Commission of the European Communities

Opinion of the Scientific Committee on Cosmetology (11/86 – 10/90)

Commission Decision 78/45/EEC of 19 December 1977 concerning the institution of a Scientific Committee on Cosmetology (OJ L 13, 17.1.1978, p. 24)

Scientific Committee on Cosmetology

Directorate-General
Employment, Industrial Relations and Social Affairs

Directorate-General
Environment, Nuclear Safety and Civil Protection

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FOREWORD

The Scientific Committee on Cosmetology was set up by Commission Decision 78/45/EEC on 19 December 1977 (OJ N° L 13 of 17 January 1978, p. 24) in order to provide the Commission with informed opinions on any scientific and technical problems arising in connection with cosmetic products, and in particular on the substances used in their manufacture, on their composition and on the conditions for their use.

The members of the Committee are independent scientists highly qualified in the fields of medicine, toxicology, biology, chemistry or other similar disciplines.

The Committee is serviced by the Consumer Policy Service.

C L A S S I F I C A T I O N O F I N G R E D I E N T S

Ingredients are classified by the Scientific Committee on Cosmetology into four groups on the basis of assessment of the information provided in accordance with the guidelines.

GROUP A

Contains ingredients for which full data have been provided, and on the basis of which they do not present any health hazard, and which therefore may be used in cosmetic products for the purpose stated and at concentrations not exceeding the limits recommended.

GROUP B

Contains ingredients which, in the light of the data provided so far, do not appear to present a health hazard and which therefore may be used temporarily in cosmetic products for the purpose stated and at concentrations not exceeding the limits recommended ; additional information is needed for complete assessment.

GROUP C

Contains ingredients which have been submitted to the Scientific Committee on Cosmetology but which, in the light of the data provided, could not be evaluated, or for which the additional information requested under Group B has not been received within the prescribed time limit and, as a result cannot at present be recommended for use in cosmetic products.

GROUP D

Contains ingredients which, on the basis of the information provided, present a health hazard and which are therefore not recommended for use in cosmetic products.

Assessment have been made on the basis of the concentrations of ingredients requested by industry.

CONTENTS

Forword V

Classification of ingredients VI

32nd meeting of the SCC - November 6-7, 1986 3
33rd meeting of the SCC - February 2, 1987 31
34th meeting of the SCC - June 6, 1987..... 85
35th meeting of the SCC - October, 1987 109
36th meeting of the SCC - January 19, 1988 175
37th meeting of the SCC - April 12, 1988 183
38th meeting of the SCC - October 10-11, 1988 273
40th meeting of the SCC - January 16, 1989 367
42th meeting of the SCC - October 30-31, 1989 419
43rd meeting of the SCC - January 23, 1990 439
45th meeting of the SCC - October 10-11, 1990 453

CONTENTS

38th meeting of the SCC - October 10-11, 1988

- Resorcinol - A11 277
- 4-Chlororesorcinol - A12 291
- 2-Hydroxy-4- aminotoluene - A27 297
- 2-(2'-4'-Diaminophenoxy) ethanol hydrochloride - A42 306
- Methylresorcinol - A44 315
- Oxadine A - P75 322
- Glutaraldehyde - P76 328
- 2,4,6-Trianilino-(p-carbo-2'-ethylhexyl -1'oxy)-
  -1,3,5 triazine - S69 337
- Stablizating agents of 1,1,1-trichloroethane 346

ATTENDANCE LIST

SCIENTIFIC COMMITTEE ON COSMETOLOGY

38th MEETING - 10/11 OCTOBER 1988

Present

Mr AGACHE

Mr DE GROOT

Mrs DONY

Mrs ENJOLRAS

Mr FIELDER

Mr GOULDING

Mr KAPOULAS

Mrs KNAAP

Mr LOPRIENO

Mr O'MAHONY

Mr RAMOS MORGADO

Mr SCHOU

Excused

Mr GRANADOS JARQUE

Mr MUSCARDIN

Mr STÜTTGEN

COMMISSION

Mrs MASSE

Mr GONTIER (DG XI/C/2)

Mr COLLIN (expert)

SUMMARY

Opinions expressed on October 10-11, 1988 concerning the use of :

- HAIR DYES (X1/663 - 88 ; X1/716/88)

1. RESORCINOL (A11)
2. 4 - CHLORORESORCINOL (A12)
3. 2 - HYDROXY - 4 - AMINOTOLUENE (A27)
4. 2 - (2'-4'-DIAMINOPHENOXY) ETHANOL HYDROCHLORIDE (A42)
5. METHYL RESORCINOL (A44)

- PRESERVATIVE AGENTS

1. OXADINE A (EEC n° 60 ; P75)
2. GLUTARALDEHYDE (EEC n° 26 ; P76)

- UV FILTERS (X1/823/88)

2, 4, 6 TRIANILINO - (p-CARBO-2'-ETHYLHEXYL-1'OXY) -
1, 3, 5 TRIAZINE (S69).

- STABILIZATING AGENTS

1 - 1 - 1 - TRICHLOROETHANE

REPORTS BY THE SCIENTIFIC COMMITTEE ON COSMETOLOGY

ON THE USE OF CERTAIN HAIR DYES

. THE COMMITTEE'S MANDATE

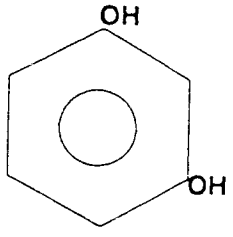
To give its opinion on the question whether the use of certain hair dyes :
- Resorcinol
- 4 - chlororesorcinol
- 2 - hydroxy - 4 - aminotoluene
- 2 - (2' - 4' - diaminophenoxy) ethanol hydrochloride
- methyl resorcinol

is admissible from the health point of view.

1. RESORCINOL

. FORMULA AND SYNONYMES

C1.: 76505
CAS: 108-46-3
Colipa A 11



Mol.w.110.11

RESORCINOL
1,3-DIHYDROXYBENZENE
1,3-BENZENEDIOL
m-BENZEDIOL
m-DIHYDROXYBENZENE
m-DIOXYBENZENE
3-HYDROXYCYCLOHEXADIEN-1-ONE
m-HYDROXYQUINONE
3-HYDROXYPHENOL

. USE

Oxidative hair dye

max. use : 2.5%

1.25% in combination with H_2O_2

0.5% in hair lotions and shampoos

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral (rat) : 370-980 mg/kg

dermal (rabbit) : 3360 mg/kg

ORAL TOXICITY

Short term oral : Rat, 20 mg/kg/d., 5d./wk 12 wks.: no-effects.

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

Eye irritation

Rabbit : Mild conjunctival redness (1 h, 2.5 w/v in water with 0.5 sodium sulphate into 1 eye. Rabbit (24, 0.1 g into 1 eye) : severe conjunctivities, iritis, corneal opacities occluding most of the iris, corneal ulcerations extending over the entire corneal surface, keratoconus and pannus formation with no improvement during 14 days of observation.

Dermal irritation

Rabbits : 2.5% w/v for 24 h, not irritating on shaven skin (reading at 72 h) ; 0.5 g in saline solutions : negative ; 1000-7950 mg/kg or 2500-19000 mg/kg dermal : irritation and necrosis dose-related ; 3% in ointment : canthose, hypergranulosis, oedema (ear and shaven flank daily for 14 days).

Sensitization

Guinea pig : 3% in water with 2% Natrosol 250, 2% Tween 80 and 0.05% sodium sulphite : no inflammation and no allergic reactions (3 weeks treatment and 2 wks challenge reaction).

Human-skin sensitization

Resorcinol : (1% in paraffin solution) showed positive reaction in 2.1% (1.9% males and 2.2% females) of 877 persons suffering from primary contact dermatitis. Resorcinol causes primary irritation of skin and eye in doses above 10%. Resorcinol (0.2% in salicyl-alcohol) given positive response in a patch test in eczematous subject (erythema) and acute dermatitis with salicyl-resorcinol solution in alcohol applied dermally. Resorcinol (5% in water) showed allergic reactions in 7.9% of 340 patients (eczematous) tested. Resorcinol given positive reaction for cross-sensitivity/patch test with resorcinol monoacetate ; hydroquinone ; pyrocatecol ; phenol ; pyrogallol ; hydroxy-quinone ; phoroloroglucinol ; hexylresorcinol ; orcinol ; cathecol ; 4-phenyl cathecol ; pyrogallic acid ; 3,5-dihydroxybenzene ; resorcinol mono methyl ether ; resorcinol dimethyl ether ; and floroglucinol. Resor - cinol caused ochronosis and myxoedema in a patient with received 12% in ointment on the leg ulcers for 13 years before died. In a case of resorcinol poisoning in a young children (7 weeks) the compound given severe haemolytic anaemia with haemoglobinuria and a generalized papulo-squamous eruption. Resorcinol (3-10-35% in vaseline) caused urticaria in 4 patients treated dermally : 3/4 fever and headache ; 2/4 showed articular pain. One person died after cutaneous applications of an ointment with 20% resorcinol.

Dermal asorption

Rhesus monkey : 0.2% ; guinea pig : 0.6%. Rat (back skin, 30 min.) Resorcinol (1.5% and 3.0%) + p-TDA (1.5% and 3.0%) 1:1 H_2O_2 : 2.77% (ie. 75.5 nM/cm ^{2}, 332.5 µg/kg) and 4.58% (i.e. 62.46 nM/cm ^{2}, 270 µg/kg) after 96 h.

Short term dermal

Rat : 2 50 mg/kg s.c. x 14 or 30 days : no-effects. Rat (154 mg/kg/d. in arachis oil x 47 days s.c.) : goitrogenic action after 69 day (irregular absorptions could have been involved. Guinea-pig (1% and 3% in unguentum on unshaven ears and flank of male for 14 d.) : acanthosis (max. 8 d.), hypergranulosis and orthohyperkeratosis, oedema on ears and flank, and papillomatosis only ears (max. 8 d.). Female rat, Resorcinol diacetate (800 mg/kg s.c. 2 times/d.x 12-19 d. or 22-25 d.) : goitrogenic action.

Human-skin absorption

In commercial hair dye (1.225% of resorcinol) applied (dose not reported) for 25-28 min penetrated 0.076% (as % of total dose excretion). Resorcinol 2% in hydroalcholic vehicle, dermal topical applications (2 time/d., 6 d/wk x 4 wks, i.e. 48 appl.) on 3 human volunteers (0.30 mg/cm2, 12 mg/kg/day) : 2.87% (i.e. 0.34 mg/kg/day), flux rate 0.37 µg/cm /h. It has been reported a skin absorption of 0.7% In vitro : 0.86 µg/cm2/h. (46).

MUTAGENICITY AND GENOTOXICITY

The compound has been tested for the induction of gene mutations in vitro and found negative in Salmonella spot test and in two studies plate test and in E.coli. The compound did not induce micronuclea on mice treated with a total dosage of 600 mg/kg in two equal oral doses separated by an interval of 24 hours.

CARCINOGENICITY

Mice : Skin painting throughout life span (50 animal/group) : 1 squamous cell papilloma on dorsal skin (dose : 5%) ; 1 squamous cell carcinoma on ear (dose : 25%).

Rabbit : 0.02 ml of 5-10-50% 2 time/week during life-time : no adverse signs or tumours were observed.

Mice, rat : dermal topical applications (0.2% to 2.0% containing formulation) : No adverse effects.
Mice, rats : feeding study in progress at NCl.

TERATOGENICITY AND REPRODUCTION

Teratogenicity

Rat : (until to 500 mg/kg oral : no-effects. Rat (40-80-250 mg/kg, 6-15 days gestation) : slightly lower maternal b.w. gain (250 mg/kg) ; no-effects : 80 mg/kg. Rabbit (25-50-100 mg/kg oral, 6-18 days gestation) slightly lower maternal b.w. gain (100 mg/kg) ; no effects : 50 mg/kg. Mice (28-35 mg/kg s.c. appli., 5-7 d, 8-10 d. and 11-14 d. gestation) : no-effects. Mouse, rat and rabbit : dermal topical apllications with formulations containing resorcinol (from 0.2% to 2.0%).

Mouse (1.7%) : significant decrease in b.w. and increase number of unossified caudal and vetebral centra and an unossified bones in feet ; (1.7%). Rat (1.7%) : significant increase of skeletal anomalies (notched and short ribs). Rabbit : no-sign of maternal toxicity only focal alopecia.

Embryotoxicity

HET test : It had no-effect-level, including systemic effects at doses ca. 1-5 ppm. Chicken embryons (3 days) : ED50 2.4 µmol/egg ; LD50 2.9 µmol/egg ; and malformed fetuses vs. 3% control (0.9-7.3 µmol/egg).

Multireproduction

Rat : 3-generations (0.2% to 2.0% in formulations) : negative.

MISCELLANEOUS INFORMATIONS

Metabolism

Rat : (10-50-100 mg/kg s.c.) : peak in plasma after 15 min. ; ca. 90% compound equivalent eliminated after 2 h : half-lives : 23 min-8.6 h (50 mg/kg) ; 18 min-10.5 h (100 mg/kg) ; peak in liver and kidney at 1 h (0.2%-0.3%). 10 mg/kg : 7% in gastrointestinal tract (1 h), 1.4% gastrointestinal tract and feces (24 h), 93.6% in urine (24 h). Rat (2 x 50 mg/kg, 6 h interval, for 14-30 d. and 50 mg/kg with trace 14-C-Resorcinol) : after 2 h plasma level declined to ca. 90% at 15-30 d. ; half-lives : 32 min (14-30 day, fast phase) - 5.0h (14 days) and 7.0 h (30 day) slow phase.

Thyroid and Liver fixation

Rat : dermal topical applications (30 min, intervals of 30-40 d.) of hair dye solution (1.5% (=136 nM) radiolabelled compound, mixed 1:1 with H_2O_2) : trace (2.2 µg) in liver and any fixation has been observed in thyroid 4 day treatment.

Excretion

14% as sulphate, 52% as glucuronide and 11% free resorcinol, in urine of rabbit after oral dose of 100 mg/kg.

Immunosuppressive effect

Mixed lymphocytes cultures, suppression of humural responses in vitro and inhibition of the production of circulating antibodies against red blood cells in vivo : negative.

Thyroid

Antihyroid effects might be ascribed to inhibition of thyroid peroxidase.

CONCLUSION

The SCC does not consider the use of Resorcinol in the hair dyes to be linked to any particular toxic risk for consumers.
National Toxicology Program's results might confirm the absence of any type of toxic risk in the use of Resorcinol.

Classification : B.

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SKIN IRRITATION
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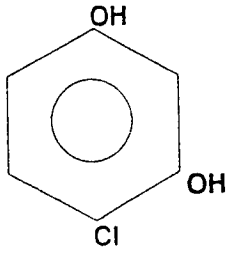
2. 4-CHLORORESORCINOL

. FORMULA AND SYNONYMES

C.1.: 76510
CAS : 95-88-5
Colipa A12



4-CHLORORESORCINOL
1,3-DIHYDROXY-4-CHLOROBENZENE

Mol.w. 144.56

. USE

Oxidation hair dye
Max. use : 3%
1.5% upon application

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral (rat) : 369 mg/kg

ORAL TOXICITY

Short term oral

Rat : 20 mg/kg bw/day per os for 12 wks. (daily/5 times weekly) : no effects. Rat : 40 mg/kg bw/day for 3 wks (5 day/wk) oral gavage : slight activation of thyroid epithelium ; slight decrease in the triiodothyronin in the serum.

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

Eye irritation

Rabbit : 2.5% w/v into one eye : negative, mild conjunctival irritation only (2/3 animals).

Dermal irritation

Rabbit : 2.5% w/v for 24 h : negative (reading at 72 h).

Sensitization

Guinea pig : 3% epicutaneously, daily (6 d/wk) for 3 weeks : no reaction.

Dermal absorption

Rat : 1%, 2% and 3% in formulations (1:1 H_2O_2) for 30 min (clipped skin) : 0.216%, 0.217% and 0.367 in 72 h. Rat : 2% in formulation (1:1 H_2O_2) : 0.006% as $^{14}CO_2$ in expired air after 8 h. Rat : 1% in formulation (1:1 H_2O_2) for 30 min. (unclipped skin) : 0.088% in 72 h. Rat : 300 mg in acqueous sol. for 30 min. (clipped skin) : 5.47% (0.11 mg/cm^2) in 72 h.

Short term dermal

Rabbit : 2% in formulation with 6% H_2O_2 for 13 wks : no-effects.

Chronic toxicity

Mice : skin painting, 2% in formulation, 21 months : no toxicity or carcinogenicity effect.

MUTAGENICITY AND GENOTOXICITY

The compound has been tested for the induction of gene mutations in vitro and found negative in Salmonella at spot test and in several studies at plate test and in E.coli. The compound did not induce micronuclea on mice treated with a total dosage of 600 mg/kg in two equal oral doses separated by an interval of 24 hours ; the compound was negative for the induction of chromosome aberrations on human lymphocytes in vitro.

CARCINOGENICITY

A long term study is in progress at National Toxicology Programm.

TERATOGENICITY

Rat : 2 ml/kg bw in formulation on day 1-4-7-10-13-16-19 of gestation. Rat : 50, 100, 200 mg/kg oral gavage (6 to 15 of gestation), 200 mg/kg : significant decrease in maternal weight gains, embryolethal (increase in resorptions), slight increase in fetal anomalies (not statistically significant). Minor anomalies (wavy ribs) and variations (incomplete ossification of the sternebrae, rudimentary and bilateral 14th ribs) of skeletal ; 100 mg/kg : no-effects.

MISCELLANEOUS INFORMATIONS

Organ distribution

Rat : 2% in formulation, cutaneous appl. : No marked affinity for any particular tissue (highest conc. found after 35 min 1h). Rat: oral 50 mg/kg in acqueous sol. : No special affinity to any organ.

Excretion

Rat : 50 mg/kg bw s.c. : ≥ 96% (urine and feces) in 24 h. Rat : 50 mg/kg oral : predominantly in urine in 24 h. ; 19.3% of oral dose in bile within 3 h.

Immunosuppressive effects

4-Chlororesocinol was found negative for the induction of immunosuppressive action evaluated by plate test and hemagglutination test when s.c. administered to 6 NMRI mice four time at the maximal tolerated dose of 1.5 mg.

CONCLUSION

The Committee requires :
- a study on skin penetration
- information on the (90-day) oral study in rats (Henkel) to establish the NEL on the thyroid
- the results of the aberration test on mammalian cells.

The Committee is waiting for the ongoing long-term study for carcinogenicity of NTP - USA.

Classification : B.

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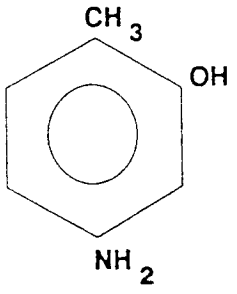
3. 2-HYDROXY-4-AMINOTOLUENE

. FORMULA AND SYNONYMES

CAS: 2835-95-2

Colipa A27



p-AMINO-o-CRESOL
1-METHYL-2-HYDROXY-4-AMINOBENZENE
2-HYDROXY-4-AMINOTOLUENE
4-AMINO-2-HYDROXYTOLUENE

Mol.w. 123.1

. USE

Oxidative hair dye
Max. use : 3%
1.5% with H_2O_2

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral (rat) : 3600 → 10000 mg/kg

ORAL TOXICITY

Short term oral

Rat : up to 2700 mg/kg oral intubation for 2 weeks (5 days a wk): disseminated white lesions in the pancreas (dose-related), no other dose-related toxic effects.

Rat : oral intubation, 300 to 2700 mg/kg bw/day for 13 weeks (5 day wk) : dose-related adverse effects at all doses (nervous system symptoms, decrease in the hematological values, increase in the relative weight

of the liver) ; at high doses (≥300 mg/kg) dose-related but not significant : increase or decrease in organ weights, histopathological changes in liver, stomach, pancreas, kidney and spleen ; liver, renal and adrenals enlargement).
Rat : oral up to 180 mg/kg bw/day for 13 weeks (5 days a week, by gavage) : no-effects.

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

Eye irritation

Rabbit : 2.5% w/v for 10 sec. : negative, mild conjunctival irritation only.

Dermal irritation

Rabbit : 2.5% for 24 hours : negative. Another study the compound resulted mild irritant.

Sensitization

Guinea pig : 3% in CMC and Tween 80, epicutaneously daily for 13 weeks (6 day a week) and other 2 weeks : weak sensitization in 4/19 animals.

Dermal absorption

Rat : 1% in formulation (1:1 with H_2O_2) for 30 min : 3.62 $\mu g/cm^2$ (male) and 5.64 $\mu g/cm^2$ (female).

Human absorption

1% in formulation (1:1 with H_2O_2) to dry hair at a lotion/hair ratio of 1.5-2, worked into hair of 3 human volunteers for 5-8 min. and left on for other 20 min., total urinary excretion : 0.2% applied dose.

MUTAGENICITY AND GENOTOXICITY

The compound was tested for gene mutation and found positive on Salmonella and negative in E.coli. The compound did not induce micronuclea on mice treated orally by gastric intubation.
Negative results obtained in several studies on Salmonella were performed at lowest doses respect to doses used in positive studies.
A formulation containing 2-hydroxy-4-aminotoluene have been tested for SCE in vivo in lymphocytes of human volunteers exposed for different times produced negative results.

TERATOGENICITY

Rat : oral up to 180 mg/kg bw/day (0.5% in CMC, 10 ml/kg) from day 6 to 15 of gestation : no embriotoxicity, embryoletalithy or teratogenicity effects.

MISCELLANEOUS INFORMATIONS

Immunosuppressive effects

Mouse : 4 x 7.75 mg s.c. injection, by plate-test (cell-mediated immunity) and haemagglutination test (humorally mediated immunity): negative.

CONCLUSION

The Committee requires a study on chromosomal aberration test in mammalian cells in vitro.

It appears to be judicious to look forward to a warning caution on the package following the high rates of sensitization (19%) which are observed in the animal studies.

Classification : B.

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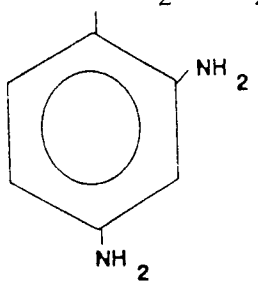
4. 2(2'-4'-DIAMINOPENOXY) ETHANOL HYDROCHLORIDE

. FORMULA AND SYNONYMES

CAS: 66422-95-5

Colipa A42



2,4-DIAMINOPHENOXYETHANOL DIHYDROCHLORIDE

2-(2'-4'-DIAMINOPHENOXY) ETHANOL DIHYDROCHLORIDE

(DIAMINO-2',4'-PHENOXY)-2-ETHANOL DICHLORIDRATE

Mol.w. 241

1-β-HYDROXYETHYLOXY-2,4-DIAMINOBENZENE

. USE

Oxidative hair dye

Max. use 4%

2% in combination with H_2O_2

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral (rats) : 1113 - 1160 mg/kg

(mice) : 1745 mg/kg

ORAL TOXICITY

Short term oral

Rat : 56 mg/kg bw/day oral for 3 months : no-effects. Mouse and rat: up to 0.2% oral for 12 weeks, toxic effects in mice (0.1%) and rats (0.2%). No effects level : 0.05% (mice) and 0.1% (rats).

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

Eye irritation

Rabbit : 4% aqueous solution (pH 2,5), without rinsed : pratically not irritating (reading at 24, 48, 72 h. and days 4,7).

Skin irritation

Rabbit : 4% in proplene glycol for 24 h : mildy irritating.

Sensitization

Guinea pig : compound with 50% Freud's complete adjuvant, challenge reaction (25% in petrolatum for 24 h) : 3/10 sensibilized (middle potential of allergenicity).

Dermal absorption

Hairless rat : 0.40% (= 23.65 nM) in commercial vehicle + 20 vol H_2O_2 for 40 min.: 5.05 nM (= 0.84 $\mu g/cm^2$) ; 0.33% (= 23.65 nM) in complete formulation : 2.83 nM (= 0.47 $\mu g/cm^2$) ;

(a) 0.40% (23.65 nM)

(b) 0.80%

(c) 1.20% in commercial vehicle (1:1 with 20 ml H_2O_2)

for 40 min : (a) 0.84 $\mu g/cm^2$ (5.03 nM)

(b) 1.34 $\mu g/cm^2$ (7.95 nM)

(c) 1.58 $\mu g/cm^2$ (9.42 nM) after 4 days.

MUTAGENICITY AND GENOTOXICITY

The compound has been tested for gene mutations in vitro in several experiments on Salmonella typhimurium and found negative on 5 studies with 1 only positive study.

Negative results were also obtained in Salmonella with a urinary assays on rat treated oral, i.p. and by topical applications, and on mice treated dermal. Negative data for gene mutations in vitro were obtained on reversions systems in E.coli (2 studies) and S. cerevisiae XV185-14C, forward mutation assay in S.pombe P1 and V79 hamster cell line (HPRT). Negative data were also obtained in vivo on D.melanogaster and in mouse spot-test with up to 1500 mg/kg.
The compound was anable to induce chromosome aberrations in vitro on CHO cell line and in human lymphocytes, micronuclea and dominant lethals on mice in vivo. The compound did not induce gene conversion on S. cerevisiae D4 (2 studies), UDS on HeLa human cell line.

CARCINOGENICITY

Mouse (0.07% and 0.04% in water) and rat (0.1% and 0.05% in water) for 104 weeks : no carinogenic effects.

TERATOGENICITY

Rat : 50-100-200 mg/kg/d. gastric intubation, 6 to 15 of pregnancy : increase in the incidence of skeletal anomalies and the proportion of litters containing such foetuses (dose-related, statistically significant at all doses), an increase in skeletal variants (extra rib and variant sternebrae) and lower values, not statistically significant, for litter and mean foetal weight at 200 mg/kg. Mouse up to 1500 mg/kg topically on shaven skin (6 to 15 post-fertilization): negative.

CONCLUSION

The SCC does not consider the use of 2,4-Diaminophenoxyethanol in the hair dyes to be linked to any particular toxic risk for consumers.

It appears to be judious to look forward to a warning caution on the package following the high rates of sensitization (30%) which are observed in the animal studies.

Classification : A.

