

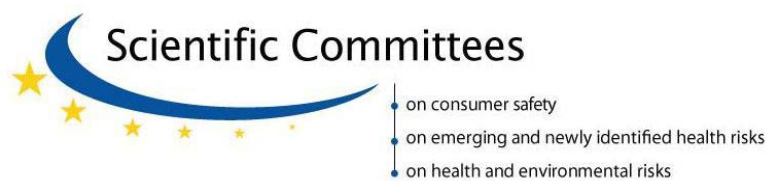


Scientific Committee on Consumer Safety

SCCS

**OPINION ON
p-Aminophenol**

COLIPA n° A16



The SCCS adopted this opinion at its 13th plenary meeting
of 13 – 14 December 2011

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 Agency (EMA), 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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ISSN 1831-4767

ISBN 978-92-79-30728-7

Doi:10.2772/22799

ND-AQ-11-021-EN-N

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http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

ACKNOWLEDGMENTS

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Keywords: SCCS, scientific opinion, hair dye, p-aminophenol, A16, directive 76/768/ECC, CAS 123-30-8, EC 204-616-2

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on p-aminophenol, 13-14 December 2011

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1. BACKGROUND

Submission I for p-Aminophenol was submitted in August 1980 by COLIPA^{1, 2}.

Submission II for this substance was submitted in June 1985 by COLIPA².

Submission III for this substance was submitted in March 1987 by COLIPA².

Submission IV and V for this substance was submitted in July 2001 by COLIPA².

p-Aminophenol is classified as a mutagen category 2 (CLP) substance.

The Scientific Committee on Consumer Products (SCCP) adopted at its 3rd plenary meeting the 15 of March 2005 the opinion (SCCP/0867/05) with the conclusion that:

"The SCCP is of the opinion that the information submitted is inadequate to assess the safe use of the substance. Before any further consideration, the following information is required:

- *adequate analytical characterisation as well as the physico-chemical profile, including information on solubility, purity and impurities;*
- *data on the genotoxicity/mutagenicity following the relevant SCCNFP opinions and in accordance with the Notes of Guidance."*

Submission VI for this substance was submitted by COLIPA in July 2005. Complete physical/chemical dossiers were submitted. p-Aminophenol is a hair dye precursor. It is incorporated in oxidative hair colouring formulations at a maximum concentration of 1.8%, and is typically mixed in 1:1 ratio with an oxidative agent thereby reaching a concentration of 0.9% for in use application.

Submission VI 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 SCCS consider p-Aminophenol safe for use as an oxidative hair dye with an on-head concentration of maximum 0.9% taken into account the scientific data provided?*
2. *Does the SCCS recommend any further restrictions with regard to the use of p-Aminophenol in oxidative hair dye formulations?*

¹ COLIPA - European Cosmetics Toiletry and Perfumery Association

² According to records of COLIPA

3. OPINION

3.1. Chemical and Physical Specifications

The following evaluation is based on the physical chemical dossier submitted in Submission IV of 2005.

Declaration by the applicant in submission IV of 2005, concerning physico-chemical properties of p-aminophenol:

"All pivotal toxicological safety evaluations in previous submissions were conducted with a test material that corresponds to the specifications described in the physical chemical dossier."

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

p-Aminophenol (INCI name)

3.1.1.2. Chemical names

1-hydroxy-4-aminobenzene (IUPAC)	phenol, 4-amino-
phenol, p-amino-	p-hydroxyaniline
4-hydroxyaniline	4-amino-1-hydroxybenzene
4-hydroxy-1-aminobenzene	1-amino-4-hydroxybenzene
p-aminophenol	4-aminophenol
4-hydroxybenzenamine	p-hydroxyphenylamine
4-hydroxyphenylamine	

3.1.1.3. Trade names and abbreviations

Activol; Azol; BASF Ursol P Base; Benzofur P; Certinal; Cetal; Citol; Durafur Brown RB; Fouramine P; Fourrine 84; Fourrine P Base; Furro P Base; Imexine OB (Hydrochloride); Nako Brown R; PAP; Paranol; Pelagol Grey P Base; Pelagol P Base; Renal AC; Rodinal; Tertral P Base; Unal; Ursol P; Ursol P Base; Zoba Brown P Base

COLIPA n° A16

Colour index: CI 76550 (Oxidation base 6)
CI 76551 (Oxidation base 6a — Hydrochloride)

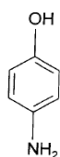
PAP: p-aminophenol

APAP: paracetamol

3.1.1.4. CAS / EC number

CAS: 123-30-8 (free base)

EC: 204-616-2 (free base)

3.1.1.5. Structural formula**3.1.1.6. Empirical formula**Formula: C₆H₇NO**3.1.2. Physical form**

White, odourless crystals or powder, turning brownish on exposure to air/humidity (commercial product usually pink)

3.1.3. Molecular weight

Molecular weight: 109.13 g/mol

3.1.4. Purity, composition and substance codes

Raw material presentation based on the analysis of p-aminophenol of batches 913341, 2070155, 3G037, I36483, 3A049604

Chemical characterisation by elemental analysis, NMR, MS, IR, UV,

Purity: >98% (w/w) determined by potentiometry (neutralisation of the amine function with 0.1N perchloric acid in a medium of acetic acid)

>99% by HPLC (Area%, without response factor, UV detection, irrespective of residual solvents, and other non-detectables)

Impurities:

o-Aminophenol: < 100 µg/g

m-Aminophenol: < 100 µg/g

Aniline: < 10 µg/g

4,4'-Oxidianiline: < 20 µg/g

As, Sb, Hg: < 5 µg/g each

Cd: < 10 µg/g

Pb: < 20 µg/g

Ash content: < 1 g/100g

Opinion on p-aminophenol

p-Aminophenol (A016)

Batches 2070155, 913341, 3G037, I36483, 3A049604

Comparative table of the main results

	A016				
	2070155	913341	3G037	I36483	3A049604
Appearance	A white to beige crystalline powder				
Titre (g/100g) Potentiometry	99.3	99.5	101.4	101.4	101.6
Water content (g/100g)	< 0.1				
Melting point DSC (°C)		188			
H.P.T.L.C. Profile	In accordance with the specification				
H.P.L.C. Profile (%) ⁽¹⁾		> 99	99.6	99.2	99.6
Impurities by H.P.L.C. (µg/g) ⁽²⁾					
o-Aminophenol		< 1000 (ND)	< 100 (ND)	< 100 (ND)	< 100 (ND)
m-Aminophenol		< 500 (ND)	< 100 (ND)	< 100 (ND)	< 100 (ND)
Aniline		< 2 (ND)	< 10 (ND)	< 10 (ND)	< 10 (ND)
4,4'-Oxidianiline		< 100 (ND)	< 20 (ND)	< 20 (D)	< 20 (D)
UV Spectrum	In accordance with the proposed structure				
IR Spectrum	In accordance with the proposed structure				
Mass spectrum		In accordance with the proposed structure			
¹H and ¹³C N.M.R. Spectrum		In accordance with the proposed structure			

ND : Not detected – D: Detected

⁽¹⁾ UV detection : UV purity - Area %, without response factor.
Irrespective of residual solvents, salts and other non detectable products

(2) in the raw material presentation, the results of batch 913341 were not taken into account
Elemental analysis was performed of batches 3G037, I36483, 3A049604

Comment

Quantification of p-aminophenol is performed by potentiometric titration, which is the neutralisation of the amine function with 0.1 N perchloric acid in a medium of acetic acid. Quantification of p-aminophenol is not performed by using a reference standard.

3.1.5. Impurities / accompanying contaminants

See 3.1.4

3.1.6. Solubility**(g/100 ml at 23°C after 24 hours)**

Water*: S < 1
Ethanol: S < 1 (different than reported earlier!)
DMSO: 1 ≤ S ≤ 10

Comment

Water solubility was not determined by EU Method A.6

3.1.7. Partition coefficient (Log Pow)

Log Pow Calculated: 0.25 (C log P v.4.2 – C. Hansch)

Log Pow Experimental: -0.09 ± 0.01 at 25 °C

3.1.8. Additional physical and chemical specifications

Organoleptic properties:	/
Melting point:	186-190 °C
Boiling point:	/
Flash point:	/
Vapour pressure:	/
Density:	/
Viscosity:	/
pKa:	/
Refractive index:	/
UV/Vis spectrum:	λ_{max} at 234 nm and 301.4 nm

HPLC procedure and features were provided. HPTLC, IR, UV-Vis, MS and NMR spectral characteristics are also available for identification purposes.

3.1.8. Homogeneity and stability / additional physico-chemical specifications

A 2 mg/ml p-aminophenol suspension in aqueous carboxymethylcellulose, conditioned in sealed glass bottles under argon atmosphere, was sampled at 3 different levels (top, middle and bottom) and analysed in duplicate by HPLC. The suspension was shown to be homogeneous (coefficient of variation 1.5%) with respect to p-aminophenol concentration. The stability of p-aminophenol in the same preparation, as described above, was determined after storage at 5 h at room temperature and after 2, 4 and 7 days storage at 4°C. p-Aminophenol was shown to be stable (<5% variation in the concentration of p-aminophenol in the preparation) during the study period.

General comments on analytical and physico-chemical characterisation

- * Quantification of p-aminophenol is performed by potentiometric titration, which is the neutralisation of the amine function, with 0.1 N perchloric acid in a medium of acetic acid. Quantification of p-aminophenol is not performed by using a reference standard. It is insufficient to determine the purity by potentiometric titration; it is not State-of-the-Art.
- * Solubility of p-aminophenol in various solvents was not properly characterised in the dossier.
- * Batch number and/or chemical purity were not stated in some toxicity study reports.
- * Stability of p-aminophenol in typical hair dye formulations has not been reported

3.2. Function and uses

p-Aminophenol is an oxidative hair dye precursor. It is incorporated in oxidative hair dye formulations and in the bottle on the market at a maximum concentration of 1.8% and is typically mixed in a 1:1 ratio with an oxidative agent thereby reaching a concentration of 0.9% for in use application.

p-Aminophenol is also used as an agent in the production of dyes and medicines, notably paracetamol.

Ref.: A.2

3.3. Toxicological Evaluation

Taken from SCCP/0867/05

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Oral median lethal dose values of p-aminophenol (PAP) previously reported in Submission I were:

Rats

671 mg/kg bw (Lloyd, 1977)

1270 mg/kg bw (Lloyd, 1977)

Ref. C26

375 mg/kg bw (Ind. Bio-Test Lab. Inc., 1975)

Ref. C23

Mice

1550 mg/kg bw (Segré, 1977),

Ref. C43

In a review report, an acute oral toxicity study in rats where the lethal median dose in male rats was 393 mg/kg bw and that for females was 1139 mg/kg bw. It also reports an oral lethal median dose in rabbits of between 4 and 10 g/kg bw.

Ref.: A2

A 5% suspension of p-aminophenol in a 3% starch solution was administered orally to albino rats. Deaths were reported within 48 hours of compound administration. A median lethal dose of 0.5 g/kg bw was established for p-aminophenol. Methaemoglobin concentration was less than 10% of the total haemoglobin concentration.

Ref.: A1

3.3.1.2. Acute dermal toxicity

The dermal median lethal dose for cats was calculated to be 37 mg/kg bw.

Ref.: C29

The medial lethal dose in mice was calculated to be 470 mg/kg bw.

Ref.: A2

The dermal median lethal dose for rabbits was calculated to be 10,000 mg/kg bw.

Ref.: C23

A separate study reported a dermal median lethal dose of >8000 mg/kg bw in rabbits. In rats, a dermal lethal median dose of >5000 mg/kg bw has been reported.

Ref.: A2

3.3.1.3. Acute inhalation toxicity

The median lethal dose for rats (1 hour exposure) was calculated to be >0.005 mg/l.

Ref.: C23

In separate studies, median lethal doses of >5.91 mg/l (1.3 hour exposure) and >3.42 mg/l (4 hour exposure) were calculated for rats.

Ref.: A2

3.3.2 Irritation and corrosivity

Taken from SCCP/0867/05

3.3.2.1. Skin irritation

A 2.5% aqueous solution of p-aminophenol containing 0.05% sodium sulphite was mildly irritant when applied to the skin of New Zealand White (NZW) rabbits.

Ref.: C26

Three protocols for evaluating the skin irritation of p-aminophenol were compared. 0.5 g of p-aminophenol as powder was applied to clipped skin of 6 New Zealand White rabbits under occlusive and semi-occlusive patches [occlusive - Official French Cosmetic (OFC) method and Association Française de Normalisation (AFNOR) method; semi-occlusive OECD method]. The occlusive patches were applied on both scarified and intact sites. Patches were removed at 4 hours (AFNOR, OECD) or at 23 hours (OFC). Macroscopic changes were recorded up to 42 hours (OFC, AFNOR) or 78 hours (OECD).

Ref.: A1

Conclusion

p-Aminophenol showed irritancy on rabbit skin under the conditions of the above experiments.

3.3.2.2. Mucous membrane irritation

A 2.5% aqueous solution of p-aminophenol containing 0.05% sodium sulphite was not considered to be irritant when instilled into the rabbit eye and then rinsed with water after ten seconds. Mild conjunctival irritation was observed in two animals.

Ref.: C26

100 mg/kg bw of p-aminophenol in the form of dry powder were instilled into one eye of each of six albino rabbits. The Ocular Irritation Index was estimated to be 17/110 after 24 hours, 4.5/110 after 48 hours, and 0/110 after 72 hours.

Ref.: C23

100 mg of p-aminophenol were instilled into the eyes of 9 New Zealand White rabbits. The eyes of 3 rabbits were rinsed after a 20 second exposure. The Ocular Irritation Index was estimated to be 6/110. Barely perceptible to mild conjunctival erythema, chemosis and secretion were observed; these signs improved over time. p-Aminophenol was considered to be non-irritant under the conditions of this study.

Ref.: A2

Conclusion

p-Aminophenol was irritant to mucous membranes under the conditions of the above experiments.

3.3.3. Skin sensitisation

Taken from SCCP/0867/05

Animal studies

In an open epicutaneous test in Pirbright white guinea-pigs, p-aminophenol at a concentration of 3% was applied on the flank six days per week for three weeks. Two weeks later, a challenge application was performed on the opposite untreated flank of the animals.

A single application of the test compound produced an allergic reaction at the challenge application site in 4 of 20 animals.

Ref.: C42

Using 24-hour occlusive patches, induction of Hartley albino guinea pigs with 2% p-aminophenol in petrolatum was performed on the flank (scapular area). Four patches were applied on alternate days. Following a 14-day waiting period, animals were challenged with a concentration of 2%, 1%, 0.5%, or 0.1% p-aminophenol on the opposite flank using a Finn chamber (closed technique). Dose-dependent elicitation was observed: 9/10 animals were positive at 2%, 6/10 animals at 1%, 5/10 animals at 0.5%, and 3/10 animals at 0.1%.

Ref.: C25

A 3% preparation of p-aminophenol in "Schultz Hamburg vehicle II" did not evoke a sensitization reaction in Hartley albino guinea pigs after induction using 18 applications of 0.5 ml of the test compound followed by a 0.5 ml challenge dose administered on the opposite flank two weeks later.

No study details were available to the SCCP.

Ref.: A2

The potential for cross sensitization between the industrial allergen 2-amino-4-chlorophenol (ACP) and p-aminophenol was studied in guinea pigs (strain not indicated). Fifteen guinea pigs were injected intra-cutaneously with ACP and then challenged with ACP and p-aminophenol. No reaction to p-aminophenol was elicited by challenge with 1.0% p-aminophenol for up to six weeks.

