



Scientific Committee on Consumer Products

SCCP

OPINION ON
2-Methyl-1-naphthol
(including 1-acetoxy-2-methylnaphthalene, A153)

COLIPA n° A156



The SCCP adopted this opinion at its 18th plenary of 16 December 2008

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Products (SCCP), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Evaluation Agency (EMEA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCP

Questions concerning the safety of consumer products (non-food products intended for the consumer).

In particular, the Committee addresses questions related to the safety and allergenic properties of cosmetic products and ingredients with respect to their impact on consumer health, toys, textiles, clothing, personal care products, domestic products such as detergents and consumer services such as tattooing.

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1. BACKGROUND

Submission I for 1-acetoxy-2-methoxynaphthalene/2-methyl-1-naphthol was submitted in September 2003 by COLIPA¹.

The two ingredients are chemically related and although the substance 1-acetoxy-2-methoxynaphthalene is used in the manufacture of the product, it hydrolyses completely into 2-methyl-1-naphthol in the formulation under alkaline conditions.

According to the current submission II, the 1-acetoxy-2-methoxynaphthalene/2-methyl-1-naphthol is used as a precursor for dying products in oxidative hair dye formulations.

The final concentration can be up to 2.0%.

2. TERMS OF REFERENCE

1. *Does the Scientific Committee on Consumer Products (SCCP) consider 2-methyl-1-naphthol safe for use in oxidative hair dye formulations with a concentration on-head of maximum 2.0% taking into account the scientific data provided?*
2. *Does the SCCP recommend any restrictions with regard to ensure the complete hydrolyses of 1-acetoxy-2-methoxynaphthalene into 2-methyl-1-naphthol in oxidative hair dye formulations?*

¹ COLIPA - European Cosmetics Toiletry and Perfumery Association

3. OPINION

Based on the chemistry of 1-acetoxy-2-methylnaphthalene (A153) and its *in situ* conversion to 2-methyl-1-naphthol, the relevant material for consideration in the context of the consumer risk assessment for hair dye products is 2-methyl-1-naphthol. Therefore, the toxicological evaluation only of 2-methyl-1-naphthol is relevant.

The physicochemical properties of 1-acetoxy-2-methylnaphthalene (A153) as well as the evaluation of the tests performed with this substance are reported in the Annex 1 to this opinion.

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

2-Methyl-1-naphthol (INCI)

3.1.1.2. Chemical names

1-Hydroxy-2-methylnaphthalene
Naphthalene, 1-hydroxy, 2-methyl

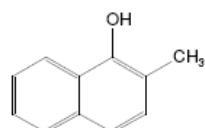
3.1.1.3. Trade names and abbreviations

/

3.1.1.4. CAS / EINECS number

CAS: 7469-77-4
EINECS: 231-265-2

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: C₁₁H₁₀O₂

3.1.2. Physical form

White powder

3.1.3. Molecular weight

Molecular weight: 158.20

3.1.4. Purity, composition and substance codes

Test substance: GTS03958
 Batch n°: 1083099
 Storage: (-20 ± 10°C), protected from light
 Identification: 2-methyl-1-naphthol by FTIR, NMR and elemental analysis
 Purity determined by HPLC (15.11.04): 100 ± 3.39% (98.2-105.0%)
 Purity determined by HPLC (17.2.05): 98.8 ± 0.91% (97.7-99.9%)

3.1.5. Impurities / accompanying contaminants

Loss on drying: 0.168%
 Ignition residue: < 0.1%
 1-Naphthol: 0.417%

3.1.6. Solubility

Water: 0.28 – 0.42 mg/ml
 Ethanol: 139 – 208 mg/ml
 DMSO: 275 – 413 mg/ml

* Solubility determined after 15 minutes of sonication

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: 3.17 ± 0.19 (calculated)

3.1.8. Additional physical and chemical specifications

Melting point: 63.7 – 64.5 °C
 Boiling point: /
 Flash point: /
 Vapour pressure: /
 Density: /
 Viscosity: /
 pKa: /
 Refractive index: /
 pH: /

3.1.9. Homogeneity and Stability

Stable when stored at -20 ± 10°C.

Undergoes air-oxidation when stored at room temperature

Stable under the alkaline conditions of the hair dye product matrix (no documentation provided).

2-Methyl-1-naphthol (Batch 1083099), stored at -20 ± 10°C and protected from light, was stable throughout the course of the toxicological investigations performed with this substance.

PEG-400 solutions of 2-methyl-1-naphthol (0.5 and 200 mg/ml) were shown to be stable (within ± 6% of the initial value) for 24 hours when stored at room temperature and for 15 days when stored at 5 ± 3°C. PEG-400 solutions of 2-methyl-1-naphthol (400 mg/ml) were

shown to be stable (within \pm 3.2% of the initial value) for 7 days when stored at -20 \pm 10°C.

DMSO solutions of 2-methyl-1-naphthol (0.005 and 200 mg/ml) were shown to be stable (within \pm 3.5% of the initial value) for 8 days when stored at -20 \pm 10°C.

General Comments to physico-chemical characterisation

- The test material used in several studies is not specified and no information was provided on its purity.
- Solubility in water is not determined by the EU method.
- No documentation is provided for the reported stability of 2-methyl-1-naphthol in marketed products.
- The P_{ow} strongly depends on the pH, especially for ionisable molecules, zwitterions etc. Therefore, a single calculated value of Log P_{ow} , without any reference to the respective pH, cannot be correlated to physiological conditions and to the pH conditions of the percutaneous absorption studies.

3.2. Function and uses

2-Methyl-1-naphthol is used in oxidative hair dye formulations at levels up to 4% (2% on-head).

3.3. Toxicological Evaluation

The coded test substance used in many of the submitted studies was not specifically identified as 2-methyl-1-naphthol.

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline:	/
Species/strain:	Sprague Dawley rats (Crl:CD (SD) BR)
Group size:	12 total (6/sex), 6/group (3/sex)
Test substance:	2-methyl-1-naphthol
Batch:	C7634/342B (A156)
Purity:	not specified
Dose:	500 or 2000 mg/kg bw in 0.5% methylcellulose. Volume of 40 ml/kg bw delivered in 2 x 20 ml/kg bw doses, 4 hours apart. No control group was included.
Route:	oral gavage
Observation period:	14 days
GLP:	in compliance
Study period:	30 May 1994

Two groups of six rats (3/sex) were fasted and orally dosed with 2-methyl-1-naphthol at dose levels of 500 and 2000 mg/kg bw. The rats were observed at 1, 4 and 24 hrs after dosing and once daily through day 14 for pharmacological and toxicological effects. Viability was checked daily. Body weights were recorded at study initiation and on days 7 and 14 or when found dead. All surviving rats were sacrificed by CO₂ asphyxiation at termination of the study and necropsy was performed.

Decreased activity was observed at 500 mg/kg bw. Clinical signs observed at 2000 mg/kg bw included decreased activity, abnormal stance, dyspnea, abnormal gait and prostration. None of the animals in the 500 mg/kg bw dose group died. All animals in the 2000 mg/kg bw dose group died on the day of dosing. Necropsy of these animals indicated distended and/or fluid filled stomachs and fluid filled intestines. No visible lesions were noted in any animals at terminal necropsy.

C7634/342B was found to be non-toxic at 500 mg/kg bw and toxic at less than 2000 mg/kg bw. Therefore, the LD50 ranged between 500 and 2000 mg/kg bw.

Ref.: 6

3.3.1.2. Acute dermal toxicity

No data submitted

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline:	/
Species:	New Zealand White Rabbit
Group:	6 male; 3 per group
Substance:	C7634/342DD
Batch:	/
Purity:	/
Site of Application:	intact skin 6.25 cm ² on dorsal area of trunk for 4 hours semi-occluded patch
Dose:	Group 1: 0, 0.5, 1, 5 and 10% in 0.5% methylcellulose Group 2: 0, 0.5, 1, 5 and 10% in a prototype hair dye base
Reading Times:	30 minutes, 24, 48, 72 and 96 hours and 7, 14 and 21 days post patch removal
GLP:	not in compliance
Study period:	25 July – 22 August 1996

Group 1: At concentrations up to 1% in methylcellulose, no irritation was observed. This increased to very slight to well defined erythema and very slight oedema at 5% and very slight to moderate-severe erythema and very slight to slight oedema at 10%. All sites were clear by day 21.

Group 2: Very slight to well defined erythema was observed at 0.5% in the prototype hair dye base. Very slight to well defined erythema and very slight oedema was observed at 1%. Well defined erythema and very slight to slight oedema was observed at 5%. Moderate-severe to severe erythema and moderate to severe oedema was observed at 10%. Blanching, desquamation, fissuring and possible necrotic areas and/or possible scar tissue were also observed with the 10% mixture of test material in the prototype hair dye base. At concentrations up to 1%, all sites were clear by day 21. At 5%, 1 animal presented with very slight erythema on day 21. At 10% all animals presented with very slight erythema and very slight to slight oedema on day 21.

Ref.: 9

Comment

Opinion on 2-methyl-1-naphthol (including 1-acetoxy-2-methylnaphthalene)

It appeared that the irritation potential of C7634/342DD was influenced by the base in which it was applied. The test substance was not specifically identified as 2-methyl-1-naphthol in the test report.

