days
GLP:	in compliance
Study period:	5/1989 to 6/1989

The test substance (suspended in 0.5% w/v aqueous carboxymethylcellulose) was administered daily by gavage for 28 consecutive days to groups of 20 Sprague-Dawley rats (strain crl: CD [SD] BR) (10/sex) at doses 0 (group I), 2 (group II), 5 (group III) and 15 (group IV) mg/kg bw/d. The age of the animals at the start of the study was approximately 4 – 5 weeks and the body weight range was 141 – 188 g for males and 111 - 157 g for females. Food and water was provided *ad libitum*.

Clinical observation and mortality was checked daily. Ophthalmoscopic examination was performed before and during week four. Body weights, food and water consumption were recorded weekly.

Haematology, blood biochemistry and urinalysis was investigated during week four. Necropsy, main organ weights, histopathological examination were done at sacrifice.

Results

Reduced bodyweight gains were recorded in males and females of group IV and in females of group II and III. An increased plasma glucose level was recorded in males group IV. Increases in absolute (not significant) and relative (significant) thyroid weights were recorded in females group IV

Conclusions

The reduced bodyweight gains are not considered to be related to treatment as there is no dose relationship. The thyroid weight changes are also considered by the study authors of questionable toxicological importance as no histopathological evidence was related.

Ref.: 37 (Subm. IV)

Comment

Due to the thyroid weight changes a NOAEL of 5 mg/kg bw/d is set.

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

3-Month oral toxicity in rats

1-Hydroxy-2-aminobenzene was administered orally to 20 Sprague-Dawley rats (10 males and 10 females) at a dosage of 50 mg/kg bw/d as a 1% solution in propylene glycol during 3 months. A group of 20 animals receiving 1 ml of a 1% propylene glycol solution per 100 g body weight served as a control. No mortality was observed. Body weight gain, haematological parameters, blood biochemistry and urine examination revealed no difference between the treated and the control group (exception alkaline phosphatase). Histopathological examination showed broncho-pulmonary injuries which did not permit to affirm the toxicity of the substance.

Ref.: 4 (Subm. I)

Comment

In this study, only a single dose level of 50 mg/kg bw/d was tested, therefore the results are inconclusive.

13-Week dermal toxicity study of a hair dye formulation

A hair dye formulation containing 0.3% o-aminophenol was mixed with an equal volume of 6% H₂O₂ and applied twice weekly for 13 weeks at a dosage of 1 ml per kg to 12 adult New Zealand white rabbits (six of each sex). A group of 12 rabbits was used as a control. No evidence of toxicity was seen and no effect on body weight and urinalysis was observed. Scattered statistically significant differences in clinical chemistry and haematological values were seen which were considered of no toxicological significance.

Ref.: 7 (Subm. V)

Comment

The value of this study is limited due to the low dose applied (topical application of an oxidation hair-dye mixture containing 0.15% of o-aminophenol after mixing with hydrogen peroxide).

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Gene mutation test in bacteria (Ames test) (1979)

o-Aminophenol was investigated for the induction of gene mutations in *Salmonella typhimurium* strains TA100 (with and without metabolic activation) and TA98 (with metabolic activation only) at concentrations of 25 to 500 µg/plate. Liver S9 fraction from Arachlor-1254-induced, male Fisher-344 rats was used as exogenous metabolic activation system. o-Aminophenol was checked by thin layer chromatography (TLC) for purity; putative impurities were separated using preparative TLC. Under the experimental conditions used, a dose dependent increase in the number of revertants was found in TA100 in the presence of metabolic activation.

Ref.: 19 (Subm. V)

The test is reported in a paper from the open literature in which several aminophenyl and nitrophenyl ethers, sulfides and disulfides were tested in the Ames test. Since the test has several deficiencies compared to the minimal current requirements for a genotoxicity test, it can only be used as supportive evidence.

Gene mutation test in bacteria (Ames test) (1976)

o-Aminophenol was investigated for the induction of gene mutations in *Salmonella typhimurium* strain TA98 with and without metabolic activation at concentrations of 15 to 150 µg/plate. Under the experimental conditions used, treatment with o-aminophenol did not result in an increase in the number of revertants without and with metabolic activation.

Ref.: 9 (Subm. I)

The test is reported in a paper from the open literature (abstract only, translated from Japanese) in which around 300 compounds were tested in the Ames test. Since the test has several deficiencies compared to the minimal current requirements for a genotoxicity test and the reporting is poor, the test has only limited value.

Gene mutation test in bacteria (Ames test) (1979)

o-Aminophenol, dissolved in DMSO, was investigated for the induction of gene mutations in *Salmonella typhimurium* strains TA98 and TA100 both without and with metabolic activation at concentrations of 0.5 to 2.0 µmol/plate. Liver S9 fraction from polychlorinated biphenyl (Kanechlor 500)-induced Wistar rats was used as exogenous metabolic activation system. The pre-incubation method was used with 30 minutes pre-incubation and 48 h incubation time both without and with S9-mix. The experiment was repeated 3 times using duplicates. Under the experimental conditions used, both without and with metabolic activation, treatment with o-aminophenol did not result in an increase of revertants in both strains.

Ref.: 20 (Subm. V)

The test is reported in a paper from the open literature in which several metabolites of 4-aminobenzene were tested in the Ames test. Since the test has several deficiencies compared to the minimal current requirements for a genotoxicity test, it can only be used as supportive evidence.

Gene mutation test in bacteria (Ames test) (1983)

o-Aminophenol was investigated for the induction of gene mutations in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 both without and with metabolic activation at concentrations from 10-10,000 µg/plate. Test concentrations were based on the level of toxicity in a pre-experiment with *Salmonella* strain TA100 up to a concentration of 10 mg/plate. Toxicity was evaluated on the basis of reduced viability on complete medium, of a reduction in the number of revertant colonies and/or a clearing of the bacterial background lawn. Liver S9 fraction from Aroclor 1254-induced male Sprague-Dawley rats and male Syrian hamsters was used as exogenous metabolic activation system. The pre-incubation method was used with 30 minutes pre-incubation and 48 h incubation time both without and with S9-mix. The experiments were performed at 3 different laboratories and at each site repeated twice using triplicates. Negative and positive controls were included.