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90-DAY ORAL STUDY

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(Mai 1982)

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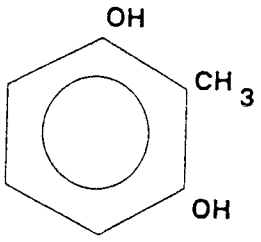
Report April 15,1983

5. METHYL-RESORCINOL

. FORMULA AND SYNONYMES

CAS: 608-25-3

Colipa A44



2-METHYLRESORCINOL
1,3-DIHYDROXY-2-METHYL-BENZENE
1,3-BENZENEDIOL-2-METHYL
2,6-DIHYDROXYTOLUENE

Mol.w.124

. USE

Oxidative hair dye
Max.use : 2.0%
1% in combination with H_2O_2

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral (rats) : > 5000 mg/kg (0.2% in hair dye basic cream)
(mice) : 390 mg/kg

ORAL TOXICITY

Short term oral

Rat : Up to 180 mg/kg/day oral (5 times a week) for 12 weeks : no-effects.

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

Eye irritation

Rabbits : 5% w/v in water : mild conjunctival irritation (after 24 h., disappeared after 7 days) ; cornea and iris : no irritation.
Rabbit : 2% in basic cream (6% H_2O_2) for 10 sec. : slightly irritating.

Dermal irritation

Rabbit : 10% for 4 h. : mild reversible dermal irritation.
Rabbit : 2% in basic cream (6% H_2O_2) for 4 h. : not irritating.
Mouse : 10% in aqueous, 2 appl./day for 5 days on same skin area : mild skin redness after 5 appl.

Human dermal irritation

5 human volunteers, 10% aqueous solution on forearm skin for 30 min. (30 sec. intervals), open method : not irritating. 5 human volunteers, 10% aqueous solution on upperarm skin for 2 h., occlusive conditions : no irritation until to 24 h.

Sensitization

Guinea pig : 5% aqueous : negative. Guinea pig : 2% in basic cream (6% H_2O_2) : negative up to 72 h. (challenge 1%, 21 and 28 day).

Dermal absorption

Rat : 16.9 mg (= 0.136 mMol) / 6.25 g basic cream + 6% H_2O_2 (1:1): 0.48% in 24 h.

Short term dermal

Rabbit : 1% in formulation (1:1, 6% H_2O_2) dermal topical application twice weekly for 13 wks. : no-affects (slight thickening in treated skin).

MUTAGENICITY AND GENOTOXICITY

The compound was tested for gene mutation in vitro and found negative on Salmonella. The compound did not induce chromosome aberrations in vitro on CHO cells and ; was inable to induce micronuclea in vivo on mice with oral doses up to 2 x 350 mg/kg.

TERATOGENICITY

Embrytoxicity

Chicken-embryo : HET test : moderately toxic, no-evidence of teratogenicity : LD50 = 6.1 mg/egg (day 1) - LD50 = 1.08 mg/egg (day 5).
Rat : 1% in formulation, 2% ml/kg topical applications on day 1-4-7-10-13-16-19 of gestation. Study inadequate (treatment every 3 days ; only one dose tested).

MISCELLANEOUS INFORMATIONS

Excretion study

Rat : 20 mg in water s.c. : > 90% as glucuronide or sulphate after 24 h. (urine and feces), no expired air. Rat : 40 mg in water, oral : completely absorbed by the intestine ; > 90% in 24 h. (urine).

CONCLUSION

Is required a teratogenicity study.

Classification : B.

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REPORTS BY THE SCIENTIFIC COMMITTEE ON COSMETOLOGY

CONCERNING THE USE OF TWO PRESERVATIVE AGENTS

IN COSMETIC PRODUCTS

. THE COMMITTEE'S MANDATE

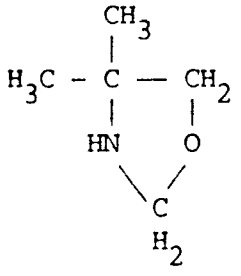
To give its opinion whether the use of two preservative agents :
- 4,4-dimethyl-1,3-oxazolidine up to 0.1%
- glutaraldehyde up to 0.1%

is admissible from the health point of view.

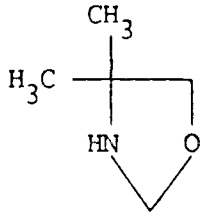
1. 4,4-DIMETHYL-1,3-OXAZOLIDINE

. FORMULA AND SYNONYMES

EEC n° 60
Colipa P75
dimethyl oxazolidine
Oxadine-A
Oxaban-A



or



$C_5H_{11}NO$

MW 101

. CHARACTERISTICS

The material is described as an aqueous mixture of two active ingredients, viz. 4,4-dimethyl-1,3-oxazolidine or 4,4-dimethyl-1-oxa-3-azacyclopentane and (as an impurity, generated during synthesis)

3,4,4-trimethyl-1,3-oxazolidine or 3,4,4-trimethyl-1-oxa-3-azacyclopentane. The levels of the two active ingredients in Oxaban-A are c. 76% and c. 2% respectively. The commercial product also contcains c. 3% 2-amino, 2-methyl-1-propanol, traces of formaldehyde and c. 19% water. The concentration of active ingredients is c. 87.4% or c. 78%. The material is soluble in water and in oil.

. USE

Use level of the commercial mixture up to 0.1% both in rinsed-off-, and in non-rinsed off cosmetics.

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral (rat) : 956 mg/kg
(mouse) : 890 mg/kg (ref.11)
dermal (rabbit): 970-2000 mg/kg

Because the oral and dermal values are comparable, dermal absorption is probably high.

LC50 (rat) : 11,66 mg/l (-1-hour inhal.)

ORAL TOXICITY

In a 28-day study, groups of 6 rats/sex were orally dosed with 0, 50, 200 or 600 mg Oxaban A/kg bw/day, 5 times weekly. The top dose caused clinical symptoms and growth depression, changes in several haematological characteristics (decreases in Hb and PCV, and increases in WBC, neutrophils and thrombocytes) and decreases in serum levels of total protein and albumin. At autopsy, 10/12 rats in the top-dose group showed gross changes in the stomach. The relative liver weight showed increases with 200 and 600 mg/kg, while the relative weights of the spleen, adrenals and testicles were increased with 600 mg/kg. Of the 5 organs weighed and examined microscopically only the stomach of top-dose rats showed treatment-related changes (Ref. 16).

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

An 87.4% solution was severely irritating to the rabbit eye and the changes produced showed no recovery over 72 hours. Dilutions of 1000 ppm and 5000 ppm of the commercial solution (equal to 0.08% and 0.4% of the active material) were not irritant to the eye (Ref. 2, 3 and 4).

In rabbits a primary skin irritation test, using 0.5 ml of the 87.4% aqueous solution on an occlusive patch for 24 hrs, caused slight to severe signs of oedema, erythema and deep tissue reaction (Ref.5). A test on rabbit skin with 0.5 ml applied for 4 hrs, produced signs of irritation but not necrosis (Ref. 14). A repeated skin application test with 3% aqueous emulsion produced irritation in 70 out of 101 human volunteers (Ref. 15).

The material showed skin sensitizing properties in the guinea pig (Ref. 6). In a well-conducted study in 101 human volunteers no evidence of sensitization was found in repeated tests with a 3% aqueous solution (Ref. 7, 15).

In a sub-chronic (13-week) dermal toxicity test in rats with Oxadine-A at dose levels of 0, 1.95, 19.5 and 195 mg a.i./kg, applied daily 5 days/week in a 1:1 ethanol/water solution, the top dose induced increased haematopoietic activity, as well as severe skin irritation. No other treatment-related abnormalities were found despite extensive clinical-chemical and histological examination (Ref. 9).

MUTAGENICITY

Results from an Ames test showed a reproducible dose-related increase in revertants in TA 98 and TA 100, but this was less than twice background and was not significant (Ref. 10 ; Ref. 18). Negative results were also obtained in a second assay using TA 98 and TA 100, that has been briefly reported (Monte et al., Fd Chem. Tox. 21(5)695-696(1983).

A fluctuation test in the S. typhimurium strains was negative (Ref.17). A chromosomal aberration test in human lymphocytes in vitro with and without S-9 mix was positive at all doses (Ref. 19). An in vitro test in CHO cells showed dose-related increases in activity, and a mouse lymphoma assay was also positive (file dated 11-5-83). A chromosomal aberration test in vivo in bone marrow cells of rats treated intraperitoneally with 20, 40 or 80 mg/kg was negative (Ref.20).

TERATOGENICITY

A teratogenicity study with 4 groups of 16 rabbits, treated dermally with 0, 30, 100 or 300 mg Oxaban-A/kg for 6 hr per day during days 7 through 19 of pregnancy, did not reveal signs of systemic embryonal or maternal toxicity or teratogenicity (Ref. 21).

CONCLUSION

The product was found to be only moderately toxic in short-term oral and dermal studies in rats. The no-effect levels were 50 and 25 mg/kg respectively (expressed as commercial product). Studies on dermal absorption are not available but data on acute oral and dermal toxicity suggest dermal absorption to be considerable. No evidence of embryotoxicity or teratogenicity was found in rabbits treated dermally with up to 300 mg/kg. Genotoxic properties were established in several in vitro tests, but one in vivo study for chromosomal aberrations in rats was negative. Further testing is necessary to confirm absence of genotoxic potential in vivo. It should be noted that the material is nitrosatable.

Classification : B.

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2. 1,5-PENTANEDIOL

. FORMULA AND SYNONYMES

EEC n° 26
Colipa P76
CAS Reg n° 111-30-80
GLUTARALDEHYDE

$CHO - CH_2 - CH_2 - CH_2 - CHO$ $C_5H_8O_2$

MW: 100,13

. CHARACTERISTICS

Readily soluble in water and most organic solvents. The substance reacts with amino groups and forms cross links.

. USE

Used as a preservative in rinsed off and non-rinsed off cosmetics up to 0.1%.

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50	oral (rats)	:	60 - 650	mg/kg	
	dermal(rabbits)	:	735	mg/kg	(50% concentr)
			900		(40% concentr)
			2000 - 3200		(25% concentr)
LC50	(inhal - rats)	:	12,6	mg/l	(8h to saturated vapour)
			23,5 - 40	ppm	(4h to 50, 20 and no ppm)

ORAL TOXICITY

Short-term

Oral administration of 0.1, 0.5, 1.0 and 1.6 g/kg bw/day to rats with the diet for 7 days produced growth depression and decreased weights of liver and kidneys with 1.6 g/kg. No changes were seen with 1.0 g/kg (Colipa ref. 27). Two-week oral treatment of rats with 100 or 1000 ppm in drinking water (13 and 103 mg/kg bw/day respectively) revealed hyperplasia of mucous glands in the stomach with 103 mg/kg, while 13 mg/kg was a NEL (ref. 28). A 13-wk study in rats with up to 100 mg/kg bw in the drinking water showed growth retardation at the top-dose. There were no other relevant changes and the NEL was 25 mg/kg bw for males and 37.5 mg/kg bw for females (ref. 47).

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

Eye irritation

In rabbits was severe with concentrations above 1%, with concentrations between 0.25 and 1% only slight and with 0.1% absent.

Skin irritation

In rabbits with a 45% solution was severe and was accompanied by oedema and necrotic foci. With 2% only mild erythema occurred and with 1% no effects were seen. In humans a 5% solution caused severe skin reactions after 2 occluded applications. Repeated open applications of 5% or less did not induce skin reactions in humans, but in another study 0.5% was slightly irritant. Irritation seemed more likely if the applications were to areas where the stratum corneum is thin.

Sensitization

Was examined in several studies in humans. Thirteen open applications of 5% a.i. to 20 healthy subjects followed by an open challenge with 5% produced no reactions. With 7 occluded applications of 1% a.i. and 3 occluded applications of 2%, followed by a challenge with 2% also occluded produced mild reactions in 6/20.
Applications of 0.1%, 0.2% and 0.5% under occlusion for 48 hrs, 10 times to 109 subjects, followed by a challenge to the same concentration as that of the induction did not produce reactions with 0.1 and 0.2%. With 0.5% a few reactions occurred during induction, and 2/109 showed reactions to the challenge.
There are several reports of sensitization to the a.i. in medical and paramedical personnel.

Dermal treatment

Of mice with 50 µl of various aqueous dilutions on 9 days during 2 weeks caused death of all animals treated with 25 or 12.5 mg a.i. per mouse, and growth retardation with 2.5 mg a.i. per mouse. No significant changes were seen with 1.25 mg a.i./mouse, which was equivalent with 80 mg/kg bw/day.

Dermal absorption

Of 0.075%, 0.75% or 7.5% (1,5) -^{14}C-glutaraldehyde, applied in vivo under occlusion for 24 hrs, was between 10 and 53% in the rabbit and less than 9% in the rat. The highest absorption occurred with the high concentration, due to the skin damage. The percentages absorption mentioned, do not include the considerable amounts of radioactivity which remained in the stratum corneum under the area of application. This retention may explain the long $t_{\frac{1}{2}}$ of 112 hrs in rats and of 77 hrs in rabbits observed upon dermal dosing, whereas after intravenous dosing the $t_{\frac{1}{2}}$ was only 9-11 hrs in rats and 12-18 hrs in rabbits. Dermal dosing resulted in reasonably uniform tissue distribution. Up to 80% was excreted as CO_2 and up to 20% in the urine (ref. 33).

MUTAGENICITY

Mutagenicity

Several gene mutation test were conducted with S.typhimurium strains, but none was positive. The exposure concentrations were low, due to toxicity of the compound. No indications of mutagenicity were observed in a HGPRT test with CHO-cells treated in vitro with up to 0.1 mg/ml (ref. 40). In a SCE test with CHO-cells exposed to various concentrations up to 0.1 mg/ml, a significant increase in exchanges was seen only with 0.05 mg/ml (not with 0.1 mg/ml).
The positive result was considered a chance event (ref. 40). An UDS test with rat hepatocytes did not induce positive effects but the positive control substances were sometimes negative also (ref. 40).
In a dominant lethal assay, mice received one oral treatment with 30 or 60 mg/kg (respectively one tenth and one fifth of the LD_{50}).
No increased lethality of embryos was observed (ref. 41).

Teratogenicity

Was examined in mice, treated by gavage with a 2% commercial product in amounts providing 16, 20, 24, 40, 50 and 100 mg a.i./kg bw/day on days 6-15 of pregnancy. Increased incidences of resorptions and of malformed foetuses were seen with 40 mg/kg and more. With 24 mg/kg and less no increased incidence of malformations was seen, but signs of toxicity occurred at all dose levels (ref. 42).
In a second teratogenicity study in mice (dosed orally with 3.3, 10 or 30 mg/kg bw/day on days 7-12 of pregnancy) the percentage of resorbed and dead foetuses were relatively high in the 2 high-dose groups. The number of malformations was relatively high in all dose groups although the significance of these findings was not clear (ref. 44). Rats dosed orally with 25 or 50 mg/kg bw/day, on days 6-15 of pregnancy, did not show increases in resorptions, deaths or malformations, although the top-dose induced decreased body weight gain (IBT-study ; ref. 43).

MISCELLANEOUS INFORMATIONS

Short term inhalation

By rats of 0.2, 1.0 and 3.0 ppm in the atmosphere for 9 days, caused reduced intake of food and water, weight loss and mortality with 3.0 ppm, haematological and clinical-chemical changes with 1.0 and 3.0 ppm. A few signs of toxicity were seen with 0.2 ppm (ref. 31).
A 90-day inhalation study in rats at 0.02, 0.05 and 0.2 ppm, 5 hrs/day, 5 days/week, revealed growth depression, and clinical signs of mucosal irritation (perinasal wetness and discharge) at 0.2 and 0.05 ppm. Microscopic examination showed lesions of the heart in 3/20 males of the high-dose group (which the authors did not consider to be treatment-related). The NEL was probably 0.02 ppm, but individual data were not provided (ref. 32).

CONCLUSION

The available data showed that in short-term animal studies the substance was highly toxic by inhalation and moderately toxic when administered orally. Dermal absorption in animals was considerable. Teratogenic properties were observed at oral dose levels which produced maternal toxic effects, but foetal toxicity occurred at lower dose levels, and a no-effect level was not identified. Genotoxicity test in vitro were generally negative.
No information was available on chromosomal aberration. A dominant lethal test in mice was negative. Long-term studies were not available The substance has sensitizing properties.
A further teratogenicity study in mice is needed to identify a NEL, since this seems to be critical in assessing safety factors in use. The mutagenicity data should be supplemented with a chromosomal aberration test in vitro with mammalian cells.

Classification : B. (but not to be used in aerosol sprays)

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REPORT BY THE SCIENTIFIC COMMITTEE ON COSMETOLOGY

CONCERNING THE USE OF

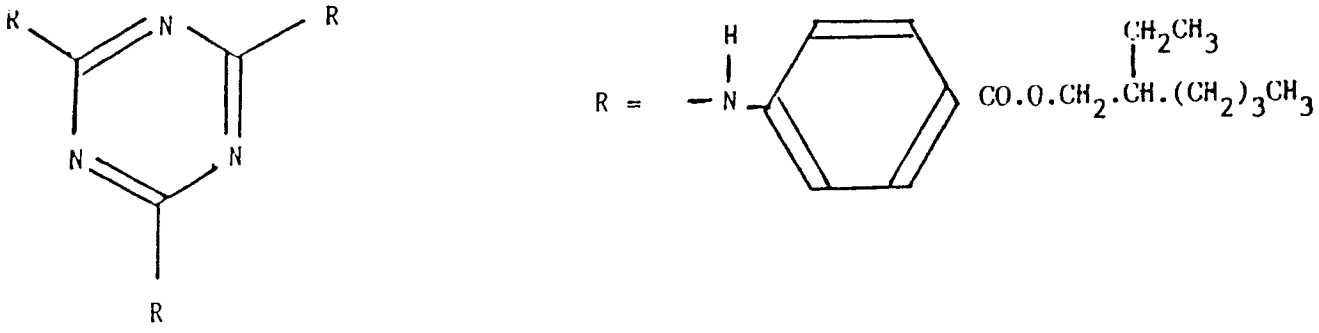
2,4,6-TRIANILINO-(p-CARBO-2'-ETHYLHEXYL-1'-OXY)-1,3,5-TRIAZINE

. THE COMMITTEE'S MANDATE

To give its opinion on the question whether the use of this compound (S69) as UV filter at the maximal concentration of 5% in sunscreen preparatives is admissible from the health point of view.

. FORMULA AND SYNONYMES

Colipa : S69
UVINUL
2,4,6-trianilino-(p-carbo-2'ethylhexyl-1'-oxy)-1,3,5-triazine.



$C_{48}H_{66}N_6O_6$ MW: 823,1

. CHARACTERISTICS

Stated by manufacturer to be more than 98% pure.
Insoluble in water ; soluble in isopropyl myristate, olive oil, ethanol.
Absorption maximum : 312 nm.

. USE

Used as a stabiliser in light-sensitive plastics, dyes, etc...
Proposed use level in suncreen preparations : up to 5%.

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral (rat and mouse) : > 10 gr/kg bw
dermal (rat) : > 2 gr/kg (ref. 1 → 5)

ORAL TOXICITY

Thirteen-week study. Groups of 10 male and 10 female rats were given 0, 1000, 4000 and 16000 ppm in the diet. There was a dose related increase in the weights of the liver in female rats only ; there was no evidence of damage on histological examination, and clinical chemistry tests were normal. The authors therefore did not consider the liver findings to be significant, and the no-effect level was put at 16000 ppm, or about 1150 mg/kg bw/day (ref. 19).

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

Four standard Draize eye test are reported. Evaluation is uncertain in two of the experiments because of doubts about the concentrations use. Slight changes were found with a 10% solution in olive oil, with and without rinsing. Findings were normal after 48 hours.
A suspension of (probably) 50% in saline caused no abnormality.
A suspension, probably of 41%, caused slight changes only. In another study, up to 50% was used in olive oil ; no abnormalities were found.

Overall, the substance appears to be only slightly irritating, if at all (ref. 6→10).

Chick chorio-allantoic membrane

The probable concentrations of a.i. used were 0.64% and 6.4%. No abnormalities were found at either concentration (ref. 11).

Skin irritation

Two groups of 6 rabbits were used, one group with scarified skin and one group without. A 10% solution of a.i. in olive oil was applied for 24 hours with occlusion. There was definite erythema in 4/6 rabbits with sacrification, and slight erythema in 2/6 rabbits with intact skin. No abnormality was found after 7 days.

In another experiment, groups of 3 male and 3 female NZW rabbits were used. A 50% suspension of a.i. in physiological saline was applied to intact and scarified skin with occlusion for 24 hours. Vehicle controls were used. No abnormality was found.

In another experiment, groups of 6 males and 6 females were used 3 of each sex had scarification of the area of application. Concentrations of up to 2% of the a.i. were applied for 24 hours with occlusion ; the material was formulated in various o/w creams, as emulsions, and in the form of commercial preparations.

The commercial preparations had no adverse effects, but the concentrations of a.i. were only 0.9% and 1.8%. The emulsions and o/w preparations showed slight erythema and oedema in the first few days, but the maximum Draize score at any one reading was 2.

In another experiment, groups of 3 male and 3 female rabbits were tested with intact and scarified skin. The maximum concentration tested was 50% in olive oil. Applications were for 24 hours with occlusion. There was slight oedema for the first 2 or 3 days at 25% and 50% ; the highest score at any time was 2.

In another experiment, a 50% suspension in water was applied under semi-occlusive conditions for 24 hours to 3 animals. There was no evidence of irritation (ref. 9, 12, 13, 14, 16).

Guinea-pig

A commercial preparation containing 2% of a.i. was applied daily for 5 days. No abnormality was found (ref. 9).

Man

Fifty subjects were tested, 28 males and 32 females. Concentrations of 5% and 10% were applied as emulsions and as oily solutions for 24 hours with occlusion. There was one reaction to the 5% solution in oil. Otherwise no abnormality was found (ref. 15).

Sensitization

Guinea-pig

A commercial formulation containing 2% of a.i. was used. It was applied daily to the skin, 5 days a week for 3 weeks. After a 2 week rest, the same preparation was applied 3 times to a fresh site. No reaction was found (ref. 9).

A Magnusson-Kligman maximisation test was carried out in 40 animals, 20 test and 20 controls. The induction concentration of a.i. was 5% in olive oil intradermally, and 60% dermally, with occlusion for 48 hours. The challenge was made with 40% solution in olive oil. There were no significant differences between control and test groups (ref. 17).

Man

Sixty subjects were tested by applications of a commercial preparation containing 2% a.i. applied for 24 hours with occlusion. Of the 60 original subjects, 10 had the test material applied to the same sites 5 times at intervals of 48 hours with occlusion. This procedure was repeated after 10 days. No reaction was seen (ref. 9).

Photosensitization

Guinea-pig

Two groups of 9 animals were used. The concentration of a.i. was probably 0.5%. The positive control was 3,3',4,5-tetrachlorosalicylanilide. The applications were followed by 15 minutes of UV irradiation and the procedure was repeated 5 times. After a 10 day rest, 2 challenge applications were made, followed by irradiation as before. Later, 2 compounds were applied at a lower concentration and irradiation was again carried out. There was no evidence of photosensitization. The positive control, however, gave either very weak reactions or none ; and the maximum concentration of a.i. tested was probably only 0.5% (ref. 24).

Allergic effects

Man

A 1% solution of a.i. in olive oil was applied in a panel of 8 subjects known to be allergic to para-aminobenzoic acid derivatives. It is stated that no reaction was produced, but no details are given (ref.18).

Tolerance on repeated use

Man

In 45 subjects, of whom 14 had sensitive skin and allergic conditions a commercial formulation containing 2% a.i. was applied daily. During 3 weeks of exposure no adverse reaction was seen (ref. 9).

Percutaneous absorption

Man

A 0.5% solution of a.i. in ehtanol/hexane was applied to the forearms of 8 subjects. After 30 minutes the area was repeatedly stripped, 20 times in all. The concentration of a.i. in each stripping was estimated by HPLC.

Since the amount of percutaneous penetration can be estimated from the degree of penetration of a substance into the stratum corneum in the first 30 minutes after application, it should be possible to calculate the amount of percutaneous absorption of the a.i. from the figures given. However, there are various difficulties in doing this from the data given in the report. It is probable, however, that percutaneous absorption is likely to be considerable.
An investigation of the effect of vehicle on the permeation of a.i. into the stratum corneum, under occlusion, was made using a photo-acoustic method. It is difficult to evaluate this, since the technique is experimental, but possibly permeation is greater from a w/o emulsion than an o/w emulsion (ref. 20).

MUTAGENICITY

A standard Ames test was carried out. The maximum concentration used was 5000 microgram/plate (limit of solubility was 500 microgram). There was no increase in revertants, with or without activation (ref.21).

Mouse

Micronucleus test : The test dose was 2100 mg/kg bw. No adverse effect was found (ref. 22).

EMBRYOTOXICITY

Two series of Chick embryos were used, injections being made on day 1 and day 5 of incubation, respectively. The doses were lower on day 5 than on day 1. At the highest doses applied, nearly 50% of the embryos died, and the LD50 was about 45 mg and 25 mg respectively. Mortality in each dose group was dose related. There was a significant increase in the length of the metatarsus in the group given 10 mg on the fifth day. There were also some changes in blood chemistry values (ref. 23).

MISCELLANEOUS INFORMATIONS

Rat : doses up to 500 mg/kg bw by mouth had no effect on the blood pressure, nor on carrageenan-induced oedema of the paw (ref. 25-26).

Conclusion

CONCLUSION

The Committee felt that the concentrations of a.i. used in some of the tests for irritation of mucous membranes and for sensitization were low in relation to the proposed use level. On the whole, however, these tests were acceptable. Tests for acute oral and dermal toxicity were acceptable. Tests for mutagenicity were acceptable. The tests for phototoxicity and photosensitization were unsatisfactory.

Further testing for percutaneous penetration would be required ; if it is marked, consideration of the need for studies in pharmacokinetics and teratology/reproduction would be required.

The data provided were not such as to suggest a need for tests for inhalational toxicity or short term dermal toxicity.

Classification : B.