Guideline: /
 Species: New Zealand White Rabbit
 Group: 3 female and 3 males
 Substance: TM#171
 Batch: /
 Purity: /
 Site of Application: five applications on consecutive days of 0.3 ml test material to intact skin sites approximately 6 cm² on the dorsal area of the trunk for 6 hours under occluded patch conditions.
 Dose: 2 and 5% test substance in 0.5% methylcellulose
 Reading Times: Prior to test material application and following test material removal
 GLP: not in compliance
 Study period: 1997

Concentration of TM#171 (%)	Observations
<i>Sham control</i>	<i>Very slight erythema and very slight oedema</i>
<i>0.5% methylcellulose control</i>	<i>No skin reactions</i>
2.0	Very slight erythema and very slight oedema in 4 animals - cleared in 3/4 animals by day 6 Moderate to severe erythema with moderate oedema in 1 animal - not cleared by day 6
5.0	Very slight erythema with very slight oedema in 3 animals - cleared by day 6 in 2/3 animals Well defined erythema with moderate oedema in 2 animals - not cleared by day 6

Histopathological evaluation indicated both treatment and dose related changes in the dermis and epidermis as characterised by epidermal hyperplasia, parakeratosis, epidermal degeneration, leukocyte infiltration of the epidermis and dermis and congestion of the dermis.

Ref.: 10

Comment

TM#171 was irritant to rabbit skin under the described conditions. The test substance was not specifically identified as 2-methyl-1-naphthol in the test report.

3.3.2.2. Mucous membrane irritation
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Guideline: /
 Species: New Zealand White Rabbit
 Group: 1 female
 Substance: C7634/342D
 Batch: /
 Purity: /
 Site of Application: Conjunctival sac of the right eye
 Dose: 0.01 g
 Reading Times: 1, 24, 48 and 72 hours, 4, 5 and 6 days post instillation
 GLP: not in compliance
 Study period: 7 – 13 April 1995

Diffuse deep crimson red to beefy redness with obvious swelling was observed at the 1 hour reading and continued through to 48 hours post instillation. These effects subsided over the

following 3 days and had resolved by day 6. Slight iritis was noted at 24 hours only and slight corneal effects were noted at 72 hours only.

Ref.: 7

Comment

Neat C7634/342D caused reversible eye irritation in the rabbit. The test substance was not specifically identified as 2-methyl-1-naphthol in the test report.

3.3.3. Skin sensitisation

Buehler Test, study 1

Guideline: /
 Species: Hartley Guinea Pigs
 Group: 48 total (24 male, 24 female) (20 in the test group, 10 in the vehicle control group, 10 in the re-challenge control group, 8 in the pilot irritation screen group)
 Substance: C7634/342
 Batch: Lot # LRS7938-29
 Purity: /
 Vehicle: acetone
 Concentration: Epidermal induction: 25% test material in acetone
 Epidermal challenge: 0.5% test material in acetone
 Epidermal re-challenge: 0.5% test material in acetone
 Dose: 0.3 ml
 GLP: in compliance
 Study period: 9 January – 22 February 1995

The test material was applied, under occlusion, in 25 mm Hilltop chambers at the same site once every 7 days for a total of 3 exposures. After an interval of 14 days, 0.3 ml of 0.5% test material in acetone was applied to a naïve skin site using 25 mm Hilltop chamber.

Interval between induction and challenge application: 14 days

During induction, skin sites were examined approximately 24 hours after each application. Following challenge, skin sites were examined 24 and 48 hours after application.

Following the challenge application, the incidence and severity of the skin responses in the test group were greater than those produced by the vehicle control group thus indicating that sensitisation had been induced. Subsequent re-challenge with the same concentration confirmed this result.

Sensitisation rate: Sensitizer - 4/20 positive responses.

Ref.: 11

Comment

C7634/342 is a skin sensitizer. The test substance was not specifically identified as 2-methyl-1-naphthol in the test report.

Buehler Test, study 2

Guideline: /
 Species: Hartley Guinea Pigs
 Group: 82 total (41 male, 41 female) (20 in the test group, 10 in the vehicle control group, 40 in the re-challenge control group, 12 in the pilot irritation screen group)
 Substance: C7634/342A
 Batch: Lot # LRS7938-29
 Purity: /
 Vehicle: acetone

Opinion on 2-methyl-1-naphthol (including 1-acetoxy-2-methylnaphthalene)

Concentration: Epidermal induction: 5% test substance in acetone
 Epidermal challenge: 0.5% test substance in acetone
 Epidermal re-challenge: 0.05% test substance in acetone
 Dose: 0.3 ml
 GLP: in compliance
 Study period: 28 February – 31 May 1995

The test material was applied, under occlusion, in 25 mm Hilltop chambers at the same site once every 7 days for a total of 3 exposures. After an interval of 14 days, 0.3 ml of 0.5% test material in acetone was applied to a naïve skin site using 25 mm Hilltop chamber. Subsequent re-challenge was with 0.3 ml of 0.05% test material in acetone. During induction, skin sites were examined approximately 24 hours after each application. Following challenge, skin sites were examined 24 and 48 hours after application. Following the challenge application, the incidence and severity of the skin responses in the test group (4/20 = 20%) were greater than those produced by the vehicle control group thus indicating that sensitisation had been induced. Subsequent re-challenge with a 10-fold lower concentration also produced a higher number of positive responses (1/20 = 5%) versus the control.

Sensitisation rate: Sensitiser – 0.5% challenge = 4/20 positive responses; 0.05% re-challenge = 1/20 positive responses.

Ref.: 12

Comment

C7634/342A is a skin sensitizer. The test substance was not specifically identified as 2-methyl-1-naphthol in the test report.

Local Lymph Node Assay

Guideline: /
 Species: CBA/J Mice
 Group: 25 females total (5 per group)
 Substance: TM#171
 Batch: /
 Purity: /
 Dose: 3 applications of 25 µl to each ear
 Dilutions: 1, 3, 6 and 10% in acetone
 GLP: in compliance
 Study period: 19 June – 15 July 1996

Topical application was with 12.5 µl to the ventral and dorsal surfaces of each ear with a total of 3 applications of 25 µl total on consecutive days. Concentrations of 1, 3, 6 and 10% in acetone were used.

On day 6, animals received an intravenous injection of 20 µCi of ³H-thymidine. Approximately 5 hours later, they were killed and the draining auricular lymph nodes removed, pooled and analysed via liquid scintillation counting.

The stimulation indices are tabulated below:

Concentration (% w/w)	Stimulation Index (SI)
1	1.63
3	4.27
6	6.42
10	8.70

Sensitisation Rate: TM#171 produced evidence of stimulating allergic contact sensitisation in this study at levels of 3% as indicated by mean stimulation indices greater than 3 at these doses.

Ref.: 13

Comment

TM#171 is a strong sensitisier. An EC3-value of 1.28% was calculated. The test substance was specifically not identified as 2-methyl-1-naphthol in the test report.

Repeat Insult Patch Test, study 1

Guideline: /
 Species: Human
 Group: 101; 9 male, 92 female
 Substance: TM#1016 (2-methyl-1-naphthol and 3 methyl-PAP) with TM#1018
 Batch: /
 Purity: /
 Dose: Not stated
 Application: application to inner forearm under occlusive patch conditions for 60 minutes three times a week for 3 consecutive weeks
 GCP: /
 Study period: 11 November – 20 December 1996

A hair dye formulation was applied to the inner forearm under occlusive patch conditions for 60 minutes three times a week for 3 consecutive weeks. After an interval of 2 weeks, there were 2 applications of the same material to a naive site under occlusive patch conditions for 120 minutes applied 48 hours apart.

Skin observations were made prior to each induction patch application and at 48 and 96 hours after challenge patch application.

Results

Six subjects elicited skin reactions indicative of an induced contact allergic response during the challenge phase of this study.

The formulation TM#1016 with TM#1018 induced contact allergic sensitisation under the conditions of this study. However due to the presence of other hair dye ingredients the causative ingredient could not be positively identified.

Ref.: 13 (subm. 2003)

Comment

The concentration of the test ingredients was not described in the study.
 SCCP considers that HRIPT-studies are unethical.

Repeat Insult Patch Test, study 2

Guideline: /
 Species: Human
 Group: 54; 1 male, 53 female
 Substance: C7634/1016 (1.5% 2-methyl-1-naphthol with 3 methyl-PAP) dye base mixed 1:1 with C7634/1018 hydrogen peroxide developer
 Batch: /
 Purity: /
 Dose: Not stated
 Application: application to inner forearm under occlusive patch conditions for 60 minutes three times a week for 3 consecutive weeks

Opinion on 2-methyl-1-naphthol (including 1-acetoxy-2-methylnaphthalene)

GCP: in compliance
 Study period: 22 July – 30 August 1996

A hair dye formulation was applied to the inner forearm under occlusive patch conditions for 60 minutes three times a week for 3 consecutive weeks. After an interval of 2 weeks, there were 2 applications of the same material to a naive site under occlusive patch conditions for 120 minutes applied 48 hours apart.

Skin observations were made prior to each induction patch application and at 48 and 96 hours after challenge patch application.

Results

No skin reactions were elicited during the induction and challenge phases of this study. This product containing 0.75% 2-methyl-1-naphthol did not cause contact allergy under the conditions of this study.

Ref.: 14 (subm. 2003)

Comment

SCCP considers that HRIPT-studies are unethical.

3.3.4. Dermal / percutaneous absorption

Guideline:	OECD 428
Species:	Human female epidermal; 5 donors, post mortem
Chambers:	12 chambers, Diffusion; 2.54 cm ²
Integrity of membranes:	electrical resistance >10KΩ
Substance:	4% 2-methyl-1-naphthol in an oxidative hair dye base containing no other dye precursors.
Batch:	custom synthesised material, lot code 1083099
Purity:	99.4%
Radiolabel:	[¹⁴ C]-radiolabelled 2-methyl-1-naphthol incorporated into the dose to give 1 x 108 to 1 x 109 dpm/ml
Dose:	20 mg/cm ² ; final concentration applied following dilution with peroxide developer = 2% 2-methyl-1-naphthol
Duration of contact:	30 minutes followed by rinsing
Sampling:	0.5, 1, 2, 4, 6, 24, 29, and 48 hours after application
Receptor fluid:	4% polyoxyethylene-20-oleyl ether in PBS
Solubility in receptor:	> 1mg/ml
Stability in receptor:	/
Analysis:	liquid scintillation counting
GLP:	in compliance
Study period:	12 – 25 October 2004

Results

The amount of 2-methyl-1-naphthol considered absorbed (receptor fluid + epidermis) was $3.07 \pm 1.47 \mu\text{g}/\text{cm}^2$ (range 1.02 – 5.73 $\mu\text{g}/\text{cm}^2$). This is equivalent to $0.762 \pm 0.366\%$ (range 0.254 – 1.42%) of the applied dose.