Result

A dose dependent increase in the number of revertants was observed in strain TA100 without and with both rat and hamster metabolic activation. An increase in the number of revertants was not found in the other strains tested both without and with metabolic activation.

Conclusion

Under the experimental conditions used o-aminophenol was genotoxic (mutagenic) in this gene mutation test in bacteria.

Ref.: 17 (Subm. V)

The present test is reported in a paper from the open literature in which 250 chemicals were tested in the Ames test.

Gene mutation test in bacteria (Ames test) (1983)

o-Aminophenol, dissolved in DMSO, was investigated for the induction of gene mutations in *Salmonella typhimurium* (strains G46, TA1535, TA100, C3076, TA1537, D3052, TA1538 and TA98) and *Escherichia coli* (strains WP2 and WP2uvrA-). Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. The maximum test concentration was 1000 µg/ml of agar. Negative and positive controls were included. o-Aminophenol treatment induced an increase in the number of revertants in TA100 but was negative in the other strains. Under the experimental conditions used o-aminophenol was mutagenic in this gene mutation test in bacteria.

Ref.: 18 (Subm. V)

The present test is reported in a paper from the open literature in which 45 monosubstituted aniline compounds were tested in the Ames test. Since the test has several deficiencies compared to the minimal current requirements for a genotoxicity test, no result data are given and the reporting is poor, the test has only limited value.

Gene mutation test in bacteria (Ames test) (1984)

o-Aminophenol was investigated for the induction of gene mutations in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA 1538 both without and with S9-mix using duplicate or triplicate plates. Test concentrations were not mentioned but various concentrations were used started from its solubility or toxicity limit. Liver S9 fraction from Aroclor 1254-induced Sprague-Dawley rats was used as exogenous metabolic activation system. The plate incorporation method was used. Negative and positive controls were included. Both without and with metabolic activation o-aminophenol did not induce an increase in the number of revertants in any strain tested. Under the experimental conditions used o-aminophenol was not mutagenic in this gene mutation test in bacteria

Ref.: 22 (Subm. V)

The present test is reported in a paper from the open literature in which 135 chemical compounds of different classes were tested in the Ames test. Since the test has several deficiencies compared to the minimal current requirements for a genotoxicity test, the test concentration was not given, the result was reported as "negative" without data and the reporting was poor, the test has only limited value.

Gene mutation test in bacteria (Ames test) (1984)

o-Aminophenol was investigated for the induction of gene mutations in *Salmonella typhimurium* strains TA97 and TA102 both without and with S9-mix using duplicate or triplicate plates. Test concentrations not mentioned but various concentrations were used started from its solubility or toxicity limit. Liver S9 fraction from Aroclor 1254-induced Sprague-Dawley rats was used as exogenous metabolic activation system. The plate incorporation method was used. Negative and positive controls were included. o-Aminophenol exposure did not induce an increase in the number of revertants in strain TA102 whereas the result in TA97 was equivocal.

Ref.: 21 (Subm. V)

The present test is reported in a paper from the open literature in which 30 chemical compounds of different classes were tested in the Ames test. Since the test has several deficiencies compared to the minimal current requirements for a genotoxicity test, the test concentration was not given, the result was reported as "negative" or " \pm " without data and the reporting was poor, the test has only limited value.

Gene mutation test in yeast (1983)

o-Aminophenol was investigated for the induction of forward gene mutations in *Schizosaccharomyces pombe* strain ade6-60 rad10-198 h⁻ (P1 strain). Liver S9 fraction from phenobarbital/ β -naphthoflavone-induced rats was used as exogenous metabolic activation system. o-Aminophenol dissolved in culture medium was tested at 5, 20, 30 and 50 mM without metabolic activation and at 5, 20 and 30 mM with metabolic activation for 16 hrs. The positive controls were 2 mM cyclophosphamide (with activation) and 3.2 mM epichlorohydrine (without activation). Treatment with o-aminophenol did not result in an increase of mutant colonies both without and with metabolic activation. Under the experimental conditions used o-aminophenol was not mutagenic in this gene mutation test in yeast

Ref.: 15 (Subm. II)

The description of the test appears to be the abstract of a report. Since relevant issues of the protocol and raw data are included, the test is acceptable.

DNA repair test in bacteria (1984)

o-Aminophenol was investigated in a DNA repair test using 3 isogenic *Escherichia coli* strains: WP2 (wild-type, repair-proficient), WP67 and CM871. Liver S9 fraction from Aroclor 1254-induced Sprague-Dawley rats was used as exogenous metabolic activation system. Test concentrations were not mentioned but various concentrations were used started from its solubility or toxicity limit. Negative and positive controls were included. O-Aminophenol appeared slightly active demonstrated by the DNA repair potency of 0.005 Δ MICs (minimal inhibitory concentration) per nmole.

Ref.: 22 (Subm. V)

The present test is reported in a paper from the open literature in which 135 chemical compounds of different classes were tested in the DNA repair test. The test is of limited value.

Recombination assay in *Bacillus subtilis* (1979)

o-Aminophenol was investigated in a recombination assay using *Bacillus subtilis* strains H45 and H17. o-Aminophenol was tested in a concentration of 10^{-2} M in an aqueous solution of 50 µl. Under the experimental conditions a low DNA damaging activity was found.

Ref.: 32 (Subm. V)

The test is reported in a paper on genotoxic activity of reactions systems containing nitrite and butylated hydroxyanisole, tryptophan, or cysteine. o-Aminophenol was used as an additional compound. Due to its poor description and reporting the test is of limited value.