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Evaluation of the safety of stabilizers for 1,1,1-trichloroethane as a propellant in aerosol cans

		Classification
1.	1,2-butylene oxide	D
2.	dimethoxymethane	C
3.	nitromethane	C
4.	nitroethane	A
5.	1-nitropropane,	-
6.	methoxypropane, 2 ol	A
7.	n-propanol	A
8.	isobutanol	D
9.	tertiary butyl alcohol	C
10.	tertiary pentyl alcohol	A
11.	2-methyl-3-butyn-2 ol	C

1. 1,2-butyleneoxide = 1,2-epoxybutane CAS Reg.No 106-88-7

1 ppm = 2.94 mg/m^3 1 mg/liter = 340 ppm

Maximal use level 1 % of the propellant gas

This substance is moderately anaesthetic and irritating to the skin and eyes, but skin irritation is not likely to follow vapor exposure alone.

The acute toxicity is moderate: the oral LD_{50} in rats was 500 mg/kg, an inhalation LC_{50} in rats was 4000 ppm.

In sub-acute inhalation studies in rats and mice, deleterious effects including mortality occurred with 1600 ppm, while 800 ppm was without effect. However, in reproduction studies in rabbits, mortality occurred with 250 and 1000 ppm.

In sub-chronic inhalation studies in rats and mice, 150 ppm was a no-toxic effect level (NEL), while 600 ppm caused growth retardation and changes of the nasal mucosa.

Mutagenic properties were established in the Ames test and in the mouse lymphoma test. The substance also induced chromosomal aberrations and sister chromatid exchanges in CHO-cells in vitro, and was positive in Drosophila (NTP TR329, 1988).

In long-term inhalation studies, the substance was found to be carcinogenic in rats, not in mice (NTP TR329, 1988).

Evaluation

In view of its mutagenic and carcinogenic properties, this substance should not be used as a stabiliser in aerosol cans.

Classification: D

October, 1988

2. Dimethoxymethane = dimethylformal = methylal CAS Reg.No 109-87-5

TLV: 1000 ppm or 3100 mg/m^3. STEL: 1250 ppm

Maximal use level: 2.0 % of the propellant

This substance is slightly anaesthetic and moderately irritating to the skin and to mucous membranes. No sensitisation properties have been observed.

The acute toxicity is low: the oral LD_{50} was 5.7 g/kg in rabbits. The inhalation LC_{50} in rats was 15.000 ppm, in mice and guinea pigs around 18.000 ppm.

Short-term inhalation exposure of mice and guinea pigs to 11.000 ppm during 7 h. on 15 days, induced irritation of mucous membranes, anaesthesia, fatty degeneration of the liver and kidneys, inflammation of the lungs, CNS-effects and high mortality.

The substance is well-absorbed through the skin, and metabolized to methanol, which can be detected in the blood.

Evaluation

No information is available on sub-chronic-, or chronic toxicity, or on possible effects on reproduction or teratogenicity. Results of mutagenicity tests are also lacking. An evaluation of this substance is therefore, not possible.

Classification: C.

October, 1988

3. Nitromethane CAS Reg.No 75-52-5

TLV 100 ppm = 250 mg/m^3; STEL 150 ppm = 375 mg/m^3.

Maximal use level in the propelling agent is 1.0 %.

The acute toxicity is considerable: the oral LD_{50} in rats and mice was c. 950 mg/kg. Inhalation exposure for brief periods to levels above 10.000 ppm may induce symptoms of irritation, narcosis, weakness, and salivation. Microscopic changes were seen in the liver and kidneys.

In a 6-month inhalation study, rats and rabbits exposed to 98 or 745 ppm showed slightly depressed haemoglobin levels. The high dose induced growth depression and decreased haematocrit and haemoglobin levels in rats. In rabbits, the high dose induced decreased thyroxine levels in the blood, and increased thyroid weights, suggesting goitrogenic activity.

No mutagenic properties were observed in an Ames test, a Drosophila test and a micronucleus test.

The substance has been selected for extensive examination by the NTP. The studies will be initiated early in 1988.

Evaluation

On the basis of available toxicity data, evaluation is not possible.

Classification: C.

October, 1988.

4. Nitroethane CAS Reg.No 79-24-3

TLV 100 ppm = 310 mg/m^3; STEL 150 ppm = 465 mg/m^3

Maximal use level in the propellant is 1.0 %

The acute toxicity is considerable: the oral LD_{50} in rats was 1100 mg/kg and in mice 860 mg/kg. The LC_{50} in rabbits was between 1000 and 5000 ppm but in guinea pigs c. 10.000 ppm.

This substance is more irritating to the respiratory tract than is nitromethane. Inhaled nitroethane results in increased blood levels of nitrate. It is rapidly metabolized and is completely eliminated from the body in 30 h., mainly via the lungs.

An Ames test with and without metabolic activation was positive especially with the strain TA 100. A micronucleus test in mice was negative.

In a chronic inhalation study, rats were exposed to concentrations of 100 or 200 ppm, 7 hr/day, 5 days/week for 2-yr. There were no effects of exposure on survival,haematology, clinical chemistry, organ weights, or on non-neoplastic or neoplastic pathology. Body weights were slightly lower in exposed rats. (Griffin et al., Ecotoxicol. Environ. Saf. 16(1)11-24(1988)).

In a project on photochemical smog, a number of inhalation studies has been conducted with a mixture of diethylhydroxyl-amine (DEHA 10-20 ppm), nitroethane (c.10 ppm) and diethylamine hydrogen sulfite (at its vapour pressure). A chronic (2.5-yr) study in rats did not reveal changes of any significance (Heicklen et al., Environm. Res. 26 (1981) 258-273).
Chronic (2-yr) inhalation exposure of mice to the mixture, resulted in an increased tumour incidence of marginal statistical significance in male mice, whereas in females the total incidence of tumours was significantly decreased (Heicklen et al., Environm. Res. 27 (1982) 277-289).
A reproduction study in mice exposed to the mixture over 3 successive generations did not reveal any deleterious effect on survival, numbers of litters and pups borne per litter or on the microscopy of the organs examined (Heicklen et al., Environm. Res. 20 (1979) 450-454).

A teratology study on mice indicated that the exposure to the mixture (DEHA 9 ppm, nitroethane 14 ppm, diethylamine HS at vapour pressure) produced no effect on the dams, and there was no evidence of treatment-related terata, embryotoxicity, or inhibition of fetal growth and development (Beliles et al., Environm. Res. 17 (1978) 165-176).

Evaluation

A concentration of 1 % stabiliser in the propellant gas of aerosol cans may result in a daily human exposure to 105 mg, if the can contents contain 35 % propellant, and the consumer uses up to 30 g can contents per day. If it is assumed that the amounts of stabiliser used, is present in 1 m^3 of air surrounding the consumer, the concentration is 34 ppm, which is considerably below the TLV. Because long-term exposure of rats and mice to c.10 ppm did not result in overt toxicity it is unlikely that daily exposure of humans to the can contents for a few minutes would be harmful.

Classification: A.

October, 1988.

5. 1-nitropropane CAS Reg.No 108-03-2

Use level 1 % in propellant gas

TLV 25 ppm = 90 mg/m^3 STEL (35 ppm) = (135 mg/m^3)

This substance is more toxic upon acute exposure than NE and NM. The oral LD_{50} varied with the species between 250 and 800 mg/kg. The inhalation LC_{50} for rats was 3100 ppm/8 h. Exposures at 5000 ppm for 3 h. killed rabbits and guinea pigs.

Irritating properties are noticed by humans at concentrations above 100 ppm after brief periods of exposure. Headache and nausea are noted upon prolonged exposure to excessive levels.

One out of two Ames tests conducted was negative, the other was marginally positive. A micronucleus test in mice with up to 300 mg 1-nitropropane/kg bw was negative, as was 2-nitropropane in the same test. (Kliesch and Adler, Mutation Research 192 (1987) 181-184).

In a long-term (21 1/2 months) inhalation study in rats, daily exposure to 100 ppm 7 hr/day, did not induce changes in appearance or behaviour, growth rate, organ weights, serum chemistry or haematology, or in the gross or microscopic appearance of the organs. If rats were exposed to 100 ppm 2-nitropropane (CAS Reg.No 79-46-9) instead of 1-nitropropane, an increased incidence of hepatocarcinomas was observed (Griffin et al. Toxicol. Environm. Safety 6 (1982) 268-282).

Evaluation

A use level of 1 % in the propellant may result in a daily human exposure to 105 mg stabilizer. If this amount is present in 1 m^3 of air around the user, it would be an exposure concentration of 29 ppm, which is close to the TLV. Daily exposure to such a level for only a few minutes is not considered to represent a significant health hazard.
Although the information on mutagenicity is incomplete, the absence of carcinogenic potency in the chronic study may justify acceptance of this compound. However, because no specification was available, and no information on possible contamination of 1-nitropropane by the more toxic coumpound 2-nitropropane, classification was postponed.

Classification: No classification.

October, 1988.

6. Methoxypropane, 2 ol = propyleneglycol monomethylether CAS Reg.No 107-98-2

TLV 100 ppm or 360 mg/m^3 STEL 150 ppm
(1 ppm = 3.68 mg/m^3; 1 mg/l = 272 ppm)

Oral LD_{50} values (in mg/kg bw) for rats vary between 5.2 and 11.9, the value for mice is 10.8, for dogs 9.2, for rabbits 5.3. The LC_{50} for rats and guinea pigs was 10.000 ppm during respectively 5-6 and > 7 h. exposure. The dermal LD_{50} in rabbits was 13-14 g/kg.

The substance causes only very mild skin irritation after prolonged contact. Eye irritation is mild to moderate.

Dermal absorption is rather high (1.17 mg/cm^2/h) and dermal application of 7 or 10 ml/kg for 90 days has caused narcosis and mortality in rabbits, and mild narcosis also with 2 and 4 ml/kg. Increased kidney weight was the only organ change observed with 10 ml/kg.

In sub-chronic oral studies in rats and dogs at dose levels of 0.5 up to 4.0 ml/kg b.w./day, depression of the CNS was observed in a dose-related manner. In rats, liver damage occurred with 2.0 and 4.0 ml/kg. Minor kidney damage was seen in both the rats and the dogs at the higher doses (Rowe and Wolf. In: Pathy 1981, pp 3977-3981).

Sub-chronic inhalation studies in rabbits and monkeys with 800 ppm (2.910 mg/m^3) did not induce adverse effects. In rats and guinea pigs no ill-effects were seen with 1500 ppm (5460 mg/m^3). At higher levels there was slight growth depression, slight liver and lung changes and mild CNS-depression.

Teratogenicity studies in mice, rats and rabbits with 0.04 up to 2.00 ml/kg b.w. by gavage or by s.c. injection revealed only minor foetotoxic effects. Inhalation teratogenicity studies in rats and rabbits with 500, 1500 and 3000 ppm induced growth retardation and CNS depression, but no statistically significant increases in malformations. Both 500 and 1500 ppm were no-effect levels.
A reproduction study over 2 generations is being conducted by NTP.
No indications of mutagenic properties were observed in the Ames test or in a chromosomal aberration test in CHO cells. There was no induction of unscheduled DNA synthesis in rat hepatocytes in vitro.

Evaluation

The substance is low in both single- and repeated-dose oral, and inhalation toxicity, but can be absorbed through the skin in toxic amounts if exposure is extensive and prolonged. Because repeated exposures are very disagreeable, as a result of their irritant action on the eyes and mucous membranes, the hazard from inhalation is negligible.

Classification: A.

October, 1988.

7. n-propanol = 1-propanol = propylalcohol CAS Reg.No 71-23-8

TLV 200 ppm = 500 mg/m^3 (skin) STEL 250 ppm = 625 mg/m^3

Use level in propellant 2 %

Oral LD_{50} values for rats vary from 1.87 to 6.5 g/kg. The s.c. and i.v. LD_{50} for mice was 3.2 and 1.1 g/kg respectively. A 4-h. LC_{50} for rats was c. 4000 ppm. Signs of intoxications are ataxia, paralysis, hypothermia, dyspnoea and narcosis.

Irritation to the skin is moderate, but contact with the eye causes moderate to severe irritation.

Sub-acute (4-day) oral administration of 2160 mg/kg/day to 6 rats did not induce mortality or gross liver lesions. Liver damage occurred however, in long-term oral rat studies (Rowe and Mc Collister 1981. In: Patty pp 4556-4561). The substance is well-absorbed through the skin, the intestinal wall and the lungs.

An Ames test was negative. A test in E.coli was positive. Tests for gene mutations, SCE's and micronuclei in mammalian cells in vitro were negative. (Obe and Ristow, Mut. Res. 56 (1977) 211-213; Lasne et al., Mut. Res. 130 (1984) 273-282; von der Hude et al. Env. Mutagenesis 9 (1987) 401-410; WHO Food Add. Series (1980) no. 16).

In long-term studies with limited numbers of rats treated either orally or by subcutaneous injection, an increased incidence of tumours was reported. A long-term dermal study in mice, with applications of 40 mg/mouse (or 800 mg/kg b.w.), 3 x/week, was negative (NTP-study).

Inhalation of 10.000, 7.000 or 3.500 ppm by groups of 15 pregnant rats on days 1-19 of pregnancy, for 7 hr/day, caused lowered food intake and body weights of the dams, and implantation losses, retardation of foetal growth and

increased incidences of external - and skeletal malformations in the two high dose groups. Visceral malformations were increased in the highest dose group. The low dose group only showed slightly reduced food intake of the dams. Thus n-propanol was teratogenic at concentrations that also produced maternal toxicity (Nelson et al., Fd Chem. Toxicol. 26 (1988) 247-254).

Evaluation

The substance is low in acute oral and inhalation toxicity, but markedly injurious and irritating to the eye of rabbits. Inhalation of toxic levels was teratogenic in rats, but 3500 ppm was not toxic and not teratogenic.
A use level of 2 % in propellant gas may result in a daily human exposure to 210 mg stabilizer. If this amount is present in 1 m^3, the exposure level in 84 ppm, which is less than half the TLV. Daily exposure to this level for only a few minutes is not considered a health hazard.

Classification: A.

October, 1988.

8. Isobutanol = 2-methyl-1-propanol = isobutyl alcohol CAS Reg.No 78-83-1

TLV 50 ppm = 150 mg/m^3 STEL (75 ppm) = (225 mg/m^3)

The oral LD_{50} for the rat was 2.46 g/kg and for the rabbit 3.4 g/kg; the dermal LD_{50} for the rabbit was 4.24 g/kg. In inhalation tests, rats survived 16.000 ppm for 2 h. suggesting a 4 h. LC_{50} of around 10.000 ppm. Isobutanol acts primarily as a narcotic.

The substance is not considerably irritating to the skin of rabbits, but, may cause severe eye irritation.

Short-term oral exposure of rats to 7.4 % in drinking water for 4 months induced an enlarged stomach filled with food and gas, and signs of constipation and bleedings of the small intestine. Increasing the concentration to 15 % for 2 months induced decreased liver size and decreased contents of fat, glycogen and RNA of the liver (WHO/IPCS Environmental Health Criteria for Isobutanol, 1985).

A long-term oral rat study with a total dose of 29 ml/rat (0.2 ml/rat twice weekly during 495 days) and a long-term subcutaneous rat study with a total dose of 9 ml (0.05 ml/kg, twice weekly for 544 days) were reported to have shown an increased incidence of malignant tumours, viz. in 3 out of 19 test rats in the oral study, and 8 out of 24 test rats in the subcutaneous study. Control rats had no malignant tumours. In the oral study one test rat had a forestomach carcinoma, and a liver cell carcinoma, another had a forestomach carcinoma and myelogenous leukaemia, and the third a myelogenous leukemia. In the subcutaneous study the tumours comprised two forestomach carcinomas, two liver sarcomas, one spleen sarcoma, one mesothelioma and two retroperitoneal sarcomas. A total of three benign tumours was recorded (Patty, p. 4580).

A mutagenicity test in E.coli CA274 was positive.

Humans exposed to low concentrations, show irritation of the respiratory tract and the eyes. Chronic inhalation of 3 mg/m^3 and 0.5 mg/m^3 affects the CNS. Oral ingestion of alcoholic drinks induce behavioural and subjective changes (WHO/IPCS Environmental Health Criteria for Isobutanol, 1985).

Evaluation

Animal studies have shown the substance to be low in acute oral toxicity, appreciably irritating to the eyes, not appreciably irritating to the skin, and low in acute inhalation toxicity. Systemically, isobutylalcohol has a narcotic action. Long-term studies with small numbers of rats suggest a carcinogenic potential. Since, moreoveer, a mutagenicity test was positive, and very low levels in the air have been reported to affect the NS of humans, it was not considered justified to accept this substance as a stabiliser in pressure cans without further studies.

Proposed classification: D.

November, 1988.

9. Tertiary butylalcohol = 2-methyl-2-propanol = trimethyl carbinol

CAS Reg.No 75-65-0

Use level 3 %

TLV 100 ppm = 300 mg/m^3 STEL 150 ppm = 450 mg/m^3
1 mg/l = 330 ppm 1 ppm = 3.03 mg/m^3

The oral LD_{50} in the rat was 3.5 g/kg, in the rabbit 3.6 g/kg. The i.v. LD_{50} for the mouse was 1.5 g/kg. The primary effect of the substance is that of a narcotic. The oral narcotic dose (ND_{50}) for rabbits was 1.4 g/kg.

The substance was not irritating to the skin of rabbits. When applied to human skin, only slight erythema and hyperemia were observed. Excessive exposure of humans to vapour may cause irritation of the airways, and headache, nausea, fatigue and dizziness.

Information on systemic toxicity is not available.

No mutagenic properties were detected in a test with Neurospora crassa.

Oral studies in rats and mice with 0.5, 1.0 and 2.0 % in drinking water are in progress in the framework of NTP. The program also includes inhalation studies.

Evaluation

The absence of information on systemic toxicity and the nearly complete absence of mutagenicity data, makes an evaluation impossible. It is suggested to await the results of ongoing studies by NTP.

Classification: C.

March, 1988.

10. Tertiary pentyl alcohol = tertiary amyl alcohol = 2-methyl-2-butanol = tertiary pentanol CAS Reg.No 75-85-4

Use level 2 %

1 mg/l = 278 ppm 1 ppm = 3.60 mg/m^3

Oral LD_{50} values are 1.0 g/kg and 1-2 g/kg for rats, 2.0 g/kg for rabbits. The dermal LD_{50} for rabbits was 1.7 g/kg. The LC_{50} for rats (exposed 6 h.) was between 3000 and 5700 ppm; with 3000 ppm all rats became unconcious but recovered and survived; with 1100 ppm only slight motor incoordination was observed. The substance exerts a narcotic action. The narcotic dose 50 (ND_{50}) in rabbits was 0.7 g/kg.

Topical, non-occluded application to the rabbit skin did not induce irritation, but severe narcosis occurred when applied under occlusion.

Marked eye irritation occurred in rabbits and was accompanied by signs of pain, swelling of the lids, corneal cloudiness and iritis. Healing was complete after 14 days.

A sensitization test in guinea pigs by a modified Maguire method was negative, whereas all 10 animals of a positive control group were sensitized.

Short-term (4-wk) dermal toxicity in rabbits was examined at dose levels of 0, 344 and 3440 mg/kg/day, 5 days a week. No adverse effects occurred in the low-dose group, except the expected local skin changes. The high-dose caused more extensive skin injury and serious systemic effect. Three of each sex became comatose and died or were sacrificed when moribund. Gross and microscopic examination did not reveal morphologic lesions. Decreases in body size, adipose reserves, and glycogen of hepatocytes were the only changes detected.
Short-term (7-day) inhalation exposure of rats to 0, 150, 500 or 1500 ppm, 6 h./day, for 7 consecutive days revealed motor incoordination and lethargy with 1500 ppm. Liver and kidney weights were increased.

Sub-chronic (90-day) inhalation exposure of rats, mice and dogs, to 0, 50, 225 or 1000 ppm, 6 h./day, 5 days/wk, caused transient motor incoördination in dogs and rats of the high dose group, and an increased alkaline phosphatase activity in top-dose dogs. No mortality occurred. Extensive microscopic examination did not reveal treatment-related changes (Rowe and Mc Collister (1981). In: Patty, pp 4602-4608).

The metabolism of tert. amylalcohol is very slow. An appreciable amount of absorbed material is excreted unchanged in the expired air.

Mutagenicity tests with Salmonella typhimurium (three strains) and with Saccharomyces cerevisiae were negative.

Evaluation

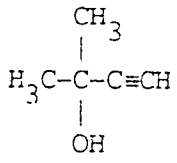
The substance is of moderate acute oral toxicity, is severely irritating to the eye and moderately irritating to the skin. Toxic amounts may pass the skin. Repeated inhalation of 100 ppm, induced relatively minor effects in the dog, lesser effects in the rat, and none in the mouse. No systemic effects were established. The substance is very slowly metabolized and largely excreted in the expired air. It was not found mutagenic in tests with microorganisms. Other mutagenicity tests or a long-term study are not available.
A 2 % concentration of the stabilizer in the propellant gas of aerosol cans and the use of 30 g can contents/day results in a potential daily exposure to 210 mg. If this amount is present in 1 m^3 of air the concentration is 58 ppm. In view of the available data, this level of exposure is not likely to present a health hazard.

Classification: A.

October, 1988.

11. 3-Butyn-2 ol, 2 methyl =dimethylacetyl carbinol=2-hydroxy-2-methyl-3-butyn

CAS Reg.No 115-19-5



Used at a concentration of 2 % as stabilizer in TCE.

LD_{50} values in mice are 1.8 g/kg oral, 2.34 g/kg s.c., and 3.6 g/kg i.p.

No sensitization occurred when the substance was tested in guinea pigs by the Landsteiner/Draize method.

Because no further information is available an evaluation is not possible.

Classification: C.

March, 1988.

REFERENCES
- H.G. verscuuren, C.G. De Rooij "Health Risk assessment of environmental exposure to 1,1,1-TCE" Regulatory Toxicology & Pharmacology 11, 90-99 1990
- Abstracts on developmental toxicity of 1,1,1-trichloroethane in CD rats. Studies done at Research Triangle Institute NIEHS/NTP Sponsored Chemical Mixture Projects/Studies Annual Plan for the fiscal year 1989
- Federal Register Volume 54 N°163 Aug. 23, 1989

CONTENTS

40th meeting of SCC - January 16, 1989

- Hexamidine - P8 369
- Benzethonium chloride - P70 374
- CI 13065 378
- CI 42045 384
- CI 42535 388
- CI 44045 390
- Solvent yellow 98 394
- Ethoxylated Ethyl-4-aminobenzoate - S3 398
- Ethylhexyl-p-dimethylaminobenzoate - S8 402
- 2-Ethylhexylsalicylate - S13 408
- 2-Hydroxy-4-methoxybenzophenone-5-sulphonic acid - S40 ... 413

REPORTS BY THE SCIENTIFIC COMMITTEE ON COSMETOLOGY

40th REUNION

JANUARY 16th, 1989

LIST OF PARTICIPANTS

Present

Mr. DE GROOT
Mrs. DONY
Mr. FIELDER
Mr. GOULDING
Mrs. KNAAP
Mr. LOPRIENO
Mr. MUSCARDIN
Mr. O'MAHONY
Mr. PONS-GIMIER
Mr. RAMOS-MORGADO
Mr. SCHOU
Mr. STUTTGEN
Mr. SOMOGYI

Not Present

Mr. AGACHE
Mrs. ENJOLRAS
Mr. KAPOULAS

COMMISSION

Mrs. MASSE
Mr. GONTIER (Serv. "Politique des Consommateurs")
Mr. COLLIN (expert)

REPORTS BY THE SCIENTIFIC COMMITTEE ON COSMETOLOGY

CONCERNING THE USE OF TWO PRESERVATIVE AGENTS

. THE COMMITTEE'S MANDATE

To give its opinion on the question whether the use of :

- HEXAMIDINE at levels of 0.1% and 0.3%
- BENZETHONIUM CHLORIDE at the level of 0.1%

in cosmetic products is admissible from the health point of view.

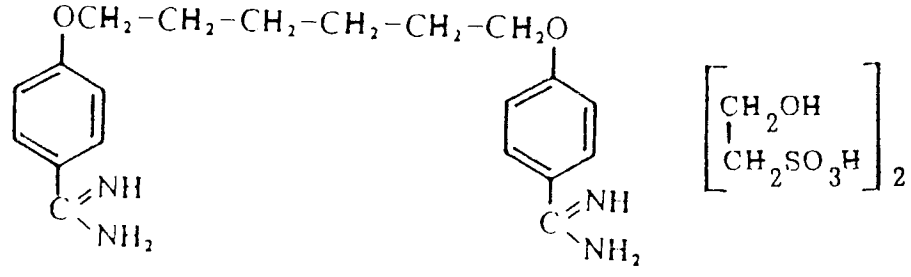
1. HEXAMIDINE SCC/204/89

. FORMULA

EEC n° 20

Colipa P8

1,6-di(4-amidino phenoxy)-n-hexane and its salts including di-isethionate and di(p-hydroxybenzoate)



$C_{20}H_{26}N_4O_2$

. CHARACTERISTICS

Soluble in water

Insoluble in organic solvent

. USE

Hexamidine is used in cosmetics as a preservative at a maximum dose level of 0.1%, and for uses at concentrations up to 0.3% in non-rinsed skin products.

. RECAPITULATION OF THE STUDIES OF TOXICITY

The acute toxicity of hexamindine is considerable.

LD50	oral	(rat)	:	750 mg/kg bw
		(mouse)	:	710 - 2500 mg/kg
		(rabbit)	:	500 mg/kg
	i.p.	(rat)	:	57 mg/kg
		(mouse)	:	17 - 51 mg/kg
	i.v.	(rat)	:	8 mg/kg
		(mouse)	:	17 mg/kg
	dermal	(rat)	:	> 4000 mg/kg

ORAL TOXICITY

A recent short-term (4-wk) oral study was conducted by gavage administration of 50, 100 and 200 mg/kg bw/day to groups of 5 rats/sex. All test animals showed post-treatment symptoms (salivation, wet fur, brown oral staining). The top-dose rats also showed abnormal position and locomotion, and increased counts of white blood cells and lymphocytes. In the two higher dose groups there were increases in the values for GPT, GOT and calcium in blood plasma. All treated rats showed caecal enlargement. The lungs, heart, liver, kidneys and caecum did not reveal treatment-related microscopical changes. Other organs (including spleen and adrenals) were not examined.

The clinical signs and the caecum enlargement were not considered to be of toxicological significance. The no-toxic effect level was established at 50 mg/kg, but the study showed several deficiencies (Colipa subm. III, ref. 10).