Ref.: 14

Comment

As only a total of 12 chambers were used, the Amax of 5.73 $\mu\text{g}/\text{cm}^2$ (1.42% of the applied dose) may be used for calculating the MOS.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (14 days) oral / dermal / inhalation toxicity

Guideline:	OECD 407
Species/strain:	Sprague Dawley rats (Crl:CD(SD)IGS BR)
Group size:	50 total (25/sex), 10/group (5/sex)
Test substance:	GTS03958
Batch:	1083099
Purity:	100 %
Dose:	0, 10, 40, 150 and 500 mg/kg bw/group in PEG 400
Vehicle:	PEG-400
Route:	oral
Observation period:	16 days
GLP:	in compliance
Study period:	21 June 2004 – 27 January 2005

Male and female Crl:CD(SD)IGS BR rats were assigned to five groups (five animals/sex/group). Each group received dose preparations containing the vehicle (Polyethylene glycol 400) or 10, 40, 150, or 500 mg of test article/kg bw/day at a dose volume of 5 mL/kg bw. The animals were observed twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain or distress. Detailed clinical observations were performed and body weights recorded once prior to treatment and on days 1, 4, 8, 11, and 15. Food consumption data were measured from days 1 to 4, 4 to 8, 8 to 11, and 11 to 15. Blood and urine samples for haematology, coagulation, clinical chemistry, and urinalysis were collected at the scheduled sacrifice. Blood samples were also collected from the animal sacrificed on day 11. On day 16, surviving animals were anaesthetized, weighed, and necropsied. At necropsy, macroscopic observations were recorded, selected organs weighed, and selected tissues collected and preserved.

Results

One female in the 500 mg/kg bw/day dose group was sacrificed in a moribund condition on day 11. This condition was not related to the test substance. There were no test article-related effects on mean body weights, body weight changes, or food consumption. GTS03958 administration at dose levels up to 150 mg/kg bw/day had no effect on clinical pathology test results. At 500 mg/kg bw/day, GTS03958 administration was associated with mildly lower haemoglobin values for males, moderately higher absolute reticulocyte count for males and females, and mildly lower urine pH for males. Of uncertain relationship to test article administration was the occurrence of discoloured urine (red) for two males and one female given 500 mg/kg bw/day. No test article-related mortality, organ weight changes or macroscopic findings were observed in this study.

Based on the results of this 14-days oral toxicity study, the study authors derived an NOAEL 500 mg/kg bw/day.

Ref: 15

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

Guideline:	OECD 408
Species/strain:	Sprague Dawley rats (Crl:CD(SD)IGS BR)
Group size:	vehicle (40/group, 20/sex), 6 mg/kg bw/day (30/group, 15/sex), 25 mg/kg bw/day (30/group, 15/sex), 100 mg/kg bw/day (40/group, 20/sex),
Test substance:	GTS03958
Batch:	1083099

Purity: 98.8-100%
Dose: 0, 6, 25, 100 mg/kg bw/day
Vehicle: PEG-400
Route: oral, via gavage
Observation period: 91 days
GLP: in compliance
Study period: 26 July – 22 November 2004

In this study, male and female Sprague Dawley rats were assigned to five groups (15-20 animals/sex/group) and exposed to 6 (30/group, 15/sex), 25 (30/group, 15/sex) or 100 (40/group, 20/sex) mg/kg bw/day GTS03958 or the vehicle control PEG-400 (40/group, 20/sex) at a dose volume of 5 ml/kg bw. The animals were observed twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain or distress. Detailed clinical observations were performed prior to treatment, weekly thereafter, and on the day of each scheduled sacrifice. Neurobehavioral observations were performed weekly; handheld and open-field expanded clinical observations were done pre-study and during Weeks 4, 8, and 13; elicited behaviour observations were done pre-study and during Week 13; and motor activity data were collected pre-study and during Week 13. Ophthalmic examinations were done prior to treatment and during Week 13. Body weights were collected twice prior to treatment, on Day 1, and weekly thereafter. Food consumption was measured weekly. Vaginal cytology data were collected once daily for 21 consecutive days, beginning after Week 10. Blood and urine samples for haematology, coagulation, clinical chemistry, urinalysis, and urine chemistry were collected prior to each scheduled sacrifice. On Day 95 (males) or 96 (females), up to 15 animals/sex/group were anesthetized, weighed, and necropsied. On Day 124, all surviving animals were anesthetized, weighed, and necropsied. At each necropsy, macroscopic observations were recorded, selected organs weighed, and selected tissues collected and preserved. The four females found dead on Days 12 (Group 3), 32 (Group 1), and 64 (Groups 1 and 4) were also necropsied, but organ weights were not recorded. At each scheduled sacrifice, male reproductive assessment (sperm motility, morphology, and count) was done by Pathology Associates, Inc. Microscopic examination of tissues was done, and a pathology peer review was conducted.

Results

Two control females, one female given 25 mg/kg bw/day, and one female given 100 mg/kg bw/day, were found dead. There were no test article-related macroscopic or microscopic findings for the treated animals that would attribute these deaths to effects of the test article. All males and all remaining females survived to their respective scheduled sacrifice. No test article-related effects on clinical, expanded clinical or ophthalmic observations were noted.

No statistically significant differences in mean body weights of animals were noted during the treatment or recovery period. Statistically significant differences in mean body weight gains during treatment and recovery for males were sporadic and not considered toxicologically relevant; no statistically significant differences were noted for females. When compared with controls, mean food consumption was decreased for all treated male groups. Differences were statistically significant for two or three groups at 10 of the 14 weekly intervals during treatment and at 2 of the 4 intervals during recovery for males given 100 mg/kg bw/day; however, these differences did not correlate with adverse effects on mean body weights and were not considered toxicologically important. No statistically significant differences were noted for females during the treatment or recovery period.

GTS03958 administration was associated with minimally higher absolute reticulocyte count for males given 100 mg/kg bw/day and minimally higher urinary chloride excretion for males and females given 100 mg/kg bw/day. Both of these findings were reversible, and neither was considered adverse. GTS03958, given by oral gavage to male and female rats for at least 91 days, resulted in no test article-related macroscopic or microscopic changes. Early deaths occurred in four females, but none of these were attributed to the test article; one was determined to have been gavage-related, and a second may also have been

gavage-related. No target organs/tissues were identified for examination from the lower dose groups or recovery sacrifice animals.

The mean percent sperm motility, caudal epididymal sperm count, and sperm morphology were not affected by treatment with GTS03958 at dosage levels of 6, 25, and 100 mg/kg bw/day. No biologically meaningful differences were observed between the study groups.

Conclusion

Based on the results of this study, the NOAEL was 100 mg/kg bw/day.

Ref.: 16

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial Reverse Mutation Test

Guideline:	OECD 471 (1983)
Species/strain:	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537 and TA1538
Replicates:	triplicates in a single experiment both in the presence and absence of metabolic activation
Test substance:	C 7634/342
Solvent:	DMSO
Batch:	/
Purity:	/
Concentrations:	3.3, 10, 33, 100, 333 and 1000 µg/plate without and with S9-mix
Treatment:	direct plate incorporation with 48 - 72 h incubation
GLP:	in compliance
Study period:	October - November 1994

C 7634/342 was investigated for the induction of gene mutations in *Salmonella typhimurium* (Ames test). Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the level of toxicity in a dose range-finding study up to the prescribed maximum concentration of 5000 µg/plate on toxicity and precipitation. Negative and positive controls were in accordance with the guideline.

Results

Both in the presence and absence of S9-mix clear toxicity, reported as extremely reduced or even absent bacterial background lawn, was observed at the highest dose (1000 µg/plate). No positive responses were observed in any of the test strains either in the presence or absence of metabolic activation.

Conclusion

Under the experimental conditions used C 7634/342 was not mutagenic in this gene mutation tests in bacteria.

Ref.: 21 (subm. 2003)

Comment

The test substance was not specifically identified as 2-methyl-1-naphthol in the test report.

Bacterial Reverse Mutation Test

Guideline:	OECD 471 (1997)
Species/strain:	<i>Salmonella typhimurium</i> TA98, TA100, TA1535 and TA1537; <i>Escherichia coli</i> WP2uvrA(pKM101)
Replicates:	di- or triplicates in two independent experiments both in the presence and absence of metabolic activation
Test substance:	GTS03958 (2-methyl-1-naphthol)
Solvent:	DMSO
Batch:	1083099
Purity:	99.4 %
Concentrations:	<p>Experiment 1: 2.5, 5, 20, 50, 200, 500, 2000 and 5000 µg/plate, without and with S9-mix</p> <p>Experiment 2: 10, 25, 50, 100, 250, 500, 1000 and 3000 µg/plate, without and with S9-mix</p> <p>Experiment 3: 5, 10, 25, 50, 75, 100, 150 and 300 µg/plate, with S9-mix, TA98 only</p> <p>Experiment 4: 10, 25, 50, 75, 100, 150, 300 and 1000 µg/plate, with S9-mix, TA 98 only</p>
Treatment:	<p>experiment 1 and 3: preincubation method with 20 ± 2 minutes preincubation and 60 ± 12 h incubation</p> <p>experiment 2 and 4: direct plate incorporation method with 60 h incubation</p>
GLP:	in compliance
Study period:	June 2004- November 2005

2-methyl-1-naphthol was investigated for the induction of gene mutations in *Salmonella typhimurium* and *Escherichia coli* (Ames test). Liver S9 fraction from Aroclor™-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the results of a toxicity test up to the prescribed maximum concentration of 5 mg/plate with all strains and both without and with S9-mix. Toxicity was evaluated on the basis of a reduction in the number of revertant colonies and/or qualitative evaluation of the bacterial background lawn. Experiments 1 and 3 were performed with the pre-incubation method, experiment 2 and 4 with the direct plate incorporation method. Negative and positive controls were in accordance with the OECD guideline.