Test on Inhibition of Replicative DNA Synthesis (1991)

o-Aminophenol was investigated in a test on inhibition of replicative DNA synthesis in V79 Chinese hamster cells. V79 cells were grown in [^{14}C]TdR (0.01 µCi/ml) medium for 24 h and then exposed to o-aminophenol for 30 minutes. The concentration of o-aminophenol used, although not given, was based on preliminary studies over a wide concentration range. After exposure the incorporation of [^3H]TdR (4 µCi/ml) during a 10 minutes pulse labelling period was measured. The incorporation of [^{14}C]TdR and [^3H]TdR into DNA was determined by liquid scintillation counting. The ration between [^{14}C]TdR and [^3H]TdR for exposed cells are expressed as percent of the control cells. o-Aminophenol exhibited a high activity inhibition rate of [^3H]TdR incorporation and thus a strong inhibition of replicative DNA synthesis.

Ref.: 26 (Subm. V)

The test is reported in a paper on inhibition of replicative DNA synthesis of paracetamol analogues. The test concentration was not given.

***In vitro* unscheduled DNA synthesis test (1983)**

o-Aminophenol was investigated for the induction of unscheduled DNA synthesis (UDS) in primary hepatocytes isolated from Fischer 344 rats. Eight concentrations over a range of 0.5 -1000 nmol/ml were tested. The experiment was repeated with hepatocytes from a different rat. o-Aminophenol treatment of primary hepatocytes isolated from rats did not result in an increase in unscheduled DNA synthesis, measured as nuclear silver grain counts, in primary hepatocytes.

Ref.: 18 (Subm. V)

The present test is reported in a paper from the open literature in which 45 monosubstituted aniline compounds were tested in the UDS test. The test has several deficiencies compared to the minimal current requirements for a genotoxicity test, the test protocol, results and reporting are poor. Therefore the test has only limited value.

***In vitro* Sister Chromatid Exchange (SCE) test (1983)**

o-Aminophenol was investigated for the induction of sister chromatid exchanges in CHO cells. The toxicity of o-aminophenol was determined in a pretest. After exposure to o-aminophenol for 1 h at concentrations of 0.4, 0.8 and 1.6 mg/ml, fixation was carried out after further incubation for 24 h in 5-BrdUrd containing medium. Colcemid (0.5 ml/10 ml, 0.00028%) was added to the culture medium the last 2 h of incubation to block cells at metaphase of mitosis.

Exposure to o-aminophenol did not increase the number of sister chromatid exchanges per cell.

Under the experimental conditions used o-aminophenol was not genotoxic in this SCE test in CHO cells.

Ref.: 12 (Subm. I)

Since the test has several deficiencies compared to the minimal current requirements for a genotoxicity test, it has only limited value.

In vitro Sister Chromatid Exchange (SCE) test (1981)

o-Aminophenol was investigated for the induction of sister chromatid exchanges in male human fibroblasts. o-Aminophenol was purified by recrystallization twice in absolute ethanol. The fibroblasts were treated for 2 h with 0.01, 0.03, 0.1 and 0.3 mM o-aminophenol. Thereafter the medium was replaced and the cells were incubated for 48 h in medium containing 10 µM BrdU. Then colcemid (0.1 µg/ml) was added to the culture medium to block cells at metaphase of mitosis and the cells were harvested 4.5 h later. For cell cycle analysis, and thus an indication of putative proliferation inhibition, 200 metaphases per culture were examined as being in the first, second or third replication cycle. The mitotic index was derived from 1000 nuclei per culture to investigate cytotoxicity. o-Aminophenol induced a dose related increase in the number of SCEs per metaphase. At the highest dose tested (0.3 mM) o-aminophenol was found cytotoxic whereas also an inhibition of the cell cycle was observed

Under the experimental conditions used o-aminophenol was genotoxic in this SCE test in male human fibroblasts.

Ref.: 28 (Subm. V)

The present test is reported in a paper from the open literature in which aniline and its metabolites were tested in the sister chromatid exchange test in male human fibroblasts.

In vitro Sister Chromatid Exchange (SCE) test (1982)

o-Aminophenol was investigated for the induction of sister chromatid exchanges in human lymphocytes. Heparinized venous blood from 4 healthy volunteers was cultured in medium with phytohaemagglutinin for stimulation of the lymphocytes for 72 h. BrdU (83 µmol) for differential staining was added for the total time of cell culture. o-Aminophenol in final concentrations of 1.6, 3.3 and 6.6 µg/ml was also present for the total period of 72 h. Two hours before the end of cell culture colcemid (10 µg/ml) was added to the culture medium to block cells at metaphase of mitosis.

o-Aminophenol induced an dose-dependent increase in the number of SCEs/cell in all cultures of the 4 volunteers.

Under the experimental conditions used o-aminophenol was genotoxic in this SCE test in human lymphocytes.

Ref.: 31 (Subm. V)

The present test is reported in a paper from the open literature in which m-, p- and o-aminophenol were tested in the sister chromatid exchange test in human lymphocytes.

In vitro Sister Chromatid Exchange (SCE) test (1981)

o-Aminophenol was investigated for the induction of sister chromatid exchanges in Chinese hamster cells (V79) at concentrations of 0.5 - 2.0 × 10⁻⁵ M. o-Aminophenol was mutagenic in this sister chromatid exchange test.

Ref.: 29 (Subm. V)

The test is only reported as an abstract without any details. As such this study is considered inadequate and not suitable for evaluation.

***In vitro* chromosome aberration test (1982)**

o-Aminophenol has been investigated for the induction of chromosomal aberrations in CHO cells. The toxicity of o-aminophenol was determined in a pretest. CHO cells were exposed at concentrations 0.4, 0.8 and 1.6 mg/ml (1 h treatment and fixation times 6, 12 or 16 h after start of treatment) and 0.0025, 0.005 and 0.01 mg/ml (6 h, 12 h and 16 h treatment with fixation immediately after the end of treatment). Colcemid (0.5 ml/10 ml, 0.00028%) was added to the culture medium the last 2 h of incubation to block cells at metaphase of mitosis.

A biologically relevant dose dependent increase in the number of cells with chromosome aberrations was not found under any treatment condition. Under the experimental conditions used o-aminophenol was not genotoxic (clastogenic) in this chromosome aberration test in CHO cells.

Ref.: 16 (Subm. II)

Since the test has several deficiencies compared to the minimal current requirements for a genotoxicity test, it has only limited value.