Ref.: A1

In a comparative study using guinea pigs (strain not indicated) and humans, 1% p-aminophenol in Vaseline produced no sensitization in the guinea pigs tested versus 36% of humans tested. 0.1% p-aminophenol in Vaseline produced no sensitization in the guinea pigs compared with 14% of the humans tested.

Ref.: A2

In a comparative study using two methods of induction, Hartley guinea pigs were tested for sensitization with p-aminophenol (>99%). For the first method, Freund's Adjuvant was injected into the foot pad of the hind paw and 0.18 mmol/l p-aminophenol was administered topically twice (over two days). For the second method, a preparation containing a 1:1 ratio of Freund's Adjuvant and 0.18 mmol/l p-aminophenol in distilled water was injected into the foot pad of the hind paw. After a 16-day waiting period, both groups of animals were challenged with a dose of 0.09 mmol/l in the lumbar region. Animals tested under the first method of induction exhibited no sensitization reactions, while 40% of those tested under the second method of induction were positive for sensitization.

Ref.: A2

A photosensitization test with 10% p-aminophenol in 80% DAE (40% dimethylacetamide, 30% acetone and 30% ethanol) was performed in Hartley guinea pigs. 5% musk ambrette was used as the positive control. The induction phase was 3 weeks long. During the first week, it consisted of the topical administration of 4 daily doses of 0.1 ml of the p-aminophenol solution followed by irradiation with ½ MED of UVA. Scoring was performed 24 hours after each dose. During weeks 2 and 3, it consisted of the topical administration of 4 daily doses of 0.1 ml of the p-aminophenol solution followed by irradiation with 1 MED of UVB. On the first and third days of the second and third weeks of induction, 0.1 ml Freund's complete adjuvant was injected intradermally surrounding the topical application site. Challenge was performed two weeks after the final induction dose was given, using the same application site and using 0.1 ml of 5% p-aminophenol for 3 consecutive days. One part of the application site was irradiated with ½ MED UVA, one part with ½ MED UVB, and one part received no irradiation. Sites were scored 24 hours after treatment. Rechallenge

consisted of a single application of a dye containing 7.5% p-aminophenol 9 days after the initial challenge. Sites were irradiated and scored 24 hours after application. No photosensitization was observed.

Ref.: A1

Comment

The studies were not performed according to standard methods.

Human studies

Various tests reported in previous submissions have demonstrated cross-reactivity between p-aminophenol and other aromatic amines in humans.

The following reports on human sensitization to p-aminophenol were compiled in 1995 (BCI, 1995, ref. A2):

- Among 60 patients from a dermatology clinic who were tested with 1% p-aminophenol, 7 (12%) were positive.
- Between 1973 and 1977, 4600 patients were tested for sensitization to benzidine. Of the 5.0% who were positive, 16.4% also had positive reactions to para-amino compounds. 1% of the patients (n=46) had a positive reaction to p-aminophenol.
- Between 1974 and 1984, 32 professional hairdressers with hand dermatitis due to use of hair dyes were patch tested for sensitization to these products. Twenty-two subjects had a positive reaction to hair dyes and 25% of these were positive when tested with p-aminophenol.
- 408 patients with eczema were patch tested for reactions against p-aminophenol. In response to the application of 1% p-aminophenol in Vaseline, 3% of the patients were positive.
- Of 13 female cosmetologists with hand, face, and/or axillary dermatitis, 4 were patch tested with a concentration of 1% p-aminophenol in Vaseline using standards approved by the International Contact Dermatitis Research Group. Of these, one person tested positive for sensitization with p-aminophenol.
- Two groups of hairdressers were tested for sensitization to p-phenylenediamine (PPD). 32 were negative for sensitization and 7 were positive. When the same subjects were tested for sensitization to p-aminophenol, the 32 who were negative with PPD were also negative with p-aminophenol. One of the 7 who was positive with PPD was also positive with p-aminophenol.

Conclusion

p-Aminophenol is a strong sensitizer.

3.3.4. Dermal / percutaneous absorption

Taken from SCCP/0867/05

In vitro studies

p-Aminophenol was among several para-substituted phenols for which percutaneous penetration was evaluated *in vitro* in full thickness hairless mouse skin. 4 µg/cm² p-aminophenol in acetone were applied to a surface measuring 3.1 cm². After evaporation of the solvent, the diffusion chamber was perfused with phosphate-buffered normal saline for a period of 48 hours. Transport of 72 ± 6% of the applied dose across the skin was attained after 24 hours, with the time of maximum flux occurring three hours after compound administration.

Ref.: Col. 73

The *in vitro* percutaneous penetration and metabolism of ^{14}C -labeled p-aminophenol in ethanol/water or acetone was studied in rat and human skin. 10% of the dose of 20 mg/ml p-aminophenol was absorbed through dermatomed rat skin when applied as an infinite dose over a 24-hour period. Skin stripping (removal of the stratum corneum) increased the absorption to 40% over 24 hours. In human skin, absorption resulting from the application of 20 mg/ml p-aminophenol was measured as 0.5 – 6.0% over 24 hours. Skin stripping increased absorption up to 27% of the dose administered. Results pertaining to the metabolism of p-aminophenol by the skin during percutaneous penetration were inconclusive.

Ref.: Col 74

Percutaneous absorption *in vitro* (oxidative and non oxidative conditions)

Guideline:	/
Tissue:	Human skin (abdomen, cadaver)
Membrane integrity	Tritiated water
Method:	static diffusion cell; membrane sizes 1.04 – 1.54 cm ²
No. of chambers:	1-6 replicates from 3-4 donors per experiment.
Test substance:	p-aminophenol in hair dye formulations: Formulation A (TM#1277) mixed with peroxide; p-aminophenol 0.42% Formulation B (TM#1278) mixed with water; p-aminophenol 0.42%
Batch:	/
Purity:	/
Radiolabel	4-amino [U- ^{14}C]phenol
Radiolabel purity	99% HPLC
Area Dose:	20 mg formulation/cm ²
Receptor fluid	PBS
Solubility in receptor	/
Stability	/
Analyses	liquid scintillation
Date	1997
GLP:	/

The percutaneous absorption of two test formulations containing ^{14}C -labeled p-aminophenol was examined *in vitro* using human abdominal skin. Three experiments were performed, each with:

Formulation (A) containing 0.84% ^{14}C -labeled p-aminophenol as well as other primary intermediates and couplers used to produce a reddish-brown shade.

Formulation (B) containing ^{14}C -labeled p-aminophenol as the sole dye constituent.

Formulation A was mixed with an equal volume of hydrogen peroxide; formulation B was mixed with an equal amount of water. The final concentration of p-aminophenol was 0.42% in each formulation.

20 mg/cm² of the test compounds were applied to heat-separated epidermal membranes for 30 minutes, after which the application sites were rinsed with water. Samples of receptor fluid were taken at 1, 2, 4, 6, 8, 24, 30, and 48 hours following dye application.

	Formulation A	Formulation B
Number Donors/acceptable replicates per donor	4/ 6, 5, 2, 1	3/ 6, 5, 2
Applied dose of formulation (mg/cm ²) ± SD	20.8 ± 3.3	20.2 ± 4.1
Applied dose of dye (µg/cm ²) ± SD	87.4 ± 13.86	84.8 ± 17.22
In receptor fluid (µg/cm ²) ± SD		
24 Hr	0.118 ± 0.130	0.316 ± 0.322
48 Hr	0.127 ± 0.125	0.457 ± 0.50
In receptor fluid (%) ± SD		
24 Hr	0.130 ± 0.137	0.320 ± 0.264
48 Hr	0.141 ± 0.132	0.456 ± 0.400
Amount retained in skin (µg/cm ²) ± SD	1.398 ± 0.839	2.20 ± 1.89

Mass Balance (% applied dose)

30 minute rinse	84.62 ± 10.87	91.69 ± 10.63
Terminal rinse, cell rinse, blot	1.73 ± 1.86	3.34 ± 3.34
Skin	1.60 ± 0.96	2.60 ± 2.23
Receptor fluid	0.14 ± 0.13	0.457 ± 0.40
Total (% applied dose)	88.09	98.09

Ref.: Col. 75

Comment

Complete original data is not available. The concentration of p-aminophenol used was 0.42% when the intended maximum on-head concentration is 0.9% in oxidative dyes. Accordingly, the amount considered to be absorbed from formulation A is considered to be 2 x ((receptor at 24 hours mean + 2SD) + (retained in skin mean + 2SD)) µg/cm².

This is 2 (0.378 + 3.076) = 6.9 µg/cm² or 7.84%, which may be used in calculating the MOS.

Taken from SCCP/0867/05*In vivo* studies

Sprague-Dawley rats of both sexes were treated with a single dermal application of either an aqueous solution (8%) or one of several hair dye formulations (1%, 2% or 3% p-aminophenol mixed with 6% hydrogen peroxide) containing ¹⁴C-labelled p-aminophenol for 30 minutes, followed by rinsing of the application site. The doses applied were 0.3 ml for the aqueous solution and 45, 90, and 135 mg/kg bw p-aminophenol for the 1%, 2%, and 3% hair dye solutions, respectively. The shaved application site measured 9 cm², except in one group of animals receiving the 2% hair dye formulation where the skin was not shaved and the application site measured 16 cm². Additionally, a single dose of 37.5 mg/kg bw of a 1% aqueous solution of p-aminophenol was administered either orally or subcutaneously to Sprague-Dawley rats. The urine and faeces of all animals were collected over a period of 72 hours. The animals were then killed, and urine, faeces and organs were evaluated for the presence of ¹⁴C-labelled p-aminophenol using liquid scintillation counting.

Results

The levels of absorption observed were as follows: 12.5 µg/cm² (0.627%) for the aqueous solution, 0.8 µg/cm² (0.08%) for 1% p-aminophenol, 5.4 µg/cm² (0.27% for 2% p-aminophenol), and 2.7 µg/cm² (0.09%) for 3% p-aminophenol. The absorption observed at unshaved application sites was lower (0.103% with 2% p-aminophenol). 95% of the radioactivity was found in the rinsing waters. The maximum amount of p-aminophenol in organs was found 35 minutes after compound administration. Excretion was primarily via the urine regardless of the route of administration.

Ref.: C21

Female hairless Wistar rats were administered a single dose of 0.14, 0.69, or 3.44 µM/cm² of a 0.75% solution of ¹⁴C-labelled p-aminophenol mixed with an equal volume of 20 vol. hydrogen peroxide on the skin of the back. The application site measured 10 cm². After 30 minutes of exposure, the application site was rinsed. Urine, faeces, skin and viscera were evaluated for p-aminophenol content over/after 4 days.

Results

The amounts of p-aminophenol absorbed were 15.9 nm/cm², 52.04 nm/cm² and 58.4 nm/cm².

In another set of experiments, penetration was measured for 3.44 $\mu\text{m}/\text{cm}^2$ of ^{14}C -labelled p-aminophenol alone, ^{14}C -labelled p-aminophenol admixed with a non-radioactive coupler and the ^{14}C -labelled indamine structure (a benzoquinoneneimine) produced from the reaction between p-aminophenol and its coupler. At this concentration, the level of ^{14}C -labelled p-aminophenol detected in the skin was approximately the same as that of ^{14}C -labelled p-aminophenol applied with the non-radiolabelled coupler. Penetration of the ^{14}C -labelled benzoquinoneneimine was $\sim 17\times$ less than that of ^{14}C -labelled p-aminophenol alone. The investigator concluded that indamine structures do not effectively cross the skin barrier.

Ref.: C46

The percutaneous absorption of p-aminophenol was evaluated in a study using 5 human volunteers. 2 $\mu\text{g}/\text{cm}^2$ of ^{14}C -labelled p-aminophenol in 95% ethanol was applied to the bend of the forearm over a total surface area of 2.5 cm^2 . The application time was not specified. Urine from each subject was collected over a seven day period and the radioactivity recovered was quantified. The dermal absorption of p-aminophenol was thus determined to be 13% of the applied dose. In a subsequent study performed by the same investigators using similar dosing and urine collection regimens, penetration was determined to be 6-8%.

Ref.: A2

3.3.5. Repeated dose toxicity

Taken from SCCP/0867/05

3.3.5.1. Repeated Dose (13 days) oral

Wistar rats were fed 55 mg/kg bw/day p-aminophenol in the diet for 13 days. No compound-related toxicity was observed.

Ref.: A2

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

Oral

p-Aminophenol was administered to male and female Wistar rats by oral gavage at a dose of 20 mg/kg bw/day for 12 weeks. The control group received a 1% carboxymethylcellulose solution only. No death, clinical signs, or histopathological findings could be attributed to compound administration.

Ref.: C18

A 13-week study on the dietary toxicity of p-aminophenol was conducted in Sprague-Dawley rats. The doses administered were 0, 47, 133, and 467 mg/kg bw/day. At 467 mg/kg bw, both sexes were observed to have reduced body weight gain and food consumption. Female rats had significantly lower haemoglobin and erythrocyte counts and increased corpuscular haemoglobin levels, but these changes were without clinical significance. Increased relative liver, kidney, and brain weights in both sexes and increased relative pituitary and thyroid weights in females were attributed to compound administration. Nephrosis was observed in all groups, including controls, but the incidence and severity of this change increased in a dose-dependent fashion.

Ref.: A2

The same doses (0, 47, 133 and 467 mg/kg bw) were administered daily in the diet to groups of Sprague-Dawley rats for a period of 6 months. To evaluate reversibility, 20 additional male rats were administered the dose of 467 mg/kg bw/day over a 20 week period and then observed for 7 weeks without treatment. Control, high dose and recovery animals were evaluated. A significant decrease in body weight gain was observed at 467 mg/kg bw/day as in the three month study; the males that had been allowed a reversibility

period also had decreased body weight gain. There were no significant clinical pathology changes. Increased relative liver, kidney, and brain weights were attributed to compound administration; these changes were not observed in rats allowed a reversibility period. Nephrosis, characterized by eosinophilic droplets in renal tubules, was diagnosed in both sexes at 467 mg/kg bw/day (including recovery males). The severity of this finding was dose-dependent, as in the three month study.

Ref.: A2

Guideline: OECD 408 (1981)
 Species/strain: Sprague-Dawley rats, CrI CD (SD) BR strain, 80 rats (40 males and 40 females)
 Group size: 10 animals per sex and per dose group
 Test substance: p-aminophenol
 Batch: 2070155
 Purity: 99.3% (potentiometric)
 Vehicle: 0.5% aqueous colloid emulsion of carboxymethylcellulose
 Dose levels: 0, 10, 30 and 100 mg/kg bw/day
 Dose volume: 5 ml/kg bw/day
 Route: oral (gavage)
 Administration: daily, 7 days per week over a period of 92 or 93 days
 GLP: in compliance
 Study period: 8 March – 9 June 1994

The daily administration of p-aminophenol by gavage at the doses of 10, 30 or 100 mg/kg bw/d for 13 weeks induced at all doses a not dose-related lower body weight gain and ptyalism at 100 mg/kg bw/d, in females only.

The major finding consisted of the tubular nephrosis observed with a dose-related incidence at the two-highest doses in both sexes (in 5/10 males and 2/10 females given 30 mg/kg bw/d and in 9/10 males and 10/10 females given 100 mg/kg bw/d).