Results

Toxicity was observed in experiment 1 at 500 µg/plate and above without S9-mix and at 250 µg/plate and above with S9-mix. In experiment 2, toxicity was reported from 1000 µg/plate and above both without and with S9-mix. In experiment 3 and 4, performed with TA98 with S9-mix only, toxicity was seen at 300 and 1000 µg/plate.

Except for TA98, both in experiment 1 and 2, a biological relevant increase in revertant colonies was not seen in any of the remaining tester strains following treatment with 2-methyl-1-naphthol neither in the absence nor in the presence of S9-mix. In experiment 1 and 2, a 2.1- and 2.0-fold increase in the number of revertant colonies was seen in strain TA98. However, the increase was not dose dependent and therefore did not meet the criteria for a positive response. In two confirmatory assays (experiments 3 and 4), no positive increases in the mean number of revertants per plate were observed with test strain TA98 in the presence of S9-mix.

Conclusion

Under the experimental conditions used 2-methyl-1-naphthol was not genotoxic (mutagenic) in this gene mutation tests in bacteria.

Ref. 17 (subm. 2005)

In vitro Mammalian Chromosome Aberration Test

Guideline: /
 Cells: CHO-K1 cells
 Replicates: duplicates in a single experiment
 Test substance: C7634/342G
 Solvent: Acetone
 Batch: LRS7988/69
 Purity: 95-97%
 Concentrations: 20, 40 and 60 µg/ml without S9-mix
 5, 7.5 and 10 µg/ml with S9-mix
 Treatment: 20 h treatment and harvest time 20 h after start of treatment without S9-mix
 4 h treatment and harvest 20.7 h after start of treatment with S9-mix
 GLP: in compliance
 Study period: August 1995 - September 1996

C7634/342G has been investigated in the absence and presence of metabolic activation for the induction of chromosomal aberrations in CHO cells. Test concentrations were based on the results of an initial toxicity assay on toxicity, as indicated by a decline in cell growth, and mitotic index. Approximately 2 h before harvest, each culture was treated with colcemid (final concentration 0.1 µg/ml) to block cells at metaphase of mitosis. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Chromosome (metaphase) preparations were stained with Giemsa and examined microscopically for chromosomal aberrations and the mitotic index. The known clastogen cyclophosphamide was used as positive control.

Results

In the presence of S9-mix, doses scored for chromosomal aberrations were 5, 7.5 and 10 µg/ml. Higher doses could not be evaluated due to excessive toxicity. Based on trend analysis, the test material in the presence of metabolic activation induced a statistically significant and dose dependent increase in the percentage of metaphase cells containing at least one chromosomal aberration. Additionally an increased frequency of metaphase cells containing at least one aberration was present at 7.5 and 10 µg/ml based on a pair-wise comparison with the concurrent control. In the absence of S9-mix, clastogenic activity was evaluated at 20, 40 and 60 µg/ml. Higher doses could not be evaluated due to excessive toxicity, Based on trend analysis in the absence of metabolic activation, the test material did not induce a statistically significant nor dose dependent increase in the percentage of metaphase cells containing at least one chromosomal aberration. However there was a statistically significant increase in clastogenic damage at 20 and 40 µg/ml based on a pair-wise comparison with the concurrent control. This clastogenic response was considered equivocal.

Conclusion

Under the experimental conditions used C7634/342G was clastogenic in this chromosomal aberration test *in vitro*.

Ref.: 18 (subm. 2005), 22 (subm. 2003)

Comment

The test substance was not specifically identified as 2-methyl-1-naphthol in the test report. Reference 22 of submission 2003 is identical to reference 18 of submission 2005 and describes the *in vitro* chromosome aberration test in CHO cells for A156.

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

Guideline: OECD 476 (1997)
 Cells: L5178Y Mouse lymphoma cells (clone 3.7.2C)
 Replicates: single cultures per concentration in two independent experiments
 Test substance: GTS03958 (2-methyl-1-naphthol)
 Solvent: DMSO
 Batch: 1083099
 Purity: 99.4%
 Concentrations:
 Experiment 1: 1, 5, 10, 20, 40, 50, 60 and 70 µg/plate without S9-mix
 10, 15, 20, 25, 30, 35, 40 and 45 µg/plate with S9-mix
 Experiment 2: 5, 10, 15, 20, 25, 30, 35 and 40 µg/plate without S9-mix
 2.5, 5, 15, 25, 25, 30, 35 and 40 µg/plate, with S9-mix
 Treatment:
 Experiment 1: 4 h treatment without and with S9-mix; expression period 48 h and selection period of 13 days
 Experiment 2: 24 h treatment without S9-mix; expression period 48 h and selection period of 13 days
 4 h treatment with S9-mix; expression period 48 h and selection period of 13 days
 GLP: in compliance
 Study period: August 2004 - July 2005

2-methyl-1-naphthol was assayed for gene mutations at the *tk* locus in mouse lymphoma cells. Test concentrations were based on the results of a toxicity test measuring the reduction in cell growth relative to the concurrent vehicle control cell cultures. The concentrations for the main tests were chosen to cover a toxicity range from 10-20% survival to no apparent effect on growth compared to the concurrent vehicle control. In the initial experiment (experiment 1), cells were treated for 4 h and in the confirmatory experiment (experiment 2) for 24 h (without S9-mix) or 4 h (with S9-mix), followed by an expression period of 48 h to fix the DNA damage into a stable *tk* mutation. Toxicity was determined as relative total growth. Liver S9 fraction from AroclorTM 1254-induced rats was used as exogenous metabolic activation system. Negative and positive controls were in accordance with the OECD guideline.

Results

There was no relevant shift in pH values nor in osmolarity measured at the highest concentration.

In all experiments the appropriate level of toxicity (10-20% survival after the highest dose) was reached.

In experiment 1 and in experiment 2 with S9-mix a dose dependent increase in mutant frequency was observed. For the tests with S9 the mutant frequency (just) exceeded the range of the historical control data. These increases were due to an increase in the number of small colonies indicating to a putative clastogenic effect of 2-methyl-1-naphthol. Since the mutant frequency of one or more of the doses tested did not exhibit a mutant frequency which is greater or equal to 90 mutants per 10^6 clonable cells over the concurrent background frequency, the response was considered not biologically relevant.

Conclusion

Under the experimental conditions used 2-methyl-1-naphthol is not genotoxic (mutagenic) at the *tk* locus of mouse lymphoma cells.

Ref. 19 (subm. 2005)

Comment

Although the positive results did not meet the criteria for a positive result as used in the report, the SCCP considers 2-methyl-1-naphthol genotoxic in this mouse lymphoma assay.

Exposure to 2-methyl-1-naphthol clearly resulted in a dose dependent increase in the mutant frequency which reached values outside the range of the historical control data.

In vitro unscheduled DNA synthesis test in rat primary hepatocytes

Guideline:	OECD 482 (1986)
Cells:	hepatocytes from male Sprague-Dawley rats
Replicates:	triplicates in a single experiment
Test substance:	C7634/342U
Solvent:	acetone
Batch:	LRS-798869
Purity:	95.97 %
Concentrations:	10, 15, 20, 25 and 30 µg/ml
Treatment	18 - 20 h treatment
GLP:	in compliance
Study period:	August 1995 - February 1996

C7634/342U was investigated for the induction of unscheduled DNA synthesis (UDS) in primary hepatocytes isolated from rats. Test concentrations were based on the results of a preliminary cytotoxicity assay on lactate dehydrogenase release as an indicator of cytotoxicity. Cells were treated for 18-20 h with C7634/342U and 10µCi ³H-thymidine/ml and then progressed for autoradiography. Evaluation of autoradiography was done after 5-12 days exposure and hematoxylin-eosin staining. UDS was measured by counting the number of grains in each nucleus and subtracting the average number of grains present in 3 equal-sized adjacent cytoplasmic areas (net nuclear grain). The mean net nuclear grain count for each dose level and the percentage of cells in repair (cells with > 5 net nuclear grains) were determined. DMBA was used as positive control.

Results

Microscopic examination of the hepatocytes cultures indicated a high to moderate level of toxicity at 30 and 40 µg/ml. Due to excessive toxicity the 40 µg/ml dose level could not be evaluated. None of the doses between 10 and 30 µg/ml caused a significant increase in the mean net nuclear counts when compared to the solvent control.

Conclusion

Under the experimental conditions used C7634/342U did not induce unscheduled DNA synthesis and, consequently, is not genotoxic in rats in this *in vitro* UDS test.

Ref.: 23 (subm. 2003)

Comment

The test substance was not specifically identified as 2-methyl-1-naphthol in the test report.

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Mammalian Erythrocyte Micronucleus Test

Guideline:	/
Species/strain:	CD-1® albino mice (sub-strain Crl:CD-1® (ICR)BR)
Group size:	5 mice/sex/dose group
Test substance:	C7634/342E
Batch no:	/
Purity:	/
Dose level:	15, 75 and 150 mg/kg bw
Route:	intraperitoneal injection
Vehicle:	0.5% methylcellulose
Sacrifice times:	24, 48 and 72 h after treatment.