In a 90-day oral study in male rats, daily doses of 400 and 800 mg/kg by gavage induced mortality, growth depression, signs of anaemia, increased liver weight and decreased liver and kidney function. The lower dose of 200 mg/kg was not a clear no-effect level.

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

A concentration of 0.1% was slightly irritating to the skin and eye of the rabbit.

Hexamidine did not produce sensitization in guinea pigs and was not photosensitizing.

A subacute (28-day) dermal toxicity study in rabbits showed that solutions of up to 2% were only slightly irritant. Daily application of 4 ml/kg bw of a 0.05, 0.1 and 2.0% solution revealed no systemic toxicity.

A 90-day dermal study in rabbits with the very low dose level of 16 mg/kg bw revealed no systemic toxicity.

MUTAGENICITY

An Ames test with 4 S. typhimurium strains (TA 1535, 1537, 98 and 100) using concentrations up to 500 µg/plate was negative (ref. 11).

No evidence of clastogenic activity was observed in an in vitro test for chromosomal aberrations in CHO cells exposed up to 34 µg/ml in the absence of metabolic activation and 420 µg/ml in its presence. The negative result was, however, not convincing, because the effect found at the low dose cannot be disregarded (ref. 12).

CONCLUSION

Information on dermal absorption is not yet available, but a dermal absorption study is ongoing and will be finished by the end of this year (1989).

Classification : B.

REFERENCES

1. to 8. Manufactured data.

9. Toxicité à tèrme chez le Rat, d'une suspension d'Hexamidine diisothio nate, per os (14.05.1974) Communication personnelle de Mesdames SCHMUTS et GHAYE et de Messieurs J. GAILLOT et A. BIDER ,des Laboratoires de Recherche de la Société THERAPLIX réf. LP. LRB N°9415

10. MX 202 Toxicity to Rats by repeated oral administration for 4 weeks. Huntingdon Research Center.14.07.1987.

11. Ames metabolic activation test to assess the potential mutagenic effect of MX 202.
Huntingdon Research Centre, January 12, 1987.

12. Analysis of metaphase chromosomes obtained from CHO celles cultured in vitro and treated with MX 202.
Huntingdon Research Centre, April 10, 1987.

2. BENZETHONIUM CHLORIDE

COMPLEMENTARY OPINION (April 11, 1989)

PRECEDING OPINION EXPRESSED ON APRIL 12, 1988

CONSULT THE REPORT OF THE 37th REUNION

. OTHER NAMES

EEC n° 15
Colipa P70
Hyamine 1622
Phemerol chloride

4'-(1,1,3,3-tetramethylbutyl)phenoxy-ethoxyethylene-dimethyl-benzyl-ammonium chloride

. USE

As a preservative agent in cosmetics at the level of 0.1%.

. PRECEDING CONCLUSIONS (11-4-88)

Classification C.
Adequate information on dermal absorption was needed.

. FURTHERING INFORMATIONS

A supplementary 28-day study in rats with the same feeding levels was conducted to verify and extend certain findings in the previous study. The results confirmed most of the changes seen at the top-dose, including caecal enlargement.

The latter finding was not accompanied by histopathological changes. Decreased levels of serum-P seen at the two higher levels in the previous study did not occur in the present study. Therefore, 500 ppm was the NEL in the supplementary study (Subm. IV, ref. 15).

CONCLUSION

No modification :

The available data from teratogenicity studies in rats indicate that benzethonium chloride produces both maternal toxicity and adverse effects on the developing embryo at oral doses of 3 mg/kg and above.
The NEL was around 1 mg/kg. The safety factor resulting from cosmetic use and assuming a NEL of 1 mg/kg and 10% dermal absorption is very low (around 20). Although the actual extent of absorption through intact skin is likely to be low, it is essential that vertual absence of dermal absorption is adequately demonstrated.

Classification : C.

Reference 1 : S. DE FLORA
"Study of 106 Organic and Inorganic Compounds in the Salmonella/microsome Test"
Carcinogenesis, Vol. 2, n° 4, (1981), pp. 283-298

Reference 2 : Isao KARUBE et al
"Preliminary Screening of Mutagens with a Microbial Sensor"
Anal. Chem., 53, (1981), pp. 1024-1026

Reference 3 : Rohm and Haas
Report n° 82R-265, 16/12/1982

Reference 4 : Hazleton laboratories America, Inc,
November 16, 1977

Reference 5 : Report from July 16, 1976
J.C. KILLEEN, W.E. RINEHART
Bio/dynamics - New Jersey

Reference 6 : Report from May 5, 1976
J.C. KILLEEN, W.E. RINEHART
Bio/dynamics - New Jersey

Reference 7 : Report from December 2, 1976
G.K. HOGAN, W.E. RINEHART
Bio/dynamics - New Jersey

Reference 8 : Report from July 16, 1976
J.C. KILLEEN, W.E. RINEHART
Bio/dynamics - New Jersey

Reference 9 : Report from April 22, 1976
J.C. KILLEEN, W.E. RINEHART
Bio/dynamics - New Jersey

Reference 10 : CIR Final Report - June 5 1984

Reference 11 : Reference : 32 :
Expert opinion on :
Studies of Benzethonium Chloride in pregnant rats and rabbits
by E. MARSHALL JOHNSON. July 17, 1985

Reference 12 : Dermal absorption study on 14C Hyamine 1622, 1974

Reference 13 : Rat Maternal and Fetal Absorption of 14C-BTC - February 23, 1977

Reference 14 : CIVO INSTITUTES TNO, ZEIST, NETHERLANDS
Report n° V 86.537/260702
Study director : Dr. H.P. TIL
October 1986

Reference 15 : "Addendum to Report n° 86.537/260702" Subacute (28-day) oral toxicity study with Benzethonium Chloride in rats"
Dr. H.P. TIL et al. TNO-CIVO institutes Zeist - the Netherlands - May 1988

REPORTS OF THE SCIENTIFIC COMMITTEE ON COSMETOLOGY CONCERNING THE USE OF CERTAIN COLOURING AGENTS IN COSMETICS

. THE COMMITTEE'S MANDATE

To give its opinion on the question whether the use of the following colouring agents in cosmetic products is admissible from the health point of view.

CI 13065
CI 42045
CI 44045
CI 42535
Solvent Yellow 98

1. CI 13065

COMPLEMENTARY OPINION

For preceding informations and opinions, consult :

- Reports of SCC, seventh series, EUR 11303 P.23-25
- Report of the 35th reunion October 13th, 1987.

. PRECEDING CONCLUSION : Classification : D.

. USE

Up to 0.5% in now rinsed off products.
Up to 0.1% in rinsed off products.

. FURTHERING INFORMATIONS

In a third short-term (31-day) oral study, conducted in 1988, groups of 10 rats/sex were treated by gavage with 0, 10, 30, 100 or 300 mg/kg/day (in a hydrogel of 2% polysorbate 80, sterilized water and 0.01% activated dimeticone as an antifoam). The top-dose induced decreased growth rate and food intake, ocular changes, changes in haematology, blood biochemistry, urinary ocnstituents, and changes in the weight of several organs. Liver enlargement occurred with 300 and 100 mg/kg. Ocular changes (haemorrhages, cataract and/or opacity) were seen with 300, 100 and 30 mg/kg. Histopathological changes were seen in the liver and bone marrow. The NEL was 10 mg/kg bw/day (ref. 18, subm. VI).

Skin absorption test

An in vitro test with human abdominal epidermis, in which 35 mg of 0.05% colourant in a hair dye formulation was applied to 2.0 cm^2 for 30 min., only 0.045% of the applied dose was recovered in the penetration chamber (ref. 19, subm. VI).

CONCLUSION

The substance has shown considerable systemic toxicity and was found to induce several changes, e.g. testicular and ocular damage. Different oral studies in rats showed marked differences in the toxic effects observed. Chromosomal aberrations and sister chromatid exchanges were reported both from in vivo, and from in vitro studies. In a negative chromosomal aberration test reported recently only very low concentrations (1.6 ug/ml) could be used. In view of the high toxicity of this colourant, included the ability to induce catarract at 30 mg/kg bw within 30 days, and testicular toxicity, and the in vivo genotoxic properties, its continued use as a colourant in cosmetics is not justified.

Classification : D.

Reference 1 : Dr. Leuschner, Hamburg
"Akute orale Toxizität von C-ext Gelb 10 an Sprague-Dawley-Ratten"
Lab. für Pharmakologie W. Toxikologie
Report dated Gebruary 15, 1971

Reference 2 : G. Tonfoy 1978
"Sel de sodium de l'Acide (Anilino-4 Pheylazo)
-1 Benzene
Sulfonique-3, détermination de la DL 50 par voie orale chez le rat"

Laboratory of "Institut Français de Recherches et Essais Biologiques" (IFREB) St-Germain s/l'Arbresle
Report IFREB - R 801261 of 25 January 1978

Reference 3 : Dr. Leuschner, Hamburg
"Uber die schleimhautverträglichkeit von C-ext Gelb 10 bein Kaninchen"
Lab. für Pharmakologie u. Toxikologie
Report dated February 10 1971

Reference 4 : J.P. Guillot 1978
"COLORANT N° 63
Sel de sodium de l'Acide (Anilino-4 Phenylazo) -1 Benzene Sulfonique
(CI 13065 Acid Yellow 36)
Numéro du lot : AJ 7436 - 15.9.77"

Laboratory of "Institut Français de recherches et essais Biologiques (IFREB) St-Germain s/l'Arbresle
Report IFREB - R 801239 of January 1978

Reference 5 : Dr. Leuschner, Hamburg
"Prüfung der lokalen Verträglichkeit von C-ext Gelb 10 an Kaninchen (Patch-Test)"
Lab. für Pharmakologie u. Toxikologie
Report dated December 20 1970

Reference 6 : J.P. Guillot 1978
"COLORANT N° 63
Sel de sodium de l'Acide (Anilino-4 Phenylazo) -1 Benzene Sulfonique-3
(CI 13065 - Acid Yellow 36)
Numéro du Lot : AJ 7436 - 15.9.77"
Laboratories of "Institut Français de recherches et essais Biologiques (IFREB) St-Germain s/l'Arbresle
Report IFREB - R 802265 of 2 March 1978

Reference 7 : Testicular lesions induced by metanil yellow in mice
GB SINGH and SK KHANNA
Exp. Path. Bd. 9, S. 251-255-1974

Reference 8 : Haematological studies in rats fed with Metanil Yellow
NK. Mehrotra, SK Khanna cend 6 B Singh
Environ. Physiol. Biochem. 1974 4, 232-235

Reference 9 : S. Ivancovic, R. Preussmann
"90 Tage Test"
Deutsches Krebforschungszentrum, Heidelberg
Report dated November 4 1973

Reference 10 : Mutagenicity test of dyes used in cosmetics with the Salmonella / Mammalian-microsome
Test by Jeanne M. Muzzall and Warren L. Cook
Department of Biology, Georgia State University,
Atlanta GA 30303 USA
(Accepted 11 December 1978)

Reference 11 : C. Gloxhuber, F. Bartnik, F. Wingen
C-ext Gelb CI 13065 : Resorptionsscreening an Humanhaut in vivo
Henkel KGaA, 12.5.1980

Reference 12 : Prof. Dr. Gloxhuber, Dr. Bartnik
C-ext Gelb 10, CI 13065
Resorptionscreening mittels der TesafilmabriB-Methode nach Applikation einer CI 13065 enthaltenden Basiscreme an Humanhaut in vivo
Henkel KGaA, 11.1.1983

Reference 13 : Khanna, S.K., Singh, G.B.
"Anti-testicular effect of Metanil Yellow in Guinea pigs"
J. Food Sci. Techn. 1973, 10, 75-76

Reference 14 : Jolly, P.N., Michael, W.R.
20 day Percutaneous study
Procter & Gamble Internal. Report V-1439-20,
August 2 1965

Note : The report on this study was stored on micro-fiche. The resulting photocopy is of poor quality. It has nevertheless been submitted but in addition the relevant pages have been retyped.

Reference 15 : Jolly, P.N., Michael, W.R.
90 day Percutaneous study
Procter & Gamble Internal. Report V-1439-1
August 8, 1965

Reference 16 : Niewenhuis, R.J., Jolly, P.N.
2 year Mouse Painting study
Procter & Gamble Internal Report V-1606-1
December 15 1964

Reference 17 : M. McConville, D.B. McGregor
Inveresk Research International Report to
Procter & Gamble Ltd. March 1979

Reference 18 : C. Bezancon et al 1988
"Evaluation de la toxicité du produit "jaune Acetacid 4R Extra" administré chez le rat pendant 1 mois par voie orale"
Laboratoires d'Etudes et de recherches SYNTHELABO (LERS) - Gargenville - FRANCE
Report 1352 TMR/88.047 - 29 August 1988

Reference 19 : H. Schaefer 1988
"Pénétration in vitro du Colorant CI 13065"
Centre International de recherches dermatologiques (CIRD) - Valbonne - FRANCE
Report 28 August 1988

Reference 20 : A.T. Natarajan & F.H. Sobels 1988
"Evaluation of compound CI 13065 in the chromosome aberration test with chinese hamster ovary cells (in-vitro)"
Department of radiation genetics and chemical Mutagenesis
University of Leiden, THE NETHERLANDS
Report 13 June 1988

REPORT BY THE SCIENTIFIC COMMITTEE ON COSMETOLOGY

CONCERNING THE USE OF

THE COLOURANT CI 42045

COMPLEMENTARY OPINION EXPRESSED ON JUNE, 20th 1989.

For preceding informations and expressed opinion, consult :

- Reports of SCC, seventh series, EUR 11303 P79.

. FORMULA AND SYNONYMES

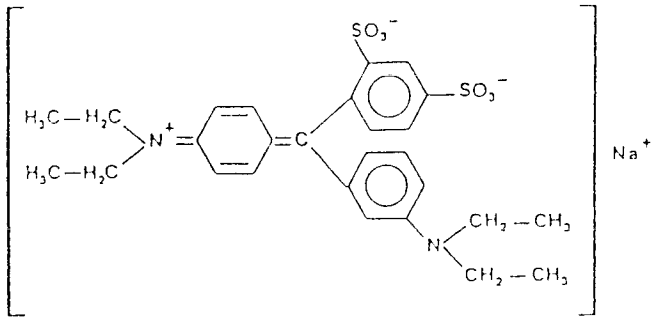
CAS Reg. n° 129-17-9

Acid Blue 1

C-ext Blau 13

Blue VRS

4,4'-bis (N-diethylamino)-2",4"-disulfotriphenyl methane, sodium salt



$C_{27}H_{32}N_2O_6S_2,Na$

MW 567

. USE

Use level 0.1% both for rinsed off and for not rinsed off products.

. PRECEDING CONCLUSIONS (1st July 1986)

Further information on genotoxicity is needed.
Moreover a sensitization test should be conducted and a short-term dermal toxicity study is needed to examine local skin changes as well as systemic effects.

Classification : A.

. FURTHER INFORMATIONS

Since the 40th reunion (April 11th, 1989), Classification A was in suspense for the time being because the Committee was waiting for information about incidences of leucemia in tested female animals.
Only very short and insufficient informations were found on the increased incidences of leucemia in treated mice. This effect has not been confirmed.

A recently conducted chromosomal aberration test with CHO cells in vitro using concentrations of 625 up to 5000 µg/ml, was also negative (Colipa subm. II, ref. 13).

CONCLUSION

Although, tumours occurred in rats at the site of subcutaneous administration, the induction of injection site tumours has little relevance for hazard assessment in man. Because the oral toxicity is low and mutagenicity tests were negative, the use of only 0.1% of this colourant in cosmetics is considered acceptable.

Confirmation of Classification : A.

REFERENCES

1. Dr. Weygand, Gewerbe und Arzneimittel
   Toxikologie Farbwerke Hoechst.
   Report from 10.7.1961

2. Dr. E. Hall, I.F. Gaunt, M. Fanner, P. Grasso
   British industrial Biological Research Ass., Woodmansterne Road
   Carshalton, Surrey, England
   Fd; Cosmet. Toxicol. Vol 5 pp. 165-170
   Pergamon Press 1967

3. Mannuell, W.A. & Grice, H.C. (1964)
   Chronic toxicity of brilliant blue FCF, blue VRS and green S in rats
   J. pharm. Pharmacol. 16, 56-59

4. Grasso, P. & Golber, L. (1966)
   Early changes at the site of repeated subcutaneous injection of
   food colourings
   Fd. Cosmet. Toxicol. 4, 269-282

5. Grasso, P. Gangolli, S.D., Golberg, L. & Hooson, J. (1971)
   Physicochemical and other factors determining local sarcoma
   production by food additives
   Fd. Cosmet. Toxicol 9, 463-478

6. Hooson, J., Grasso, P. & Gangolli, S.D. (1973)
   Injection site tumours and preceding pathological changes
   in rats treated subcutaneously with surfactants and carcinogens
   Brit.J. Cancer, 27, 230-244

7. Grice, H.C. & Mannell, W.A. (1966)
   Rhabdomyosarcomas induced in rats by intramuscular injections of
   blue VRS
   J. nat. Cancer Inst. 37, 845-857

8. Grice, H.C., Dupuis, I., Dennery, M. & Mannell, W.A. (1966)
Blue VRS induces rhabdomyosarcomas (Abstract N° 25)
Toxicol. appl. Pharmacol. 8, 342-343

9. The technique of bladder implantation further results and an assessment
D.B. Clayson, J.A.S. Pringle, G.M. Bonsor and M. Wood
from the department of experimental Pathology and Cancer Research, The school of Medicine, Leeds 2
Br. J. Cancer 22, 825

10. S.D. Gangolli, P. Grasso, L. Golberg, J. Hooson
"Protein binding by food Colourings in relation to the Production of subcutaneous Sarcoma"
Fd. Cosmet. Toxicol. Vol. 10, pp. 449-462
Pergamon Press (1972)

11. S.D. Gangolli, P. Grasso, L. Golberg
"Physical Factors Determining the eraly local Tissue Reaction produced by food Colourings and other Compounds Injected Subcutaneously"
Fd. Cosmet. Toxicol. Vol. 5, pp. 601-621 (1967)

12. Prof. Kramer - Dr. Weigand
Stellungnahme zu Karzinogenität von Acid Blue 1
Hoechst. 19 January 1978
Statement on the carcinogenicity of Acid Blue 1

13. Blue solid W 6007 CI 42045 - OECD 473 metaphase analysis in CHO cells in vitro
Safepharm Laboratories Limited P.O. Box n° 45 - Derby U.K.
January 1988

2. CI 42535

COMPLEMENTARY OPINION

For preceding informations and expressed opinions consult :

- Reports of SCC, seventh series, EUR 11303 P90-91
- Report of the 35th reunion ; October 13th, 1987.

. PRECEDING USE AND CONCLUSIONS

It was used at levels up to 0.1% in rinsed off and non rinsed off cosmetics, and up to 2.5% in hair setting lotions.

Classification : D: Because the colourant was chemically not well defined. Results of tests depend on the source of the sample (samples contain CI 42555 a colourant with carcinogenic and genotoxic properties).

. NEW PROPOSED USE LEVEL

It is proposed to use only 0.01% and only in hair colouring products.

. FURTHERING INFORMATIONS

A recent Colipa submission (IV, November 1988) included a new Ames test conducted with a commercial sample of CI 42535 (containing 5% CI 42555) and a purified sample (not containing CI 42555). Using test levels up to 0.1 mg per plate the results with the strain TA 1537 were positive both with the purified and the non-purified material, in the absence as well as in the presence of the metabolic activation system (ref. 14).

Negative results were obtained in a recent chromosomal aberration test with CHO cells exposed in vitro up to 1.6 µg/ml. Higher levels were not tested because these did not permit 50% survival of the cells (Colipa subm. IV, ref. 15).

CONCLUSION

However, positive results were obtained also with the purified material in the Ames test, the use of 42535 has to be banned in cosmetics.

Classification : D.

3. CI 44045

COMPLEMENTARY OPINION

For preceding informations and expressed opinons consult :

- Reports of SCC, seventh series, EUR 11303 P97-98
- Report of the 35th reunion, October 13th, 1987.

. PRECEDING CONCLUSION

In the only short-term study available, no treatment-related changes were observed at an exposure level of 10 mg/kg bw on 5 days/wk. Because higher levels were not examined this study did not meet the requirement that several levels should be administered of which at least one should exert an effect.

Classification : C.

. USE

Use level 0.1% in rinsed off and non rinsed off cosmetics and up to 0.5% in hair dye formulations.

. FURTHERING INFORMATIONS

A second oral study in rats with 0, 10, 30, 90 or 270 mg/kg/day for 30 days showed 100% mortality in the top-dose group. With 90 mg/kg 7/20 rats died, and haematological changes and increased relative weights of the liver and adrenals were observed. With 30 mg/kg 2/20 rats died.
A blue discolouration of some organs, and histiocytosis of lymph nodes occurred with 30 mg/kg and more.
No changes were seen with 10 mg/kg (Subm. IV, ref. 10).

Dermal penetration was examined in an in vitro test with human abdominal epidermis, exposed to 40 mg 0.1% formulation on 2.0 cm^2 for 30 minutes. Only 0.02% passed into the penetration chamber (Subm. IV, ref. 11).

CONCLUSION

The colourant showed considerable systemic toxicity upon short-term oral exposure of rats to 30 mg/kg and more but 10 mg/kg was a NEL. An in vitro test for dermal absorption showed negligible penetration. Mutagenicity tests were negative.

Classification : A.

Reference 1 : U. HACKENBERG, D. KUHN
Inbifo Institut für biologische Forschung, Köln
Report from 18.1.1978

Reference 2 : G. RONDOT (1978)
"Détermination de la DL50 par voie orale chez le rat -
Chlorure de (N',N'-Dimethyl)amino-4' (N"-Phenyl) amino-4"
naphto (N,N-dimethyl)fuchsonimonium-4 "
Laboratories of Institut Français de Recherches et Essais
Biologiques (IFREB), Saint Germain s/L'Arbresle
Report IFREB-R 801227 - 10th January 1978

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Inbifo, Institut für biologische Forschung, Köln
Report from 17.1.1978

Reference 4 : U. HACKENBERG, D. KUHN (1978)
Inbifo, Institut für biologische Forschung, Köln
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"Détermination de l'Indice d'Irritation Cutanée Primaire,
chez le lapin -
Colorant n° 65 : Chlorure de (N', N'- Dimethyl) amino-4'
(N"-phenyl)amino-4" naphto(N,N-dimethyl) fuschsonimunium-4
(CI 44045 Basic Blue 26)
Numéro de lot : AE 7542 - 15.9.77"
Laboratories of "Institut Français de Recherches et d'Essais
Biologiques" (IFREB) - Saint Germain s/L'Arbresle
Report IFREB-R 802264 - 2nd March 1978

Reference 6 : J.P. GUILLOT et al, (1979)
"Evaluation of the Sensitizing Potential of a Test Substance by Topical Applications in the Guinea-Pig - IFG 69-79 : Colorant (Prelt : 8.1.0889)"
Laboratories of "Institut Français de Recherches et d'Essais Biologiques (IFREB) - Saint Germain s/L'Arbresle
Report IFREB-R 911365 - 27th November 1979

Reference 7 : P.E. FOURNIER (1987)
"Etude de Toxicité Chronique chez le Rat d'un Produit Dénommé Bleu Victoria".
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Report 13 January 1987

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Report 27.11.1986

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Laboratoires d'Etudes et de Recherches SYNTHELABO (LERS) - GARGENVILLE - FRANCE
Report 1310 TMR/030.88 - 6 June 1988

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"Pénétration In Vitro du Colorant CI 44045"
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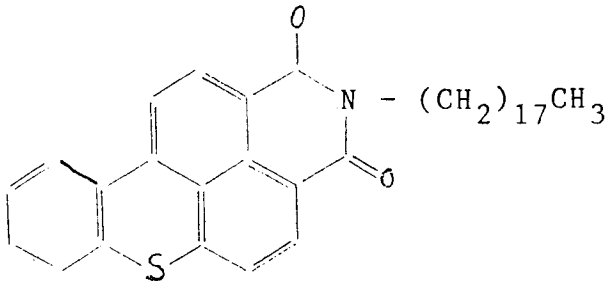
4. SOLVENT YELLOW 98

. FORMULA AND SYNONYMES

HOSTASOL - GELB 3G

1H-thioxantheno [2,1,9-def]-isoquinoline-1,3(2H)dione, 2-octadecyl



MW: 543

. CHARACTERISTICS

Insoluble in water.
Soluble in organic solvents and mineral oils.

. USE

This new colourant is intended for use only in nail varnish at a concentration of 0.5%.

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral (rats) : 15 gr/kg

ORAL TOXICITY

A dose of 15 gr/kg did not cause any signs of intoxication. The colourant was excreted with the faeces.
A short-term (30-day) feeding study was conducted with 3 groups of 10 rats/sex fed a basal diet with 0, 1, or 5% of the colourant. There was no mortality or any other sign of intoxication. Growth rate, composition of blood and urine, or the weights of 6 of the major organs were not affected. A yellow discolouration of the depot fats and liver was observed in test animals at autopsy. Microscopy of the liver, kidneys, adrenals, heart, spleen and lungs did not reveal treatment-related changes (summary report only).

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

An eye irritation test

Test in rabbits with 0.1 ml of a 5% or a 10% dilution in sesame oil did not produce any response.

Skin irritation

Did not occur in rabbits treated by intracutaneous injection of 0.02 ml of dilutions in sesame oil varying from 0.001% to 10%. Repeated topical application of the 10 and 5% dilution daily for 5 days was also negative.

A phototoxicity

Test in mice by a single intraperitoneal injection of 2.5 g/kg bw of a 25% dilution in sesame oil, followed by UV irradiation for 6 hours, on the same day and on the next day, did

not provide indications of phototoxicity as compared with a control group treated with the test substance in the same way but without subsequent irradiation. A similar test was conducted in rabbits by application of 1 ml 10% dilution in sesame oil to the back skin, followed by UV irradiation for 8 hours. Two control rabbits received only irradiation on the neck skin. There were no indications of phototoxicity.

CONCLUSION

The substance has shown little systemic toxicity. An evaluation is not possible because information on genotoxicity or sensitization is not available. An Ames test and a maximization test in the guinea pig should be conducted.

Classification : C.

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First version July 1984.

Revision August 1987.

Revision March 1988.