As the dose of 10 mg/kg bw/d induced only a lower body weight gain (-16% as compared to the control) in females, this dose level was considered to be close to the NOEL.

Ref.: Col. 69

Comment

The SCCS considers the dose of 10 mg/kg bw/day as the NOAEL since the decrease in body weight gain was not dose related.

Dermal

Three hair dye formulations containing 0.04%, 0.2% or 1.0% p-aminophenol and mixed with 6% hydrogen peroxide were applied topically to the clipped skin of rabbits twice weekly for 13 weeks. Rabbits in three independent control groups were clipped as were treated animals, but no dyes were applied. No compound-related findings were observed. Scattered variations in clinical pathology parameters were observed, but none were considered to be of toxicological significance.

Ref.: C7

3.3.5.3. Chronic (> 12 months) toxicity

Taken from SCCP/0867/05

p-Aminophenol dissolved in corn oil was fed to 12 Sprague-Dawley rats at a concentration of 0.087% (approximately 43.5 mg/kg bw) in the diet for 9 months. No compound-related histopathological effects were observed.

Ref.: C30

The chronic toxicological and carcinogenic potential following skin painting in Swiss Webster mice was evaluated for three oxidative formulations of p-aminophenol (see also Section Carcinogenicity).

Ref.: C8

Chronic dermal toxicity was evaluated in a multi-generation reproduction study in Charles River CD rats (see Section Reproductive Toxicology). No compound-related dermal toxicity was observed.

Ref.: A1

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

Bacterial Reverse Mutation Test

Guideline:	/
Species/Strain:	<i>Escherichia coli</i> WP2uvrA/pKM101
Replicates:	triplicates in 2 individual experiments
Test substance:	p-aminophenol
Solvent:	distilled water
Batch:	/
Purity:	/
Concentration:	700, 900, 1100, 1300 and 1500 µg/plate without S9-mix
Treatment:	pre-incubation method with 20 minutes pre-incubation without S9-mix
GLP:	/
Date:	publication from 1998

p-Aminophenol was investigated for the induction of gene mutations in *Escherichia coli* WP2uvrA/pKM101. The test was performed in the absence of metabolic activation only according to the pre-incubation method with 20 minutes pre-incubation. Concurrent positive controls were not included.

Results

Toxic effects were not reported. Exposure of *E. coli* to p-aminophenol resulted in a biologically relevant and dose dependent increase in revertant colonies.

Conclusion

Under the experimental conditions used p-aminophenol was mutagenic in this gene mutation tests in *E. coli* in the absence of S9 metabolic activation.

Ref.: 81

Comment

The test is reported as a publication in the open literature. The test is considered inadequate due to the following reasons: purity and batch number were not reported, the test has not been performed in the presence of activation systems and no concurrent positive control was included, the assays were not conducted in compliance with GLP or with OECD guidelines.

***In vitro* Mammalian Cell Gene Mutation Test (*hprt* locus)**

Guideline: /
 Cells: CHO - K₁-BH₄ cells
 Replicates: /
 Test substance: p-Aminophenol
 Solvent: /
 Batch: /
 Purity: /
 Concentrations: 0, 2.5, 5, 7.5 and 10 µg/ml without S9-mix
 0, 4, 6, 8, 10, 12, 14 and 16 µg/ml with S9-mix
 Treatment: treatment time not reported; expression period 9 days.
 GLP: /
 Date: publication from 1990

p-Aminophenol was assayed for mutations at the *hprt* locus of CHO cells both in the absence and presence of metabolic activation. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Treatment times were not reported, the expression period was 9 days. Positive controls were not included.

Result

A biologically relevant increase in mutant frequency compared to concurrent controls was not observed, either in the absence or in the presence of S9-mix.

Conclusion

Under the experimental conditions used, p-aminophenol did not induce mutations at the *hprt* locus of CHO cells and, consequently, is not genotoxic (mutagenic) in this gene mutation test.

Ref.: 82

Comment

The test is reported as a publication in the open literature. The test is considered inadequate due to the following reasons: purity and batch are not reported, treatment times were not mentioned, concurrent positive controls were not included and the assays were not conducted in compliance with GLP or OECD guidelines.

***In vitro* Mammalian Cell Gene Mutation Test (*tk* locus)**

Guideline: /
 Cells: L5178Y *tk*^{+/-} mouse lymphoma cells
 Replicates: 2 replicates in 2 independent experiments
 Test substance: p-aminophenol
 Solvent: culture medium
 Batch: /
 Purity: /
 Concentrations: 0, 4, 6, 8, 10 and 12 µg/ml without S9-mix
 Treatment: 4 h without S9-mix; expression period 48 h, selection growth 9-11 days.
 GLP: /

Date: publication from 1995

p-Aminophenol was assayed for gene mutations at the *tk* locus of mouse lymphoma cells in the absence of S9 metabolic activation. Mouse lymphoma cells were treated for 4 h followed by an expression period of 48 h to fix the DNA damage into a stable *tk* mutation. Toxicity was measured as percentage relative total growth of the treated cultures relative to the survival of the solvent control cultures.

Result

The appropriate level of toxicity (10-20% survival after the highest dose) was reached. p-Aminophenol treatment for 4 h without biological activation resulted in a biological relevant and dose dependent increase in the mutant frequency in these mouse lymphoma cells. The mutant colonies were distributed over a wide range of sizes but with increasing concentrations, the smaller colonies were predominant, indicating a clastogenic effect of p-aminophenol.

Conclusion

Under the experimental conditions used, p-aminophenol was genotoxic in this mouse lymphoma assay at the *tk* locus. Since at higher concentrations smaller colonies dominate, the results point to a clastogenic effect.

Ref.: 83

Comment

The data are from a publication in the open literature. The test has only limited value and can only be used for confirmation purposes. The test was only performed without metabolic activation. The purity and batch number are not given and a concurrent positive control was not included. The test was not conducted in compliance with GLP or OECD guidelines.

***In vitro* Mammalian Cell Gene Mutation Test (*hprt* locus)**

Guideline:	/
Cells:	CHO - K ₁ -BH ₄ cells and L5178Y <i>tk</i> ^{+/-} mouse lymphoma cells
Replicates:	2 replicates in 2 independent experiments
Test substance:	p-aminophenol
Solvent:	culture medium
Batch:	/
Purity:	/
Concentrations:	CHO cells: 0, 10, 15, 20, 25 and 30 µg/ml without S9-mix mouse lymphoma cells: 0, 4, 6, 8, 10 and 12 µg/ml without S9-mix
Treatment:	4 h without S9-mix; expression period 9 days, selection growth 9-11 days.
GLP:	/
Date:	publication from 1995

p-Aminophenol was assayed for gene mutations at the *hprt* locus of CHO cells and mouse lymphoma cells in the absence of S9 metabolic activation. Cells were treated for 4 h followed by an expression period of 9 days to fix the DNA damage into a stable *hprt* mutation. Toxicity was measured as percentage relative total growth of the treated cultures relative to the survival of the solvent control cultures.

Result

The appropriate level of toxicity (10-20% survival after the highest dose) was reached. Both in CHO and in mouse lymphoma cells, p-aminophenol treatment for 4 h without biological activation did not result in a biological relevant and dose dependent increase in the mutant frequency at the *hprt* locus.

Conclusion

Under the experimental conditions used, p-aminophenol was not genotoxic in these gene mutation tests at the *hprt* locus.

Ref.: 83

Comment

The data are from a publication in the open literature. The test has only limited value and can only be used for confirmation purposes. The test was only performed without metabolic activation. The purity and batch number are not given and a concurrent positive control was not included. The test was not conducted in compliance with GLP or OECD guidelines.

***In vitro* Mammalian Chromosomal Aberration Test**

Guideline:	/	
Cells:	CHO - K ₁ -BH ₄ cells and L5178Y <i>tk</i> ^{+/-} mouse lymphoma cells	
Replicates:	duplicate cultures, 2 independent experiments	
Test substance:	p-aminophenol	
Batch:	/	
Purity:	/	
Concentrations:	CHO cells:	0, 5, 10, 15, 20, 25 and 30 µg/ml without S9-mix
	mouse lymphoma cells:	0, 4, 6, 8, 10 and 12 µg/ml without S9-mix
Treatment:	22 h treatment and harvest immediately after the end of treatment	
GLP:	/	
Date:	publication from 1995	

p-Aminophenol has been investigated for induction of chromosomal aberrations in both CHO and mouse lymphoma cells. Cells were treated for 22 h and harvested immediately after the end of treatment. Approximately 2 h before harvest, each culture was treated with colcemid (final concentration 0.08 µg/ml) to block cells at metaphase of mitosis. Chromosome (metaphase) preparations were stained with 2% aceto-orcein and examined by phase-contrast microscopy. A positive control was not included.

Results

Exposure of cells was demonstrated by a decrease of the relative mitotic index. Both in CHO cells and in mouse lymphoma cells the mitotic index was >50% at the highest dose only. In both cell lines a biological relevant and dose-dependent increase in cell with chromosome aberrations was observed after 22 h treatment with p-aminophenol. An increase in polyploidy and endoreduplication was seen in CHO cells and occasionally in mouse lymphoma cells.

Conclusion

Under the experimental conditions, p-aminophenol was genotoxic (clastogenic) both in CHO and mouse lymphoma cells in the chromosomal aberration test.

Ref.: 83

Comment

In the figure and the table showing the mitotic index in CHO cells different results at 20 µg/ml are reported. If the figure is correct then the positive result found in this chromosome aberration test is restricted to cytotoxic doses (MI > 50 %).

The data are from a publication in the open literature. The test has only limited value and can only be used for confirmation purposes. The test was only performed without metabolic activation. The purity and batch number are not given and a concurrent positive control was not included. The test was not conducted in compliance with GLP or OECD guidelines.

***In vitro* Single Cell Gel electrophoresis assay (Comet assay)**

Guideline:	/
Cells:	CHO - K ₁ -BH ₄ cells and L5178Y <i>tk</i> ^{+/-} mouse lymphoma cells
Replicates:	duplicate cultures, 2 independent experiments
Test substance:	p-aminophenol
Batch:	/
Purity:	/
Concentrations:	CHO cells: 0, 5, 10, 15, 20, 25 and 30 µg/ml without S9-mix mouse lymphoma cells: 0, 4, 6, 8, 10 and 12 µg/ml without S9-mix
Treatment:	not reported, possibly 22 h
GLP:	/
Date:	publication from 1995

p-Aminophenol has been investigated for induction of DNA strand breaks in both CHO and mouse lymphoma cells in the *in vitro* single cell gel electrophoresis assay (Comet assay). Although treatment times were not reported, the cells were probably treated for 22 h. The slides were stained with ethidium bromide (final concentration 10 µg/ml) in distilled water. Cells were scored for the presence of a tail. A positive control was not included.

Results

In both cell lines a biological relevant and dose-dependent increase in the percentage of damaged cells was observed.

Conclusion

Under the experimental conditions, p-aminophenol was genotoxic both in CHO and mouse lymphoma cells in the *in vitro* Comet assay.

Ref.: 83

Comment

The data are from a publication in the open literature. The test is considered inadequate due to the following reasons: the test was only performed without metabolic activation. The purity, batch nr and treatment time are not given and a concurrent positive control was not included. The test was not conducted in compliance with GLP or OECD guidelines.

***In vitro* Mammalian Chromosomal Aberration Test**

Guideline:	OECD 473
Cells:	Human lymphocytes from a single, male, healthy donor
Replicates:	Duplicate culture in a single test
Substance:	p-aminophenol
Solvent:	DMSO
Batch:	9040158
Purity:	/
Concentrations:	0, 13.0, 19.0 and 25.0 µg/ml without S9-mix 0, 960.4, 1372 and 1960 µg/ml with S9-mix
Treatment:	20 or 44 h treatment without S9-mix; harvest time 20 or 44 h after the start of treatment. 3 h treatment with S9-mix; harvest time 20 or 44 h after the start of treatment
GLP:	in compliance
Date:	15 September 1989 – 21 November 1989

p-Aminophenol has been investigated for induction of chromosomal aberrations in human lymphocytes withdrawn from 1 male volunteer. Phytohaemagglutinin was included at a concentration of 0.375 ml/10 ml culture to stimulate the lymphocytes to divide. Liver S9 fraction from Aroclor™ 1254-induced rats was used as the exogenous metabolic activation system. Cells were treated for 20 or 44 h without S9-mix or for 3 h with S9-mix with several concentrations ranging from 1 – 85 µg/ml without S9-mix and from 79.09 – 4000 µg/ml with S9-mix. Cells were harvested 20 and 44 h after the start of treatment both without and with S9-mix. The three concentrations which were finally analysed were selected by evaluating the effect of treatment on mitotic index. The top dose for analysis was to be one at which a 50-80% reduction in mitotic index occurred.

Approximately 1.5 h before harvest, colchicine was added to each culture (final concentration 1 µg/ml) to block cells at metaphase of mitosis. Chromosome (metaphase) preparations were stained with 4% filtered Gurr's Giemsa R66 and examined microscopically for chromosome aberrations and mitotic index. Negative and positive controls were in accordance with the OECD guideline.

Results

Both in the absence as well as in the presence of metabolic activation, at a harvest time of 20h, a biologically relevant increase in the number of cells with structural chromosome aberrations was observed as compared to the corresponding solvent control for all concentrations tested. In the absence of S9-mix the increase was clearly dose dependent. The frequency of numerical aberrant cells was within the historical negative control range for all doses.

As positive results were found at the 20 h harvest time, the cultures of the 44 h harvest time were not analysed.

Conclusions

Under the experimental conditions used, p-aminophenol was genotoxic (clastogenic) in human lymphocytes *in vitro*.

Ref.: 87

Comment

The purity of the batch was not reported.

3.3.6.2. Mutagenicity/Genotoxicity *in vivo*

In vivo gene mutation test in transgenic mice

Guideline:	/
Species:	Muta™Mouse mice
Group sizes:	/
Test substance:	4-aminophenol
Batch:	/
Purity:	/
Dose levels:	214.5 mg/kg bw
Route:	oral gavage
Vehicle:	/
Sacrifice times:	7 days after treatment
GLP:	/
Date:	abstract from 1996

4-Aminophenol has been investigated for induction of mutations in the *lacZ* gene in the liver of mice (Muta™Mouse). 4-Aminophenol is considered as a promutagen converted into labile mutagen metabolites in the liver. Mice were treated once orally with 214.5 mg/kg bw which is 50% of the maximum tolerated dose. Mice were euthanized and livers collected 7 days after treatment. Mutations in the *lacZ* gene were measured using *E. coli gal E* and phenyl

galactoside for positive staining of mutants. *N*-ethyl-*N*-nitrosourea, applied intraperitoneally, was used as positive control.

Results

Exposure of mice carrying the *lacZ* gene of *E. coli* to 4-aminophenol, did not result in an increase in the mutant frequency at the *lacZ* gene in the liver collected 7 days after treatment.

Conclusion

Under the experimental conditions, 4-aminophenol was not genotoxic (mutagenic) in this *in vivo* gene mutation test with transgenic mice.

Ref.: 85

Comment

The data are from an abstract presented at a conference. The test is considered inadequate. The purity, batch nr, group sizes and vehicle are not reported. The expression time (7 days) is much too short to detect gene mutations in the liver in this model; in the draft OECD guideline (in press) an expression time of 28 days with 3 day recovery is recommended. The test was not conducted in compliance with GLP or draft OECD guideline.

Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *in vivo*

Guideline:	/
Species/strain:	Wistar rat
Group size:	5 males/group
Test substance:	p-Aminophenol
Batch:	9040158
Purity:	/
Dose levels:	0, 285, 1425 mg/kg bw
Route:	oral gavage
Vehicle:	corn oil
Sacrifice times:	4 h and 12 h after dosing
GLP:	in compliance
Date:	16 September 1989 – 18 December 1989

p-Aminophenol was investigated for the induction of unscheduled DNA synthesis (UDS) in hepatocytes of rats. Test doses were based on the results of an initial range finding study on toxicity. Rats were treated orally with doses up to 2000 mg/kg bw and examined for mortality and clinical signs. In the main experiment mice were exposed orally to 0, 285 and 1425 mg/kg bw (\pm 80% of the LD50). Two sampling times 4 and 12 h post-treatment were selected.

Hepatocytes for UDS analysis were collected by perfusion with collagenase approximately 4 h and 12 h after administration of p-aminophenol. At least 90 minutes after plating the cells were incubated for 4 h with 10 μ Ci/ml ³H-thymidine followed by overnight incubation with unlabelled thymidine. Evaluation of autoradiography was done after 14 days. UDS was reported as net grain counts/nucleus (the nuclear grain count subtracted with the number of grains in a nuclear sized area adjacent to each nucleus), the % cells responding or in repair and the population average of the nuclear grains for the sub-population of cells in repair. Unscheduled DNA synthesis was determined in 50 randomly selected hepatocytes on 2 replicate slides per rat. Although OECD guidelines are not cited, the positive controls (AAF and DMN) are in accordance with the actual OECD guidelines.

Results

In the initial range finding study with treatments up to 2000 mg/kg bw, 2 rats died at 2000 mg/kg bw and one rat from the 1500 mg/kg bw group was killed in extremis. Hence a dose of 1425 mg/kg bw was chosen as the appropriate high dose.

p-Aminophenol at doses of 285 and 1425 mg/kg bw yielded group mean net nuclear grain (NNG) values less than 0 for both sacrifice times and caused no significant increases in mean nuclear grain counts, as compared to controls. The percentage of cells in repair did not significantly differ from the control group and was well below the percentage required for a positive response.

Conclusions

Under the experimental conditions, it is concluded that p-aminophenol did not induce DNA-damage leading to unscheduled DNA synthesis and, consequently, is not genotoxic in this *in vivo* UDS test in rats.

Ref.: 86

Comment

The purity of the batch was not reported.

Mammalian Erythrocyte Micronucleus Test

Guideline:	OECD 474 (1983)
Species:	Swiss OF1 mice
Group sizes:	5 mice/sex/group
Material:	p-aminophenol
Batch:	913341
Vehicle:	carboxymethylcellulose
Purity:	/
Dose levels:	0, 170, 250 and 500 mg/kg bw
Route:	single oral dose
Sacrifice times:	24 h and 48 h after treatment for all concentrations
GLP:	In compliance
Date:	26 November 1991 – 5 May 1992

p-Aminophenol has been investigated for induction of micronuclei in bone marrow cells of male and female mice. Test doses were based on the results of a preliminary study in which mice were treated orally with 500 and 1000 mg/kg bw p-aminophenol and examined for acute toxic symptoms. In the main experiment mice were exposed orally to 0, 170, 250 and 500 mg/kg bw. Bone marrow cells were collected 24 h or 48 h after dosing.

Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and normochromatic erythrocytes (PCE/NCE). Negative and positive controls were in accordance with the OECD guideline.

Results

In the preliminary study, to select the doses for the main study, 2 of 6 mice treated with 1000 mg/kg bw died within 24 h and 1 of 6 mice demonstrated hypokinesia in the 500 mg/kg bw group. Consequently, 500 mg/kg bw was defined as the maximum dose for the main study. In the main test no clinical signs were observed in any of the treated mice. The PCE/NCE ratio 24 hours after treatment was not altered after treatment as compared to the concurrent control values, reflecting a lack of cytotoxicity of p-aminophenol at 24 hours sacrifice time. However, at 48 h after dosing a dose dependent and statistically significant decrease in the PCE/NCE ratio was observed indicating to cytotoxicity of the test agent to the bone marrow and thus to exposure of the bone marrow cells.

At 24 h after dosing a biologically relevant and statistically significant increase in the incidence of polychromatic erythrocytes with micronuclei was observed as compared to the concurrent vehicle control values. The amplitude of the increase is much higher in females than in males.

At 48 hours a slight but still statistically significant increase in the incidence of micronucleated polychromatic erythrocytes over the concurrent vehicle control values was found. It is also a dose dependent increase with less marked inter sex difference.

Conclusions

Under the experimental conditions used p-aminophenol induced an increase in the number of erythrocytes with micronuclei in treated mice and, consequently, p-aminophenol is genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice.

Ref.: 88

Mammalian Hepatocyte Micronucleus Test

Guideline:	/
Species:	male CD1/CR mice
Group sizes:	5 mice/group
Material:	4-Aminophenol
Batch:	/
Vehicle:	0.5% aqueous solution of methylcellulose
Purity:	/
Dose levels:	0, 53, 107 and 214 mg/kg bw/day
Route:	intraperitoneal injection
Sacrifice times:	144 h after start of treatment
GLP:	/
Date:	publication from 1989

4-Aminophenol has been investigated for induction of micronuclei in hepatocytes of male mice. Mice received 2 intraperitoneal injections 24 h apart with 53, 107 and 214 mg/kg bw (= 12.5, 25 and 50 % of the LD50 determined 7 days after treatment). Two-thirds of the liver is removed 24 h after the last treatment. The proliferative stimulus of partial hepatectomy is necessary since adult liver has a very low proliferation which is essential for expression of DNA damage into micronuclei. Hepatocytes for micronucleus analysis were collected by perfusion with 0.5 mg/ml collagenase 96 h after partial hepatectomy. The cell suspension was rapidly spread on slides by cytopspin. Hepatocyte slides were stained with May-Grünwald/Giemsa and examined microscopically for micronuclei. Only undamaged cells were scored, i.e. cells with intact nuclear and outer membranes and with intact and vacuole-free cytoplasm. For each dose 1000 hepatocytes were examined.

Results

Exposure of mice to 4-aminophenol resulted in a statistically significant increase in the number of hepatocytes with micronuclei as compared to the concurrent controls. However, the increase was not dose-dependent since the highest increase was found at the mid dose (107 mg/kg bw/day).

Conclusions

Under the experimental conditions used 4-aminophenol induced an increase in the number of hepatocytes with micronuclei in treated mice and, consequently, 4-aminophenol is genotoxic (clastogenic and/or aneugenic) in hepatocytes of mice.

Ref.: 89

Comment

The data are from a publication in the open literature. The test is considered inadequate due to the following reasons: the purity and batch nr are not reported and a concurrent positive control was not included. The test was not conducted in compliance with GLP or OECD guidelines. Moreover, a micronucleus test in hepatocytes after partial hepatectomy is not commonly used, not validated and not generally accepted.

Mammalian Splenocyte Micronucleus Test

Guideline:	/
Species:	male CD1 mice
Group sizes:	5 mice/group
Material:	4-Aminophenol
Batch:	/
Vehicle:	0.5% aqueous solution of methylcellulose
Purity:	/
Dose levels:	0, 53.6, 107.2 and 214.5 mg/kg bw/day
Route:	single oral dose
Sacrifice times:	2 and 14 days after treatment
GLP:	/
Date:	publication from 1994

4-Aminophenol has been investigated for induction of micronuclei in splenocytes of male mice. Mice received 2 intraperitoneal injections 24 h apart with 0, 53.6, 107.2 and 214.5 mg/kg bw (= 12.5, 25 and 50 % of the LD50 determined 7 days after treatment). Mice were killed by cervical dislocation and the spleen was collected. Splenocytes were isolated by stripping the spleen using a syringe needle. The cell suspension was then cultured for 44 h at 37 °C in a humidified atmosphere containing 3% CO₂ in air. Cytokinesis was blocked by adding cytochalasin B (final concentration 3 µg/ml) 24 h after the beginning of the culture. After the 44 h culture the number of cells was counted and processed onto slides by cytopsin. Splenocyte slides were stained with May-Grünwald/Giemsa. Microscopic scoring of micronuclei was limited to binucleated splenocytes only. Toxicity and thus exposure of the target cells was determined by calculating the percentage of binucleated splenocytes in order to give the splenocytes proliferating index.

Results

A 2-day exposure of mice to 4-aminophenol resulted in a dose dependent and statistically significant increase in the number of splenocytes with micronuclei as compared to the concurrent controls. Simultaneously, the number of binucleated splenocytes dose-dependently increased indicating that 4-aminophenol was not toxic for the splenocytes. At 14-day exposure the number of binucleated splenocytes dose dependently decreased, indicating cytotoxicity of 4-aminophenol. The effect of this toxicity is obvious by the higher, dose dependent and statistically significant increase in the number of splenocytes with micronuclei as compared to the concurrent controls.

Conclusions

Under the experimental conditions used 4-aminophenol induced an increase in the number of splenocytes with micronuclei in treated mice and, consequently, 4-aminophenol is genotoxic (clastogenic and/or aneugenic) in splenocytes of mice.

Ref.: 90

Comment

The data are from a publication in the open literature. The test has only limited value and can only be used for confirmation purposes. The purity and batch nr are not reported and a concurrent positive control was not included. The test was not conducted in compliance with GLP or OECD guidelines. Moreover, a micronucleus test in splenocytes is not commonly used and not validated.

Mammalian Erythrocyte Micronucleus Test

Guideline: /
Species: Crl Cd (SD) BR strain of Sprague Dawley rats
Group sizes: 6 rats/sex/group
Material: p-aminophenol
Batch: 2070155
Vehicle: 0.5% aqueous carboxymethylcellulose
Purity: /
Dose levels: 0, 12 and 30 mg/kg bw/day
Route: daily by gavage for more than 91 days
Sacrifice times: 91 days after the start of treatment
GLP: In compliance
Date: September – December 1994

p-Aminophenol has been investigated for induction of micronuclei in the bone marrow cells of male and female rats. Dose levels were determined by the sponsor. p-Aminophenol was administered daily by a single intragastric gavage during 13 weeks. Clinical signs were observed for each animal at least once a day. All animals were checked at least twice a day for mortality and signs of morbidity. Food consumption and body weight were recorded once a week.

In the micronucleus test, toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and normochromatic erythrocytes (PCE/NCE). Bone marrow preparations were stained with May-Grünwald/Giemsa and examined microscopically for the PCE/NCE ratio and micronuclei. Five mice/sex/group were analysed; the remaining 6th animals of each group were only evaluated in case a mouse died spontaneously. No mention is made regarding concurrent negative and positive controls.

Results

Except from orange coloured urine noted in all the animals of both sexes given 30 mg/kg bw/day from week 10/11 to the end of the treatment period, no other treatment-related clinical signs were recorded. No mortality was noted during the treatment period. Mean food consumption and mean body weight gain did not differ between treated and untreated animals.

Treatment with p-aminophenol did not result in substantially decreased PCE/NCE ratios compared to the untreated controls indicating that p-aminophenol did not have cytotoxic properties in the bone marrow. The orange coloured urine observed in rats treated with 30 mg/kg bw/day indicates systemic bioavailability of p-aminophenol. A biologically relevant increase in the number of erythrocytes with micronuclei over the concurrent vehicle control values was not observed in both sexes given 12 or 30 mg/kg bw.

Conclusions

Under the experimental conditions used, 13 week treatment with p-aminophenol did not induce an increase in the number of erythrocytes with micronuclei in rats and, consequently, p-aminophenol is not genotoxic (clastogenic and/or aneugenic) in this micronucleus test.

Ref.: 91

Comment

Since this protocol only allows the application of a lower dose compared with a single application, it may be less suited to detect the mutagenic potential of a test substance. However, the results might be useful in the context of risk estimation.

Mammalian Bone Marrow Chromosomal Aberration Test

Guideline:	OECD 475 (1997)
Species:	Wistar rats
Group sizes:	5 rats/sex/group
Material:	p-aminophenol
Batch:	99 B 197
Purity:	99.9%
Vehicle:	0.5% carboxymethylcellulose
Dose levels:	0, 200, 400 and 800 mg/kg bw
Route:	oral gavage
Sacrifice times:	24 h after treatment for all concentrations, 48 h for the high dose only.
GLP:	In compliance
Date:	14 December 1999 – 10 May 2000

p-Aminophenol has been investigated for induction of chromosome aberrations in the bone marrow cells of male and female rats. Test doses were based on the results of a pre-experiment on acute toxicity. Two animals per sex and test group were treated orally with p-aminophenol up to 1000 mg/kg bw and examined for acute toxic symptoms at 1, 6, 24 and 48 h after treatment. In the main experiment animals were exposed orally to 0, 200, 400 and 800 mg/kg bw. Prior to sacrifice (2.5 hours) animals were injected intraperitoneally with the spindle inhibitor colcemid (final concentration 2 mg/kg bw) to arrest cells in metaphase. The animals of the vehicle control group and the highest dose groups were bled. The blood was collected in heparinised vessels and centrifuged to receive blood plasma. The plasma samples were stored until an eventual chemical analysis on the p-aminophenol plasma levels. Toxicity and thus exposure of the target cells was determined by measuring the mitotic index. Negative and positive controls were in accordance with the OECD guideline.

Results

In a pre-experiment on acute toxicity 1 of the 4 animals of the 1000 mg/kg bw group and 3 of the 4 of the 900 mg/kg bw group died within 24 h. Until death these animals as well as the surviving animals regularly showed reduction of spontaneous activity, abdominal position, eyelid closure and apathy. Reduction of spontaneous activity, eyelid closure and apathy were also observed in the 800 and 600 mg/kg bw group. Since no animals from the 800 mg/kg bw group died, this dose was chosen as the highest dose. Clinical signs observed in the main experiment were not reported.

The mitotic indices dose-dependently decreased after p-aminophenol treatment indicating a cytotoxic effect of p-aminophenol on bone marrow cells and thus to exposure of the target tissue.

Under the conditions of the test, a dose dependent increase in the number of cells with chromosome aberrations was observed. However, since the mean number of cells with chromosome aberrations per dose, even for 800 mg/kg bw which is the only one that is statistically significant, was still below 2%, which is the upper limit of an acceptable negative control, and since the actual negative control was unusually low, the positive results found may be of no biological relevance.

Conclusions

Under the experimental conditions used p-aminophenol did not induce a biologically relevant increase in the number of erythrocytes with micronuclei and, consequently, p-aminophenol is not genotoxic (clastogenic and/or aneugenic) in this micronucleus test in rats.

Ref.: 92

Sex-linked recessive lethal test in *Drosophila melanogaster* (SLRLT)

Guideline: /
Species: *Drosophila melanogaster*; females M5 (Muller 5), males KO (a subline of Karsnäs)
Test substance: p-aminophenol
Batch: /
Vehicle: feeding: water containing 5% sucrose
injection: 0.9% NaCl
Purity: /
Dose levels: feeding: 130 mmol/l
injection: 30 (trial 1) and 15 (trial 2) mmol/l
GLP: /
Date: publication from 1990

p-Aminophenol was assayed for gene mutations in the sex-linked recessive lethal test in *Drosophila melanogaster* (SLRLT). Dose levels were based on the results of a toxicity test to determine dose levels without causing severe reduction in fertility. When 4-5 days old, the flies were treated either by feeding or by injection. After feeding the males were immediately mated whereas after injection the males were allowed to recover for 24 h. Each male was crossed with groups of 3 virgin M5 females for consecutive 3, 3 and 4 day periods in trail 1 and for consecutive 2, 3 and 3 day periods in trail 2. Where possible, 10 single daughters of the F1 generation of each brood and each parental male were transferred to fresh vials together with two M5 males in order to produce the F2 generation. The F2 generation was examined for the presence of phenotypically wild-type males from individual F1 males. If 10 or more M5 males were present but no wild type male, the F1 female was considered to have carried a lethal mutation in the wild type X-chromosome. A retest was performed when there were fewer than 10 M5 males and no wild- type males.