Table: Summary of the results of the *in vitro* genotoxicity tests with o-aminophenol

Test	Doses	S9-mix	Result	Comment	Date	Ref.
GM test in bacteria	0-500 µg/plate	- and +	positive, TA100 only	paper, supportive	1979	8/I 19/V
GM test in bacteria	0-150 µg/plate	- and +	negative	paper, limited value	1976	9/I
GM test in bacteria	0-2.0 µmol/plate	- and +	negative	paper, supportive	1979	10/I 20/V
GM test in bacteria	0-10000 µg/plate	- and +	positive, TA100 only	paper	1983	14/II 17/V
GM test in bacteria	0-500 µg/ml agar	- and +	positive, TA100 only	paper, limited value	1983	18/V
GM test in bacteria	doses below solubility of toxicity limit	- and +	negative	paper, limited value	1984	33/III 22/V
GM test in bacteria	doses below solubility of toxicity limit	- and +	negative TA102, questionable TA97	paper, limited value	1984	21/V
GM test in yeast	0-50 mM -S9 0-30 mM +S9	- and +	negative	abstract, acceptable	1983	15/V
DNA repair in bacteria	not mentioned	- and +	slightly active	paper, limited value	1984	33/III 22/V
Rec assay in <i>Bacillus subtilis</i>	10 ⁻² M	-	low damaging activity	paper, limited value	1979	32/V
Inhibition Replicative DNA synthesis	not mentioned	-	strong inhibition	paper	1991	26/V
UDS test	0-1000 nmol/ml	-	negative	paper, limited value	1983	18/V
SCE test in CHO cells	0-1.6 mg/ml	-	negative	limited value	1983	12/I
SCE test in human fibroblasts	0-0.3 mM	-	positive	paper	1981	17/II 28/V
SCE test in human lymphocytes	0-6.6 µg/ml	-	positive	paper	1982	18/II 27/V 31/V
SCE test in V79 cells	0-2x10 ⁻⁵ M	-	positive	paper, inadequate, not suitable	1981	19/II 29/V

Test	Doses	S9-mix	Result	Comment	Date	Ref.
CA test in CHO cells	0-1,6 mg/ml (1h exposure) 0-0.01 mg/ml (long exposures)	-	negative	limited value	1982	16/II

Abbreviations

GM: gene mutation test; Rec: recombination test; UDS: unscheduled DNA synthesis test; SCE: sister chromatid exchange test; CA: chromosome aberration test.

3.3.6.2 Mutagenicity / Genotoxicity *in vivo*

In vivo Unscheduled DNA Synthesis (UDS) test in rat

Guideline: /
 Species/strain: Wistar rats
 Group size: 5 male rats/group
 Test substance: o-Aminophenol
 Batch: /
 Purity: 99.7%
 Dose level: 400 and 2000 mg/kg bw
 Route: oral gavage, once
 Vehicle: 0.01 M HCl
 Sacrifice times: 4 h and 12 h after dosing
 GLP: In compliance
 Date: 24 November 1988 - 8 November 1989

o-Aminophenol was investigated for the induction of unscheduled DNA synthesis (UDS) in hepatocytes of rats. Test concentrations were based on a toxicity range-finder study with doses up to the highest recommended dose of 2000 mg/kg bw. In the main experiment male rats were treated with 400 and 2000 mg/kg bw. To enable a manageable number of animals to be processed at a time, dosage was carried out on 2 separate days treating 3 animals from each dose group on each occasion. Hepatocytes for UDS analysis were collected at 4 h and 12 h after administration of o-Aminophenol by perfusion with collagenase. After plating the cells were incubated for 4 h with 10 µCi/ml ³H-thymidine. Evaluation of autoradiography was done after 14-15 days exposure.

UDS was measured by determining nuclear grain count (the number of nuclear grains minus the number of grains in a adjacent area of the cytoplasma of the same size with the apparently highest number of grains and the mean and percentage cells in repair (cell with a net grain count larger than 5). Unscheduled synthesis was determined in at least 50 randomly selected hepatocytes from at least 2 replicate cultures per rat. Negative and positive controls were included.

Results

In the absence of toxicity in the range-finder study, the top dose of the main experiment was chosen to be 2000 mg/kg bw. In the main test one of the animals treated with 2000 mg/kg bw for the 12 h group died prior to perfusion.

Both for the 4 h and the 12 time-points after treatment an increased mean net nuclear grain count in the hepatocytes as compared to the untreated controls was not observed. Also the number of in repair never reached the necessary criterion of 10% above the percentage found for the untreated control.

Conclusion

Under the experimental conditions used o-Aminophenol did not induce unscheduled DNA synthesis and, consequently, is not genotoxic in rats in this *in vivo* UDS test.

Ref.: 38 (Subm. IV)

Comment

The test was performed before the OECD guideline for the *in vivo* UDS test was developed. Batch number, viability of the isolated hepatocytes and indications of biological availability of o-aminophenol were not reported.

Sister Chromatid Exchange (SCE) test in root cells of *Vicia faba* (1990)

o-Aminophenol was investigated for the induction of sister chromatid exchanges in root cells of *Vicia faba*. Seedlings with main roots of 3-6 cm in length were used for the experiment. Three day old seedlings were incubated in a BrdU solution (100 µM) for 17 h in the dark. The seedlings were exposed to 10^{-4} , 5×10^{-4} and 10^{-3} M o-aminophenol in distilled water for 3 h in the dark. After rinsing they were incubated in an aerated thymidine solution (100 µM dThd) in tap water for 25 h in the dark. The seedlings were then immersed in 0.05% colchicine solution for 2 h and the root tips were fixed. At least 50 metacentric chromosomes and 250 subtelocentric chromosomes were analysed per treatment. The SCEs occurring at the secondary constrictions of metacentric chromosomes and at the centromeres of both metacentric and subtelocentric chromosomes were counted. Positive and negative controls were included. A dose dependent increase in the number of chromosomes with SCEs was observed after treatment with o-aminophenol at both the metacentric and subtelocentric chromosomes.