REPORTS BY THE SCIENTIFIC COMMITTEE ON COSMETOLOGY CONCERNING THE USE OF CERTAIN U.V. FILTERS

. THE COMMITTEE'S MANDATE

To give its opinion on the question whether the use of the following U.V. filters :

- S_3 Ethoxylated ethyl-4-aminobenzoate — up to 10%
- S_8 2-ethylhexyl-p-dimethylaminobenzoate — up to 8%
- S_{13} 2-ethylhexylsalicylate — up to 5%
- S_{40} 2-hydroxy-4-methoxybenzophenone-5-sulphonic acid — up to 5%

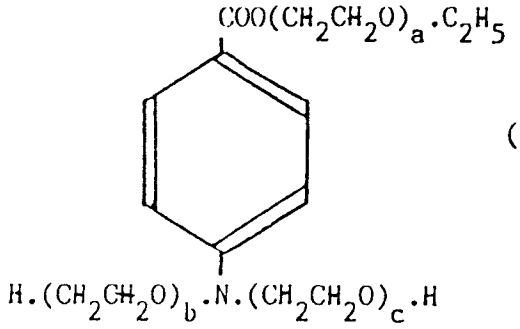
in cosmetic products is admissible from the health point of view.

1. ETHOXYLATED ETHYL-4-AMINOBENZOATE

. FORMULA AND SYNONYMES

EEC n° 2.2

Colipa S3



(a + b + c) = 25

. CHARACTERISTICS

A clear slightly viscous yellow liquid at room temperature. Absorption maximum 307 nm. Soluble in water, poorly so in ethanol or anhydrous isopropanol.
The compound is manufactured by reacting the ethyl ester of para-aminobenzoic acid with ethylene oxide.
Free ethylene oxide is then blown away by a stream of nitrogen. Purity : not less than 99%.

. USE

Proposed use level : up to 10%.

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral (rat) : $>$ 1.9 gr/kg
i.p. (mouse) : $>$ 1.9 gr/kg

Exposure of rats to air saturated with a.i. for up to 8 hrs gave no abnormality.

CUTANEOUS AND MUCOUS TOLERANCE

Irritation

In the rabbit, 3 sets of experiments were carried out, using undiluted and diluted a.i., applied both to scarified and intact skin, for up to 24 hrs. Some animals showed slight erythema ; the tests were otherwise negative.
Two animals had 5 applications within a week of a 50% aqueous solution to an area of 36 cm^2 of skin. Each application was allowed to act for 8 hrs. There was no evidence of irritation.

In 20 human subjects, undiluted and diluted a.i. was applied to areas of 1 cm^2 for 20 hrs. No irritation was produced. A test for mucous membrane irritation has been carried out. This was found to be satisfactory.

Sensitization

Ten guinea pigs were used. Induction was by application of a 50% solution in acetone to the skin. An 80% solution was then applied 5 days a week for 9 days. After a 12-day rest, a challenge application of a 50% solution was made.
No reaction was found.

Phototoxicity

A 10% aqueous solutiona of a.i. was applied to the skin of 10 human subjects. Control applications were also made. The treated areas were exposed to UV radiation, increasing in a step-wise manner to determine the m.e.d. There was no evidence of phototoxicity. The compound had a protective effect.

Photosensitisation

Ten male and ten female guinea pigs were used. A daily application of 0.5 ml of a 20% aqueous solution of a.i. was applied to the shaved skin of one flank for 5 days. Each application was followed by 15 mins of UV irradiation from a lamp with a maximum at 260 nm. The procedure was repeated on the opposite flank after a 10-day rest.
The test was negative.

MUTAGENICITY

The substance tested was designed "LUSANTAN 25" (stated to be ethoxylated ethyl-4-aminobenzoate).
An Ames test was normal.

CONCLUSION

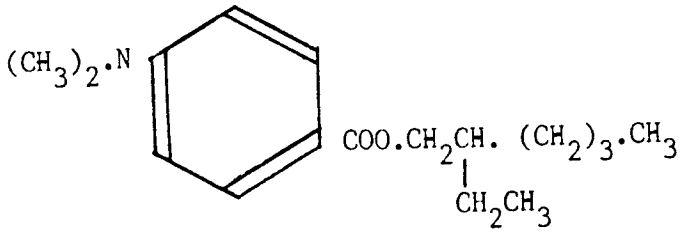
The Committee was satisfied with the data presented on acute toxicity, inhalational toxicity, and tests for capacity to irritate mucous membranes. It is noted that the tests for sensitisation were not carried out using a maximisation technique. The test described as a photosensitisation test should more properly be regarded as a test for photo-allergenicity or phototoxicity. A test for chromosomal aberration in vitro should be carried out.
It will then be possible to consider whether tests for carcinogenesis are necessary. It is understood that a 90 day oral test and a test for percutaneous absorption are in progress. When the result of the latter is available, the need for tests for teratogenicity and pharmacokinetics can be decided. Ethylene oxide should be below the limits of detection.

Classification : C.

2. 2-ETHYLHEXYL-p-DIMETHYLAMINOBENZOATE

. FORMULA AND SYNONYMES

EEC n° 2.5
Colipa S8
"Padimate O"
"Escalol 507"



. CHARACTERISTICS

Yellow fluid, stated by manufacturer to contain not less than 98.5% of a.i.
Maximum absorption 310 nm.
Not known to polymerise.
Soluble in isopropyl alcohol, mineral oil, and ethanol.
Insoluble in water.

. USE

Use level up to 8%.

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral (rat) : 3-15 gr/kg bw.

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

Irritation of mucous membranes

A Draize test in the rabbit at concentrations of 2% and 5% in mineral oil showed slight transient irritation.

Irritation of skin

Rabbit : Solutions of 5% a.i. were applied to both intact and abraded skin for 24 hrs under occlusion. The test was negative.

Man : Occlusive patch tests with 5% a.i. in yellow soft paraffin were applied for 48 hrs. The test was negative.

Sensitisation

Guinea pig : Ten male animals had an initial intracutaneous injection of 0.05 ml of a 0.1% solution of a.i. in saline, followed by 9 injections of 0.1 ml 3 days a week. After a 12 week rest period, a challenge dose of 0.05 ml was given. There were no adverse effects. The report is somewhat ambiguous as to the concentration used : it is possible that a 0.005% solution was used.

Man : (a) Fifteen applications of a 4% solution of a.i. in soft paraffin were made under occlusion over 3 weeks. A challenge application was made after a 2 week rest. There was no adverse reaction.

(b) A mixture of 7% a.i. with 3% oxybenzone was used in 150 subjects in a repeated insult patch procedure. No abnormality was found.

(c) Ninety subjects were similarly tested using 8% a.i. and 3% benzophenone. The test was negative, although there were occasional slight irritant responses during the induction.

(d) A panel of 156 subjects was similarly tested with 7% a.i. in soft paraffin. The test was negative.

Phototoxicity

Guinea pig : The ears of 10 animals were stripped and a formulation containing 7% a.i. and 3% oxybenzone was applied several times to one ears with vigorous rubbing. The untreated ear served as a control ; 2 of the animals had 8-methoxypsoralen applied as a positive control. Thereafter the animals were exposed to UV radiation (wavelenght not stated) for 2 hr. The test was negative ; the positive controls showed marked effects.
In another test, a similar preparation was applied to the nuchal area with occlusion for 2 hrs. This was followed by irradiation with 3 J/cm^2 at 320-400 nm. Suitable positive and negative controls were used. The test was negative.
Man : In a poorly reported tes, a mixture of 7% a.i. and 3% oxybenzone was tested in 26 human subjects. No adverse effects were seen.
In another similar test, a 5% ehtanolic solution was used. At 30 J, the control area showed more damage than the test area. Ten fair-skinned subjects were treated with a mixture of 7% a.i. and 2% oxybenzone under occlusion for 24 hrs. A control was similarly applied. After removal of the patches a further application was made to the skin and irradiation was carried out using 1 m.e.d. of UVB followed by 12 minutes of UVA. The test was negative

Percutaneous absorption

Man : An 8% ethanolic solution of ^{14}C a.i. was applied over 100 cm^2 of forearm skin in 4 female subjects. After the ethanol had dried, the areas were covered with a gauze pad for 24 hrs. No radioactivity was found in the blood ; the urine containded between 1.2% and 2.5% of the applied radioactivity.

Subchronic toxicity

A 13-week dermal toxicity study was carried out in groups of 20 rabbits at dose levels of 140 and 280 mg/kg bw. No significant abnormality was detected.

MUTAGENICITY AND GENOTOXICITY

A standard Ames test was negative. A second similar test is also reported negative, but figures are given only for plates with activation.
A micronucleus test was carried out in the mouse, using a dose which caused disorders of gait and hypotonicity. The a.i. was given intraperitoneally in a dose of 5000 mg/kg bw to 3 groups of 10 animals. Positive and negative control groups were included. Sacrifice was at 30, 48 and 72 hrs. The test was negative.

TERATOGENICITY

Rat : Dermal applications of 2 ml/kg bw of a preparation (concentration of a.i. not specified) were made daily from days 6 to 16 of pregnancy. In the test group 7/56 foetuses had bilateral wavy ribs and 2/56 had unilateral wavy ribs. There were no such findings in the control group. This effect is not regarded as indicating teratogenic activity, and is a common finding in rats of this strain, but the reason for its appearance in foetuses of the test group only is unexplained.

CONCLUSION

The Committee notes that sensitisation tests were carried out at levels less than the proposed use level. Testing up to irritant level would have been desirable to reveal any potential for sensitisation. Tests for phototoxicity and photosensitisation are poorly reported, but seem to be negative ; since the compound is widely used, they may be accepted. Tests for absorption suggest that the amount absorbed may be about 1 mg/kg bw/day.
A chromosomal aberration test and a 28-day or 90-day oral toxicity study should be carried out.

Classification : C.

REFERENCES
++++++++++

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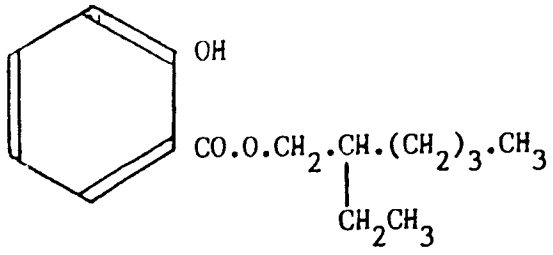
3. 2-ETHYLHEXYLSALICYLATE

. FORMULA AND SYNONYMES

EEC n° 2.6
Colipa S13
OCTYLSALICYLATE
"SUNAROME"



$C_{15}H_{22}O_3$ MW 250·33

. CHARACTERISTICS

Clear odourless liquid.
Insoluble in water.
Soluble in ethanol, mineral oil, other organic solvents.
Specific gravity 1.013 - 1.022.
Maximum absorbance at about 300 nm.
There is evidence that this compound dissociates only very slowly and to an extent of less than 0.5% at pH values of 8.9 ; there is no dissociation below pH 8.0, approximately (8).

. USE

Proposed use level : up to 5%.

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral (rat) : 4.8 ± 0.3 gr/kg bw.

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

Irritation of mucous membranes

A Draize test in the rabbit was negative. The concentration of a.i. used is not clear from the report ; it may have been used undiluted.

Irritation of the skin

In the rat, an 18.5% solution caused irritation in the sub-chronic dermal test noted above.
In man, a 5% solution of a.i. in mineral oil was applied for 24 hrs with occlusion in 10 subjects. After a 7-day rest, the procedure was repeated. No adverse effect was found.
A repeated insult patch test was carried out in 25 subjects. The initial application was allowed to remain on the skin for 48 hrs ; thereafter 24 hr applications were made every second day until 10 applications had been made. The concentration of a.i. used is not clear from the report : it may have been undiluted. After a 10-14 day rest, a 48 hr test patch was applied. There was no adverse reaction.

Phototoxicity

In 10 human subjects, a 5% solution of a.i. in ethanol was applied for 1 hr on stripped skin, and for 24 hrs on intact skin. In each case, the area was then exposed to UV radiation at 322-410 nm. There were no adverse effects. Declomycin as a positive control under the same conditions caused phototoxicity.

Photocontact allergy

Twenty-five subjects were tested with an ointment containing 15% of a.i. Applications of 10 $\mu l/cm^2$ were applied for 24 hrs with occlusion. After removal, the areas were exposed to 3 m.e.d. of simulated solar radiation from a xenon arc. This sequence was repeated after 48 hrs and thereafter twice weekly for 3 weeks, always at the same site.
After a 10-day rest, applications of 5 $\mu l/cm^2$ were made to a fresh site with occlusion for 24 hrs, followed by irradiation. No adverse reactions were seen.

Subchronic toxicity

A 13-week dermal study was carried out in the rat. The a.i. was applied as an 18.5% ethanolic solution at doses of 0, 55.5, 277 and 555 mg/kg bw. There were changes in biochemical values in the animals of the intermediate and top dose groups, suggesting adverse effects on the liver, but there was no histological evidence of liver damage. At the low dose, in females, the absolute and relative weights of the kidney and spleen were increased, and the absolute weight of the heart ; in males, the relative weith of the lungs was increased. At higher doses, both sexes showed changes, with a fall in absolute weight of the liver in males and of the kidney and heart in females. There was a dose-related effect on the skin : histological changes of hyperkeratosis were found at the low dose, progressing to marked hyperkeratosis and inflammatory foci at the top dose.

MUTAGENICITY

An Ames test was negative.

CONCLUSION

Are required :
- a subchronic oral toxicity test
- a test for percutaneous absorption
- investigation of pharmacokinetics and teratogenic activity
- a chromosomal aberration test in vitro
- photomutagenicity test
- inhalation toxicity (if used in aerosols and confirmed spaces).

Classification : C.

REFERENCES
++++++++++

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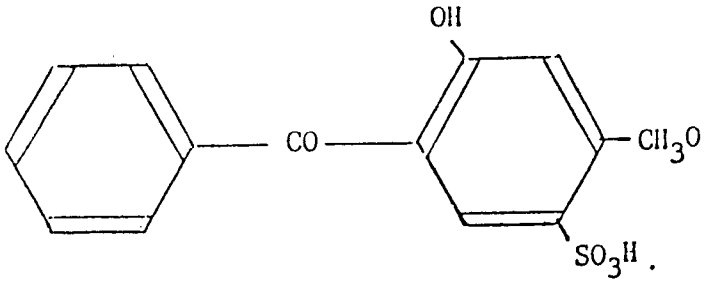
4. 2-HYDROXY-4-METHOXYBENZOPHENONE-5-SULPHONIC ACID

. FORMULA AND SYNONYMES

EEC n° 2.19

Colipa S40

Sulisobenzone (INN)



$C_{14}H_{12}O_6S$

MW = 308·3

. CHARACTERISTICS

A free-flowing powder, soluble in water and alcohol.
Maximum absorbance at 285 nm.

. USE

Use level : up to 5%.

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral (rat) : > 6.4 gr/kg bw.

CUTANEOUS AND MUCOUS TOLERANCE

Irritation of mucous membranes

The compound was non-irritant in a 4% solution in dimethylphthalate and slightly irritant at 8%. A 5% aqueous solution was non-irritant. A 16% solution in dimethylphthalate was markedly irritant.

Irritation of skin

Solutions of 4%, 8% and 16% of a.i. in dimethylphthalate were tested on the skin of the rabbit. There was slight irritation at the highest concentration.
In 14 human subjects, an occluded patch test was carried out. There was no reaction at 4% at 8%, 1/14 showed irritation, and at 16% 4/14 showed irritation.
A Shelanski repeated insult patch test was carried out in 50 subjects, using a 5% aqueous solution. Occluded patches were applied for 24 hrs, repeated every 48 hrs, to a total of 15 applications. After a 2 week rest, a challenge application was made.Ther was no evidence of irritation or sensitisation. Daily application of a 1% solution in alcohol for 21 days was non-irritant.

Sensitisation

The Shelanski test, noted above, was negative.
A 10% alcoholic solution applied as a cumulative test over 21 days showed the substance to be a marked contact allergen. (No further details given).

MUTAGENICITY

An Ames test was carried out using 7 batches of a.i. Each batch was tested for toxicity, using filter paper discs, in two strains, TA 1537 and TA 100. Concentrations ranged up to 10,000 µg/plate. One sample (400) showed inhibition at both 1000 and 10,000 µg.

The highest concentration used in mutagenicity testing was 1000 µg/plate. Sample 400 showed toxicity, with and without activation, as low as 100 µg, but not consistently.

Sample D-49 also showed some toxicity as low as 100 µg, but again, not consistently. Marked increases in revertants were found in some strains with samples numbered N-35 (without activation) and D-49 (with activation).

The toxicity of samples D-49 and 400 confined testing to concentrations of 10 to 100 µg/plate and less.

A standard chromosomal aberration test was carried out with Chinese hamster ovary cells. There was no evidence of clastogenic activity with or without metabolic activation.

CONCLUSION

A test for percutaneous absorption should be carried out.

The problem of batch effects in the Ames test should be resolved, and testing carried out up to toxic concentrations in each strain.

A 28-day or 90-day oral study at suitable dose levels, and tests for phototoxicity and photosensitisation, should be carried out. The full report of the positive allergenicity test in man should be provided, and further tests carried out if necessary.

Classification : C.

REFERENCES
++++++++++

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Hazelton - Biotechnologies - The Netherlands - April 29, 1988

CONTENTS

42nd meeting of SCC - October 30-31, 1989

- Chlorphenesin - P4 421
- Diazolidinylurea - P79 423
- Decominol - P87 425
- CI 73900 (46500) 429
- CI 74180 432
- Solvent yellow 98 435

43rd meeting of SCC - January 23, 1990

- Bergasol suntan products containing 5-MOP 441

REPORTS BY THE SCIENTIFIC COMMITTEE ON COSMETOLOGY

42nd REUNION

OCTOBER 30th - 31st, 1989

LIST OF PARTICIPANTS

Present

Mr. AGACHE
Mrs. ENJOLRAS
Mrs. DONY
Mr. FIELDER
Mr. GOULDING
Mr. KAPOULAS
Mr. LOPRIENO
Mr. O'MAHONY
Mr. PONS-GIMIER
Mr. SCHOU
Mr. STUTTGEN
Mr. SOMOGYI
Mr. RAMOS-MORGADO

Not Present

Mrs. KNAAP
Mr. MUSCARDIN

COLIPA : Dr. WILSON, Dr. TAYLOR, Mrs. RICHOLD,
Dr. STERZEL, Mrs. VANDEVORST

COMMISSION

Mrs. MASSE
Ms. DE CRAWHEZ
Mr. GONTIER

1. CHLORPHENESIN

. FORMULA AND SYNONYMES

Chlorphenesin

EEC n°3
Colipa P4
p-chlorophenyl-glycerol ether
3-(p-chlorophenoxy) propane-1,2-diol

COMPLEMENTARY OPINION

For preceding informations and expressed opinions consult :
- Report of the 37th reunion, April 12th, 1988.

. PRECEDING CONCLUSION (12-4-88)

Although short-term studies have been conducted, insufficient information on the studies were available as required :

- a repeated-dose oral study (with special attention for the immune system)
- a chromosomal aberration test in vitro
- information on dermal absorption
- a teratogenicity study (depending on the degree of dermal absorption).

Classification : C.

. FURTHERING INFORMATIONS

Proposed use : up to 0.3%.
A chromosomal aberration test with human lymphocytes exposed in vitro up to 0.325 mg/ml was negative (subm. IV, ref. 22).

CONCLUSION

Although short-term studies have been conducted, insufficient information on these studies is available. A repeated dose oral study should be conducted with special attention for the immune system. Information on dermal absorption is needed. Depending on the degree of dermal absorption a teratogenicity study may be needed.

Classification : C.

2. DIAZOLIDINYLUREA

. FORMULA AND SYNONYMES

EEC n°17
Colipa P79
Germall II

COMPLEMENTARY OPINION

For preceding informations and expressed opinions consult :
- Report of the SCC, fifth series, EUR 11080 (25-6-85)
- Report of the 34th reunion June 30th 1987.

. PRECEDING CONCLUSION (30-6-87)

Classification : B.

In the previous discussion on this substance (June 1987) the Committee requested an oral teratogenicity study and a gene mutation test in mammalian cells.

. FURTHERING INFORMATIONS

In a teratogenicity study using the dermal route, rats were exposed to 30, 95 or 300 mg/kg body wt/day on days 6-15 of gestation. Compound was applied directly to the shaved skin as a 30% solution in deionised water (no occlusion). The only toxic effects noted were due to a direct effect on the skin (erytherma and scabbing). Foetuses of 26 gravid rats in each group were examined for abnormalities in bone and soft tissue. Only one soft tissue abnormality was found by the Wilson technique, and this was in a control foetus.

There were no significant differences in skeletal variations among the groups. Germal II was not teratogenic or embryotoxic under the conditions of the test (Subm. X, ref. 29).

In a chromosomal aberration test with CHO cells the highest concentration tested was only 1.5 µg/ml because even 1 µg/ml caused 70% mitotic inhibition (Submission X, ref. 27). The positive control substances triethylene amine and cyclophosphamide were tested at 50 and 0.5 µg/ml respectively. Under the conditions of the test Germal II was negative (Submission X, ref. 28).

CONCLUSION

Industry has now provided a dermal teratogenicity study and a chromosomal aberration test. The dermal study is of little significance, because there is no information on dermal absorption, which is probably very low. The chromosomal aberration test is of little value for the evaluation because the levels tested were very low, while negative results had already been obtained at very high dosages in an in vivo micronucleus test. Moreover the SCC requested a gene mutation test instead of a chromosomal aberration test.

Classification : B. confirmed.

3. DECOMINOL

. FORMULA AND SYNONYMES

Colipa P87
3-decyloxy-2-hydroxy-1-aminopropane-hydrochloride
Ster 4
PVA 44

COMPLEMENTARY OPINION

For preceding informations and expressed opinions consult :
- Report of the 33rd reunion, February 20th 1987.

. PRECEDING CONCLUSION (20-2-87)

The Committee required informations on :
- percutaneous absorption
- results of tests of chromosomal aberration and mutagenicity in mammalian cells.

Classification : C.

. FURTHERING INFORMATIONS

Dermal absorption of ^{14}C-Decominol was examined in young rats by single application of 5 mg a.i./kg bw in water or in a water/oil emulsion to groups of 3 males and 3 females for 24 h. One similar group of 3 rats/sex received 5 mg/kg intravenously in aqueous solution. Urine faeces and expired air were collected at intervals over 96 h. Dermal absorption from the emulsion was 7.2 and 10.7% for males and females respectively, while for the aqueous solution these figures were 2.4% and 4.1%.

The major route of elimination was the urine ; faeces and expired air were minor routes. The mean recoveries of the dose were 91.3% for the emulsion, 95.3% for the aqueous solution and 83.8% for the intravenous dose.
Radioactivity occurred in all tissues after percutaneous administration in either of the formulations mainly in the liver and residual carcass (ref. 16).

A chromosomal aberration test in human lymphocytes in vitro was also negative. Toxicity of the compound precluded testing of concentrations higher than 0,1 mg/ml (ref. 17).

CONCLUSION

The tests presented show a compound which has some irritant action on the skin and mucous membranes at 1%, and which may also cause liver and kidney damage when given systemically. In an oral 13-week study in the rat, the NEL was less than 10 mg/kg bw/day with local irritant effects on the gastric mucosa being seen at this level ; uraemia was seen at 40 mg/kg. The material does not seem to be well absorbed from the gastrointestinal tract.
Percutaneous absorption in rats was relatively low (<11%) from an oil emulsion and lower from aqueous solution. Tests for genotoxic properties in vitro and in vivo were negative, but only low concentrations could be examined in vitro due to the toxicity of the compound. The use of 0.5% Decominol in all types of cosmetics may result in a human dermal exposure of up to 2.5 mg/kg bw/day. Assuming 10% dermal absorption, the systemic exposure may be up to 0.25 mg/kg bw/day. The NEL is below 10 mg/kg effects being limited to local irritation of the gastric mucosa at this level. There was evidence of nephrotoxicity (uraemia) at 40 mg/kg, and also hepatoxicity at 140 mg/kg.

Classification : A.

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10. i.v. TOXIPHARMA - MONTROUGE

11. 90-day TOXIPHARMA - MONTROUGE

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DUFOUR Labor. EVIC-CEBA 3-1984

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"Absorption, distribution and excretion following percutaneous and I.V. administration to the rat".

Report n° 5778.324/3 1/89.

17. HAZLETON. Institut Français de Toxicologie Test pour l'évaluation de l'induction d'aberrations chromosomiques dans le lymphocytes humains.

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Report n° 804462 29/4/88.

REPORTS OF THE SCIENTIFIC COMMITTE ON COSMETOLOGY

CONCERNING THE USE OF CERTAIN COLOURANTS

. THE COMMITTEE'S MANDATE

To give its opinion on the question whether the use of certain colourants :

- CI 73900 (46500)
- CI 74180
- Solvent Yellow 98

is admissible from the health point of view.

1. CI 73900 (46500)

. COMPLEMENTARY OPINION

For preceding informations and expressed opinions consult :
- Reports of SCC, seventh series, EUR 11303 P103
- Report of SCC 35th reunion October 13th 1987.

. PRECEDING CONCLUSION (13-10-87)

Information on possible sensitizing properties, and a chromosomal aberration test in vitro were required as well as a detailled report on the short-term oral study.

Classification : B.

. FURTHERING INFORMATIONS

In a second short-term (33-day) oral study, groups of 8 rats/sex were fed diets with 0, 1, 5 or 10% of a purified form of the colourant to examine systemic toxicity (Five rats of each group were also used for examining chromosomal aberrations and 4 males were added to each diet group for an UDS test in hepatocytes. See mutagenicity data).
There was no growth depression even at the high dose level, but food intake was increased in top-dose males.
A large number of haematological and clinical chemical characteristics were examined in week 2 and 4, but there were no treatment-related changes.
Organ weights and gross and microscopic examinations failed to reveal abnormalities attributed to the colourant (Subm. III, ref. 8).

An in vivo chromosomal aberration test conducted with bone marrow cells from rats fed 0, 1, 5 or 10% of the purified colourant for 33 days was negative (Subm. III, ref. 8).

An in vivo/in vitro test for unscheduled DNA synthesis with hepatocytes from rats fed 0, 1, 5 or 10% of the purified colourant for 14 days did not show an increased number of nuclear grain counts, while rats dosed in a similar way with the positive control substance 2-acetylaminofluorene showed significant increases over that in the negative controls (Subm. III, ref. 9).