Results

Altogether 5088 chromosomes were tested for "lethal"-carrying chromosomes in the negative controls and 4299 chromosomes after treatment. The latter number is too limited to detect small mutagenic effects. Consequently, this sex-linked recessive lethal test in *Drosophila melanogaster* did not significantly demonstrate that p-aminophenol has genotoxic properties.

Moreover, the material does not indicate any marked mutagenic effect of p-aminophenol in this test in comparison to the somatic mutation and recombination test which is discussed next.

Conclusion

Under the experimental conditions used, p-aminophenol was not genotoxic in this sex-linked recessive lethal test in *Drosophila melanogaster*.

Ref.: 84

Comment

The data are from a publication in the open literature. The test has only limited value and can only be used for confirmation purposes. The purity and batch nr are not given, a concurrent positive control was not included. The test was not conducted in compliance with GLP or OECD guidelines.

Somatic mutation and recombination test in *Drosophila melanogaster* (SMART)

Guideline: /
Species: *Drosophila melanogaster*; females *mwh/mwh*, males *flr³/TM3*, *Ser*
Test substance: p-aminophenol
Batch nr.: /
Vehicle: feed
Purity: /
Dose levels: both trial 1 and 2, 20 mmol/l
GLP: /
Date: publication from 1990

p-Aminophenol was assayed for somatic mutation and recombinations in *Drosophila melanogaster*. About 150 females and a small surplus of males were mated for at least 1 day in flasks with ordinary culture medium and thereafter transferred to new flasks with agar covered with yeast suspension. Oviposition took place for 8 h. Larvae were flushed from the flasks 72 h after oviposition and were divided into test vials (50/vial). The larvae were allowed to feed in the vials until pupation and remained there until emergence. Wings from surviving adult flies were stored in 70% alcohol. A similar number of male and female wing-carrying slides were evaluated. In both trials the *mwh/flr3* genotype was the main subject of analysis; however, in trial 1 *mwh/TM3*, *ser* wings were studied as well. Three categories of spot types were recognised: small single spots (with 1 or 2 affected cells), large single spots (with 3 or more affected cells) and twin spots (containing both *mwh* and *flr* cells).

Result

In both trials, a large number of twin spots was found after treatment indicative for a very potent recombinogenic activity of p-aminophenol. In addition, a significant increased frequency of *mwh* single spots, both small and large, was found on wings of both genotypes. A major proportion of these spots is the consequence of somatic recombination indicating that p-aminophenol has a relative high recombinogenic potential in *Drosophila*. The results of this somatic mutation and recombination test demonstrate that p-aminophenol has genotoxic properties.

Conclusion

Under the experimental conditions used, p-aminophenol was genotoxic (recombinogenic) in this somatic mutation and recombination test in *Drosophila melanogaster*.

Ref.: 83

Comment

The data are from a publication in the open literature. The test has only limited value and can only be used for confirmation purposes. The purity and batch nr are not given; a concurrent positive control was not included. The test was not conducted in compliance with GLP or OECD guidelines.

3.3.7. Carcinogenicity**Taken from SCCP/0867/05****In vitro cell transformation**

p-Aminophenol has induced morphological transformation of Syrian hamster embryo cells (A), but did not transform the C3H/10T1/2 mice cell line (B).

Animal studies

Guideline: OECD Guideline 451
Species/strain: Sprague-Dawley rats
Test substance: p-aminophenol suspended in 0.5 w/w aqueous carboxymethylcellulose
Batch: 2070155, purity stated 99% ± 0.5%
Concentrations: 2, 5, 12 and 30 mg/kg bw/d administered daily by gavage
GLP: in compliance

Sprague-Dawley rats (approximately 6 weeks old), groups of 50 males and 50 females received p-aminophenol daily by gavage, at dose levels of 0, 2, 5, 12 and 30 mg/kg bw/d for at least 101 weeks. The dose levels selected on the basis of results of a previous 13-week toxicity study where daily oral doses of 30 and 100 mg/kg bw/d produced minimal to marked tubular nephrosis. The dose level of 30 mg/kg bw/d was considered to be the MTD. The test substance was administered as a suspension in 0.5% w/w aqueous carboxymethylcellulose.

The study was scheduled for at least 104 weeks, however, since the survival in the control groups was only 26% in males and 32% in females in week 101, it was decided to terminate the study and all surviving animals were killed. Representative organs were weighed and the animal submitted to microscopic necropsy observations. No major difference from the controls was noted in the mean bodyweight of treated males and females or in the survival except for a lower survival of the high dosed females (20% compared to 32% at week 101). Orange coloured urine was noted from week 10 in almost all the animals given 30 mg/kg bw/d.

The number of animals with more than one primary neoplasm and the number of benign and malignant tumours were comparatively similar in all groups including the control group, except for a marginal increase in the number of malignant lymphoma in males given 30 mg/kg bw/d (3 cases of heterogeneous malignant lymphoma compared to 1 in the control group and 1 in the low dose group). It was concluded that the test substance showed neither a carcinogenic potential nor an effect on the incident of spontaneously occurring tumours at any dose level.

Ref.: Col. 93

Dermal study

A two-generation reproduction study, which included a chronic toxicity-carcinogenicity study, was conducted with Sprague-Dawley rats receiving topical applications of six oxidative hair- colouring formulations. p-Aminophenol (1.0%) was present together with 2,5-diaminotoluene sulphate (6.0%) in one of the formulations tested. Each formulation was mixed with an equal volume of 6% hydrogen peroxide prior to application. In the reproduction study, p-aminophenol at a concentration of 1% in the formulation was applied twice weekly throughout the growth, mating, gestation and lactation phases of the F0 parents to the weaning of the F1a and F2b litters. Weanlings selected from the F1a litters were the subjects for the lifetime carcinogenesis study. For 24 months, they received topical administrations of the formulation containing p-aminophenol twice weekly.

Five animals/sex/group were killed at 12 months; the remainder of the animals were killed at 24 months. All were necropsied; their tissues were subjected to histological evaluation. No compound-related increases in neoplasms were observed. It is noted that 2,4-diaminoanisole (2 and 4%) was also negative in the experiments.

Ref: Col. 72

Mice

Swiss Webster mice (8 – 10 weeks old), groups of 50 males and 50 females, were painted weekly for 21 or 23 months. The study consisted of 12 treatment groups and 3 negative control groups. Nine oxidative hair dye formulations and 3 semipermanent hair dye formulations were studied. The 9 oxidative hair dye formulations were mixed with an equal volume of 6% hydrogen peroxide just before use and applied within 15 min after mixing. A 0.05 ml sample was used for application. p-Aminophenol was present in 3 of the oxidative hair dye formulations in concentrations of 0.04, 0.2 and 1.0%. Animals found dead or sacrificed in moribund conditions or at termination of the study were necropsied and evaluated histopathologically. Comparison of incidence of tumours and of non-tumour pathology among the various treatment and control groups revealed no biologically significant differences. The authors state that toxicological and carcinogenic effects were not induced by the hair dye formulations. It is noted that 2,4-diaminoanisole (2% and 4%) was also negative in the experiments.

Ref.: Col. 8

The composition of the formulations used was given in a separate publication.

Ref.: Col. 7

Swiss mice (8 weeks old), groups of 60 males and 60 females, were painted weekly in the case of 2 oxidative and 3 times weekly in the case of 12 non-oxidative hair dye formulations for 20 months. Aliquots of 0.05 ml were delivered to an area of skin in the interscapular region. The mice were shaved 24 hours before treatment as needed. Two control groups were shaved only and received no treatments. The oxidative dye solutions were mixed with an equal volume of 6% H₂O₂ just prior to application. One of the oxidative hair dye formulations contained 1.5% p-aminophenol. A gross necropsy was performed on all mice. The application of hair dyes did not have an adverse effect on average body weight gains or survival of any group. Body weights were not depressed more than 10% in any group compared with the controls. No unusual tumours developed in any of the groups. Significant increases in malignant lymphomas over those in control group 2 (12%; 7/60) were observed in 3 treated groups of females (p-aminophenol group [32%; 19/60], a non-oxidative hair dye group [30%; 18/60], a non-oxidative hair dye group [38%; 23/60]. The authors state that the observed increases were possibly due to a low control value in control group 2. The percentage of animals with malignant lymphomas in control group 1 was 22% [13/60]. Moreover, the average in 3 previous control groups was 33%. Otherwise, no increased tumour frequencies were found. It is noted that both the oxidative hair dye formulations contained p-phenylenediamine and one contained 4-amino-2-nitrophenol. Two of the non-oxidative hair dye formulation contained Disperse Blue 1.

Ref: Jacobs et al., 1984

Comments

An oral rat study with p-aminophenol has been performed according to OECD Guideline No. 451. The mortality in the control group was very high. The study was negative.

One dermal study with p-aminophenol has been performed with rats and 2 dermal studies have been performed with mice. They were all negative. It should be noted that several hair dye formulations were tested in the dermal studies and some of the formulations contained

substances classified as carcinogenic either by EU or the German MAK commission (2,4-diaminoanisole, EU carcinogen category 1B; Disperse Blue 1, EU carcinogen category 1B; p-phenylenediamine, MAK commission carcinogen category 3B; 4-amino-2-nitrophenol, MAK commission carcinogen category 3B). This indicates that the sensitivity of the dermal carcinogenicity studies has not been sufficient to identify possible carcinogenic effects of hair dye formulations. No conclusions regarding carcinogenicity can be drawn from these studies.

3.3.8. Reproductive toxicity

Taken from SCCP/0867/05

In vitro Reproductive Toxicology

In an *in vitro/in vivo* Hen's egg test, 20 ng to 50 mg of p-aminophenol dissolved in egg albumen were onto the egg's chorion allantois membrane during preincubation or on the fifth day of incubation. All nonviable embryos and hatched chicks were examined for gross abnormalities and other signs of toxicity. p-Aminophenol induced dose-related mortality, with a median lethal dose of 18.6 mg/egg (~170 ppm) on day 1 and 10.5 mg/egg (~20 ppm) on day 5. Developmental retardation and significant variations in blood chemistry were observed up to 25 mg/egg. Hatched chicks had dose-dependent increases in absolute and relative heart weights. The No Effect Level for the study was considered to be between 1 and 5 ppm.

Ref.: C27

Peri/postnatal reproductive toxicity

A single dose of 0, 100, 333, 667, or 1000 mg/kg bw p-aminophenol was administered by oral gavage to pregnant female Sprague-Dawley rats on day 11 of gestation. Maternal body weight was significantly reduced after 24 and 72 hours in animals receiving 667 or 1000 mg/kg bw p-aminophenol. Associated with this maternal toxicity were perinatal loss at 1000 mg/kg bw, decreased pup weight on days 1 and 6 postpartum, and tail abnormalities and/or paralysis of the hind limbs in 50% of the pups at 667 mg/kg bw and 37.5% of the surviving pups at 1000 mg/kg bw.

Ref.: Col. 71

3.3.8.1. Two generation reproduction toxicity

Multi-generation reproduction toxicity

A multi-generation reproduction study was performed using three oxidative hair dye formulations, one of which was a mixture of 1.0% p-aminophenol and 0.7% meta-aminophenol (MAP). Freshly prepared formulations were mixed with equal volumes of 6% hydrogen peroxide and applied twice weekly to the clipped back and neck areas of groups of 40 male and 40 female Charles River CD rats. The initial dose was 0.2 ml per application; this was increased incrementally by 0.1 ml weekly to a dose of 0.5 ml per application. Three control groups were clipped regularly but received no other treatment. Treatment was administered through the growth, mating, gestation and lactation periods through the weaning of the F1B, F2B, and F3C litters of the respective generations. Selected animals from the F1B and F2B litters were used as parents for the F2 and F3 generations, respectively. There were no compound-related findings observed in any generation of the study.

Ref.: C48

In a combination reproduction/chronic toxicity/carcinogenicity study in Sprague-Dawley rats, p-aminophenol mixed with hydrogen peroxide (final concentration: 1% p-aminophenol) was applied topically twice weekly throughout the growth, mating, gestation and lactation phases of the F0 parents to the weaning of the F1a and F2b litters. Fertility, gestation, foetal viability indices, and foetal body weights were evaluated and compared with controls.

No adverse effects on fertility of males or females, or on gestation, lactation or weaning indices were observed.

Ref.: Col. 72

3.3.8.2. Teratogenicity

Groups of pregnant female Charles River CD rats were treated with topical applications of three hair dye formulations containing 0.04%, 0.2%, or 1% of p-aminophenol on days 1, 4, 7, 10, 13, 16, and 19 of gestation. The formulations were mixed 1:1 with 6% hydrogen peroxide just prior to application to mimic normal use. No compound-related effects were observed in this study.

Ref.: C7

p-Aminophenol dissolved in distilled water was administered by gavage at doses of 0 (vehicle control), 25, 85, or 250 mg/kg bw/day to pregnant female Sprague-Dawley rats from day 6 to day 15 of gestation. Vitamin A (15 mg/kg bw/day) was administered by gavage as a positive control. A reduction in body weight gain was associated with skeletal malformations, anophthalmia and hydrocephalus at 250 mg/kg bw/day. Reduced body weight gain was also observed at 85 mg/kg bw/day, but no embryotoxic or teratogenic effects were observed at this dose level. The No Effect Level of the study was 25 mg/kg bw/day.

Ref.: C38

Syrian Golden hamsters were treated with p-aminophenol in acidified isotonic saline on day 8 of gestation using three different routes of administration. Animals treated using intraperitoneal injection received a single dose of 100, 150, or 200 mg/kg bw. Those treated using intravenous injection received a single dose of 100, 150, 200, or 250 mg/kg bw. Hamsters treated using oral gavage were administered 100 or 200 mg/kg bw. Control animals received saline alone. A comparison was made between the administration of a fresh p-aminophenol solution and solutions made 1, 2, or 4 weeks prior to administration. No difference was observed related to the age of the solution administered. Animals treated using intraperitoneal or intravenous injection, were observed to have a significant increase in the frequency of litters with one or more malformed fetuses. No developmental anomalies were observed in litters from animals treated orally.

Ref.: C41

25 female Sprague-Dawley rats received concentrations of 0.07, 0.2, or 0.7% p-aminophenol in the diet for 13 weeks (corresponding to a daily intake of approximately 35, 100 or 350 mg/kg bw/day). They were then mated with untreated males. Pregnant females were once again fed the p-aminophenol -containing diet until day 20 of gestation, when they were killed. On day 0 of gestation, the body weights of animals in the 0.2 and 0.7% dose groups were lower than those of controls. From day 0 to day 20 of gestation, animals in the 0.7% dose group had significantly reduced body weight gain. Dose-related postimplantation loss was observed at 0.2 and 0.7%; this was significant at the dose level of 0.7%. No teratologic effects were observed, but some skeletal variations secondary to maternal toxicity were seen at 0.2 and 0.7% p-aminophenol.