Under the experimental conditions used o-aminophenol was genotoxic in the sister chromatid exchange (SCE) test in root cells of *Vicia faba*.

Ref.: 30 (Subm. V)

The present test is reported in a paper from the open literature in which aniline and its C-hydroxylated metabolites were tested in the sister chromatid exchange test in root cells of *Vicia faba*.

Sister Chromatid Exchange (SCE) test (1982)

o-Aminophenol was investigated for the induction of sister chromatid exchanges in male Chinese hamster bone marrow. The hamsters were treated *i.p.* with 5 mg o-aminophenol/kg bw 2 h after the subcutaneous implantation of a BrdU tablet. Two h after treatment the hamsters were injected with colchicine (concentration unknown) and again 2 h later the animals were killed and bone marrow cells collected.

Treatment with o-aminophenol did not result in an increase in the number of SCEs/cell. Under the experimental conditions used o-aminophenol was not genotoxic in this *in vivo* sister chromatid exchange (SCE) test in Chinese hamsters.

Ref.: 31 (Subm V)

The present test is reported in a paper from the open literature in which m-, p- and o-aminophenol were tested in the sister chromatid exchange test in human lymphocytes. Since the test has several deficiencies compared to the minimal current requirements for a genotoxicity test, it has only limited value.

***In vivo* micronucleus test (1981)**

o-Aminophenol was investigated for the induction of micronuclei in bone marrow cells of male Chinese hamster at concentrations of 0.5 - 2.0 mM/kg. o-Aminophenol was mutagenic in this micronucleus test.

Ref.: 29 (Subm. V)

The test is only reported as an abstract without any details. As such this study is considered inadequate and not suitable for evaluation.

In vivo micronucleus test (1983)

o-Aminophenol was investigated for the induction of micronuclei in bone marrow cells of young adult Swiss mice. 40 mice received a single *i.p.* injection with 125 mg o-aminophenol/kg bw in tricaprylin. Bone marrow was collected 12, 30, 72 and 96 h after treatment. A positive (mitomycin C, 2 mg/kg) and negative control (vehicle) were included. At a higher dose (200 mg/kg bw) a number of clinical disorders were observed which did not allow further investigation of these animals. However, these clinical signs demonstrate systemic availability of o-aminophenol. At 125 mg/kg bw no mortality was observed. Treatment with o-aminophenol did not result in an increase of bone marrow cells with micronuclei as compared to the negative controls. Under the experimental conditions used o-aminophenol was not mutagenic (clastogenic or aneuploidogenic) in this *in vivo* micronucleus test in Swiss mice.

Ref.: 20 (Subm. II)

Although the test has some deficiencies compared to the minimal current requirements for a genotoxicity test, the test is acceptable.

Chromosome aberration test in root cells of Vicia faba (1990)

o-Aminophenol was investigated for the induction of chromosome aberrations in root cells of *Vicia faba*. Seedlings with main roots of 3-6 cm in length were used for the experiment. Four day old seedlings were used. The seedlings were exposed to 10^{-3} , 2×10^{-3} and 4×10^{-3} M o-aminophenol in distilled water for 3 h in the dark. After treatment the seedlings were incubated in aerated tap water for 24 h in the dark. The seedlings were then immersed in 0.05% colchicine solution for 2 h and the root tips were fixed. Per treatment 100 metaphases were analysed for chromosome aberrations at the secondary constrictions of metacentric chromosomes. Positive and negative controls were included. A more or less dose dependent increase in abnormal cells was observed indicating sufficient exposure. A dose dependent increase in the number of chromosome aberrations per cell was observed after treatment with o-aminophenol.

Under the experimental conditions used o-aminophenol was mutagenic (clastogenic) in the chromosome aberration test in root cells of *Vicia faba*.

Ref.: 30 (Subm. V)

The present test is reported in a paper from the open literature in which aniline and its C-hydroxylated metabolites were tested in the chromosome aberration test in root cells of *Vicia faba*.

In vivo chromosome aberration test (1983)

o-Aminophenol was investigated for the induction of chromosome aberrations in bone marrow cells of Swiss Albino CD-1 mice. Mice (5 male and 5 female mice/treatment) were exposed *i.p.* to single doses of 0, 16.6, 50 and 150 mg/kg bw o-aminophenol in 0.5% carboxymethylcellulose. Three hours before sacrifice the mice were injected with 1mM colchicine. Bone marrow cells were collected 24 h after dosing. Negative (solvent) and positive (mitomycin C) controls were included. No significant increase in the number of cells with chromosome aberrations was found in treated mice as compared to untreated controls. Under the experimental conditions used o-aminophenol was not mutagenic (clastogenic) in this *in vivo* chromosome aberration test in Swiss Albino CD-1 mice

Ref.: 21 (Subm. II)

The test has some deficiencies compared to the minimal current requirements for a genotoxicity test. Although there are no indications that o-aminophenol did reach the target tissue, systemic availability of o-aminophenol following *i.p.* exposure can be assumed and the test is considered acceptable.

Sex-linked recessive lethal test with *Drosophila melanogaster* (1982)

o-Aminophenol was tested for mutagenicity in *Drosophila melanogaster* (Berlin K). Gene mutations, small deletions as well as certain types of chromosome aberrations can lead to the phenotype of a recessive lethal.

The compound was given orally by feeding adult males on glass filters placed on the test solution. Two concentrations, 25 and 50 mM, were used. Treated males, 4-5 days old, were mated individually with 3 fresh virgin Basc females for three consecutive periods of 2-3 days. Brood 1 mainly represents treated sperm, brood 2 treated spermatids and brood 3 spermatocytes (and spermatids). After 13-15 days the F1 females were mated and the progeny F2 was inspected for the occurrence of sex-linked recessive lethals. F2 cultures (progeny) with 2 or more wild type males were considered as non-lethals. The experiment was repeated twice.

No sterility and toxicity effects were revealed. There was no significant increase in the mutation frequency over controls. A small increase in the mutation frequency was observed with 50 mM o-aminophenol in the second experiment but this small effect was not persistent in all broods.