GLP: in compliance
 Study period: March – September 1995

C7634/342E has been investigated for the induction of micronuclei in bone marrow cells of mice. Test concentrations were based on the results of a preliminary toxicity study in male and female mice on mortality, pharmacotoxic signs immediately and approximately 24, 48 and 72 h after dosing and on PCE/NCE ratio. The top dose was considered the maximum tolerated dose based on the mortalities observed during the preliminary toxicity screen at 246 mg/kg and higher. In the main experiment mice were exposed by intraperitoneal injection to single doses of 0, 15, 75 and 150 mg/kg bw. Bone marrow cells were collected 24, 48 and 72 h after dosing. 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 and examined microscopically for the PCE/NCE ratio and micronuclei. The known clastogen cyclophosphamide was used as positive control.

Results

A decrease in PCE/NCE ratio was only observed for males mice at the 24 hour sacrifice time whereas a slight decrease was seen for males at the other sacrifice times and for females at 24 h. Some slight pharmacological signs only observed at the highest dose may indicate to systemic exposure.

No statistically significant or dose dependent increases in micronucleated PCEs compared to the negative controls were observed.

Conclusion

Under the experimental conditions used C7634/342E did not induce an increase in the number of micronucleated PCEs in bone marrow cells of treated mice and, consequently, C7634/342E is not clastogenic and/or aneugenic in bone marrow cells of mice.

Ref.: 24, 28 (subm. 2003)

Comment

There are only slight indications for bone marrow cell exposure. Reference 28 is identical to reference 24 and describes the *in vivo* micronucleus test for both A156 and A153.

The test substance was not specifically identified as 2-methyl-1-naphthol in the test report.

In vivo Micronucleus Test

Guideline:	OECD 474 (1997)
Species/strain:	CD-1® (ICR)BR) mice
Group size:	5 male mice/dose
Test substance:	GTS03958 (2-methyl-1-naphthol)
Batch no:	1083099
Purity:	99.4 %
Dose level:	212.25, 425 and 850 mg/kg bw
Route:	oral gavage
Vehicle:	polyethylene glycol 400 (PEG 400)
Sacrifice times:	24 and 48 h (control and high dose only) after treatment.
GLP:	in compliance
Study period:	September 2004 - November 2005

2-methyl-1-naphthol has been investigated for the induction of micronuclei in bone marrow cells of mice. Test concentrations were based on the results of a dose range-finding study in male and female mice on toxic signs and mortality with up to 48 h observation. No mortality was seen at either 850 or 750 mg/kg bw. Clinical signs of toxicity were observed in the animals dosed at 850 and 750 mg/kg including hypoactivity, laboured respiration, squinted eyes, sensitivity to touch, recumbency, ataxia, flattened posture and/or hunched posture.

Based on these findings 850 mg/kg was set as the MTD. Since no relevant differences in toxicity between sexes were observed only male mice were used in the main experiment. In the main experiment mice were exposed by gavage to single doses of 0, 212.25, 425 and 850 mg/kg bw. Bone marrow cells were collected 24 h or 48 h (control and high dose only) after dosing. 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. Plasma samples were collected from groups of three animals in the high dose group at 1, 2, 4, 6, 8, 24 and 48 h after dosing for detection of 2-methyl-1-naphthol. Negative and positive controls were in accordance with the OECD guideline.

Results

In the main experiment, all mice survived and remained healthy until the end of the experiment. Animals in the 850 mg/kg dose group displayed clinical signs of toxicity which included hypoactivity, irregular respiration, squinted eyes, hunched posture, swollen abdomen, and/or audible respiration.

Treatment with 2-methyl-1-naphthol did not result in a decreased PCE/NCE ratio compared to the untreated controls. However, toxicokinetic results confirmed biological evidence of bone marrow exposure. The peak mean plasma concentration (804 ng/ml) was observed at approximately 1 h after the single oral gavage administration of 2-methyl-1-naphthol. Elevated plasma levels were detected in plasma up to 8 h after administration.

2-methyl-1-naphthol did not induce biologically relevant or statistically significant increases in micronucleated polychromatic erythrocytes at any dose examined.

Conclusion

Under the experimental conditions used, 2-methyl-1-naphthol did not induce micronuclei in bone marrow cells of treated mice. Consequently, 2-methyl-1-naphthol is not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice.

Ref. 20 (subm. 2005)

In vivo unscheduled DNA synthesis (UDS) test

Guideline:	OECD 486 (1998)
Species/strain:	male Sprague Dawley rats
Group size:	3 rats per dose
Test substance:	GTS03958 (2-methyl-1-naphthol)
Batch:	1083099
Purity:	99.4%
Dose level:	500 and 1000 mg/kg bw
Route:	oral gavage
Vehicle:	polyethylene glycol 400 (PEG 400)
Sacrifice times:	2 - 4 h and 12 -16 h after dosing
GLP:	in compliance
Study period:	July - November 2005

2-methyl-1-naphthol was investigated for the induction of unscheduled DNA synthesis (UDS) in hepatocytes of rats. Based on the information supplied by the sponsor, the high dose was set at the maximum tolerated dose which was estimated to be 1000 mg/kg bw. Hepatocytes for UDS analysis were collected 2-4 h and 12-16 h after administration of 2-methyl-1-naphthol via liver perfusion with collagenase. Ninety to 180 minutes after plating cells were incubated for 4 h with 10 µCi/ml ³H-thymidine followed by incubation with unlabelled thymidine for 17-20 h. Evaluation of autoradiography was done after 7 days.

UDS was reported as net nuclear grain: the nuclear grain count subtracted with the average number of grains in 3 nuclear sized areas adjacent to each nucleus. For each dose level the mean net nuclear grain count and the percentage of cells in repair (cells with > 5 net

nuclear grains) were reported. Unscheduled synthesis was determined in 50 randomly selected hepatocytes on 3 replicate slides per rat. Negative and positive controls were in accordance with the OECD guideline.

Results

No mortalities occurred in either dose group. All animals appeared normal immediately after dosing. All animals in the 500 mg/kg dose group and in the 1000 mg/kg dose group and 2-4 hr treatment time appeared normal prior to harvest. All animals in the 1000 mg/kg dose group and 12-16 hr treatment time appeared normal immediately following dosing but had crusty and bloody noses prior to harvest.

Neither a biological relevant increase in mean net nuclear grain count nor in the percentage of cells in repair as compared to the untreated control was found in hepatocytes of any treated animal both for the 2-4 h and the 12-16 h treatment time.

Conclusion

Under the experimental conditions used, 2-methyl-1-naphthol did not induce unscheduled DNA synthesis and, consequently, is not genotoxic in rats in the *in vivo* UDS test.

Ref.: 21 (subm. 2005)

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

Guideline:	OECD 414
Species/strain:	rat, Crl:CD (SD)IGS BR VAF/Plus
Group size:	25 females per group
Test substance:	GTS03958 (2-Methylnaphthol)
Batch:	1083099
Purity:	100%
Dose:	0, 25, 75, 225 mg/kg bw/day
GLP:	in compliance
Study period:	4 October – 4 November 2004

Formulations of the test substance, GTS03958 (2-Methylnaphthol), and/or the vehicle, 100% polyethylene glycol 400 (PEG 400), were administered orally once daily to rats on days 6 through 20 of gestation (DGs 6 through 20) at dosages of 0, 25, 75 and 225 mg/kg bw/day. The dosage volume was 5 mL/kg bw. Viabilities, clinical observations, body weights and feed consumption values were recorded. All surviving rats were sacrificed on DG 21. The gravid uterus was excised, weighed and subsequently examined for the number and distribution of corpora lutea, implantation sites and uterine contents. A gross necropsy was performed. Foetuses were weighed and examined for gross external alterations, sex and either soft tissue or skeletal alterations. Caesarean-sectioning and subsequent foetal observations were conducted without knowledge of dosage group.

Results

Significantly increased numbers of rats in the 225 mg/kg bw/day dosage group had rales, red perioral substance, urine-stained abdominal fur, chromorhinorrhea, ungroomed coat, excess salivation and decreased motor activity. Gasping, lacrimation, cold to touch and red

perinasal substance were observed in single rats in the 225 mg/kg bw/day. No gross lesions were identified at necropsy. Mean body weight gains were reduced or significantly reduced in the 225 mg/kg bw/day dosage group on DGs 6 to 9 and 9 to 12, significantly increased on DGs 12 to 15, significantly reduced for the entire dosage period and reduced for the entire gestation period. Mean body weights were significantly reduced in the 225 mg/kg/day dosage group on DGs 12 and 13. Once corrected for the weight of the gravid uterus, mean body weight gains were significantly reduced in the 225 mg/kg bw/day dosage group for the entire dosage and gestation periods. Absolute and relative feed consumption values were significantly reduced in the 225 mg/kg/day dosage group on DGs 6 to 9 and 9 to 12, as well as during the entire dosage period. No Caesarean-sectioning or litter parameters were affected by dosages of GTS03958 as high as 225 mg/kg bw/day. No gross external, soft tissue or skeletal alterations were caused by dosages of GTS03958 as high as 225 mg/kg bw/day.

Conclusion

On the basis of these data, the maternal no-observable-adverse-effect-level (NOAEL) for GTS03958 is 75 mg/kg bw/day, and the developmental NOAEL is 225 mg/kg bw/day.

Ref.: 23

Comment

The dose levels in this study were based on the results of a dose-range finding study (reference 22). In this study animals were exposed to 0, 25, 75, 200 and 500 mg/kg bw/day GTS03958 (8 animals per group). Maternal body weight gain was decreased in the 200 and 500 mg/kg bw/d groups. Mean litter sizes and live foetuses were decreased and resorptions and the percentage of dams with any resorptions were increased in the 500 mg/kg bw/day dosage group. Three total litter losses occurred in the 500 mg/kg bw/day dosage group. Foetal body weights were reduced in the 200 and 500 mg/kg bw/day dosage groups.