Ref.: Col. 70

Twelve male and female rats per group were given 4-aminophenol (PAP) by gavage at 0, 20, 100, or 500 mg/kg bw/day. Males were dosed for a total of 49 days, beginning 14 days before mating. Females were dosed for a total of 40–60 days, from 14 days before mating to Day 3 of lactation throughout the mating and gestation periods. Four males and 2 females died at 500 mg/kg bw/day, and all surviving males and females showed brown urine at 100 mg/kg bw/day and above. Body-weight gain was lower in males and females at 500 mg/kg bw/day, and food consumption was decreased in males at 500 mg/kg bw/day and in females at 100 and 500 mg/kg bw/day. Absolute and relative weights of the testes and epididymides were decreased at 500 mg/kg bw/day. Histopathological examinations revealed decreased spermatocyte and spermatid levels in the testis, debris of germ cell in the epididymis lumen, basophilic tubules in the kidney, and deposits of hemosiderin in the red pulp and extramedullary hematopoiesis in the spleen in males at 500 mg/kg bw/day. Longer gestation period, decreased delivery index, and lower body weight of pups on postnatal day (PND) 0 and increased number of stillborns at 500 mg/kg bw/day were also observed. At this dose, the viability of pups on PND 4 was decreased markedly. No adverse effects on reproduction or development were detected at 20 and 100 mg/kg bw/day.

Ref.: Harada, 2008

Conclusion on developmental and reproductive toxicity

Despite not all studies are in compliance with acknowledged methodology it can be stated that p-aminophenol is general and reproductive/developmental toxic, but is unlikely to be teratogenic. It showed effects on fertility or on gestation, embryonic development, lactation or weanling indices in experimental animals only at high doses (500 mg/kg bw/day). Teratogenic effects were observed in one study, but only at maternal toxic doses.

3.3.9. Toxicokinetics

Taken from SCCP/0867/05

In vitro metabolism

The rates of secretion of p-aminophenol and its sulphate and glucuronide conjugates were determined in cultures of rat hepatocytes, using aniline and p-aminophenol as substrates. When hepatocytes were incubated with inorganic sulphate and 1 mM aniline, secretion of p-aminophenol or its conjugates was linear over a period of two hours. With p-aminophenol as the substrate (concentration not indicated), free p-aminophenol disappeared from the medium almost completely within 30 minutes. The secretion of conjugates was linear for 30 minutes only. There was a characteristic lag in glucuronide secretion.

Ref.: C16

Glucuronidation in cultured human skin epithelial cells, human skin fibroblasts, or homogenates from these cells was examined using 0.2 - 0.5 mM p-aminophenol mixed with ascorbic acid (to prevent auto-oxidation). Epithelial cells and the homogenate from these cells glucuronidated p-aminophenol at a rate of about 5 nmol/mg cell protein per hour. Skin fibroblasts did not glucuronidate p-aminophenol.

Ref.: C 40

A clonal strain of rat hepatoma cells (MH1C1) was used to study the metabolism of p-aminophenol in vitro. Concentrations of p-aminophenol ranging from 0.25 mM to 0.75 mM were incubated with hepatoma cells for up to 5 hours. The p-aminophenol -glucuronide rate vs. time curve did not reach linearity due to a lag which increased with increasing substrate concentrations. The highest rate of p-aminophenol -glucuronide formation was observed at a concentration of 0.5 mM, with a decrease observed at higher concentrations. This indicated the possibility of substrate inhibition of the glucuronidation process.

Ref.: A1

The metabolism of p-aminophenol in pulmonary and renal microsomes from the rat, rabbit and mouse was evaluated in the presence of another aniline metabolite. Results indicated that p-aminophenol (concentration not indicated) was not metabolized by these tissue preparations from any species.

Ref.: Col. 76

The metabolic reactivity (measured by covalent binding) of 60 µM p-aminophenol to microsomal proteins in human, rat, and mouse livers was compared to that of 1m M paracetamol (APAP: N-acetyl-p-aminophenol or acetaminophen or paracetamol) in vitro. p-Aminophenol was observed to have higher intrinsic reactivity than APAP, regardless of the species considered. Biotransformation of both substances occurs via the cytochrome P450 system, with the exception of only 50% involvement of P450 in the transformation of p-aminophenol in the mouse and no involvement of P450 in the human biotransformation of this compound. Based on the results of this study, the sensitivity to the potential electrophile generation and alkylation of p-aminophenol is greatest in the mouse, intermediate in the rat, and least in the human.

Ref.: Col. 77

The *in vitro* metabolism of 100 µM p-aminophenol by phase I enzymes in the presence of human, rat and mouse hepatic microsomes was evaluated. Incubation with glutathione was performed to identify electrophilic metabolites. Six principal metabolites were isolated – two were common to all three species, one was common to the rat and the mouse and one was shared by the rat and the human. One metabolite was specific to the rat and one to the mouse. Quantitatively, the biotransformation of p-aminophenol over a 15 minute period was most extensive in the human, intermediate in the rat, and least extensive in the mouse. The metabolites specific to the rat and mouse were electrophilic in nature, while those shared by all three species and the one common to the rat and human were not.

Ref.: Col. 78

The interspecies comparison of the *in vitro* metabolism of 100 µM p-aminophenol in the presence of human, rat, and mouse hepatocytes was evaluated. Four metabolites, common to all three species, were identified as glucuronides and sulphates of p-aminophenol and of APAP. A fifth metabolite, APAP, was identified only in human hepatocytes. These metabolites demonstrate that p-aminophenol is metabolized by phase II enzyme reactions. Biotransformation was observed to be most extensive in mouse hepatocytes, and less extensive, but comparable, in rat and human hepatocytes. Covalent binding (evidence of phase I metabolism) occurred in the same pattern among the three species evaluated. Mouse hepatocytes were observed to have a deficiency in sulfotransferase activity compared to rat and human hepatocytes, as well as a greater capacity to form reactive electrophilic metabolites.

Ref.: Col. 79

p-Aminophenol and ¹⁴C-p-aminophenol were tested in the Episkin® model of reconstructed human epidermis at a concentration of 100 µM for a 24-hour period to determine the capacity of the epidermis to metabolize the compound. Episkin® kits were obtained from a commercial supplier. After exposure of the cultures to the test compound, the culture medium was decanted and protected from auto-oxidation by the addition of ascorbic acid. The surface of the skin cultures was rinsed in PBS containing ascorbic acid. The medium, PBS rinse, epidermal cells and their collagen matrices were then frozen in liquid nitrogen and preserved until they were to be analyzed. Aliquots of culture medium were prepared for evaluation of the level of radioactivity and chromatographic analysis. The collagen matrices were extracted with methanol, and these extracts were evaluated for radioactivity. The epidermal cells were homogenized in PBS; aliquots were taken for chromatographic analysis and for determination of the levels of radioactivity, of protein inextractable radioactivity and covalent binding. The PBS rinse was also evaluated for radioactivity.

The results indicated that the epidermis quantitatively transforms p-aminophenol into APAP via N-acetylation (a phase II reaction). Virtually all the p-aminophenol applied to the skin culture (98%) was biotransformed. Only APAP was identified as a metabolite; no other metabolites were found. The biotransformation was virtually complete after 24 hours of contact. The formation of reactive electrophiles, measured by the level of covalent binding to tissue proteins, was low (0.3 ± 0.02 nmol/mg); indicating that Episkin® has limited capacity to metabolize p-aminophenol using phase I enzymes.

From the results of this study, it was concluded that exposure of Episkin® to p-aminophenol results in complete biotransformation to APAP.

Ref.: Col. 80

In vivo-toxicokinetics, human

In a human metabolism study, one subject received 200 mg and two others received 500 mg p-aminophenol hydrochloride.

The following results were obtained:

Subject	PAP (dose in mg)	PAP in Blood (mg/100 ml)					
		Unconjugated		N-acetyl-PAP		Total	
		0.5 hours	1 hour	0.5 hours	1 hour	0.5 hours	1 hour
1	200	0	0	–	–	0.41	–
2	500	0.09	0.012	0.56	0.45	0.84	0.96
3	500	0.12	0.016	0.56	0.26	0.98	0.69

The route of administration was not indicated.

Ref.: A2

In vivo toxicokinetics, rodents

Doses of 91 or 182 mg/kg bw aqueous p-aminophenol with Arabica gum were administered once orally to mice, and blood levels of p-aminophenol and its metabolites were evaluated 0.5, 1.0, 1.5, 2.0, and 2.5 hours after dosing.

The following results were obtained:

Dose (mg/kg bw)	PAP (µg/ml)	Time after Dosing (hours)				
		0.5	1.0	1.5	2.0	2.5
91	free	10	6	3	1	0.5
	conjugate	14	9	4	3	0.5
	total	24	15	7	4	1
182	free	18	10	6	3	1
	conjugate	28	27	13	6	2
	total	46	37	19	9	3

The nature of the conjugate was not identified.

Ref.: A2

Rabbits (2-3 kg in weight) were administered 1 g/animal p-aminophenol in aqueous solution by gavage, and urine levels of p-aminophenol and its metabolites were examined. The collection time(s) were not specified. 2% of the p-aminophenol was excreted unchanged. Metabolites consisted of 25% acetaminophenol, 8% aminophenylsulphate, 4% acetaminophenylsulphate, 45% aminophenylglucuronide, and 16% acetaminophenylglucuronide. Acetaminophenol and its conjugates were major metabolites of p-aminophenol. 100% of the administered compound was accounted for.

Ref.: A2

Rats (200 g) received a single dose of either 200 mg/kg bw glucuronic acid or 200 mg/kg bw glucuronamide by gavage, followed one hour later by a single gavage dose of 300 mg/kg bw p-aminophenol. Urine collected 24 hours later contained increased p-aminophenol and glucuronidated p-aminophenol compared to controls. Glucuronamide was found to be a more effective conjugating agent than was glucuronic acid.

Ref.: A2

The blood and plasma pharmacokinetics and metabolites of ^{14}C -p-aminophenol were studied *in vivo* after a single cutaneous administration (15 mg/ml ^{14}C -p-aminophenol) to female Wistar rats. Twelve rats received the test substance in a single topical administration of 12.5 mg/kg bw (18.5 MBq/kg bw; 5 mg/cm²) over 12.5% of the body surface area for a total exposure period of 24 hours. During exposure, the application site was covered with an occlusive plastic film that was held in place using light bandages. Blood samples were collected from two rats per time point at 0.5, 1, 2, 4, 8, and 24 hours during the exposure period. Plasma samples were obtained from each blood sample. Blood and plasma samples were analyzed for total radioactivity, and selected plasma samples were analyzed for metabolic patterns by HPLC/UV radioactivity determination. Metabolites were identified by comparison of peak retention times with those of known standards before and after enzyme hydrolysis.

The radioactivity in the plasma and blood samples increased from time zero to the C_{max} (498 and 313 ng-eq/g, respectively) at 4 hours after application of p-aminophenol. It then decreased until the last quantifiable time point at 24 hours (the end of the exposure period), when it was found to be 139 ng-eq/g for plasma and 106 ng-eq/g for blood.

The principal pharmacokinetic parameters (calculated using non-compartmental methods) are presented below:

Sample	t _{1/2z} (h)	λ _z (1/h)	C _{max} (ng-eq/g)	t _{max} (h)	AUC _{0-24h} (ng-eq h/g)	AUC _{0-∞} (ng-eq h/g)
Plasma	5.95	0.0626	498	4	7038	9271
Blood	4.79	0.0523	313	4	4567	6782

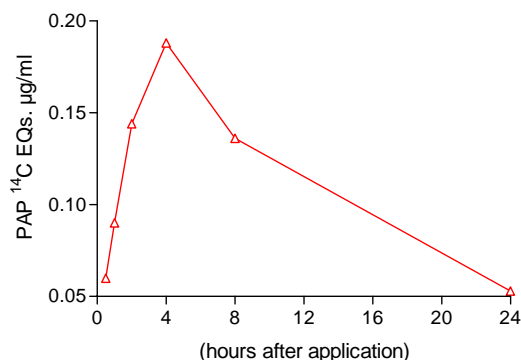
The parent compound, p-aminophenol, was undetectable in all plasma samples. Three peaks were detected in the plasma, corresponding to APAP, the glucuronic acid conjugate of APAP (shortest retention time) and the sulphate conjugate of APAP (longest retention time) (see table below). The limit of quantification was 1.04 ng-eq/g in plasma and 0.99 ng-eq/g in blood.

Time (h)	Animal No.	PAP (%)	Glucuro-APAP (%)	APAP (%)	Sulpho-APAP (%)
2	W20855	-	-	30.0	70.0
	W20856	-	-	30.0	70.0
4	W20853	-	-	45.0	55.0
	W20854	-	13.4	27.6	59.0
8	W20851	-	17.7	35.4	46.9
	W20852	-	11.7	39.8	48.5

From these results, it was concluded that p-aminophenol was not present in the plasma of rats during 24 hours of continuous exposure. All detectable radioactivity consisted of APAP and/or its metabolites.

Figure 1: Blood concentration (^{14}C -equivalents) in rats treated topically (12.5% of the body surface) for 24 hours with a single dose 12.5 mg/kg bw ^{14}C -p-aminophenol.

Opinion on p-aminophenol



The area under the curve from 0 to 12 hours reflecting the quantitative systemic exposure of the organism to APAP and/or its metabolites during this time period was calculated to be $AUC_{0-12hrs} = 1.09 \mu\text{g/ml} \times \text{hours}$ (Ref. KM3).

The area under the curve from 0 to 24 hours reflecting the quantitative systemic exposure of the organism to APAP and/or its metabolites during this time period was calculated to be $AUC_{0-24} = 7.04 \mu\text{g/ml} \times \text{hours}$ (Ref. Dressler table 2).

The area under the curve from 0 to infinite reflecting the quantitative systemic exposure of the organism to APAP and/or its metabolites during this time period was calculated to be $AUC_{0-\infty} = 9.27 \mu\text{g/ml} \times \text{hours}$ (Ref. Dressler table 2).

In vivo toxicokinetics, mini-pig

The blood and plasma pharmacokinetics and metabolites of ¹⁴C-p-aminophenol were studied *in vivo* after a single cutaneous administration (15 mg/ml ¹⁴C-p-aminophenol) to a single female Göttingen mini pig. The pig received the test substance in a single topical administration of 4.7 mg/kg bw (18.5 MBq/kg bw; 5 mg/cm²) over 12.5% of the body surface area for a total exposure period of 24 hours. During exposure, the application site was covered with an occlusive plastic film that was held in place by self-adhesive bandages and a body stocking fitted with a collar. Blood samples were collected pretreatment and at 0.5, 1, 2, 4, 6, 8, 12 and 24 hours during the exposure period. Additional samples were taken at 48 and 72 hours after application. Plasma was obtained from each blood sample. Blood and plasma samples were analyzed for total radioactivity, and selected plasma samples were analyzed for metabolic patterns by HPLC/UV radioactivity determination. Metabolites were identified by comparison of peak retention times with those of known standards.

The radioactivity in the plasma and blood samples increased from time zero to the C_{max} (11.70 and 9.23 ng-eq/g, respectively) at 12 hours after application of p-aminophenol. It then decreased until the last quantifiable time point at 72 hours post-application, when it was found to be 2.24 ng-eq/g for plasma and 2.80 ng-eq/g for blood. The limit of quantification was >1.78 ng-eq/g (7.0 Bq/g) in plasma and >1.25 ng-eq/g (4.9 Bq/g) in blood.