Under the experimental conditions used o-aminophenol was not mutagenic in this sex-linked recessive lethal test with *Drosophila melanogaster*.

Ref.: 11 (Subm. I)

Since the test has several deficiencies compared to the minimal current requirements for a genotoxicity test, it has only limited value.

Sex-linked recessive lethal test with *Drosophila melanogaster* (1985)

o-Aminophenol was tested for mutagenicity in *Drosophila melanogaster*. The compound was given by feeding (200 ppm, 3 d) and injection (100 ppm) and not found mutagenic under the study conditions.

Ref.: 34 (Subm. III)

Sperm-head abnormality test (1981)

o-Aminophenol was investigated for the induction of abnormal sperm-head in the testes of male Chinese hamster at concentrations of 0.5 - 2.0 mM/kg. o-Aminophenol was mutagenic in this Sperm-head abnormality test.

Ref.: 29 (Subm. V)

The test is only reported as an abstract without any details. As such this study is considered inadequate and not suitable for evaluation.

Table: Summary of the results of the *in vivo* genotoxicity tests with o-aminophenol

Test	Species	Doses	Route	Exposure	Result	Comment	Date	Ref.
UDS test	rat	0-2000 mg/kg bw	oral gavage	unknown	negative	report	1988	38/IV
SCE test	<i>Vicia faba</i>	0-10 ⁻³ M	water, feed??	+	positive	paper, not a common test	1990	30/V

Test	Species	Doses	Route	Exposure	Result	Comment	Date	Ref.
SCE test	chinese hamster	5 mg/kg bw	<i>i.p.</i>	unknown	negative	paper, limited value	1982	18/II 27/V 31/V
MN test	Chinese hamster	0-2 mM/kg	<i>i.p.</i>	+	positive	paper, inadequate, not suitable	1981	19/II 29/V
MN test	mouse	125 mg/kg bw	<i>i.p.</i>	+	negative		1983	20/II
CA test	<i>Vicia faba</i>	0-4 x 10 ⁻³ M	water, feed??	+	positive	paper, not a common test	1990	30/V
CA test	mouse	0-150 mg/kg bw	<i>i.p.</i>	unknown	negative		1983	21/II
SLRL test	<i>Drosophila melanogaster</i>	0-50 mM	oral/feed	unknown	negative	limited value, not a common test	1982	11/I
SLRL test	<i>Drosophila melanogaster</i>	200 ppm	feed	unknown	negative	limited value, not a common test, not available in CIRCA	1985	11/I
Sperm-head abnormality test	Chinese hamster	0-2 mM/kg	<i>i.p.</i>	+	positive	paper, inadequate, not suitable	1981	19/II 29/V

Abbreviations

UDS: unscheduled DNA synthesis test; SCE: sister chromatid exchange test; MN: micronucleus test; CA: chromosome aberration test; SLRL: sex-linked recessive lethal test.

3.3.7. Carcinogenicity

12 male Sprague-Dawley rats received a diet containing 0.117% o-aminophenol hydrochloride for 9 months. No tumours were observed after autopsy.

Ref.: 6 (Subm. I)

Cholesterol pellets containing 12.5% o-aminophenol were implanted in the bladder of 37 mice. The cholesterol control pellets caused 5 carcinomas in 55 mice (9.0%) while the pellets containing o-aminophenol caused 2 carcinomas (5.4%).

Ref.: 8 (Subm. V)

Comment

The two studies above were published in 1948 and 1958, respectively. No conclusions can be drawn from the two studies with regard to the carcinogenicity of o-aminophenol. Although the potent carcinogen 4-dimethylaminoazobenzene induced tumours already after 4 months, the sensitivity of the study is considered low. The second study is not considered relevant due to the exposure route.

Mice

Guideline:	/
Species/strain:	Swiss-Webster mice
Group size:	50 animals per sex
Test substance:	One oxidative hair dye formulation (7405) containing 0.3% o-aminophenol
Batch:	/
Purity:	/
Dose level:	0.05 ml of a solution containing an equal volume of 6% hydrogen peroxide and the hair dye formulation containing 0.3% o-aminophenol
Route:	Topical, 1 application weekly
Exposure period:	21 months
GLP:	not in compliance

Study period: Before 1980

The experiment involved 12 treatment groups (9 oxidative hair dye formulations + 3 semi permanent hair dye formulations) and 3 negative control groups.

The oxidative hair dye formulations were mixed with an equal volume of 6% hydrogen peroxide just before use and applied within 15 min after mixing.

The dye was applied topically to a 1 cm² area on a clipped (24 hours prior to application) site in the interscapular region. Mice received a dose of 0.05 ml topically without occlusion once weekly from 8 – 10 weeks of age for 21 months. The animals were observed daily for mortality and signs of toxicity, and were weighed monthly. A continuous weekly record was maintained for any skin lesions noted. After 7 months of treatment, 10 males and 10 females per group were necropsied and the study was terminated after 21 months. Skin and internal organs were evaluated histologically.

There were no overt sign of systemic toxicity in any of the dye-treated groups. Eight males and 9 females survived 21 months compared to 9 and 12 males and 11 and 14 females in the two control groups. There were no significant differences in absolute or relative liver or kidney weights in groups of 10 male and 10 female mice necropsied after 7 months. Average body weights were comparable in all groups throughout the study. There were no statistically significant differences in the distribution of tumours among treated and control groups.

The authors concluded that no evidence of carcinogenic activity was seen.

Ref.: 10 (Subm. V)

Comment

One study with o-aminophenol in an oxidative hair dye formulation (7405) involving topical application of mice has been submitted. The concentration of o-aminophenol was 0.15% (0.3% prior to mixing with hydrogen peroxide) (the maximum concentration on the human scalp is 0.6%). A number of different hair dye formulations were tested in the same studies. Although some of the formulations contained 2,4-diaminoanisol (classified as carcinogen category 2 in EU), none of the formulations induced tumours in the mice. Thus, no conclusion with regard to carcinogenicity can be made from the studies.