CONCLUSION

The purified substance did not show systemic toxicity even at 10% in the diet. Information on possible sensitizing properties is requested. A report on the bio-availability study, which was in progress already in 1987, has not yet been provided.

Classification : B.

REFERENCES

Reference 1 : Avon Acute Oral Toxcity Test
Avon Products Inc, New York, June 8th 1979

Reference 2 : Hoechst Aktiengesellschaft, Pharma Forschung Toxikologie
15th May 1979

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Avon Products Inc, New York, June 8th 1979

Reference 4 : Hoechst Aktiengesellschaft, Pharma Forschung Toxikologie
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Avon Products Inc, New York, June 8th 1979

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Full report not available

Reference 7 : Mutagenicity Evaluation of Quinacridone Red Y
Avon Products Inc, Suffern, New York
Assay n° 3885 - February 1979

Reference 8 : B.S. LEVINE
"Subchronic oral toxicity study in rats"
Microbiological associates, Bethesda, Maryland 23.12.86

Reference 9 : R.D. Curren "In vivo - in vitro rat hepatocyte unscheduled DNA synthesis assay"
Microbiological Associates, Bethesda Maryland 29.01.1988

2. CI 74180

. COMPLEMENTARY OPINION

For preceding informations and expressed opinions consult :
- Reports of SCC, seventh series, EUR 11303, P129.

. PRECEDING CONCLUSIONS (1st July 1986)

The Committee saw no objection to maintaining the use of this colouring agent for the time being in rinsed off products only. For now rinsed off products, no opinion could be expressed.
The Committee required :

- a dermal penetration test in vitro or in vivo
- a short-term oral study to establish the NEL (if penetration is considerable).

. FURTHERING INFORMATIONS

A dermal absorption test was conducted in vitro using skin from the domestic pig or the hairless rat, by applying 0.05% solutions of the labelled compound in water or in 50% aqueous ethanol in an amount of 6 $\mu l/cm^2$ (which is 0.5 μg a.i./cm^2) to an area of 5 cm^2.
The total penetration after 16 h. exposure was about 10% in rat skin and 7% in pig skin without a clear difference between the different solvents (Colipa subm. II, ref. 4).

CONCLUSION

No data are available on mutagenicity.
An Ames test should be conducted and a chromosomal aberration test with mammalian cells *in vitro*.

Classification : C.

REFERENCES

Reference 1 : K.H. LEIST
"Subacute Toxicity Studies of Selected Organic Colorants"
Ecotoxicology and Environmental Safety 6, 457 - 463
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Performed by : Industriels Français du Médicamment (IFM)
Centre d'Etudes Biologiques,
Miserey-Evreux, FRANCE

PCUK : Etude Toxicologique chez le Rat pendant 30 jours
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Reference 3 : Prof. Chr. GLOXHUBER
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Report from 17.7.1980

Reference 4 : Dr. G. KLECAK
"Penetration studies with RO 42-8247/001 ^{14}C sulfonated
Copper Phtalocyanine - CI 74 180 ^{14}C - on intact skin of
naked rat and pig "in vitro"
Hoffmann La Roche, Basle, December 29 1988

SCIENTIFIC COMMITTEE ON COSMETOLOGY

43rd MEETING

JUNE 20th, 1989

LIST OF PARTICIPANTS

Present

Mr AGACHE
Mrs DONY
Mrs ENJOLRAS
Mr FIELDER
Mr GOULDING
Mr KAPOULAS
Mrs KNAAP
Mr LOPRIENO
Mr O'MAHONY
Mr PONS-GIMIER
Mr RAMOS MORGADO
Mr SCHOU
Mr SOMOGYI

Apologies for absence
Mr MUSCARDIN
Mr STÜTTGEN

Commission
Mr GONTIER
Ms DE CRAWHEZ

3. SOLVENT YELLOW 98

. COMPLEMENTARY OPINION

For preceding informations and expressed opinions consult :
- Report of SCC, 40th reunion April 12th 1989.

. PRECEDING CONCLUSIONS (12-4-1989)

The substance has shown little systemic toxicity. An evaluation is not possible because informations on genotoxicity or sensitization is not available.
An Ames test and a maximization test in the guinea pig should be conducted.

Classification : C.

. FURTHERING INFORMATIONS

Mutagenic potential was examined in 5 strains of S. typhimurium and in E. coli WP2 uvr A at dose levels up to 10 mg/plate, with and without metabolic activation.
There were no indications of a positive response (Colipa subm. II).

CONCLUSION

A chromosomal aberration test in vitro, and a maximization test in the guinea pig should be conducted.

Classification : B.

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OPINION OF THE SCC CONCERNING

BERGASOL SUNTAN PRODUCTS CONTAINING 5-MOP

(Opinion expressed on 23.1.90)

. THE COMMITTEE'S MANDATE

5-MOP occurs naturally in oil of bergamot (up to 3000 ppm).
The use of this oil is at present allowed in suntan products by the Cosmetic Directive, but the SCC have recommended that the maximum level of 5-MOP arising from the presence of natural assences in suntan products should be 1 ppm.

The SCC have now been asked to consider data on a specific range of suntan products marketed by Bergasol and containing 15-60 ppm 5-MOP (as the natural product Bergapten). These also contain sunscreens and it is claimed that such formulations behave differently to 5-MOP alone, particularly with regard to the photomutagenicity and photocarcinogenicity which gave rise to the concern with 5-MOP itself.
Bergasol have provided a substantial amount of data to support this submission.

The question that the SCC have to consider is difficult and raises points that the Committee does not, in general, have to consider. There is a finely balanced argument regarding health risks and benefits, whereas in general the health risks of cosmetic ingredients are minimal.

In the case in question, the active melanogenic ingredient, 5-MOP, clearly gives rise to concern regarding its photomutagenic and photocarcinogenic potential.

However, sunlight itself induces skin cancer and the suntan products in question are designed to enhance tanning. More rapid tanning leads to a reduced cumulative dose of UVA and UVB radiation whilst attaining a tan that is protective against subsequent exposure.

There is also the additional problem as to how 'clearance' could be given to specific products under the Cosmetics Directive which is designed to consider either allowed or prohibited ingredients rather than products.

. SUMMARY OF THE AVAILABLE DATA ON THE TOXICITY OF 5-MOP ITSELF

5-MOP itself is well absorbed orally and may be absorbed through the skin. It is widely distributed in tissue, extensively metabolised, and rapidly excreted in the urine. It has very low oral toxicity, but is strongly phototoxic, producing marked skin reactions after exposure to UV radiation. Only limit data are available on sub-acute toxicity.
The combination of 5-MOP plus UV radiation is clearly mutagenic, much information being available on the interaction of psoralens with DNA in the presence of UVA. Linear psoralens such as 5-MOP are bifunctional, forming DNA adducts that react further producing DNA cross-linking.
5-MOP plus UVA has been shown to produce positive results in mutagenicity studies in bacteria, yeasts and mammalian cells, and this combination is also clastogenic.

Several studies have been carried out to investigate the skin carcinogenicity of 5-MOP plus UVA in animal bioassays.
Such treatment has clearly been shown to produce skin cancer in mice. There are no adequate data available to assess the carcinogenicity of 5-MOP in the absence of UV radiation, nor are any adequate data available in man.
However the combination of 8-MOP plus UVA (PUVA treatment) is clearly carcinogenic in man, and in the IARC Group I Carcinogens (i.e. known human carcinogens).

- BERGASOL PRODUCTS : DATA SUBMITTED BY BERGASOL

Studies in animals and human volunteers showed no significant skin irritation or sensitization, nor any significant photo-irritancy or photo-toxicity. Furthermore the data provided by Professor Agache indicates that the absorption of 5-MOP from such formulations is minimal (less than 1%), although greater amounts of 5-MOP were present in the stratum corneum itself.

The crux of the problem is however the potential photomutagenicity and photocarcinogenicity with respect to the induction of skin cancer and this is considered in some detail below :

Several studies have been carried out to investigate the mutagenicity of Bergasol preparations using Salmonella typhimurium TA 102, but interpretation of these are difficult since somewhat conflicting results were obtained, possible due to differences in the amount of material diffusing from the oil emulsion formulations through the agar medium.
Some studies indicated a reduction in the number of revertants but positive results were obtained with commercial preparations and mutagenic potential cannot be ruled out from these studies.

In addition it was pertinent that data from limited studies to investigate the mutagenicity of 'blister fluid' obtained from skin preparations of human volunteers treated dermally with a Bergasol preparation suggested that this had some photomutagenic potential when tested using the yeast strain Saccharomyces cerevisiae D7 (studies by Professor Dubertret).
However, in view of the very limited nature of these studies (no dose response, no repeat of the results in an independent experiment) no firm conclusions can be drawn.

Long term bioassays for skin cancer in mice have given conflicting results.
Limited data from an early study showed no protection using a UV filter (ethyl hexyl paramethoxy cinnamate). More extensive studies using a Bergasol formulation containing 5-MOP plus 2 UV filters (2 ethylhexyl, 4 methoxy cinnamate and 1.7, 7-trimethyl, 3-benzylidene-biocyclo (2,2,1) - 2 heptanone) revealed substantial protection against skin tumour induction in albino mice, but only during the treatment period (45 weeks). Animals exposed to SSR after treatment with 5-MOP plus sunscreens then went on to develop tumours sooner than those treated with sunscreen alone.
In another study in pigmented mice of the same strain, much less protection was seen, but it was argued that these mice were not an appropriate model for human pigmentation due to the absence of epidermal melanin. It is thus difficult to assess the significance of these studies or to identify the mechanisms involved. It is not possible to assess the increased risk of skin cancer in those using formulations containing 5-MOP plus UV filter. The data do not allow concerns regarding this aspect to be discounted.

More recent studies have been reported using an 'accelerated' *in vivo* model for skin tumourigenicity based on hairless mice treated with UV radiation (with or without bergapten containing 50 ppm 5-MOP) as 'initiator' for 4 months together with croton oil, as promoter, for the first 2 months. At the end of the 4 month period a similar incidence of tumours was seen following treatment with UV radiation alone, *ca* 60% incidence of skin papillomas. A marked reduction of the incidence of skin tumours was seen when sunscreens were incorporated in the treatment formulation (20% incidence) but the most impressive results were obtained by the additional incorporation of antioxidants such as BHT or B carotene or by the addition of an inhibitor of polyamine (putrescene) synthesis.

- BENEFITS OF INCORPORATION OF BERGAPTEN (5-MOP)

Considering the protective effect of 5-MOP in sunscreens, data has clearly established that the inclusion of 5-MOP enhances pigmentation. In addition the series of studies by Dr. Young showed that the 5-MOP induced tan protected against UV radiation induced DNA damage in humans as measured by unscheduled DNA synthesis (UDS) in individuals of skin type I to V.
It was particularly noteworthy that the most sensitive population for UV induced skin cancer (ie. types I and II) became like types III and IV in this regard.

However the mechanisms underlining the effects seen are not known and it is important to note that UDS is an indirect measure of damage, and a decrease may reflect a reduction in repair.

The situation is complex and it is clear that there is no direct proportionality between UDS and SSR induced DNA damage. The extent to which 5-MOP induced cross-linking is detected is unknown. It is premature to draw any conclusions from this work. No meaningful assessment can be made of the relative risk of induction of a tan by 5-MOP plus SSR compared to SSR alone.

CONCLUSIONS

In summary consideration of the health risks of products containing 5-MOP gives rises to concern. 5-MOP itself plus solar simulated radiation (SSR) is clearly mutagenic, and produces an increased incidence of skin cancer compared by SSR alone. Data provided on the Bergasol products containing 5-MOP plus UV filters gives a less clear-cut picture, but does not allow concerns about this aspect to be completely discarded.

Concerning the benefits, 5-MOP is clearly efficacious at inducing a tan. The data provided on the protective effect of this tan on subsequent DNA damage induced by SSR, as measured by unscheduled DNA synthesis, are difficult to interpret, in view of the lack of knowledge regarding the mechanisms involved. It is not possible to draw any meaningful conclusions regarding the relative risk of inducing a tan by SST plus 5-MOP plus sunscreens, as compared to SSR suscreens alone.

Much of the information provided is of research interest, but was not believed to be directly relevant to the Bergasol products on the market. For example the studies of Khettab et al relating to the incorporation of antioxidants with sunscreens. Such combinations were shown to offer complete protection against the development of skin tumours in the 4 month initiation/promotion model in hairless mice.

The Committee were concerned about procedures whereby specific cosmetic products could be considered acceptable. In this instance it was argued that certain products were less harmful tan would be expected from the 5-MOP content alone, because of the protective effect of other substances present eg. sunscreens, but this would entail a need to consider not only the concentration of the component of concern (5-MOP but also the presence of other specific compounds at specified levels. This would, in effecct, result in the need for clearance of specific products). The question of balancing health risks versus health benefits had been raised. The Committee felt that it was questionable whether it was appropriate to consider such arguments for cosmetics ; it is a requirement that cosmetic products are not harmful.

On the available data the Committee felt that it was not possible to conclude that sunscreen products containing 15-60 ppm or more 5-MOP were without risk of producing harm due to their potential for photomutagenicity and photocarcinogenic effects.
It was the opinion of the Committee that the maximum amount of 5-MOP in all sun tan products should be 1 ppm.

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Young A.R. and Walker S.L. pp. 357-66.

II. PROTECTIVE VALUE OF PSORALEN INDUCED PIGMENTATION

Enhanced skin tanning by psoralens and a sunscreen psoralens product in minature pigs.
Urbach F. and Sambuco C.P. pp. 389-97.

Comparitive photoprotection in humans by tans induced either by SSR or after a psoralen - containing sunscreen
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III. EVIDENCE FOR A PROTECTIVE EFFECT OF 5-MOP TANNING ON UV INDUCED DNA DAMAGE IN HUMANS

STUDIES BY Dr. A.R. YOUNG et al.

Pigment Cell Research I 350-4 1988

Paris Synposium 1989
A 5-MOP induced tan protects against DNA damage from a subsequent exposure to SSR in human skin pp. 431-432.

CONTENTS

45th meeting of SCC - October 10-11, 1990

- Oxaban A - P75 457
- Germall II - P79 461
- Oxaban E - P90 465
- Omadine MDS - P50 472
- CTAB - P72 475
- Chlorphenesin - P4 478
- CI 12075 484
- CI 15585 490
- CI 45170 498
- CI 26100 504
- Mexenone - S39 507
- Mexoryl SL - S59 513
- Mexoryl SD - S61 519
- Ethoxylated ethyl-4-aminobenzoate - S3 526
- Mexoryl SX - S71 528
- Bergasol suntan products containing 5-MOP 537

REPORTS BY THE SCIENTIFIC COMMITTEE ON COSMETOLOGY

45th REUNION

OCTOBER 2nd, 1990

LIST OF PARTICIPANTS

Present

Mr. LOPRIENO
Mr. CARSTENSEN
Mr. CELLENO
Mr. COTTE
Mrs. DONY
Mr. FIELDER
Mr. KAPOULAS
Mrs. KNAAP
Mr. LINA
Mr. O'MAHONY
Mr. PARRA-JUEZ
Mr. PONS-GIMIER
Mr. SHOU
Mr. SOMOGYI
Mr. WHITE

Excused

Mrs. GUEDES-BAHIA
Mrs. MASSE

Not Present

Mr. KEMPER

Commission

Mr. ANGELIS
Mr. DILLEN
Miss. de CRAWHEZ

Expert

Mr. AGACHE

COMPLEMENTARY OPINIONS CONCERNING

THE USE OF CERTAIN PRESERVATIVE

AGENTS IN COSMETIC PRODUCTS

1.	OXABAN A	(P. 75)	
2.	GERMALL II	(P. 79)	
3.	OXABAN E	(P. 90)	1st opinion
4.	OMADINE MDS	(P. 50)	
5.	CTAB	(P. 72)	
	CHLORPHENESIN	(P. 4)	

1. OXABAN A

. FORMULA AND SYNONYMES

EEC n° 60
Colipa P75
4,4-dimethyl-1,3-oxazolidine
dimethyl oxazolidine
oxadine A

. For preceding informations and expressed opinions consult :

- Reports of the SCC second series EUR 8634, 26/6/1982.
- Reports of the SCC, 38th reunion 10-11/10/1988.

. PRECEDING CONCLUSION

Classification : B.

. FURTHERING INFORMATIONS

MUTAGENICITY

Regarding mutagenicity data negative results were consistently obtained when the ability of the compound to produce gene mutation was investigated in Salmonella.
Results in one case showed a reproducible dose-related increase in revertants in TA98 and TA100, but this was less than twice background and was not significant (ref. 10 ; ref. 18). Negative results were obtained in a second assay using TA98 and TA100, that has been briefly reported (Monte et al, Fd Chem Tox 21 (5) 695-696 (1983)).
A fluctuation test in the S. typhimurium strains was also negative (ref. 17). A chromosomal aberration test in human lymphocytes in vitro with and without S-9 mix was however positive at all doses (ref. 19).

An *in vitro* test in CHO-cells showed dose-related increases in activity, and a mouse lymphoma assay was also positive (file dated 11-5-83). The compound thus has mutagenic potential, as demonstrated from *in vitro* studies ; the notifiers argue that in view of the rapid reactions with protein, this mutagenic potential would not be expressed *in vivo*.

Data from 3 *in vivo* studies are available. Negative results were obtained when the compound was investigated for its ability to produce chromosome damage in the bone marrow of rats treated with 20, 40 or 80 mg/kg using the intraperitoneal route.
Similarly negative results were obtained in a bone marrow micronucleus test the compound being given as a single oral dose of 500 mg/kg (estimated to be the MTD) and harvesting at 24, 48 and 72 hours. Thus the clastogenic potential seen *in vitro* does not appear to be expressed *in vivo*, at least in the bone marrow.
Similarly no effects were seen in a study to investigate single strand DNA breaks in testicular DNA following treatment with 5 and 50 mg/kg compound, as a single dose, or when given on 5 successive days ; (50 mg/kg was estimated to be the MTD).
Cellular preparations were obtained from the testes at 2, 6, and 24 hours post dose. No increase in the amount of low molecular weight DNA could be seen *cf* controls.
Negative results were thus obtained in this assay for DNA damage in the testes. These 3 studies indicate that the compound does not express its mutagenic potential *in vivo* in either the bone marrow or germ cells.

CONCLUSION

In conclusion the material was found to be only moderately toxic in short-term oral and dermal studies in rats. The no-effect levels were 50 and 25 mg/kg respectively (expressed as commercial product). Studies on dermal absorption are not available but on acute oral and dermal toxicity suggest dermal absorption to be considerable.
No evidence of embryotoxicity or teratogenicity was found in rabbits treated dermally with up to 300 mg/kg. The product showed clastogenic properties in mammalian cells in vitro, indicating mutagenic potential, but this was not expressed in vivo. Negative results were obtained in studies to investigate clastogenicity in bone marrow of rats, using both metaphase analysis and the micronucleus test, and using maximum tolerated doses of compound.
In addition negative results were obtained in an assay for DNA damage in the testes (alkaline elution method to detect single strand breaks).
It should however be noted that the material is nitrosatable.

Classification : A.

REFERENCES
++++++++++

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2. DIAZOLIDINYLUREA

. FORMULA AND SYNONYMES

EEC n° 17
Colipa P79
N-(hydroxymethyl)-N-(dihydroxymethyl-1,3-dioxo-2,5-imidazolidinyl-4)-N'-(hydroxymethyl) urea
GERMALL II

. For preceding informations and expressed opinions consult :

- Reports of the SCC - fifth series EUR 11080, 25/3/'85
  - 34th reunion 30/6/'87
  - 42nd reunion 31/10/'89

. PRECEDING CONCLUSIONS

Classification : B.

The confirmed NEL of 100 mg/kg justifies the continued approval of the substance Classification A. for this relatively new preservative that finds increasing use in cosmetics may be considered if results :
- of an oral teratogenicity study
- of a gene mutation test in mammalian cells become available.

. FURTHERING INFORMATIONS

A number of studies have been carried out to investigate the mutagenic potential of this compound. Its ability to produce gene mutation in bacteria has been investigated using Salmonella typhimurium TA98, 100, 1535, 1537 and 1538. Negative results were obtained in all instances except in one study with 1537, when there was some evidence of weak activity in the presence of rat S-9.

However this was not reproducible in further studies using both rat and hamster S-9 (various concentrations) and negative results were also obtained against TA97. It can be concluded that Germall II is essentially ne- negative in these assays. Negative results were also obtained in a test for chromosomal damage (metaphase analysis) in CHO-cells, but concentrations up to only 1.5 µg/ml could be used, because of toxicity (1 mg/ml caused 70% mitotic inhibition (submission X, ref. 27), limiting the value of this study. However negative results were also obtained in an in vivo bone marrow micronucleus test, using oral doses of 1200, 2000 and 2800 mg/kg and multiple harvest times (30, 48 and 72 hours), indicating that Germal II is not clastogenic in bone marrow in vivo.

CONCLUSION

The Committee had requested a study using the oral route, since this would have provided much more information on the teratogenic potential of Germal II than the dermal study actually carried out. It is likely that (eg from physiochemical considrations) the compound is poorly absorbed through the skin, although there is little data on this aspect. The results do however indicate that compound is unlikely to give rise to any concern re teratogenic effects following dermal exposure.

CLassification : A.

LABORATORY REPORT REFERENCES

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Assay n° 11858, January 28, 1981

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Assay n° 10942, November 21, 1980
Assay n° 11850, December 29, 1980

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Project n° 4230-82 ; January 28, 1983

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Assay n° 11859, December 30, 1980

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Assay n° 11861, February 4, 1981

. Contact Sensitization in Guinea Pigs ; Bio/dynamics, Inc.
Project n° 4230-82, January 28, 1983

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Assay n° 16545, December 30, 1981

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Report Study n° T2039.501, July 29, 1983

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Report Study n° T2039.501008, September 15, 1983

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Report Protocol n° CRG-38, June 4, 1982

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Personal Communication, April 30, 1984

. Phototoxicity : Unpublished Company date 81-82-84

. Additional date

. Dr. G. Foster ; Independant consultive
Safety Assessment Summary, May 14, 1985

. 90 Day Oral ; Sutton Laboratories New Jersey

. Release of formaldehyde ; J.F. Walker ; American Society

. Monograph ; Series 3rd ed ; P 471-472

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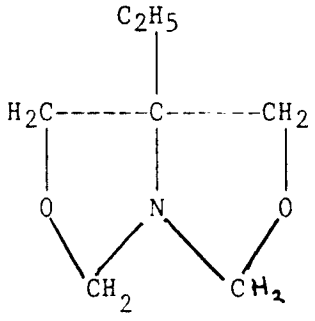
3. OXABAN E

. FORMULA AND SYNONYMES

Colipa P90
7-Ethylbicyclooxazolidine
Bioban/Amine CS-1246
Zoldine ZE
Oxazolidine E
P1601



. CHARACTERISTICS

The material is specified by COLIPA as being>97.5% pure, however the substances tested did not reach this specification, with purity being as low as 96%. The major impurities were 4,4-dimethyl oxazolidine and 4-ethyloxazolidine (2%) and water (2%).

The compound is soluble in water and most organic solvents, exceptions are cyclohexane and 1,4-dioxane.

. USE

The proposed use in cosmetics is in non-rinsed-off products, excluding oral hygiene and mucous membrance products, at concentrations up to 0.3%.

. RECAPITULATION OF THE STUDIES OF TOXICITY

The compound has low acute toxicity.
Oral LD50 values in rats are 3.7 g/kg bw (females) and 5.3 g/kg bw (males) (ref. 1).
The dermal LD50 value in rabbits was 1.95 g/kg bw (combined value for abraded and unabraded skin) (ref. 1).
An inhalation (aerosol) LC50 of 3.1 mg/litre air was obtained from a 4 h. whole body exposure of male and female rats to an aerosol mist of mass median aerodynamic particle size in the range 3.9-4.7 µm (ref. 2).
Based on the acute toxicity data, dermal and oral absorption appears to be of a similar order, though no studies of absorption/permeation were performed.

ORAL TOXICITY

In a 28 day oral study, groups of 5 rats/sex received 100, 300 or 1000 mg/kg bw/d given in deionised water. Both 1000 and 300 mg/kg bw/d produced local effects on the stomach, indicative of an irritant effect. Significant changes in many haematological and clinical chemistry parameters were seen in both sexes receiving 1000 mg/kg bw/d : evidence of anaemia, increased WCB count with neutrophil and lymphocyte numbers increased, thrombocyte numbers were increased, serum potassium and phosphate levels were increased, with glucose levels reduced.
Increases in relative oragan weights were seen for liver, adrenals, testes and kidneys ; absolute values for adrenals were increased in both sexes, despite the reduced body weight seen at 1000 mg/kg bw/d.

Microscopic examination was limited with no changes reported in spleen, liver, kidney, adrenals, heart and testes. Total protein levels were reduced in males.
In the 300 mg/kg bw/d groups there was evidence of anaemia in males and of increased thrombocyte counts in both sexes. Serum phosphate levels were increased in both sexes whilst glucose levels were reduced in both sexes (not statistically significant in females). Adrenal weight was significantly increased in females. At 100 mg/kg bw/d non significant decreases in serum glucose and WBC numbers were seen in both sexes together with evidence of increased adrenal weights. Though there was some evidence of compound related effects at the lowest dose these were not statistically significant and 100 mg/kg bw/d may be taken as the no effect level (ref. 5).

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

A preparation of unknown specification (probably >96%) was severe irritant to the rabbit eye, with washed eyes showing more damage than unwashed.
The effects persisted for 7 days. No dilutions were tested (ref. 1).

A test of primary skin irritancy in rabbits using 0.5 g of undiluted material of unknown purity produced severe irritation following 24 h. occluded exposure (ref. 1).
A 4 h. occluded exposure produced signs of mild irritation, but no tissue destruction. Repeated application (21 days) of a 0.7% solution (6.7 mg/ml) produced erythema in rats (ref. 4). Repeated application of a 0.3% solution (12 applications over 3 weeks) produced mild irritation in one of 100 human volunteers (ref. 3).