The principal pharmacokinetic parameters (calculated using non-compartmental methods) are presented below:

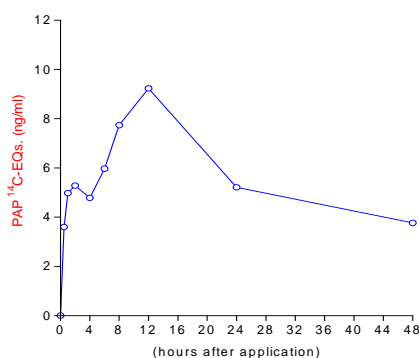
Sample	t _{1/2z} (h)	λ _z (1/h)	C _{max} (ng-eq/g)	t _{max} (h)	AUC _{0-24h} (ng-eq h/g)	AUC _{0-∞} (ng-eq h/g)
Plasma	31.3	0.021	11.70	12	389	490
Blood	53.6	0.013	9.23	12	350	566

A minor secondary peak, of unknown origin, was noted at 2 hours in both plasma and blood.

The levels of radioactivity in the plasma were very low, and thus only a single interpretable metabolic pattern for the 6 hour time point could be obtained. The one peak that was observed upon analysis for metabolites in the plasma was identified as APAP. The parent compound, p-aminophenol, was undetectable in the plasma.

The following figure shows the corresponding value for the pig treated topically with ^{14}C -p-aminophenol:

Figure 2: Blood concentration (^{14}C -equivalents) in a mini pig treated topically (12.5% of its body surface) for 24 hours with a single dose 4.7 mg/kg bw ^{14}C -p-aminophenol.



The area under the curve from 0 to 12 hours reflecting the quantitative systemic exposure of the organism to APAP during this time period was calculated to be $\text{AUC}_{0-12\text{hrs}} = 0.07 \mu\text{g/ml} \times \text{hours}$ (Ref. KM3).

The area under the curve from 0 to 72 hours reflecting the quantitative systemic exposure of the organism to APAP and/or its metabolites during this time period was calculated to be $\text{AUC}_{0-72} = 0.39 \mu\text{g/ml} \times \text{hours}$ (Ref. Dressler).

The area under the curve from 0 to infinite reflecting the quantitative systemic exposure of the organism to APAP and/or its metabolites during this time period was calculated to be $\text{AUC}_{0-\infty} = 0.49 \mu\text{g/ml} \times \text{hours}$ (Ref. Dressler).

From these results, it was concluded that p-aminophenol was not present in the plasma of the pig during 24 hours of continuous exposure. Only its metabolite, APAP could be detected.

Ref.: Col. 97

Conclusion on toxicokinetics

Topically applied p-aminophenol results in systemic exposure only to APAP and/or its metabolites in both humans and animals (SCCS/1409/11). Consequently, the systemic toxicity of p-aminophenol is mainly related to the toxicity of APAP.

3.3.10. Photo-induced toxicity

No data submitted

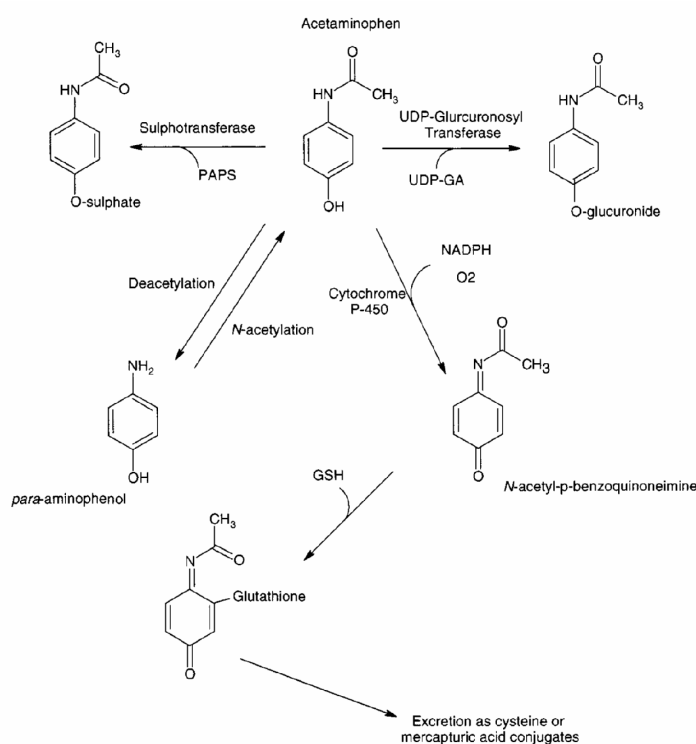
3.3.11. Human data

No data submitted

3.3.12. Special investigations

Dose-dependent metabolism of APAP

The first-pass metabolism of APAP may be dose-dependant and inducible (Prescott, 1980). APAP is largely cleared from the blood through biotransformation by hepatic microsomal enzymes. Normally 80% of a dose of APAP is conjugated, predominantly with glucuronic acid and to a lesser extent with sulphuric acid. These conjugated metabolites lack biological activity (Koch-Weser, 1976). A small percentage (<5%) of acetaminophen is deacetylated to p-aminophenol or oxidized by cytochrome P450 enzymes to the reactive metabolite N-acetyl-p-benzoquinoneimine (NAPQI). NAPQI is short lived as it binds to glutathione (GSH) and is then excreted in the urine as cysteine or mercapturic conjugates. After an excessive dose of APAP, the primary metabolic pathway is not able to process the entire drug. The cytochrome P450 isozymes then must metabolize more APAP, resulting in increasing accumulations of NAPQI. When these levels deplete glutathione stores, the toxic NAPQI then is available to bind to the hepatocytes, leading to cell death and acute liver failure, which can develop within 24 to 36 hours after ingestion of excessive amounts of APAP.



From Mc Conkey et al., 2009

Investigations with p-aminophenol

Induction of Methemoglobinemia

Several studies have examined the formation of methemoglobin after administration of p-aminophenol. A median lethal dose value of 470 mg/kg bw was obtained in a subcutaneous study in mice. A subcutaneous dose of 37 mg/kg bw p-aminophenol caused severe clinical signs and methemoglobinemia in cats, with death occurring within 30 minutes. The same dose caused similar observations in dogs, but no death; in rabbits, this dose produced no methemoglobin formation. In a separate study in cats, subcutaneous administration of 6 mg/kg bw induced a level of 37.5% methemoglobin in the blood 3.5 hours after exposure.

Normal and glucose-6-phosphate (G-6-P)-deficient human erythrocytes (35% suspension in isotonic saline, pH 7.4) were incubated with 0.001 M p-aminophenol for two hours at 37 °C. While the concentration of glutathione was not altered in G-6-P-deficient cells, red blood cell fragility and methemoglobinemia were significantly increased.

p-aminophenol was added to freshly drawn human blood in varying concentrations and the amount of methemoglobin generated was measured. At 0.1 and 0.5 mg%, no methemoglobin was detected. At 1 mg%, one blood sample was found to contain 3% methemoglobin, but another contained none. At 5 and 10 mg%, between 13.2% and 21.2% methemoglobin was found in all samples.

Ref.: A2

Human foetal and adult haemoglobin samples were incubated in 0.01 M Bis-Tris buffer in the presence of 0.5, 1, or 2M p-aminophenol per mole of haemoglobin tetramer. Under the conditions of this study, human foetal haemoglobin was more susceptible to methemoglobin formation than was adult methemoglobin.

Ref.: C52

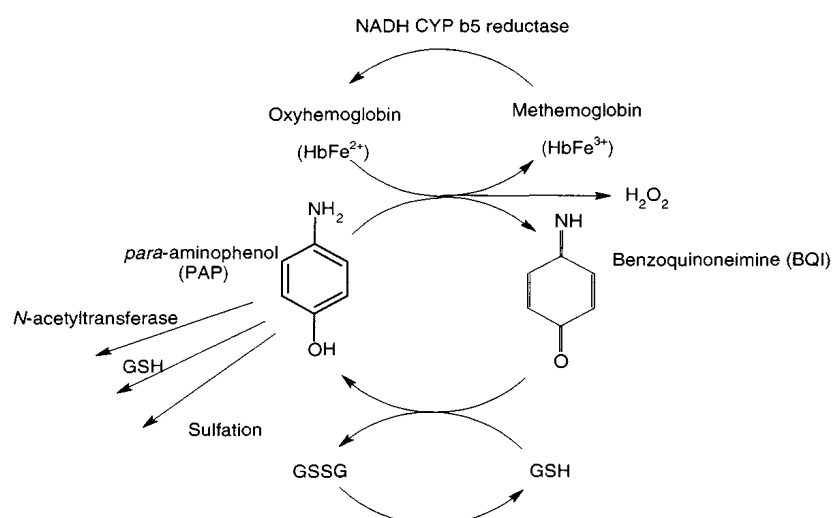
p-Aminophenol (0.5 mM in 0.2 M phosphate buffer) was found to produce methemoglobin more rapidly in purified human haemoglobin than in washed red blood cells. The investigators stated their belief that p-aminophenol binds covalently to haemoglobin.

Ref.: C15

Japanese quail received a single intraperitoneal injection of 1, 5, 10, 25 or 50 mg/kg bw p-aminophenol dissolved in water. The highest observed concentration of methemoglobin (9%) was observed after administration of 50 mg/kg bw p-aminophenol; no p-aminophenol was detectable in the blood after 30 minutes.

Ref.: C3

Recently, McConkey *et al.* (2009) have shown that p-aminophenol rather than APAP or NAPQI induces methemoglobinemia in dogs and cats. It induced more methemoglobin formation in dog and cat than rat and mouse erythrocytes. The half-life of p-aminophenol is < 5 min in the rat largely because of efficient N-acetylation. Rats and mice have 3 NAT enzymes, cats only one and dogs completely lack NAT enzymes. Dogs and cats likely experience hematotoxicity instead of hepatotoxicity because of the greater accumulation of systemic p-aminophenol in the absence of effective N-acetylation.



From McConkey *et al.* (2009)

Melanocytotoxicity

C57B1/6J black mice (7-days or 5-weeks of age) were injected with a single dose of p-aminophenol to determine whether or not it would induce depigmentation. p-Aminophenol dissolved in water was injected subcutaneously at a dose of 400 mg/kg bw or intraperitoneally at a dose of 50, 100, 200, or 400 mg/kg bw. Dose-related depigmentation was observed in mice injected intraperitoneally. Melanocytotoxicity was observed at 12, 24, 48, and 96 hours post administration in histopathologic preparations from mice injected subcutaneously.

Ref.: C5

Nephrotoxicity

Male Fischer 344 rats were administered subcutaneous injections of 25, 50, 100, or 200 mg/kg bw in water. Urine was collected for 24 hours after compound administration. Animals were killed 24 or 48 hours after compound administration and blood was collected for analysis for alanine aminotransferase (ALT) and blood urea nitrogen (BUN). The kidneys were collected and thin cortical slices were incubated in the presence of p-Aminohippurate (PAH) and ¹⁴C Tetraethylammonium (TEA). The accumulation of these substances was evaluated. Changes in renal function were observed: BUN was detected and accumulation of PAH and TEA was reduced in renal slices. No effect on hepatic function was noted.

Ref.: C32, C33

The nephrotoxicity of p-aminophenol was evaluated in male Fischer 344 rats. Three groups of rats received 0, 150, or 300 mg/kg bw p-aminophenol via intraperitoneal (IP) injection 30 minutes after pretreatment with bis (p-nitro-phenyl) phosphate or water (100 mg/kg bw) IP. Urine was collected for 24 hours, after which time the rats were killed. Blood was collected for measurement of BUN, kidneys were prepared for histopathology, and urine was evaluated for metabolites. p-Aminophenol caused tubular epithelial necrosis of the kidneys and changes in renal function. It was excreted in the urine as free or conjugated p-aminophenol or as acetaminophen.

Ref.: C34

The influence of metabolic variation on p-aminophenol toxicity was examined in homozygous and heterozygous male Gunn rats as well as albino rats. 0.5 or 1 mm/kg bw was administered intravenously to rats (tail vein); the controls received an injection of normal saline. Rats were killed 48 hours after compound administration. Tubular necrosis was observed in all treated rats, with more severe lesions occurring in the Gunn rats.

Ref.: C6

Morphological and functional studies were performed with p-aminophenol in male Sprague-Dawley rats. In the first study, a single intravenous injection of 400 mg/kg bw p-aminophenol in saline was administered and rats were killed 30, 60, or 90 minutes, or 2 hours after this administration. At necropsy, the kidneys were dark and swollen. Histopathology of the kidneys revealed damage to the proximal tubules, which on electron microscopy was shown to be cytoplasmic oedema with a reduction in the number of organelles. In subsequent studies, renal tubular pressure was measured and urine was collected after infusion of 3.5 mM p-aminophenol into the jugular vein. An isolated kidney perfusion test was also performed. In these studies, the appearance of brown material in the urine preceded a rise in tubular pressure that occurred 35 to 45 minutes after infusion. Renal blood flow did not change. Mean arterial blood pressure fell immediately after injection but stabilized thereafter.

Ref.: C13

In further investigations of nephrotoxicity in rats (strain not specified), intraperitoneal injections of 100 to 400 mg/kg bw p-aminophenol caused functional renal changes that corresponded to damage to the straight portion of the proximal tubules. A dose-dependent

reduction in renal, but not hepatic, glutathione levels was observed, and covalent binding of p-aminophenol was stronger for renal proteins than for hepatic proteins.

Ref.: Col. 95

Oxidation of p-aminophenol has been shown to be an important factor in its ability to induce nephrotoxicity. And ascorbic acid, an antioxidant, has been shown to decrease glutathione depletion and cell death in suspensions of rabbit renal tubular epithelial cells incubated with 0.5 or 1 mM p-aminophenol, as well as in rats dosed simultaneously with ascorbic acid and [ring 3H]- p-aminophenol at a 3:1 molar excess.

Ref.: Col. 94

Additional studies on the nephrotoxicity of p-aminophenol have shown that this compound causes inhibition of mitochondrial respiration, ATPase activity, oxidative phosphorylation, and renal microsomal glucose-6-phosphate activity.

Ref.: C9, C10, C11, C12, C19

Hepatotoxicity

Hepatotoxicity and nephrotoxicity due to p-aminophenol administration were investigated in male C57BL/6 mice and Sprague-Dawley rats. A single intraperitoneal dose of p-aminophenol (dose range: 100 – 700 mg/kg bw) was administered and blood samples were collected for evaluation of serum levels of glutamic pyruvate transaminase, sorbitol dehydrogenase, BUN and creatinine. Liver and kidney tissue samples were collected for histopathologic examination. Rats were dosed up to 400 mg/kg bw (lethal dose); mice were dosed up to 700 mg/kg bw. Significant changes in renal function were observed in both species at 12 hours after p-aminophenol administration, with rats being affected at doses as low as 200 mg/kg bw while mice were affected at doses of 650 – 700 mg/kg bw. Significant changes in liver function were also observed at 12 hours after exposure to p-aminophenol, but only in mice at doses of 400 – 700 mg/kg bw. Rats displayed no alterations in liver enzymes, even at the lethal dose of 400 mg/kg bw.