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

Multi-generation reproductive toxicity study of a hair dye formulation (dermal application)

A hair dye formulation containing 0.3% o-aminophenol (formula 7405) was mixed with an equal volume of 6% H₂O₂ (final concentration 0.15% o-aminophenol) and applied twice weekly through growth, mating (day 100), gestation and lactation to the weaning litters of the respective generation of Charles River rats. The dosage was 0.5 ml per animal. The group size was 40 males and 40 females. The controls were only clipped and not further treated.

F₀ parents were mated twice and selected offspring of the second mating became F₁ parents. F₁ parents were mated twice and selected offspring of the second mating became F₂ parents. They mated 3 times thus producing F_{3a}, F_{3b} and F_{3c} litters. No substance related changes were observed in parental rats and pups. With the exception of dermal reactions no toxic signs were noted. No changes in body weight gain and food consumption were noted. The mean haematological values did not differ significantly over the first months of the study. However, after 24 months, the mean values for total erythrocytes and haemoglobin in male animals were lower than those in 1 of the 3 control groups. The biological significance is doubtful since only 5 animals of the groups were investigated. The fertility,

gestation and viability indices were comparable as compared to the controls. It has to be noted that sialadenitis followed by an increased incidence of respiratory congestion was observed in some rats of each group.

Ref.: 9, 16 (Subm. V)

3.3.8.2. Teratogenicity

Study in hens

The test substance o-aminophenol suspended in egg albumen was injected in fertile hen's eggs. The test substance induced a dose-related mortality rate in chicken embryos; the LD₅₀ was 0.92 mg/egg on day 1 and 0.75 mg/egg on day 5. In general, tendencies in retardation were observed with increased dosage. The test substance had no teratogenic potential in this test.

Ref.: 22 (Subm. I)

Study in hamsters

The teratogenicity of o-aminophenol was investigated in Syrian Golden hamsters, 7 dams per dose group were used. The test substance was dissolved in acidified saline (0.5 mM HCl) and injected *i.p.* on day 8 of gestation at doses of 100, 150 and 250 mg/kg bw. The dams were sacrificed on day 13. The foetuses were examined for external gross malformations and 3 foetuses from every litter demonstrating at least one malformation were examined for soft tissue anomalies. The test substance in the absence of maternal toxicity showed embryo-lethal and teratogenic response with encephalocele, limb, tail and eye defects even at the lowest dose.

Ref.: 15 (Subm. V)

Study in rats

Guideline:	/
Species/strain:	Sprague-Dawley OFA
Group size:	20 females (mates), except group II 18 females
Test substance:	o-aminophenol
Batch:	712.0258
Purity:	99.7%
Vehicle:	hydrogel of 0.2% polysorbate 80 and 0.2% sodiumcarboxymethylcellulose
Dose levels:	0, 20, 70 and 250 mg/kg bw/d
Dose volume:	5 ml/kg bw
Route:	gavage
Administration:	Days 6-15 of pregnancy
GLP:	in compliance
Study period:	09/01 to 03/02 1989

The test substance was given daily from day 6 – 15 of gestation by oral administration of doses of 0 (group I), 20 (group II), 70 (group III) and 250 (group IV) mg/kg bw to pregnant Sprague-Dawley (OFA) rats (11 – 12 weeks old and with a mean bodyweight of 270 g on day 0 of gestation).

Clinical signs and mortality were checked daily. Body weights were measured at the beginning of the study and at day 6, 7, 10, 15, 21 and food consumption on days 6, 15, 21.

At sacrifice, hysterectomy (number of live, dead or resorbed foetuses in each uterine horn; position of the foetuses in the uterus; number of corpora lutea, number of early and late resorptions, number of implantation sites) after gross examination of all animals and of

placentas was performed. Weight, sex and gross external examination of each foetus, skeletal (2/3 of each litter) or visceral (the remaining 1/3) anomalies were recorded.

Results

Brown discolouration of urines was observed in all treated animals 24 hours after the first administration of the test substance. Reduced body weight gain (from days 7 to 10) and food consumption (from days 6 to 15) were observed in group IV. Decreased mean weight of the foetuses in group IV was observed, probably related to maternal toxicity.

2 foetuses in one litter animal of group IV shown bilateral anophthalmies associated with micrognathia and a dilatation of the lateral cerebral ventricles in one of these foetuses. These anomalies are likely related to maternal toxicity. Slight ossification retardation occurred in group IV.

Conclusions

The NOAEL for maternal toxicity and for foetal development (embryotoxicity) was 70 mg/kg bw/d.

Ref.: 39 (Subm. I)

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

Several publications dealing with effects of o-aminophenol on haemoglobin and mitochondria were provided in submission II. These studies are not considered relevant for the safety assessment.

Ref.: 27, 28, 29, 30, 31, 32, 35 (Subm. II)

3.3.13. Safety evaluation (including calculation of the MoS)

Not applicable

3.3.14. Discussion

Physico-chemical properties

The data provided for physicochemical properties of o-aminophenol are insufficient and inadequate. This especially applies to purity, homogeneity and stability of o-aminophenol and its formulations.

Irritation, sensitisation

Neat o-aminophenol as well as a 1% solution in propylene glycol applied to the clipped skin of rabbits was non-irritant. A 1% solution in propylene glycol was found slightly irritant to the rabbit eye one hour after instillation without rinsing. The effect was reversible within 24 hours.

No skin sensitizing reaction was observed in a study with guinea-pigs. However, a study with two different protocols showed no sensitization with one protocol and a 20% positive response with the other protocol. These studies are not according to modern standards and not in compliance with GLP.

In a compilation of LLNA data, 2-aminophenol was found to be a sensitisier. No study details are available.

Dermal absorption

Percutaneous absorption of o-aminophenol from a hair dye formulation was studied in rats. The study was not acceptable since it was performed in the presence of human hair and/or m-aminophenol. Furthermore, the concentration used (0.32%) was lower than the one applied for (0.6%).

Three percutaneous absorption studies were performed *in vitro* using human skin. The methodology did not follow a guideline and was not in compliance with GLP. Only epidermis was used, not the full skin. The control of the integrity was not correctly done. No data were supplied on the amount present in stratum corneum, epidermis and dermis. The variability is very high. Because of these many shortcomings, the studies were not considered suitable for safety assessment.