3.3.9. Toxicokinetics

No data submitted

3.3.10. Photo-induced toxicity

No data submitted

3.3.11. Human data

See point 3.3.3 Skin sensitisation

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)**CALCULATION OF THE MARGIN OF SAFETY****2-methyl-1-naphthol**
(oxidative / permanent)

Maximum absorption through the skin	A ($\mu\text{g}/\text{cm}^2$)	=	5.73 $\mu\text{g}/\text{cm}^2$
Skin Area surface	SAS (cm^2)	=	700 cm^2
Dermal absorption per treatment	SAS \times A \times 0.001	=	4.01 mg
Typical body weight of human		=	60 kg
Systemic exposure dose (SED)	SAS \times A \times 0.001/60	=	0.07 mg/kg bw
No observed adverse effect level (maternal toxicity, oral, rat)	NOAEL	=	75 mg/kg bw

Margin of Safety	NOAEL / SED	=	1071
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3.3.14. Discussion*Physico-chemical properties*

2-Methyl-1-naphthol is used in oxidative hair dye formulations at levels up to 4% (2% on-head).

The coded test substance used in many of the submitted studies was not specifically identified as 2-methyl-1-naphthol and no information was provided on its purity. The P_{ow} strongly depends on the pH, especially for ionisable molecules, zwitterions etc. Therefore, a single calculated value of Log P_{ow} , without any reference to the respective pH, cannot be correlated to physiological conditions and to the pH conditions of the percutaneous absorption studies. Solubility in water is not determined by the EU method. No documentation is provided for the reported stability of 2-methyl-1-naphthol in marketed products.

General toxicity

Based on the results of a 14-day oral toxicity study, the NOAEL following oral gavage administration of GTS03958 at doses of 10, 40, 150 or 500 mg/kg bw/day to rats for 15 days was 500 mg/kg bw/day. Based on the results of 3-months oral (gavage) toxicity studies an NOAEL of 100 mg/kg bw/day (highest dose tested) was established.

In a developmental study in rats, the maternal NOAEL for GTS03958 is 75 mg/kg bw/day, and the developmental NOAEL is 225 mg/kg bw/day.

A few studies were submitted which were stated in the submission to be not conducted in compliance with OECD or internationally accepted test guidelines and/or test material analytical characterisation was not documented. These studies were not considered to be in line with current requirements and do not provide additional information.

Irritation / sensitisation

The test substance (2-methyl-1-naphthol) was irritant to rabbit skin under the described conditions. The neat substance caused reversible eye irritation in the rabbit.

The test substance is a strong skin sensitizer.

Dermal absorption

The amount of 2-methyl-1-naphthol considered absorbed (receptor fluid + epidermis) was 3.07 µg/cm² (range 1.02 – 5.73 µg/cm²). As only a total of 12 chambers were used, the Amax of 5.73 µg/cm² (1.42% of the applied dose) may be used for calculating the MOS.

Mutagenicity / genotoxicity

Overall, the genotoxicity of 2-methyl-1-naphthol is sufficiently investigated for the three endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy. In the gene mutation assay in bacteria 2-methyl-1-naphthol was negative. However, in mammalian cells 2-methyl-1-naphthol exposure resulted in a dose dependent increase in mutant frequency at the *tk* locus of mouse lymphoma cells, which was due to an increase in small colonies indicating to a putative clastogenic effect. The latter was confirmed in two positive *in vitro* chromosome aberration tests. 2-methyl-1-naphthol was negative in the unscheduled DNA synthesis test.

The clastogenic effects found in the *in vitro* studies could not be confirmed in *in vivo* experiments covering the same endpoint. In two mouse bone marrow micronucleus tests, following oral and i.p. administration, 2-methyl-1-naphthol was negative. To overrule putative other genotoxic effects, 2-methyl-1-naphthol was also negative in an *in vivo* UDS test.

As the clastogenic effects found *in vitro* were not confirmed in *in vivo* tests, 2-methyl-1-naphthol itself can be considered to have no *in vivo* genotoxic potential and additional tests are unnecessary.

To reach a definitive conclusion, appropriate tests with 2-methyl-1-naphthol in combination with hydrogen peroxide have to be provided.

Carcinogenicity

No data submitted

4. CONCLUSION

Based on the chemistry of 1-acetoxy-2-methylnaphthalene (A153) and its *in situ* conversion to 2-methyl-1-naphthol, the relevant material for consideration in the context of the consumer risk assessment for hair dye products is 2-methyl-1-naphthol. Therefore, the toxicological evaluation only of 2-methyl-1-naphthol is relevant.

The physicochemical properties of 1-acetoxy-2-methylnaphthalene (A153) as well as the evaluation of the tests performed with this substance are reported in the Annex 1 to this opinion.

Assuming a complete hydrolysis of 1-acetoxy-2-methylnaphthalene, the SCCP is of the opinion that, apart from the risks associated with the use of a strong sensitisier, the use of 2-methyl-1-naphthol as an ingredient in oxidative hair dye formulations at a maximum concentration of 2.0% on the head, does not pose a risk to the health of the consumer.

When both 2-methyl-1-naphthol and 1-acetoxy-2-methylnaphthalene are present in a hair dye formulation, the maximum concentration on the head of 2-methyl-1-naphthol should not exceed 2.0%.

2-methyl-1-naphthol itself has no mutagenic potential *in vivo*.

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

5. MINORITY OPINION

Not applicable

6. REFERENCES

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Annex 1: Data submitted on 1-acetoxy-2-methylnaphthalene (A153)

1. Chemical and Physical Specifications

1.1. Chemical identity

1.1.1. Primary name and/or INCI name

1-Acetoxy-2-methylnaphthalene (INCI)

1.1.2. Chemical names

2-Methyl-1-naphthyl acetate

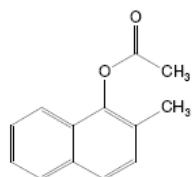
1.1.3. Trade names and abbreviations

Jarocol AMN

1.1.4. CAS / EINECS number

CAS: 5697-02-9
EINECS: 454-690-7

1.1.5. Structural formula



1.1.6. Empirical formula

Formula: C₁₃H₁₂O₂

1.2. Physical form

Off-white to cream-yellow powder

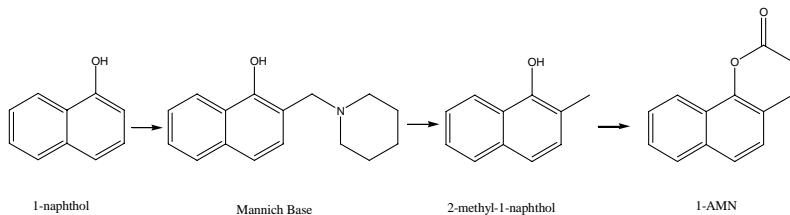
1.3. Molecular weight

Molecular weight: 200.24

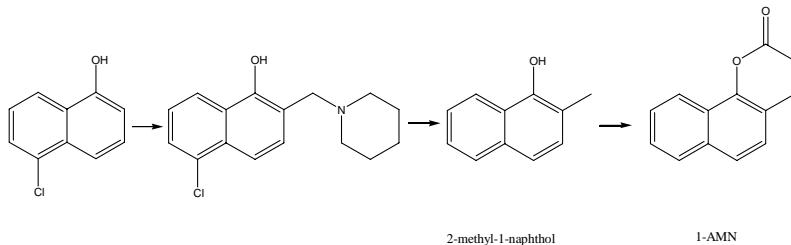
1.4. Purity, composition and substance codes

According to Summary Submission 1 of 2003, the three batches tested were synthesised by two different methods.

Process 1: Batches LRS7938-24 and LRS7988-05



Process 2: Batch MNC5E1115



Purity: 98.3% (range 95-100%), according to Summary Submission I of 2003 (without documentation)

According to Summary Submission II 2005 (without documentation):

- Identification by IR, NMR and elemental analysis
- Purity >96% determined by HPLC using an external standard

1.5. Impurities / accompanying contaminants

According to Summary Submission I of 2003 (without documentation)

Batch	HPLC Retention Time (minutes)	Proposed Structure
LRS7938-24	7.1	unknown
	8.4	
	10.96	unknown
	11.5	unknown
	18.99	
	20.9	unknown
	43.97-44.72	unknown dimer
	56.6	unknown dimer
LRS7988-05	8.4	

Opinion on 2-methyl-1-naphthol (including 1-acetoxy-2-methylnaphthalene)

	10.5	unknown
	43-97-44.72	unknown dimer
MNC5E1115	10.4	
	11.1	
	11.6	
MNC5E1115	18.99	
	20.9	unknown
	29.6	
	45.1	
	58.2	

According to Summary Submission II 2005 (without documentation):

1-naphthol	< 1000 ppm
2-methyl-1-naphthol	< 2500 ppm
1-acetoxy-2-acetoxymethylnaphthalene	< 1.5%
2-piperidinylmethyl-1-naphthol	< 1000 ppm
1-acetyl piperidine	< 1.5%
Toluene	< 1500 ppm
Arsenic	< 5 ppm

Opinion on 2-methyl-1-naphthol (including 1-acetoxy-2-methylnaphthalene)

Antimony	< 5 ppm
Lead	< 20 ppm
Cadmium	< 10 ppm
Mercury	< 5 ppm
Loss on Drying:	< 0.5%
Residue on Ignition:	< 0.5%

1.6. Solubility

According to Summary Submission I of 2003 (without method description or documentation):

Water: 0.041 mg/ml at 25°C

According to Summary Submission II of 2005 (without documentation):

Water: 0.021 – 0.031 mg/ml (after 15 minutes sonication)

Ethanol: 82 – 123 mg/ml (after 15 minutes sonication)

DMSO: 374 – 561 mg/ml (after 15 minutes sonication)

1.7. Partition coefficient (Log P_{ow})

According to Summary Submission I of 2003:

Log P_{o/w}: 2.87 at 40°C (no documentation or method description)

According to Summary Submission II of 2005:

Log P_{o/w}: 3.25 ± 0.24 (calculated)

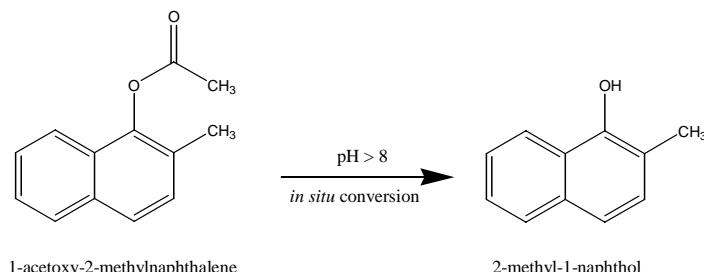
1.8. Additional physical and chemical specifications

Melting point:	78 – 83 °C
Boiling point:	/
Flash point:	/
Vapour pressure:	/
Density:	/
Viscosity:	/
pKa:	/
Refractive index:	/
pH:	/

1.9. Homogeneity and Stability

1-Acetoxy-2-methylnaphthalene (1-AMN) is unstable under the alkaline conditions of the hair dye matrix converting to 2-methyl-1-naphthol by hydrolysis [1-4].