Ref.: Col. 96

In June 2009 at a special public advisory committee meeting, the FDA refocused its concerns about the potential toxic effects of APAP. Overdoses of APA have been recognized as the primary cause of hepatotoxicity, which can lead to acute liver failure (acute liver necrosis). The number of cases of APAP-related acute liver failure increased from 28% of all acute liver failure cases in 1998 to 51% in 2003 (1600 cases of acute liver failure per year in US between 2000 and 2004).

The ingestion of more than 4g of APAP within 24h is considered a potentially toxic dose for inducing acute liver failure (Guggenheimer and Moore, 2011). However, exposure from hair dyes is much lower and less frequent.

Skin Depigmentation

An aqueous solution of 0.5% p-aminophenol was applied topically to the forearms of 13 volunteers (10 black and 3 white) over a three week period to evaluate the potential for skin depigmentation and or discoloration. 0.15 ml of the test solution was applied to the same site for 3 consecutive days during the first week and for 4 consecutive days during the second and third weeks. Sites were washed by the subjects 1 hour after application of the solution. Three days after the 11th application, the subjects' arms were evaluated for changes in appearance.

There was no evidence of change in the skin of the white subjects. In two black subjects, there was slight darkening of the skin. Slight to moderate skin irritation was also reported in one subject after three to seven applications of p-aminophenol.

Ref.: A1

Immunosuppression

The potential for immunosuppressive activity due to exposure to p-aminophenol was evaluated in NMRI mice. These animals were given four subcutaneous injections of ¼ the maximal tolerated dose. Sheep red blood cells (2×10^8) were administered with the first dose of p-aminophenol. On the fifth day of the study, a plaque test and a hemagglutination test were performed. No compound-related effects were observed.

Ref.: C17

Miscellaneous Studies

The effect of p-aminophenol on the activity of the enzymes delta-aminolevulinic acid synthetase and ferrochelatase in homogenized rat liver was evaluated. After exposure to 0.001 M p-aminophenol, there was a 33% reduction in δ -ALAS activity and a 15% increase in ferrochelatase activity.

Ref.: A2

p-Aminophenol was observed to have antimetabolic activity in the mouse small intestine, spleen and thymus after a single intraperitoneal injection of 0.05 mg p-aminophenol /mouse.

Ref.: A2

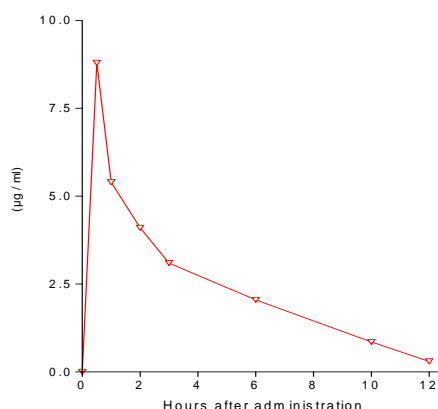
The in vitro cytotoxicity of p-aminophenol was investigated in two cell lines. In 3T3-L1 cells derived from mouse embryo fibroblasts, concentrations tested ranged from 0-1000 $\mu\text{g}/\text{ml}$. A median inhibitory dose of 28 $\mu\text{l}/\text{ml}$ was established. p-Aminophenol was ranked fifth in cytotoxic potential among the 30 substances evaluated. In V79 cells from Chinese hamsters, a median lethal concentration was established as $>1000 \mu\text{g}/\text{ml}$ after a 4-hour incubation and as 6 $\mu\text{g}/\text{ml}$ after 3 days of incubation. p-Aminophenol was ranked fourth in cytotoxic potential among the 27 substances evaluated.

Ref.: A2

3.3.13. Safety evaluation (including calculation of the MoS)

In most cases, the safety evaluation of cosmetic ingredients is based on a comparison of the calculated penetrated amount (systemic exposure dose, SED) with a NOAEL from a repeated-dose animal toxicity study yielding a nominal "margin of safety". The accepted empirical margin of safety is generally 100-fold (Ref.: KM4). In the submitted dossier, the applicant, in addition to the conventional MoS approach, proposes for p-aminophenol to base the assessment on the comparison of toxicokinetics, quantitative exposure data (plasma levels, area under curve) from animal toxicology studies (p-aminophenol applied topically) with respective human data (APAP administered orally) (Ref. KM5). In order to support their proposal they argue that p-aminophenol applied topically results in systemic exposure of APAP as shown in the in vivo toxicokinetic studies above, and the blood levels of APAP in humans after oral administration are well documented. Typical values are shown in the following figure:

Figure 3: Blood concentrations in human volunteers after oral administration of a single dose of 500 mg APAP (2)



The area under the curve from 0 to 12 hours reflects the quantitative systemic exposure of the human organism during this time period to APAP and was calculated to be $AUC_{0-12hrs} = 28.8 \mu\text{g/ml} \times \text{hours}$ (Ref. KM2, Ref. KM3).

Oral administration of 500 milligrams of APAP is recognised to be a safe human dose (Ref. KM1). APAP has been shown to be non-genotoxic in humans who received the maximum therapeutic dose of 3x1000 mg over 8 hours (Ref. KM9). Given that topically applied p-aminophenol results in systemic exposure to APAP and/or its metabolites only, in both humans and animals, the systemic exposure seen in animal studies after topical application may be compared to the systemic human exposure after a single oral dose of the drug APAP (= AUC_{man}).

The proposal from the applicant is to compare the AUC in rats (n = 12) or pig (n = 1), from 0 to 12 hours, at a high dose levels not related to NOAEL/LOAEL (p-aminophenol applied topically) to the AUC in humans following administration of therapeutic dose of APAP considered as safe (500 mg/day). However, the scientific rationale behind this approach is not completely convincing.

Assessment by the SCCS

The safety of a topically applied substance may be assessed by two principal methods: MoS calculation and the toxicokinetics based approach (cf SCCS Notes of Guidance).

The SCCS is of the opinion that for a toxicokinetic-based risk assessment, the AUC from 0 to the infinite in humans exposed at realistic doses of the hair dye should be compared to the AUC of the compound (or its active metabolite) in a sensitive animal species exposed at a dose level close to the NOAEL/LOAEL. If the ratio between both of the AUCs (AUC_{rat}/AUC_{human}) is more than 25, then the safety of the compound may be demonstrated (KM06).

In the case of p-aminophenol, in addition to the conventional MoS calculation, a different approach is used based on comparison of systemic exposures of APAP from hair dyeing to the systemic exposure from therapeutic use.

A) Usual MoS calculation:

Comparison of the calculated penetrated amount (systemic exposure dose, SED) with a NOAEL from a repeated-dose animal toxicity study yielding a nominal "margin of safety". The accepted empirical margin of safety is generally 100-fold.

CALCULATION OF THE MARGIN OF SAFETY

p-Aminophenol

(oxidative conditions)

Absorption through the skin	A (mean + 2 SD)	=	6.9 $\mu\text{g}/\text{cm}^2$
Skin Area surface	SAS	=	580 cm^2
Dermal absorption per treatment	SAS x A x 0.001	=	4.00 mg
Typical body weight of human		=	60 kg
Systemic exposure dose (SED)	SAS x A x 0.001/60	=	0.067 mg/kg bw/d
No observed adverse effect level (90-day, oral, rat)	NOAEL	=	10 mg/kg bw/d

Margin of Safety	NOAEL / SED	=	150
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B) Comparison of systemic exposures of APAP from hair dyeing to the systemic exposure from therapeutic use:

Since topically applied p-aminophenol results in systemic exposure only to APAP and/or its metabolites in both humans and animals (cf above), the toxicity of p-aminophenol is considered to be mainly related to the toxicity of APAP. Therefore, the SCCS considers it appropriate to compare the systemic dose of APAP in human following hair dyeing with p-aminophenol, to the systemic dose of APAP considered as safe for medicine.

APAP is a widely used antipyretic and analgesic drug, also in paediatric use and therefore a large data base on the safety of APAP in human is available. After oral administration of APAP at the dose of 12 mg/kg bw in healthy adults, absorption is very rapid with the mean peak plasma concentration occurring at 15 min; after a short distribution phase, concentrations decline in parallel with those after intravenous injection. Mean systemic availability of APAP calculated from the relative areas under the intravenous and oral plasma-concentration-time curves is 76%.

The therapeutic dose of APAP may be compared to the systematically available level of APAP following a hair dyeing:

Opinion on p-aminophenol

Oxidative Hair Dye: amount applied:	100 ml or 100 g
Concentration of PAP:	0.9%
Amount of PAP applied:	900 mg
Retention factor:	0.1
Amount of PAP available on the scalp:	90 mg
Assuming skin penetration (cf 3.3.4):	7.84%
Amount of PAP systematically available:	7 mg
Assuming 100% metabolism to APAP:	
Amount of APAP systematically available:	9.8 mg *

*considering molecular weights PAP = 109 and APAP = 151

The recommended human therapeutic regimen of APAP consists of multiple daily oral doses, i.e. 500 mg every four to six hours (Ref. KM1) (considering 76% systemically available). On the basis of a common therapeutic regimen of four doses of 500 mg APAP within 24 hours, the actual safety margins may be estimated to be several times higher than the nominal values calculated above.

The expected systemic exposure to APAP from hair dye use is considerably lower than normal therapeutic exposure.

3.3.14. Discussion

This opinion is an overall evaluation of the new submission IV and V and their amendments, but it also takes into account references from previous submissions I, II and III.

Regarding the classification of p-aminophenol within CMR categories in class 2 it was necessary to repeat older studies or to perform entirely new ones; these were reported in submissions IV and V and especially laid down in an appendix, covering references Col. 69-96.

Physico-chemical specifications

p-Aminophenol is an oxidative hair dye precursor. It is incorporated in oxidative hair dye formulations and in the bottle on the market at a maximum concentration of 1.8% and is typically mixed in a 1:1 ratio with an oxidative agent thereby reaching a concentration of 0.9% for in use application.

Quantification of p-aminophenol is performed by potentiometric titration, which is the neutralisation of the amine function, with 0.1 N perchloric acid in an acetic acid medium. Quantification of p-aminophenol is not performed by using a reference standard. It is insufficient to determine the purity by potentiometric titration; it is not State-of-the-Art. Solubility of p-aminophenol in various solvents was not properly characterised in the dossier.

Batch number and/or chemical purity were not stated in some toxicity study reports. Stability of p-aminophenol in typical hair dye formulations has not been reported.

Metabolism

p-Aminophenol is metabolized via sulfation, glucuronidation and N-acetylation. The latter metabolic pathway leads to the formation of APAP, which is of particular importance in the safety assessment of p-aminophenol. An *in vitro* study using the reconstructed human skin model Episkin® demonstrated that human epidermis quantitatively transforms p-aminophenol into APAP. These results corroborate the findings of an *in vivo* human study where a 2- to 6-fold ratio of p-aminophenol to APAP was found in the blood 0.5 to 1 hour

after p-aminophenol administration. The metabolism of p-aminophenol to APAP and its conjugates was also found in an *in vivo* study in rats, rabbits and guinea pigs. As a consequence, the safety of p-aminophenol has been assessed via the comparison of systemic exposure of APAP from hair dyeing to systemic exposure of APAP from the therapeutic use.

General toxicity

p-Aminophenol showed a low acute toxicity in several species and different application sites. In subchronic and chronic experiments the target organs were the kidneys and the liver. Dose-related nephrosis at 30 and 100 mg/kg bw/day were observed in both sexes. The dose of 10 mg/kg bw/day was considered as the NOAEL.

Irritation, sensitisation

p-Aminophenol showed irritancy on rabbit skin at 2.5% aqueous solution and when applied neat under semi-occlusion. It was also irritant on mucous membranes at 2.5% aqueous solution and when applied neat to rabbit eye. p-Aminophenol was shown to be a strong sensitiser.

Dermal absorption

Complete original data is not available. The concentration of p-aminophenol used was 0.42% when the intended maximum on-head concentration is 0.9% in oxidative dyes. Accordingly, the amount considered to be absorbed is considered to be $2 \times ((\text{receptor at 24 hours mean} + 2\text{SD}) + (\text{retained in skin mean} + 2\text{SD})) \mu\text{g}/\text{cm}^2$.

This is $2 (0.378 + 3.076) = 6.9 \mu\text{g}/\text{cm}^2$ or 7.84%, which may be used in calculating the MOS.

Teratogenicity

Despite not all studies are in compliance with acknowledged methodology it can be stated that p-aminophenol is unlikely to be teratogenic. It showed effects on fertility or on gestation, embryonic development, lactation or weanling indices in experimental animals only at high doses (500 mg/kg bw/day). Teratogenic effects were observed in one study, but only at maternal toxic doses.

Mutagenicity

Overall, the genotoxicity of p-aminophenol is not very well investigated. Considering the three endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy, only a properly performed *in vitro* chromosome aberration test is available. Well-performed gene mutation tests in bacteria (Ames test) and mammalian cells are lacking. Also aneuploidy is not covered. In the chromosome aberration test, a biological relevant increase in cells with chromosome aberrations is not observed. A number of tests with limited value, one chromosome aberration test and two gene mutation tests in mammalian cells, confirm the clastogenic potential of p-aminophenol, whereas indications that p-aminophenol also has the potency to induce gene mutations was not observed neither in bacteria nor in mammalian cells.

A well performed *in vivo* micronucleus test in mice confirmed the clastogenic potential of p-aminophenol found *in vitro*. The results of micronucleus test in splenocytes in mice which was considered as having limited value but which can be used to confirmation purposes, was positive as well. On the other hand in a well performed *in vivo* chromosome aberration test an increase of cells with chromosome aberrations was not found.

Although the negative *in vivo* UDS test might indicate that p-aminophenol does not induce gene mutations *in vivo*, on the results of the *in vivo* tests p-aminophenol has to be considered genotoxic.

On the other hand, APAP is the metabolite of p-aminophenol. APAP has also been shown to be clastogenic *in vitro* and *in vivo* in standard genotoxicity tests. However, the potential mechanisms of APAP-induced genotoxicity was reported to be indirect and thus thresholded (Bergman et al., 1996). Considering these mechanisms for p-aminophenol too, there should be no mutagenic risk for humans under normal use conditions. This view is supported by the negative 13 weeks micronucleus test.

Carcinogenicity

No evidence of a carcinogenic potential was found for both orally or dermal routes of application. However it has to be mentioned that all dermal studies were negative and that some of the formulations contained substances classified as carcinogenic either by EU or the German MAK commission (2,4-diaminoanisole, EU carcinogen category 1B; Disperse Blue 1, EU carcinogen category 1B; p-phenylenediamine, MAK commission carcinogen category 3B; 4-amino-2-nitrophenol, MAK commission carcinogen category 3B). This indicates that the sensitivity of the dermal carcinogenicity studies has not been sufficient to identify possible carcinogenic effects of hair dye formulations.

No conclusions regarding carcinogenicity can be drawn from these studies.

4. CONCLUSION

Based on the data provided, the SCCS is of the opinion that the use of p-aminophenol with a maximum on-head concentration of 0.9% in oxidative hair dye formulations does not pose a risk to the health of the consumer, apart from its sensitising potential.

5. MINORITY OPINION

Not applicable

6. REFERENCES

For practical reasons the references and their enumeration are given with a letter-prefix:

- A Toxicological summaries and overviews (submission IV and V)
 - B Journal articles and books (submission IV and V)
 - C References from previous submissions
 - KM Kinetics and metabolism; journal articles and special investigations
 - Col. Ongoing enumeration through all COLIPA submissions
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