General toxicity

The oral LD₅₀ of o-aminophenol in rat and mice was above 1,000 mg/kg bw.

Two subacute oral toxicity studies in rats were performed with o-aminophenol. In study 1 with treatment over 30 days at daily doses 0, 20, 80, 320 mg/kg bw, none of the three tested doses could be considered as a NOAEL. In study 2 with doses of 0, 2, 5 and 15 mg/kg bw/d, a NOAEL of 5 mg/kg bw/d was derived based on the thyroid weight changes observed.

A three-month oral toxicity study was performed in rats. As only a single dose level of 50 mg/kg bw/d was tested, the results are inconclusive. The value of the 13-week dermal toxicity study of a hair dye formulation in rabbits is limited due to the low dose applied (topical application of an oxidation hair-dye mixture with a final concentration of 0.15%). No study on sub-chronic toxicity according to the Notes of Guidance was submitted. By using an adjustment factor of 3 (30 day to 90 day study) from the data on general toxicity available, a NOAEL of 1.7 mg/kg bw/d could be deduced.

o-Aminophenol was moderately toxic in an *in vivo* hen's egg test and had no teratogenic potential in this test. o-Aminophenol administered intraperitoneally on day 8 of gestation to Syrian golden hamsters at 100, 150, 200 mg/kg bw was found to be teratogenic at all doses tested. o-Aminophenol was tested for teratogenicity by oral gavage to groups of 20 pregnant SD (OFA) rats on days 6 – 15 of gestation at 20, 70, 250 mg/kg bw/d. A brown discolouration of urines occurred in all treated animals 24 hours after the first administration of the test substance; pathological findings were only observed in 250 mg/kg treated group. Thus the NOAEL for maternal toxicity and for foetal development (embryotoxicity) was 70 mg/kg bw/d.

A reproduction study on three generations with dermal application of formulations containing a final concentration of 0.15% of the test substance showed no effect on the reproduction systems of rats. However, the dose administered was too low for hazard assessment.

Mutagenicity

Overall, the genotoxicity program on o-aminophenol investigated the three endpoints of genotoxicity: gene mutations, structural chromosome aberrations and aneuploidy. However, all tests had some or several deficiencies compared to the minimal current requirements for a genotoxicity test. The number of appropriate tests which in spite of these deficiencies can be used for evaluation of the genotoxic potential of o-aminophenol is very small. Some tests can only be used as supportive evidence, many tests had only limited value and a few tests were even not acceptable for evaluation. Therefore, the conclusion on the genotoxic potential of o-aminophenol is based on a rather limited number of (still) not optimal tests. More weight of evidence was given to the tests which are mentioned in the guidance for genotoxicity testing of hair dyes.

In some of the gene mutation tests in bacteria o-aminophenol was positive in strain TA100 but not in the other strains tested. That the positive result may be a relevant result can be deduced from the fact that o-aminophenol induced a low DNA repair activity in bacteria and was also positive in a rec assay in *Bacillus subtilis*. A gene mutation test in yeast cells was negative whereas gene mutation tests in mammalian cells were not available. In an *in vitro* UDS test of poor quality o-aminophenol also scored negative. o-Aminophenol induced sister chromatid exchanges in human cells (fibroblasts and lymphocytes) but was negative in a chromosome aberration test of poor quality in CHO cells.

Under *in vivo* conditions o-aminophenol induced both sister chromatid exchanges and chromosome aberrations in tests with *Vicia faba*. These tests in *Vicia* are not very common and as such are not part of the guidance on genotoxicity testing for hair dyes. The relevance of such tests for a genotoxic hazard for humans is uncertain. In the more common tests o-Aminophenol exposure did not result in an increase of sister chromatid exchanges nor in chromosomal aberrations. Since the micronucleus test was negative, it is not expected that o-aminophenol is a clastogenic and/or an aneugenic compound.

Finally, although the test on germ cell genotoxicity was positive, this has only limited value and was not considered suitable for evaluation.

Based on the tests evaluated it can be concluded that the positive *in vitro* findings for clastogenicity were not confirmed in *in vivo* tests for chromosome aberrations or micronuclei. Likewise, the positive *in vitro* results found for gene mutations are overruled by a negative *in vivo* UDS test. Based on these experiments, o-aminophenol might be considered as non genotoxic.

However, as stated above, these conclusions are based on the results of a few inadequate tests. Therefore, to strengthen this conclusion both a well performed gene mutation test and a chromosomal aberration/micronucleus test in mammalian cells according to current guidelines are required.

Carcinogenicity

Three studies with regard to the possible carcinogenicity of o-aminophenol have been submitted. In the first study published in 1948, the sensitivity is considered too low to draw any conclusion. The second study involved implantation of pellets containing o-aminophenol in the urinary bladder of mice and is not considered relevant. The third study involved topical application of a hair dye formulation containing o-aminophenol. Since in a parallel study containing more than 10 times higher concentrations of 2,4-diaminoanisole (classified as carcinogen category 2 in EU) no tumours were found, the sensitivity of the study is considered too low for the evaluation of the carcinogenicity of o-aminophenol.

4. CONCLUSION

Based on the submitted data, no final conclusion on the safety of o-aminophenol can be drawn.

The data provided for physicochemical properties of o-aminophenol are insufficient and inadequate; no scientifically sound expert judgement can be made on the purity of o-aminophenol batches or on the homogeneity or stability of o-aminophenol or its formulations.

Inadequate information on skin sensitising potential was provided.

The studies on dermal absorption had many shortcomings and were not considered suitable for safety assessment.

Due to inadequacy of the studies provided, a mutagenic potential cannot be excluded.

Before any further reconsideration, the following is required:

- a complete set of physico-chemical data
- a study on skin sensitisation according to the state of the art
- a dermal absorption study according to the Notes of Guidance
- a well performed gene mutation test and a chromosomal aberration/micronucleus test in mammalian cells *in vitro* according to current guidelines to exclude mutagenicity.

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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