The oxidative dye ingredient 2-methyl-1-naphthol is unstable in air and undergoes oxidation to form coloured products. It is converted *in situ* at alkaline conditions of the hair dye product base from 1-AMN to 2-methyl-1-naphthol as illustrated below. 2-Methyl-1-naphthol is stable in the alkaline matrix and does not undergo oxidation until intentionally mixed by the consumer with the hydrogen peroxide developer solution.



Depending upon the base, almost complete conversion of 1-AMN, according to a first order reaction, took 72-504 hours in the base alone and 8 min to 120 minutes in the base/developer (H_2O_2) mixture.

These studies were performed using several bases, the composition of which is not described. However, the study authors described that "in comparing the base formulation to the conversion patterns, it appears that several factors play into the conversion rate for 1-AMN including: (1) the form of the product (cream, gel, liquid), (2) the amount of water in the formulation, (3) the amount of solvent in the formulation, and (4) the amount of high HLB surfactant in the formulation. Further investigations would be needed to determine if any of these factors contributes more or less to the conversion process."

Study authors also suggested to evaluate the conversion rate of 1-AMN in bases including full shade: "Since all conversion experiments to date have been completed using monochrome batches, HPLC conditions would need to be evaluated in order to complete a conversion study on a full shade. The complexity of HPLC investigation is dependent on the number of dyes present in the shade".

General Comments to physico-chemical characterisation

- * Some physico-chemical properties of 1-acetoxy-2-methylnaphthalene reported in the Summary of Submission II are different than those described in the Summary of Submission I
 - * No documentation is provided for the chemical characterisation of 1-Acetoxy-2-methylnaphthalene or its impurities in it. Supportive documentation on the reported quantitative data on the purity and impurities is also not provided.
 - * The water solubility is not determined by an EU method
 - * Log P_{ow}: calculated values cannot be accepted as an estimate of the true physical constant without justification.

2. Function and uses

1-Acetoxy-2-methylnaphthalene is used in oxidative hair dye products at a maximum concentration of 3.2%. Due to the chemical conversion of this ingredient to 2-methyl-1-naphthol, this is approximately equivalent to 1% 2-methyl-1-naphthol on scalp.

1-Acetoxy-2-methylnaphthalene (A153) is the ingredient directly used in the formulation of some oxidative hair dye products. It is used at this stage because of its stable nature prior to and during the manufacturing process. Once formulated in the hair dye product, it irreversibly hydrolyses to 2-methyl-1-naphthol (A156) due to the alkalinity of the hair dye product matrix.

Complete hydrolysis of 1-acetoxy-2-methylnaphthalene in the marketed products has to be ensured by the manufacturer.

3. Toxicological Evaluation

3.1. Acute toxicity

3.1.1. Acute oral toxicity

An acute oral toxicity study was conducted using batch C7634/343A (A153). In this study, rats were treated with 500 or 2000 mg/kg in 0.5% methylcellulose (volume of 10 ml/kg) via oral gavage. No toxicity was observed.

Ref.: 2 (subm 2003)

Comment

In the study with A156, the dose was administered in a volume of 40 ml. In the study with A153 10 ml was used.

3.1.2. Acute dermal toxicity

No data submitted

3.1.3. Acute inhalation toxicity

No data submitted

3.2. Irritation and corrosivity

3.2.1. Skin irritation

Guideline: /

Species: New Zealand White Rabbit

Group: 3 female

Substance: C7634/343C

Batch: /

Purity: /

Site of Application: intact skin 6 cm² on the dorsal area of trunk for 4 hours unoccluded patch

Dose: Single application of 500 mg of test material

Reading Times: 30-60 minutes, 24, 48 and 72 hours post patch removal

GLP: in compliance

Study period: 6 – 9 April 1995

Very slight to well defined erythema was noted in all animals at the first reading time (30-60 minutes after patch removal). In a single animal very slight erythema was observed at the 24 and 48 hour reading times. No oedema was noted in any animal. All sites were clear within 72 hours

Ref.: 8 (subm. 2005)

Comment

Neat C7634/343C caused some transient skin irritation in the rabbit. The test substance could not be identified as 1-acetoxy-2-methyl-1-naphthol.

Guideline: /

Species: New Zealand White Rabbit

Group: 3 male

Opinion on 2-methyl-1-naphthol (including 1-acetoxy-2-methylnaphthalene)

Substance: C7634/343B
 Batch: /
 Purity: /
 Site of Application: intact skin 6 cm² on the dorsal area of trunk for 4 hours unoccluded patch
 Dose: Single application of 500 mg of test material
 Reading Times: 30-60 minutes, 24, 48 and 72 hours post patch removal
 GLP: not in compliance
 Study period: 6 – 9 April 1995

Very slight redness was observed in 2/3 animals 30-60 minutes after test material removal.
All redness had cleared by the 24 hour reading period.

Ref.: 8 (subm. 2003)

Comment

Neat C7634/343B caused some transient skin irritation in the rabbit. The test substance could not be identified as 1-acetoxy-2-methyl-1-naphthol.

3.2.2. Mucous membrane irritation

Guideline: /
 Species: New Zealand White Rabbit
 Group: 3 female
 Substance: C7634/343C
 Batch: /
 Purity: /
 Site of Application: Conjunctival sac of the right eye
 Dose: 0.01g into right eye of 1 animal, 0.1g into right eye of other two animals
 Reading Times: 1, 24, 48 and 72 hours, 4, 5 and 6 days post instillation
 GLP: not in compliance
 Study period: 7 – 13 April 1995

The animal receiving 0.01g showed no evidence of eye irritation throughout the observation period.

The animals receiving 0.1g showed conjunctival redness and swelling 1 and 24 hours after treatment and remained injected at 48 hours. One animal also displayed slight irritation of the iris. All animals had recovered by the 72 hour reading time.

Ref.: 4 (subm. 2003)

Comment

Neat C7634/343C caused reversible eye irritation in the rabbit. The test substance could not be identified as 1-acetoxy-2-methyl-1-naphthol.

3.3. Skin sensitisation**Buehler Test**

Guideline: /
 Species: Hartley Guinea Pigs
 Group: 38 total (19 male, 19 female) (20 in the test group, 10 in the vehicle control group, 8 in the pilot irritation screen group). Equal numbers of males and females per group
 Substance: C7634/343
 Batch: Lot # LRS 7938-24
 Purity: /
 Dose: 0.3ml of 50% test material in acetone
 GLP: in compliance

Study period: 9 January – 16 February 1995

The test material was applied, under occlusion, in 25 mm Hilltop chambers at the same site once every 7 days for a total of 3 exposures. After an interval of 14 days, 0.3 ml of 0.5% test material in acetone was applied to a naïve skin site using 25 mm Hilltop chamber.

During induction, skin sites were examined approximately 24 hours after each application. Following challenge, skin sites were examined 24 and 48 hours after application.

Results

Mild to moderate irritation was observed during the induction phase. No skin reactions were observed during the challenge phase. The test substance was considered non-sensitising.

Ref.: 12 (subm 2003)

Comment

The test substance could not be identified as 1-acetoxy-2-methyl-1-naphthol.

3.4. Dermal / percutaneous absorption

Guideline:	/
Species:	Human abdominal cadaver skin epidermis
Group:	3 donors
Chambers:	Diffusion; 4-5 chambers from each donor (total 14 useable); 1.04-1.54 cm ²
Integrity:	tritiated water flux < 1.5/mg cm ² /h
Substance:	1-acetoxy-2-methyl naphthalene in an oxidising base Radiolabelled 1-acetoxy-2-methyl[1- ¹⁴ C]naphthalene, specific activity of ¹⁴ C labelled material = 97.1 µCi/mg
Batch:	S12/11/95
Purity:	/
Radiolabel Purity:	99.6%
Dose:	15mg/cm ² of preparation containing ¹⁴ C-labelled 1-acetoxy-2-methyl naphthalene mixed with unlabelled dye material and incorporated into a commercial hair dye base formulation to give a concentration of 1.20% of this material. This was then added to a pre-weighed mixture containing other oxidative hair dye materials and then with a hydrogen peroxide preparation to produce a final concentration of 0.6%. Duration of Contact: 30 minutes followed by rinsing with deionized water
Sampling:	receptor fluid sampled 1, 2, 4, 6, 8, 24, 30 and 48 hours after test substance removal. Following the 48 hour receptor fluid sample, the skin was solubilised in order to determine the level of radioactivity remaining in the skin.
Receptor fluid:	PBS
Stability in receptor fluid:	/
Solubility in receptor fluid:	/
Analysis:	liquid scintillation counting
GLP:	not in compliance
Date:	1997

Total Amounts in Receptor During Application Time: Results tabulated below.