A skin sensitisation test performed by the Buehler method was defined as inconclusive (no details given) (ref. 1). A second test using the Landsteiner and Jacobs procedure (a non-adjuvant technique) showed a 0.5% solution to be non-sensitising (ref. 1). A study in 100 human volunteers found no sensitising potential following repeated applications of a 0.3% solution (ref. 3).

A 21 dermal toxicity study in rats (n=6/sex/group) at dose levels of 30, 100 or 300 mg/kg bw/d applied daily for 5d/week in a deionised water solution (0.75, 1.25 or 3.75% w/v) produced dose-related irritation. Eschar formation was observed in the majority of top dose animals and in 3 males and 2 females from the mid-dose group. Dose-related increases were seen in relative adrenal weights in females and GPT in both sexes, though these did not achieve statiscal significance. A dose-related increase in relative kidney weights was seen in females, reaching statistical significance at the top dose (ref. 4).

MUTAGENICITY

Regarding mutagenicity studies, results from a well performed Ames test showed no evidence of mutagenicity at concentrations between 6 and 600 µg/plate ± rat liver S-9 ; cytotoxicity was evident at 300 µg/plate and above (ref.6).

An assay for chromosomal aberrations in CHO cells showed no evidence of clastogenicity with rat liver S-9 mix a slight increase in the numbers of cells with aberrations and in the numbers of aberrations per cell were reported.

No details of individual cultures or types of aberrations are given, making it difficult to assess the significance of the results. The concentrations used were 0.5 to 4 µl/ml, with only slight cytotoxicity (about a 15% decrease in mitotic index) seen at the top dose. Furthermore only one harvest time was investigated, and the results were not confirmed in an independent experiment.
This study was inadequate to draw any definite conclusions (ref. 7).

An assay for unscheduled DNA synthesis in rat primary hepatocytes showed increased activity at all concentrations (0.25 - 4 µl/ml) but this was not dose-related or significant and results were negative (ref. 8).

TERATOGENICITY

In a teratogenicity study groups of 25 mated female rats received 50, 250 or 650 mg/kg bw/d on days 6-15 of gestation, by gavage in deionised water.
There were clear effects at 650 mg/kg bw/d : decreased maternal body weight and increased incidences of malformation (eg cleft palate and umbilical hernia of intestines). The fetal effects were concentrated in 4 litters including the dam with lowest body weight gain. No increases in malformations or variations were recorded at the mid and low doses. The NEL in this study was 250 mg/kg bw/day (ref. 9).

CONCLUSION

A further study to assess whether Oxeban E can produce chromosome damage in vitro in mammalian cells, by metaphase analysis, using a more rigorous protocol, to current standards is necessary before any definite conclusions can be drawn regarding the clastogenic potential of the compound.
In addition skin sensitization potential should be investigated using a more sensitive method, the magnussen - kligmann maximisation Test being the recommended method.

In addition information on the amount and rate of formaldehyde generation from this preservative should be obtained.

Classification : B.

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7. "Chromosome aberration in Chinese hamster ovary (CHO) cells", Toxicon Project No. 87G-0140. Toxicon Corporation, Woburn, Massachussetts, USA, January 6, 1988.

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4. OMADINE MDS

. FORMULA AND SYNONYMES

EEC n° 41
Colipa P50
Dithio-2,2'-bispyridine-dioxide 1,1' with magnesium sulphate trihydrate
Pyrithion disulphide + magnesium sumphate

. FOR PRECEDING INFORMATIONS AND EXPRESSED OPINIONS CONSULT :

Reports of SCC : 33rd reunion 2/2/87.

. PRECEDING CONCLUSION

Classification : D.

The toxicity of the substance, the dermal exposure and the potential dermal absorption occur with a too tight margin of safety to permit the use of this substance in cosmetics (even limited to anti-dandruff shampoos).

. FURTHERING INFORMATIONS

In a second 90-day study, rats received 15, 0.8 or 0.5 mg/kg bw/day for 13 weeks in a water vehicle. Due to signs of toxicity the top-dose was reduced to 7.5 mg/kg bw/day after 2 weeks In the top-dose group 9/15 females and 1/15 males died. At autopsy, animals from the top-dose group showed muscle wasting, reduced body weight gain, hind limb muscular atrophy, hepatocyte vacuolation, thickening of the non-glandular stomach mucosa and pancytopaenia. In the mid-dose animals, hind limb muscular wasting was seen in 2/15 females, alterations in various clinical chemistry and haematological parameters were reported. At the lowest dose reductions were seen in total serum bilirubin and leucocyte counts and the

incidence of hepatocyte vacuolation was slightly increased. Histology was limited at mid and low-doses but did include liver, muscle and sciatic nerve. The lowest dose used in this study is not a clear NEL.

- In man 1.26% of a dermal dose was recovered in the urine over 120h when 160 µg/cm2 were applied in a shampoo formulation to the forearms of 3 volunteers. The treated area was rinsed after 4 minutes. Total absorption was less than 3%.

- Dermal retention in shaved and unshaved rats receiving 0.15 ml of shampoos containing 1% omadine was 0.85% and 0.54% of the dose respectively. 17% of the dose was found bound to the hair. Treated areas were rinsed after 4 minutes with samples taken at 1h. No measurements of absorption were made.

The Company have calculated using several assumptions that intakes of omadine by a 60 kg person would be between, 1.9 and 16 µg/kg bw/shampooing. A realistic figure based on 12 ml shampoo, 1% omadine and 3% absorption would be 60 µg/kg bw/shampooing, only a small margin below a minimal effect level of 500 µg/kg bw/day in a 90-day oral study.

CONCLUSION

Omadine possesses considerable toxic potential and is possibily genotoxic. Clear adverse effects were seen at oral doses of 0.8 mg/kg bw/day and 10 mg/kg bw/day dermally with a report of foetotoxicity following dermal exposure to 5 mg/kg bw/day. The margins between proposed uses and toxic levels are inadequate to ensure safety in use, therefore Omadine MDS should not be used in cosmetic products.

Classification : D.

REFERENCES

Colipa subm VIII Dec 89.

49. OLIN CORPORATION
13-w oral tox. study (rat)
ref n° OLA/12/89
April 89 - Toxicol. Laboratories England

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HRC report n° HRC FOLN 5/89890 Sept 25, 1989

51. Deposition of 14C (on rat skin in presence and absence of hair)
HRC report n° HRC FOLN 6/89888 Sept 18, 1989
Final report by Huntingonn Research Center Ltd

5. C.T.A.B.

. FORMULA AND SYNONYMES

EEC n° 4

Colipa P72

Alkyl (C_{12}-C_{22}) trimethylammoniumbromide chloride

. FOR PRECEDING INFORMATIONS AND EXPRESSED OPINIONS CONSULT :

Reports of the SCC : 34th reunion 30/6/87

. PRECEDING CONCLUSION

Classification : B.

A chromosomal aberration test on mammalian cells was needed.

. FURTHERING INFORMATIONS

Negative results were obtained in an assay to investigate the ability of the compound to induce chromosome aberrations in Chinese hamster V79 cells both in the presence and absence of exogenous metabolic activation. Only very low concentrations could be used, up to 10 µg/ml, due to cytotoxicity. The highest concentration was associated with a 50% reduction in mitotic index.

Negative results were also obtained in a morphological transformation assay with cells of 13 day old Syrian hamster embryos, and up to 300 µg test substance/ml.

CONCLUSION

This substance has shown considerable systemic toxicity including indications of teratogenicity. However, absorption through the intestinal wall and through the skin is low. There was no evidence of mutagenicity in in vitro assays for gene mutation and chromosome damage.
Not to be used on a damaged skin.

Classification : A.

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the Netherlands, Sept 1978

Colipa Subm I April 1984

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   B. ISOMAA and K. EKMAN
   Food Cosmet. Toxicol. Vol 13 (1975) p. 331-334

Colipa Subm II February 1987

3. Ames test
   D.J.N. ROASACK and al (1977)
   HUNTINGDON RESEARCH CENTER England

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   Hauptabteilung Toxikologie
   HENKEL - Düsseldorf Germany

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6. Reverse mutation test
   U. MULLER (1976)
   Battelle Institut Frankfurt Germany

7. Terato
   C.A. LEWIS (1977)
   Consumer Product Testing Fairfield, New Jersey USA

Colipa Subm III 12-1989

8. Chromosome Aberration Assay Chinese Hamster V79 cells in vitro
   A. HEIDEMANN Cytotest cell Research GmbH & Co. KG
   In den Leppteinswiesen 19, D. 6101 ROßDORF 15-9-1989

6. CHLORPHENESIN

. FORMULA AND SYNONYMES

EEC n° 2
Colipa P4
3-(p-chlorophenoxy) propane-1,2-diol
p-chlorophenyl-glycerol ether
CAS n° 104-29-0

. FOR PRECEDING INFORMATIONS AND EXPRESSED OPINIONS CONSULT :

Report of SCC : 37th reunion 12/4/88
42nd reunion 30/10/89

. PRECEDING CONCLUSION

Classification : C.

A long-term oral study and available information on dermal absorption were required.
Depending on the results, a teratogenicity study may be required.

. FURTHERING INFORMATIONS

Full details are however available of a 28-day oral toxicity study in rats given doses of 10, 100 and 1000 mg/kg compound by gavage as an aqueous suspension. Detailed autopsies were performed at the end of the exposure period and in addition serum immunoglobulin levels and B ; T lymphocyte ratios in blood and spleen were determined.
Compound related mortality was seen at the top dose, 1/5 male animals dying. Other effects noted at this level were reduced weight gain, abnormal posture and gait, reduced spleen and thymus weight and evidence of nephrotoxicity.

The only significant effects seen at 100 mg/kg were a slight reduction in haemoglobin levels. No pathology was seen in the spleen, lymph nodes, thysmus or bone marrow at any dose level. The no effect level was 10 mg/kg with only marginal effects at 100 mg/kg.

CONCLUSION

Chlorphenesin has low acute toxiicty, no significant irritant properties and has relatively low toxicity on repeated exposure by the oral route.
No data are however available on dermal absorption.
This is needed, and depending on the results, a teratogenicity study may be required.

Classification : B.

REFERENCES

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ACUTE TOXICITY

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5. BERGER F.M. G.M. FUKUI
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MUCOUS and SKIN IRRITATION

3. 6-7-8 : HUMAN PATCH TEST 1982

40-DAY TOX.

3.

90-DAY ORAL

5.

IMMUNOSUPPRESSANT and ANTIANOPHYLACTIC ACTION

2.

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J. IMMUNOL. 107:586, 1971

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Pergamon Press 1969
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Colipa Subm IV 3/89

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Chlorphenesin - Metaphase chromosome Analysis of Human Lymphocytes cultured in vitro
Huntingdon Research Center : Rep. SR098/881424 Feb 9, 1989

Colipa Subm III 12/87

Mutagenicity

20. Ames Test
Laboratories sérobiologiques Nancy
Dr. M. Pauly 15-10-1987

21. CHO - TEST
Laboratories sérobiologiques Nancy

REPORTS BY THE SCIENTIFIC COMMITTEE ON COSMETOLOGY CONCERNING THE USE OF CERTAIN COLOURANTS IN COSMETIC PRODUCTS

. THE COMMITTEE'S MANDATE

To give its opinion on the question whether the use of :

CI 12075
CI 15585
CI 45170
CI 26100

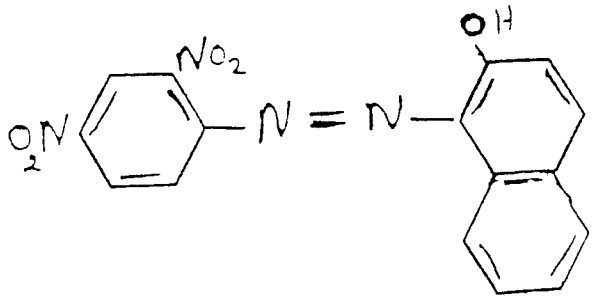
in cosmeticsproducts is admissible from the health point of view.

1. CI 12075

. FORMULA AND SYNONYMES

1-(2,4-dinitrophenylazo)-2-naphthol

(D and C Orange 17, Pigment Orange 5)



. USE

The colourant has been in use in cosmetics and drugs for external application since 1936. It is listed as an allowed colourant for all uses in the EEC Cosmetics Directive.

. RECAPITULATION OF THE STUDIES OF TOXICITY

Preamble : The Colipa submission I (May 1989) consists of a review of data and a risk assessment prepared by The Cosmetic, Toiletry and Fragrance Association, Inc of the US, dated April 15, 1983. This concentrated on long-term studies, particularly relating to carcinogenicity, and no data on acute toxicity, skin and eye irritation, sensitisation, and short-term studies for systemic toxicity were provided.
More importantly no data on the mutagenic potential of CI 12075 were given ; this is essential when assessing the relevance of the carcinogenicity data.

LONG-TERM ORAL TOXICITY AND CARCINOGENICITY

The CTFA - review contains a few details of 4 long-term studies conducted by HAZLETON Laboratories and summarised by the US-FDA in 1972.

In a rat-feeding study, dose levels of 0, 0.025, 0,1 and 1.0% were fed in the diet for 2 years. There was growth depression in the high-dose group, and dose-related haematological changes consisting of decreased haematocrit, haemoglobin content and red blood cell count. The urine showed a dose-related increase in bilirubin levels.

In a second rat-feeding study, 0, and 80 ppm was fed in the diet for 26 months, without inducing any adverse effect. This study was inadequate, in view of the low single dose used, to assess the carcinogenicity of CI 12075.

A dog-feeding study comprised diet groups fed 0, 0.025, 0.125 or 1.0% for 2 years. The top dose induced anaemia, enlargment of the thyroid, liver and spleen and changes in the skin. Increased weights of the thyroid and liver also occurred with 0.125% bilirubin excretion in urine was increased at all dose levels. There were no tumours related to treatment, but no conclusions can be drawn from this study in dogs, in view of its relatively short duration. A skin painting study in mice with a 1% aqueous suspension for 18 months produced acanthosis and hyperkeratosis, but no evidence of carcinogenicity. From these studies FDA estimated the ADI to be 0.0625 mg/kg (August 4, 1972).

More recent long-term studies were conducted by Bio/dynamics for CTFA. In one long-term study the rats were exposed from in utero by feeding diets with 0, 0.02, 0.05 and 0.1% from 60 days before mating through the gestation and lactation period and then to the descendents for 27 or 28 months, for males and females respectively. Slight growth retardation occurred with 0.1 and 0.05%. Decreased levels of creatinine and elevated levels of fasting glucose occurred in all treated groups. The incidence of lymphoreticular tumours was increased in high-dose females. (14% of 4% in controls).

A second long-term rat study was conducted in a similar was (using in utero exposure) but with only one high exposure level of 1.0%. This study lasted 26 months for the males and 30 months for the females. Growth depression, increased food intake and anaemia were observed in treated rats. A clear increase in benign hepatocellular tumours (adenomas) was seen in the female rats, with a slight increase in hepatocellular carcinomas. No increase in lymphoreticular tumours was seen in this study. There was also an increased incidence of proliferative non-neoplastic alterations in the liver (clear cell foci) as well as enlargement of liver cells.

A feeding study in mice used dietary levels of 0, 0.025, 0.25 and 1.0%. Increased mortality was observed with 0.25 and 1.0%. Anaemia occurred in the high-dose group. Pigment deposits were seen in the spleen, caecum and liver of the top-dose mice. The incidence of chronic myocarditis was increased in high-dose females. A slight increase (but not statistically significant) was seen in liver tumours (both hepatocellular adenomas and carcinomas) in the male animals only at the high dose level (1% in the diet).

The increased incidences of tumours in the foregoing studies have been evaluated in the CTFA review, which resulted in the conclusion that none of these studies indicated that D and C orange 17 is a potent or primary carcinogen. Therefore CTFA requested permanent listing of the colourant only for external cosmetics and drugs that are not subject to incidental ingestion.

DERMAL TOXICITY

Studies are briefly reported in the CTFA review on the absorption of CI 12075 through half thickness human abdominal skin using different vehicles. Rates from 0.009 ug/cm2/24 hour (estimated to represent ca 0.006% of applied dose) from a cream base to 0.0012 ug/cm2/24 hour (equivalent to ca 0.00009% applied dose) from a talc formulation.

MUTAGENICITY

However no consideration was given in the CTFA review to the mutagenicity data on this colourant. The published data are summarised below.

Several groups have reported positive results in bacterial assays various Salmonella typhimurium strains. Muzzall and Cooke (1979) obtained positive results using Salmonella typhimurium TA 98 both in the presence and absence of S9, and vs TA 100 in the absence of S9. Brown et al (1979) obtained positive results vs TA 98, 100, 1537 and 1538 both in the presence and absence of S9. Green and Pasteuka (1980) reported positive results were consistently obtained in these bacterial assays. Since activity was seen in the absence of S9, this was likely to be due to activation by endogenous nitroreductase known to be present in Salmonella. Mutagenicity data from other systems would be helpful, but no further data are available. The colourant clearly has mutagenic potential.

CONCLUSION

In summary CI 12705 clearly has mutagenic potential, as demonstrated by the induction of reverse mutations in Salmonella typhimurium, although no further data are available and it is not possible to fully assess the mutagenicity of the compound. However it has been demonstrated to have carcinogenic potential in long-term animal bioassays. In one study in rats an increase in lymphoreticular tumours was seen in females, and in a second study an increase in hepatocellular adenomas. In a study in mice an increase in liver tumours (both adenomas and carcinomas) was seen, but this was not statistically significant. It is accepted that the carcinogenicity data is relatively weak, but in view of the mutagenic potential of the compound, it cannot be discounted. The colourant should not be allowed in cosmetics.

Classification : D.

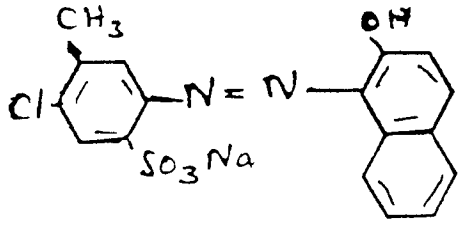
REFERENCES

"Final Review and Analysis of Scientific Studies and Risk Assessments supporting the Safety of D.C.Orange N°17 for use in External Cosmetic and Drug Products not subject to Incidental Ingestion."
CTFA , April 15, 1983.

2. CI 15585

. FORMULA AND SYNONYMES

Monosodium salt of 1-(4-chloro-O-sulpho-5-tolylazo-2-naphtol
(Pigment Red 53, C-Rot 55)



The barium salt of 1-(4-chloro-O-sulpho-5-nolylazo-2-naphtol)
D & C red n°9
Cas. Reg. n° 5160-02-01
MW: 376,7
$C_{17}H_{13}O_4N_2ClS$[1]

. USE

This colourant has been used in external cosmetics and drugs, including those subjects to incidental ingestion, for very many years (since the late 1930s).
It is used widely in lipsticks.
Use levels are up to 5%.
It is allowed as a colourant under the EEC Cosmetics Directive for all uses except around the eye.

. RECAPITULATION OF THE STUDIES OF TOXICITY

The Colipa submission I (May 1989) consists of the final review and analysis of studies on D & C Red n° 9 prepared by the Cosmetic, Toiletry and Fragrance Association (CTFA) of the US, dated, August15, 1983.

The review does not contain any information on acute toxicity, skin, or eye irritation, or sensitisation, but concentrates on long term toxicity, especially carcinogenicity, and includes mutagenicity data.

ORAL TOXICITY AND CARCINOGENICITY

A subacute (90-day) feeding study in Fischer 344 rats with 0, 0.25, 0.50, 1.0 and 2.0% in the diet revealed enlargement of the spleen in all dose groups, and abnormal red blood cells. In a 20 week study in rats using the same dietary levels splenomegaly, and low haemoglobin levels and haematocrit values were observed (in the review these changes are evaluated as 'no significant adverse effects').

The review tabulates a few details of a 2 year rat feeding study and a 2 year dog feeding study conducted before 1976.

The 2 year rat study (Osbourne Mendel strain) was conducted with dietary levels of 0, 100, 500, 2500 and 10.000 ppm. Splenomegaly and abnormal RCBs were seen at the two high dose levels. The NEL was 500 ppm, or about 25 mg/kg bw/day. No neoplasia were observed.

The 2 year dog study comprised groups fed 0, 150, 1000 or 5000 ppm.

Splenomegaly and destruction of RBCs occurred with 1000 and 5000 ppm, increased liver weights with 5000 ppm. The NEL was 150 ppm or about 4 mg/kg. Again no neoplasia were observed, but the study was of too short a duration to allow any conclusions to be drawn regarding carcinogenicity in this species.

Carcinogenicity bioassays were carried out by Battelle (1977-79) as part of the NTP programme. Fischer 344 rats were given dietary levels of 1000 and 3000 ppm, and B6C3F1 mice were given levels of 1000 and 2000 ppm. Animals were exposed to treated diet for 103 weeks and the study terminated 1 week later. D & C Red 9 was carcinogenic in male rats causing an increased incidence of sarcoma of the spleen at the top dose, and a dose related increase in neoplastic nodules of the liver ; the significance of the latter is however questionable. There was no evidence of carcinogenicity in female rats. Nor was there any evidence of carcinogenicity in the B6C3F1 mice of either sex. The significance of the spleenic tumours seen in the F344 rats was questioned, as it was argued that the mechanism by which these arose was secondary to marked toxic effects on the spleen at the high dose (fibrosis) (see later for detailed discussion).

The CTFA sponsored long-term studies conducted by Litton are considered below.

A 2 year mouse study at dose levels of 0.50, 250 and 1000 ppm produced changes in cell count, haemoglobin and haematocrit in the high dose group. Other changes were not considered related to treatment.

A second mouse study using feeding levels of 0 and 2000 ppm. The only change attributed to treatment was chronic inflammation of the stomach.

A chronic study in Sprague Dawley rats, and involving in utero exposure, used the relatively low feeding levels of 0, 100, 200 and 500 ppm for 30 months. The only change observed was an increased spleen weight accompanied by decreases in red cell parameters, at 500 ppm and observed only at 12 months. The no effect level was 200 ppm.

A second chronic (30-months) rat study again in Sprague Dawley rats and with in utero exposure used only one high feeding level of 10.000 ppm. This level caused changes of the spleen, kidney, liver, pancreas and pituitary. The spleen changes were accompanied by 4 mesenchymal tumours of the spleen in the treated animals two of which were very uncommon in the rat-strain used ; two spleenic tumours were seen in the control. The incidence was, however, not statistically significant. In the adrenals of the treated rats, the incidence of hyperplasia and of phaeochromocytoma were increased in both sexes but the increase in the latter was not statistically significant.

Mechanism of induction of spleenic tumours in rats by D & C Red 9

Chronic studies in rats have shown that exposure to high dietary levels of D & C Red 9 results in the induction of spleenic tumours, particularly in Fischer F344 animals, with a high incidence of both fibrosarcomas and angiosarcomas being observed. There was no clear evidence for an increase in any other tumour type in rats, nor of any carcinogenic effect in mice. The spleenic tumours in rats were associated with levels that also resulted in marked toxicity to the spleen (capsular and parenchymal fibrosis), effects being observed in most animals.

A number of suggestions regarding potential mechanisms have been made ; these have recently been reviewed (Bus and Popp 1987). It was suggested by Goodman et al (1984) that spleenic toxicity, arising from an initial toxicity to erythrocytes (probably by an amine metabolite), followed by sequestration of the damaged erythrocytes in the spleen, leading to haemosiderin deposition coupled with enhanced delivery of toxic metabolites in the spleen, resulted in fibrosis and subsequently the formation of fibrosarcoma. A sligthly different mechanism was proposed by Weinburger et al (1985) nameley that acute vascular congestion, resulting from spleenic scavenging of chemically damaged erythrocytes may be an important initial toxic lesion to the spleen.

The vascular congestion would lead to spleenic haemorrhage, formation of fibrous tissue mass and, again in conjunction with accumulation of toxic metabolites within the spleen (derived from scavening erythrocytes) transformation of mesenchymal cells of the spleen, resulting in the expression of spleenic fibrosarcomas and a variety of other lesions. Haemosiderin deposition was not critical for the latter hypothesis, which is pertinent as there was no evidence of increased intra-splenic accumulation of iron containing pigment in the animals treated with D & C Red 9.

These data support the hypothesis that the sarcomas seen at high dose levels were secondary to toxcity, and that a threshold thus exists.

However a crucial question in this regard is whether the compound is acting by a "non-genotoxic" mechanism.

MUTAGENICITY

There are a number of reports of this colourant being investigated for its ability to produce gene mutation in bacteria using Salmonella typhimurium. Negative results were obtained by Brown et al (1979) using TA 98, 100, 1535, 1537 and 1538, both in the presence and absence of rat S9. Duplicate plating was used, and the results were not confirmed in an independent experiment. Muzzall and Cooke (1979) reported a negative result using the spot test method and also with a plate incorporation assay with strains TA 98, TA 100, TA 1535, TA 1537 but using a lipstick containing D & C Red 9 rather than the compound itself.
This study was too limited to allow any conclusions to be drawn regarding the mutagenic potential of D & C Red 9. In a recent report on compounds tested as part of the NTP programme (Zeiger et al 1988) the colourant is reported to be weakly positive against TA 97 in the absence of S9.

However examination of the data indicates that this result was at most equivocal. Negative results were obtained with the other strains. Thus D & C Red 9 has essentially given negative results in Salmonella assays.

No data are available on the clastogenicity of D & C Red 9 either in vitro or in vivo. However the ability of the compound to produce UDS in rat hepatocytes has been investigated both in vitro and also in an in vivo/in vitro liver UDS assay using oral doses of 500 mg/kg, with perfusion of the liver and harvesting after 2 and 5 hours (Kornbrust and Barfknecht 1985).
Negative results were obtained in both cases.

There is one brief report of a positive result in a cell transformation assay using Balb/c 3T3 system, but insufficient details were given to assess this study (Trennent et al 1986).

To summarise the mutagenicity data, D & C Red 9 has given negative results in assays to investigate its ability to produce gene mutation in Salmonella. Negative results were also obtained when the compound was investigated for its ability to produce UDS in cultured primary hepatocytes, and also in an in vivo liver UDS assay using an oral dose level of 500 mg/kg. However no data are available on its clastogenic potential. Although the colourant does not appear to have significant mutagenic potential, the data are too limited to allow difinite conclusions to be drawn regarding this aspect.