Hours	Mean amounts in receptor ± SD	
	µg/cm²	% of applied dose
1	0.045 ± 0.028	0.06 ± 0.05
2	0.184 ± 0.088	0.23 ± 0.11
4	0.267 ± 0.124	0.33 ± 0.15

Hours	Mean amounts in receptor ± SD µg/cm ²	% of applied dose
6	0.295 ± 0.135	0.36 ± 0.16
8	0.300 ± 0.140	0.37 ± 0.17
24	0.326 ± 0.157	0.40 ± 0.19
30	0.322 ± 0.154	0.40 ± 0.19
48	0.359 ± 0.177	0.44 ± 0.21

After the 48 hour evaluation, the samples were removed from the chambers, blotted and the amount of test substance present in the skin determined after 'solubilisation'.

The amounts of 1-Acetoxy-2-Methyl Naphthalene in an oxidising hair formulation base present in the receptor fluid were $0.359 \pm 0.177 \mu\text{g}/\text{cm}^2$ (range $0.130 - 0.671 \mu\text{g}/\text{cm}^2$) or $0.44 \pm 0.21\%$ (range $0.14 - 0.98\%$) of the applied dose.

The amount of 1-Acetoxy-2-Methyl Naphthalene still present in the skin after 48 hours was $0.18 \pm 0.02\%$ (range $0.06 - 0.28$). The A_{\max} (receptor fluid and skin) was 1.38% of the applied dose (cell #62)

Ref.: 15 (subm 2003)

3.5. Repeated dose toxicity

3.5.1. Repeated Dose (14 days) oral / dermal / inhalation toxicity

No data submitted

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

No data submitted

3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.6. Mutagenicity / Genotoxicity

3.6.1. Mutagenicity / Genotoxicity *in vitro*

Bacterial Reverse Mutation Test

Guideline: OECD 471 (1983)
 Species/strain: *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538
 Replicates: triplicates in a single experiment both in the presence and absence of metabolic activation
 Test substance: C 7634/343
 Solvent: DMSO
 Batch: LRS 7938-24
 Purity: 95.58 %
 Concentrations: 10, 33, 100, 333, 1000 and 3333 µg/plate without and with S9-mix
 Treatment: direct plate incorporation with 48 - 72 h incubation
 GLP: in compliance
 Date: October - November 1994

C 7634/343 was investigated for the induction of gene mutations in *Salmonella typhimurium* (Ames test). Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the level of toxicity in a

dose range-finding study up to the prescribed maximum concentration of 5000 µg/plate on toxicity and precipitation. Negative and positive controls were in accordance with the guideline.

Results

Both in the presence and absence of S9-mix slight precipitation was observed at the highest dose (3333 µg/plate). No positive responses were observed in any of the test strains either in the presence or absence of metabolic activation.

Conclusion

Under the experimental conditions used C 7634/343 was not mutagenic in this gene mutation tests in bacteria.

Ref.: 25 (subm 2003)

Comment

The test substance was not specifically identified as 1-acetoxy-2-methyl-1-naphthol in the test report.

In vitro Mammalian Chromosome Aberration Test

Guideline:	/
Cells:	CHO-K1 cells
Replicates:	duplicates in a single experiment
Test substance:	C7634/343G
Solvent:	Acetone
Batch:	MNC5E1115
Purity:	95.3 %
Concentrations:	40, 60 and 80 µg/ml without S9-mix 5, 10 and 15 µg/mL with S9-mix
Treatment:	20 h treatment and harvest time 20 h after start of treatment without S9-mix 4 h treatment and harvest 20.7 h after start of treatment with S9-mix
GLP:	in compliance
Study period:	August 1995 - September 1996

C7634/343G has been investigated in the absence and presence of metabolic activation for the induction of chromosomal aberrations in CHO cells. Test concentrations were based on the results of an initial toxicity assay on toxicity, as indicated by a decline in cell growth, and mitotic index. Approximately 2 h before harvest, each culture was treated with colcemid (final concentration 0.1 µg/ml) to block cells at metaphase of mitosis. Liver S9 fraction from Arochlor 1254-induced rats was used as exogenous metabolic activation system. Chromosome (metaphase) preparations were stained with Giemsa and examined microscopically for chromosomal aberrations and the mitotic index. The known clastogen cyclophosphamide was used as positive control.

Results

In the presence of S9-mix, a statistically significant and dose dependent increase in the percentage of metaphase cells containing at least one chromosomal aberration was observed. The types of aberrations were mainly chromatid type breaks and rearrangements. In the absence of S9-mix, a statistically significant and dose dependent increase in the percentage of metaphase cells containing at least one chromosomal aberration was observed. In addition there was a statistically significant increase in clastogenic damage at these doses based on a pair-wise comparison with the concurrent control. The aberrations were mainly chromatid breaks and rearrangements.

Conclusion

Under the experimental conditions used C7634/343G was clastogenic in this chromosomal aberration test *in vitro*.

Ref.: 26 (subm. 2003)

Comment

The test substance was not specifically identified as 1-acetoxy-2-methyl-1-naphthol in the test report.

In vitro unscheduled DNA synthesis test in rat primary hepatocytes

Guideline:	OECD 482 (1986)
Cells:	hepatocytes from male Sprague-Dawley rats
Replicates:	triplicates in a single experiment
Test substance:	C7634/343U
Solvent:	acetone
Batch:	MNC5E1115
Purity:	95.3 %
Concentrations:	5, 10, 15, 20 and 25 µg/ml
Treatment	18 - 20 h treatment
GLP:	in compliance
Study period:	August 1995 - February 1996

C7634/343U was investigated for the induction of unscheduled DNA synthesis (UDS) in primary hepatocytes isolated from rats. Test concentrations were based on the results of a preliminary cytotoxicity assay on lactate dehydrogenase release as an indicator of cytotoxicity. Cells were treated for 18-20 h with C7634/343U and 10 µCi ³H-thymidine/ml and then progressed for autoradiography. Evaluation of autoradiography was done after 5-12 days exposure and hematoxylin-eosin staining. UDS was measured by counting the number of grains in each nucleus and subtracting the average number of grains present in 3 equal-sized adjacent cytoplasmic areas (net nuclear grain). The mean net nuclear grain count for each dose level and the percentage of cells in repair (cells with > 5 net nuclear grains) was determined. DMBA was used as positive control.

Results

A high level of toxicity was observed at 40 µg/ml and a moderate to low level at 30 µg/ml and as such these could not be evaluated for unscheduled DNA synthesis. None of the dose levels between 5 and 25 µg/ml caused a significant increase in the mean net nuclear counts when compared to the solvent control.

Conclusion

Under the experimental conditions used, C7634/343U did not induce unscheduled DNA synthesis and, consequently, is not genotoxic in rats in this *in vitro* UDS test.

Ref: 27 (subm. 2003)

Comment

The test substance was not specifically identified as 1-acetoxy-2-methyl-1-naphthol in the test report.

3.6.2. Mutagenicity/Genotoxicity *in vivo*

Mammalian Erythrocyte Micronucleus Test

Guideline:	/
Species/strain:	CD-1® albino mice (sub-strain Crl:CD-1® (ICR)BR)
Group size:	5 mice/sex/dose group
Test substance:	C7634/343D

Batch no: LRS 7988-05
 Purity: 100.6 %
 Dose level: 75, 375 and 750 mg/kg bw
 Route: intraperitoneal injection
 Vehicle: 0.5% methylcellulose
 Sacrifice times: 24, 48 and 72 h after treatment.
 GLP: in compliance
 Study period: March – September 1995

C7634/343D has been investigated for the induction of micronuclei in bone marrow cells of mice. Test concentrations were based on the results of a preliminary toxicity study in male and female mice on mortality, pharmacotoxic signs immediately and approximately 24, 48 and 72 h after dosing and on PCE/NCE ratio. The top dose was considered the maximum tolerated dose based on the mortalities observed during the preliminary toxicity screen at 995 mg/kg and higher. In the main experiment mice were exposed by intraperitoneal injection to single doses of 0, 75, 375 and 750 mg/kg bw. Bone marrow cells were collected 24, 48 and 72 h after dosing. 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 and examined microscopically for the PCE/NCE ratio and micronuclei. The known clastogen cyclophosphamide was used as positive control.

Results

Signs of a putative decrease in the PCE/NCE ratio was only observed for male mice at the 24 hour sacrifice time. Some slight pharmacological signs only observed at the highest dose may indicate to systemic exposure.

No statistically significant or dose dependent increases in micronucleated PCEs were observed compared to the negative controls.

Conclusion

Under the experimental conditions used C7634/343D did not induce an increase in the number of micronucleated PCEs in bone marrow cells of treated mice and, consequently, C7634/343D is not clastogenic and/or aneugenic in bone marrow cells of mice.

Ref.: 24 (subm. 2003)

Comment

Because the PCE/NCE ratio was only slightly decreased in males at the 24 h sacrifice time and there were only slight indications for systemic availability after the highest dose only in females of the 72 h dose group, there are hardly indications for bone marrow cell exposure. Therefore, this study is of limited value.

The test substance was not specifically identified as 1-acetoxy-2-methyl-1-naphthol in the test report.

Reference 24 is identical to reference 28 and describes the *in vivo* micronucleus test for both A156 and A153.

3.7. Carcinogenicity

No data submitted

3.8. Reproductive toxicity

No data submitted

3.9. Toxicokinetics

No data submitted

3.10. Photo-induced toxicity

No data submitted

3.11. Human data

No data submitted

3.12. Special investigations

No data submitted

3.13. Safety evaluation (including calculation of the MoS)

Not applicable