MISCELLANEOUS

Dermal absorption was examined by an *in vitro* test with human skin using Franz diffusion cells. The maximum absorption found was 0.06%, and the calculated total absorption of cosmetic use was *ca* ug/day, or *ca* 0.07 ug/kg/day.

CTFA calculation of ingested colourant from lipstick (assuming present at 2%) use (assuming ingestion of 50% CTFIA of the amount applied) resulted in a maximum daily intake of 0.4 mg, or 0.008 mg/kg/day. However much higher values would be obtained using other assumptions (0.01 g per application, up to 6 applications per day).

CONCLUSION

CI 15585 has been shown to be carcinogenic in rats at high dose levels producing fibrosarcomas and angiosarcomas of the spleen. No clear evidence of tumour induction at other sites was seen in the rat, nor of any carcinogenic effects in mice. Dietary levels that produced tumours of the spleen in rats was also associated with marked toxic effects (fibrosis), and the tumours may be secondary to toxicity. The no effect level in chronic studies in rats and dog was about 25 and 4 mg/kg/day respectively. The available mutagenicity data are negative in Salmonella assays for reverse mutations, and in both *in vitro* and *in vivo* assays for UDS in hepatocytes. However no information is available on its ability to produce chromosome damage either *in vitro* or *in vivo*.

In view of the importance of adequate reassurance that a genotoxic mechanism was not involved in the induction of the spleenic tumours, data form a well conducted *in vitro* assay to investigate chromosome damage in mammalian cells (metaphase analysis) are needed. In addition, the relatively low NEZ for toxicity, to the spleen (4 mg/kg) gives rise to concern. Detailed information on capsure levels are needed, and it may prove necessary to set a permitted concentration in mucous membrane products below 5%.

Classification : C.

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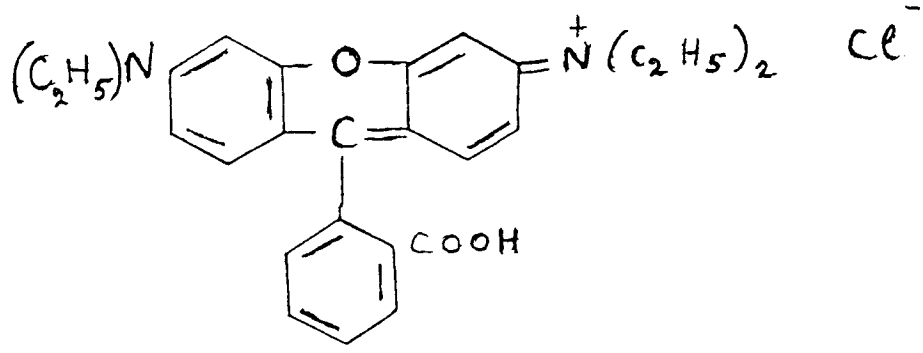
3. CI 45170

. FORMULA AND SYNONYMES

3,6-bis diethylamino - 9(2-carboxyphenyl) xanthylimonim chloride
D & C Red n° 19
C-Rot 59
Rhodamine B



. CHARACTERISTICS

The compound is a red colourant
Soluble in water and ethanol

. USE

It is allowed as a colourant in all cosmetic products under the Cosmetic Directive.

. RECAPITULATION OF THE STUDIES OF TOXICITY

The Colipa submission on this substance consists of the Final Review and Analysis of studies on D & C Red n° 19 and the risk assessment prepared for the Cosmetic Toiletry and Fragrance Association (CTFA) dated February 15th 1983. This review mainly deals with carcinogenicity studies but also briefly reviews mutagenicity data.

The CTFA review tabulates chronic studies in mice, rats and dogs conducted prior to 1976. None of these studies was adequate for an assessment of the carcinogenic potential of CI 45,170.

LONG-TERM ORAL TOXICITY

A lifetime study in mice involved the use of a single concentration given in drinking water (0.05%) for 52 weeks, with the animals then observed for their lifespan. Polyposis of the stomach was seen in 3/30 treated mice, intestinal tumours in 2 and lymphomas in 7. There was no concurrent control group.

A lifetime rat study with 0.13% in the diet given for 7 months and the 0.2% in the diet, did not produce any significant effect. However only 18 animals survived 300 days and only one survived 661 days.

A 24 month study in rats using dietary levels of 0.008, 0.025 and 0.15% showed a dose related increase in thyroid activity and weight and epithelial nodules in the thyroid at the high dose level.

A 26 month rat study using 0.004% in the diet produced pink colouration of the eye lens but no other adverse effect. The single dose level used was very low.

A three generation rat study using doses of 0,0004 and 4 mg/kg/bw/day given in the diet, did not reveal any treatment-related changes.

A 24 month study in dogs using 0,0.0008, 0.3125 and 0.1% levels in the diet showed no adverse effects.

DERMAL ABSORPTION

The CTFA report contained details of skin absorption studies carried out by Dr. Franz using human abdominal skin and the diffusion cell technique and radiolabelled material. Various formulations were investigated containing 0.001 - 0.5% compound. Absorption values for a 24 hour period were calculated to be in the range 0.002 - 1.22% (The worst-case maximum amount absorbed for all combinations of product was calculated to be 3.3 - 3.8 ug/day or 0.06 - 0.07 ug/kg bw/day).

MUTAGENICITY

Somewhat conflicting results have been obtained in assays for gene mutation using Salmonella typhimurium, due in part to differences in purity of the samples tested. Negative results were reported by Parodi et al (1981) and Muzzall and Cooke (1979). Brown et al (1974) obtained positive results with TA 1537, 1538 and 100 in the presence of rat S-9, but the compound tested was only about 20% 45,170 and it was noted that a purer sample had only 'marginal' acitivity. Nestman et al (1979) found a commercial sample (90% pure) to be positive vs TA 1538 and 98 ; purification by thin layer chromatography revealed that most of the activity was associated with an impurity, but the chromatographically pure material had some activity vs TA 1538. Negative results were obtained using laser-grade rhodamine B. (Douglas et al 1980, Weubbles & Fellan 1985). However this grade material did produce both chromosome aberrations and SCEs in mammalian cells in vitro.

Only very limited data are available from in vivo studies. Negative results were obtained in an assay for DNA fragmentation in the liver following i.p. administration of compound. No data are available from bone marrow assays for clastogenicity.

CI 45,170 clearly has mutagenic potential, particularly the technical material although the compound itself does appear to be mutagenic. Inadequate data are available to fully access this potential.

CARCINOGENICITY

More recent carcinogenicity bioassays done to acceptable protocols are described in detail in the CTFA review. These are considered below.

A mouse study used dietary levels of 0,50,200 and 200 ppm for 2 years and involved groups of 60 animals of each sex at each dose level. No significant effect were seen on mortality or growth rate. A dose-related increase in hepatocellular carcinomas was seen in the females, the incidence being 2/60 (3%) 5/60 (8%) and 14/60 (23%) as compared to 0/115 (0%) in the controls.
Effects were less clear in the males, the incidence being 13%, 9% and 15% in the low, medium and high dose group compared to 8% in the controls. No significant increase in hepatocellular adenomas was seen in either sex.

A study in rats involved dietary levels of 0,20, 50 and 200 ppm, using groups of 70 animals per sex per dose level, and involved in utero exposure (maternal animals being given these diets for 2 weeks prior to mating), followed by feeding for 30 months. No effects were seen on mortality.

A slight reduction in body weight gain was seen in the females given the high dose but no other effects were noted during the study. The only increased tumour incidence seen in any group was a slight increase incidence (but not significant) of tumours of the brain and spinal cord in the high dose males.

A second study was carried out in rats using a single higher dose level (750 ppm in the diet), following concern by the FDA that the 200 ppm level in the above study was not a maximum tolerated dose. At 750 ppm no significant effects were seen on mortality but there was a clear reduction in body weight gain in 30 males and female rats and increased incidence of both adenomas (12/56, 21%) and carcinomas (5/56, 9%) of the thyroid compared to the controls 4% (2/59) adenomas, 2% (1/57) carcinomas. It was suggested by CTFA that the thyroid tumours may be due to chronic hyperplastic stimulation as a result of thyroid hormone imbalance. No increase in tumours of the CNS or any other site was observed.

CONCLUSION

CI 45,170 clearly has mutagenic potential, but inadequate data are available on whether this can be expressed in vivo. The compound is however carcinogenic both in mice producing a clear increase in thyroid adenomas and carcinomas. It should not be allowed as a colourant for cosmetics.

Classification : D.

REFERENCE

"Final Review and Analysis of Scientific Studies and Risk Assessment supporting the Safety of D.C.Red N°19 for use in External Cosmetic and Drug products not subject to Incidental Ingestion."
CTFA, February 15, 1983.

4. CI 26100 (complementary opinion)

For preceding informations and expressed opinions consult :
- Reports of SCC, seventh series, EUR 11303 pp 68-69

. FORMULA AND SYNONYMES

CAS Reg n° 85-86-9

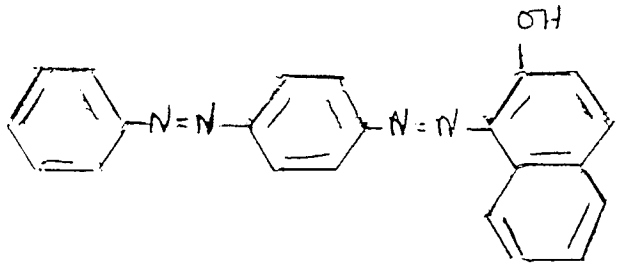
1- (4-(phenylazo) phenyl azo) -2naphthalenol

Solvent Red 23

Sudan III, C-Ext

Rot 56

. DC Red 17



. PRECEDING CONCLUSION

A short-term oral toxicity study to examine systemic effects and further information on mutagenicity were required.

. NEW PROPOSITION FOR USE

Used up to 0.5% for non-rinse applications not in contact with mucous membranes (Colipa subm II).

. FURTHER INFORMATION

A chromosomal aberration test in human lymphocytes exposed in vitro to up to 100 ug/ml was also negative (submission II, ref. 10).

CONCLUSION

CI 26100 has low oral toxicity following single and repeated exposure and marginal irritant properties. The pure material does not appear to be a skin sensitizer. There was no evidence of any mutagenic potential in studies to investigate gene mutation in Salmonella or chromosome aberrations in human lymphocytes.
No evidence of carcinogenicity, or other toxic effects were seen in chronic toxicity studies in rats and mice, using the oral and dermal route, but inadequacies in the design of these studies prevent any definite conclusion being drawn. The compound is very poorly absorbed orally, the Committee do not consider it justifiable to request additional data from a short-term oral toxicity study. It would be expected to be poorly absorbed through the skin. In view of the sensitization seen with impure compound, purity criteria are needed.

Classification : A.

REFERENCES
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REPORTS BY THE SCIENTIFIC COMMITTEE ON COSMETOLOGY

CONCERNING THE USE OF

CERTAIN U.V. FILTERS

1. MEXENONE	EEC 2.18	S39
2. MEXORYL SL	EEC 2.24	S59
3. MEXORYL SD	EEC 2.26	S61
4. ETHOYYLATED ETHYL-4-AMINOBENZOATE	EEC 2.2	S3
5. MEXORYL SX		S71

. THE COMMITTEE'S MANDATE

To give its opinion on the question whether the use of these UV filters at their usual concentration is admissible from the health point of view.

1. MEXENONE

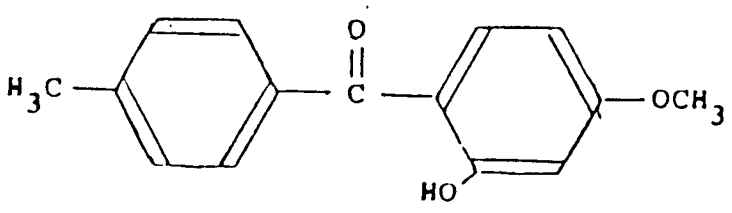
. FORMULA AND SYNONYMES

EEC n° 2.18

Colipa S39

2-hydroxy-4-methoxy-4'-methylbenzophenone

Methanone (2-hydroxy-4-methoxyphenyl)



MW: 242

. CHARACTERISTICS

White crystalline powder
Insoluble in water
Soluble in alcohol and acetone

. USE

Proposed for use as a sunscreen agent at levels up to 4%.

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral (rat) : $>$ 5 mg/kg

ORAL TOXICITY

Rat. A 28 day study was carried out in groups of 10 animals with doses of 0, 50, 250 and 1000 mg/kg bw/day, given by gavage. No dose related adverse effects were seen. The NEL is put at 1000 mg/kg bw/day.

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

Test for capacity to produce irritation of mucous membranes.
Rabbit. A test was carried out using a product, "Uvistat Sun Screen", containing 4% of a.i. Six animals were used. A dose of 0.1 ml of the product was instilled into one eye of each animal. The untreated eye served as control. There were very slight transient changes in one animal ; the test was otherwise negative. It was concluded that a 4% formulation of a.i. in a cream base was non-irritant.

Test for capacity to produce irritation of the skin.

Rabbit. The material tested was again a product containing 4% of the active ingredient : "Uvistat Sun Screen". Areas of abraded and non-abraded skin were prepared in each of 6 rabbits, and 0.5 ml of the preparation was applied to each of the areas with occlusion for 24 hours. Four animals showed well defined erythema at both sites; 2 had oedema as well. The severity of the reaction was graded 1.9 according to the US Federal Hazardous Substances Act. The formulation at 4% was judged to be a mild irritant under the conditions of the test.

Guinea Pig. A brief report states that daily application of a 1% ethanolic solution to the shaved skin of the guinea pig for 14 days did not produce irritation. No other details are available.

Test for capacity to induce sensitisation.

Man. A modified Draize-Shelanski test was carried out in 12 healthy volunteers, using the product containing 4% of the active ingredient. The induction consisted of the application of 0.5 ml of the test product to the upper arm, followed by 24 hours occlusion. This was carried out 3 times a week for 3 weeks. The challenge application (which was the same as the induction application) was made (probably) 17 days after the conclusion of the induction phase. Applications were made to new sites and to the challenge sites. There was slight primary irritation in some subjects, but no evidence of sensitization.

Test for capacity to produce phototoxicity.

Guinea pig. Twenty female Dunkin-Hartley animals were used: 10 test and 10 control. Three areas of 2 cm^2 were prepared on the back of each animal. In the animals of the test group, 0.025 ml of solutions of 2% and 4% of a.i. in polyethylene glycol were applied to two of the areas, and 0.1%

8-MOP in ehtanol/acetone to the third. The control animals had no application. Thirty minutes later, the test animals were exposed to UVA 320-400 nm, until 2.5 Jcm^2 had been administered. Control animals were not irradiated. There was no evidence of phototoxicity. The positive control areas showed slight to moderate signs of irritation.

Test for percutaneous absorption.

Man. Three male and three female volunteers were used. Areas of 20 x 20 cm were delineated on the back of each subject, and 800 mg of a cream containing 4% of a.i. was rubbed into the area. Occlusion was not used. Blood was sampled at 0, 1, 2, 4 and 6 hours. There was stated to be no evidence of the presence of a.i. in the blood.
Interpretation is difficult because the description of the method is obscure and recovery figures are not given.

MUTAGENICITY

A standard Ames test was carried out. The a.i. was dissolved in DMSO. There was no evidence of mutagenicity.
A test for the production of chromosomal aberrations *in vitro* was carried out using Chinese hamster ovary cells. There was no evidence of mutagenic activity.

CONCLUSION

The Committee was satisfied that the tests for skin and mucous membrane irritation, and for sensitisation potential, were negative, but noted that the concentrations used were the proposed use concentration (4%). Tests for *photosensitisation* and *photo-allergenic* potential would be necessary.

The test for percutaneous absorption was not satisfactory. Oral acute and 28 day toxicity studies were judged to be satisfactory. There was no 90 day study. Mutagenic studies were judged to be satisfactory, but photomutagenic studies have not been undertaken.
The Committee felt that a study of inhalational toxicity would not be required unless it was proposed to use the substance in an aerosol formulation. Acute and short term dermal studies are not suggested. When adequate date on percutaneous absorption are available, further testing may be required (e.g. for teratogenic potential).

Classification : C.

REFERENCES
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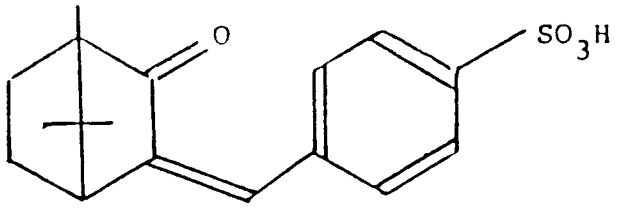
2. MEXORYL SL

. FORMULA AND SYNONYMES

EEC 2.24 (previously 2.28)
Colipa S59
Alpha'-(2-oxoborn-3-ylidene)-toluene-4-sulphonic acid and salts
3-(4'-sulpho) benzylidene bornan-2-one



$C_{17}H_{20}O_4S.\ 3H_2O$
MW 374.5

. CHARACTERISTICS

Crystalline substance
Highly soluble in water and ethanol

. USE

Proposed for use as a sunscreen agent at levels up to 6% (expressed as acid)

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral (rat) : 2,2 gr/kg (females)
3,2 gr/kg (males)
(mouse) 1,83 gr/kg (no details given)
(rat) 1,29 gr/kg (no details given)

ORAL TOXICITY

Rat. Oral 28 day study. Following a preliminary 5 day ranging study, doses of 0, 150, 300 and 600 mg/kg bw/day were chosen, to be given by gavage 7 days a week for 28 days. Groups of 5 male and 5 female animals were used. The study seems to have been properly carried out.

The chief findings were as follows. There were no drug related deaths. There was a significant reduction in weight gain in week 2 in high dose males. Food consumption was reduced in both sexes at the intermediate and high doses, particularly in the males.

There was a fall in serum sodium and an increase in chloride in high dose males. The blood glucose was lower than control in all dosed females and in high and intermediate dose males. Organ weights : in males, there was some reduction in relative liver weights at all dose levels, but the absolute changes were small. Histological examination revealed granular cytoplasm in some hepatocytes in high dose males and females. The no effect level was put at 150 kg/bw /day.

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

Test for capacity to injure mucous membranes.

Rabbit. A convential Draize test was carried out in 6 NZW rabbits. A 6% solution of a.i., neutralised with triethanolamine, was used in a dose of 0.1 ml. The opposite untreated eye served as a control. There was slight conjunctivitis which cleared in 2-3 days. The compound at this concentration was judged to be very slightly irritant.

Test for capacity to cause primary irritation of the skin.

Rabbit. Six NZW animals were used. Two areas of skin, 2.5 x 2.5 cm in area, were shaved in each animal : one was abraded and one not. A volume of 0.5 ml of a 6% solution of a.i., neutralised with ethanolamine, was applied to each area and occluded for 24 hours. No reaction was seen.
A similar test using a 4% solution is briefly reported, without details. The index of irritation was 0.12/8.

Test for capacity to produce irritation of the skin on repeated administration.

Rabbit. Guinea pig, rat. A 4% solution of the compound neutralised with treithanolamine was used. Volumes of 2.5 ml, 1 ml, and 0.5 ml, respectively, were applied daily to the shaved skin of each species. The treatement was carried out for a month. The index of aggressivity is given as : rabbit, 0.56/8 ; guinea pig, 0.2/8 ; rat, 0.5/8.
No details are given.

Test for capacity to produce sensitisation.

Guinea pig. Thirty animals of the Dunkin-Hartley strain were used, 20 test and 10 control. A Magnusson-Kligman procedure was carried out using a 6% aqueous solution of a.i. neutralised with triethanolamine.
There was no evidence of sensitisation.

Test for capacity to produce phototoxicity.

Rabbit. A brief report states that application of a 5% aqueous solution followed by ultraviolet irradiation was carried out each day for 2 weeks. The index of phototoxicity is given as 1.27/8, which is termed "slightly phototoxic".
No further details are available.

Guinea pig. Thirty female Dunkin-Hartley animals were used: 20 test and 10 positive controls. Three areas of skin were prepared in each animal, and each was covered by a patch

of filter paper. In the test animals, two of the patches were saturated with an aqueous solution of a.i. (probably 1%). The third patch was dry. In the positive control animals, two of the patches were impregnated with 20% chlorpromazine in petrolatum.
In both groups, the applications were occluded for 1.5 hours. At the end of that time, the patches were removed from one of the treated areas of skin and from the untreated area, and each animal was exposed to ultraviolett irradiation with a maximum output at 350 nm. There was no evidence of phototoxicity, but the positive control animals gave a very feeble response.

MUTAGENICITY

A standard Ames test was carried out, using an aqueous solution of a.i., neutralised with triethanolamine up to 5000 ug/plate.
There was no evidence of mutagenic effect.

CONCLUSION

The Committee was satisfied with the data on primary irritation of the skin, and irritation of mucous membranes, but noted that the concentrations used for testing were those proposed for use. The test for capacity to produce sensitisation was satisfactory. A test for capacity to produce photosensitisation should be carried out. Acute and 28 day oral toxicity tests were satisfactory. It was not felt that acute and short term dermal toxicity tests would be necessary ; inhalational toxicity tests would not be required unless aerosol preparations were proposed.

A test for percutaneous absorption is required ; depending on the results further testing may be required (e.g. for teratogenic potential). A test for chromosomal aberration in vitro should be carried out. Tests for photomutagenic acitivity had not been carried out.

Classification : C.

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7. HRC Report 861113 D/LRL 84/SS 24/10/1986

3. MEXORYL SD

. FORMULA AND SYNONYMES

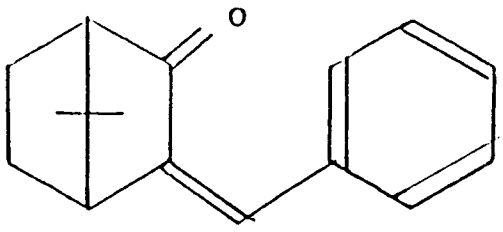
EEC n° 2.26

Colipa S61

3-benzylidene-1-camphor

3-benzylideneborman-2-one

1,7,7-trimethyl-3-benzylidene-2,2,1-bicyclo-2-heptanone



$C_{17}H_{20}O$

MW 240.4

. CHARACTERISTICS

Crystalline powder

Insoluble in water

Soluble in absolute alcohol and isopropanol

. USE

Proposed for use as a sunscreen agent at levels up to 6%

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral (rat) : $\geq$5 gr/kg

ORAL TOXICITY

Acute toxicity.

Rat. Five male and five female animals were used. The a.i. was given by gavage suspended in 1% propylene glycol in a dose of 5 g/kg bw. Observation was for 14 days. No abnormality of any kind was seen.

Subchronic toxicity.

Rat. A 13 week study was carried out in which the compound was administered by gavage in doses of 0, 100, 250 and 500 mg/kg bw/day, 5 days a week, to groups of 10 male and 10 female animals. Treatment was stopped after 6 weeks in one half of the animals in each group, and these served as recovery groups. The chief abnormality found was that there was an increase in plasma lipids at 13 weeks in female animals at all dose levels. In addition the plasma cholesterol levels were increased in males at the top dose, and in females at the intermediate and top doses. No abnormality of lipids or of cholesterol was found in any animal after 6 weeks.

Rat. A further study using groups of 20 animals was carried out, using doses of 20 and 40 mg/kg bw day in a similar manner. An abnormally high value for plasma lipids was found in females given 40 mg/kg bw/day ; the increase at the lower dose was not significant.

The NEL is probably 20 mg/kg bw/day.

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

Test for capacity to cause irritation of mucous membranes.
Rabbit. A 6% solution of a.i. in isopropyl palmitate was used. The untreated opposite eye was used as a control. The index of ocular irritation was : After administration, 5.33/10 ; day 1, 2.67/10 ; day 2, 0.67/10 ; thereafter negative. The authors judged the compound under the conditions of the test to be "mildly irritant".

Test for capacity to cause primary irritation of the skin.
Rabbit. A 6% solution of the compound in isopropyl palmitate was applied to abraded and non-abraded skin in 6 animals, with occlusion for 24 hrs. The compound was adjusted to be a mild irritant under these conditions.

Test for capacity to cause irritation with repeated application.
Rabbit. A 6% solution in isopropyl palmitate was used.
The skin was carefully clipped on both flanks and each animal had 2 ml of the test material rubbed into the flank on one side ; a similar procedure, without the solution, was carried out on the opposite side. Applications were made 5 days a week for 6 weeks. The experiment was then continued for a further week without treatment, to study recovery. The weekly mean index of irritation (maximum 8) was : 1.83 ; 1.21 ; 2.23 ; 1.13 ; 2.07 and (recovery) 1.0. The substance under these conditions was judged to be a mild irritant.

Test for capacity to induce sensitisation.
Guinea pig. Twenty albino animals of the Hartley strain were used. For induction, 0.5 gm of the compound, as a powder, was applied under occlusion for 48 hrs, 3 days a week, for 10 applications.

On days 1 and 10, an intradermal injection of 0.1 ml of Freund's compete adjuvant, 50% in saline, was given. After a 12 day rest, a challenge application, the same as the induction application, was made to a new site. No abnormality was seen.

Test for capacity to induce phototoxicity.

Rabbit. Six male and six female animals were used for the test, and 6 animals were used a vehicle controls. One site about 5 x 5 cm in area was prepared on the left flank of each animal, and 2 on the right. The compound was made up as a 5% solution in 95% ethanol. One ml of this was applied to the left flank, and to one of the sites on the right flank. The sites on the right flank were irradiated with 1 med of ultraviolet light daily for 10 days. The irradiation was carried out by exposure to an "Osram Vitalux" lamp, but its spectral characteristics are not given. There was no evidence of phototoxicity.

Test for capacity to produce photosensitisation.

Guinea pig. Twelve male and twelve female albino Hartley animals were used in 3 groups.

Induction : Animals of group 1 (test) had injections of 0.2 ml of Freund's complete adjuvant in the foot on day 1 ; on days 1, 3, 5, 8 and 11, 0.5 ml of a 4% solution of the compound in olive oil was applied to a prepared site on the back of the neck, followed by exposure for 30 minutes to 2 lamps covering the range of 285 to 450 nm. Animals of group 2 (vehicle control) were similary treated except that the applications were of olive oil only. Animals of group 3 had the Freund's adjuvant but no other treatment. Challenge. On the 22nd day of the experiment, sites on either side of the lumbar vertebrae were prepared in all animals. Applications of 10 ul of the following solutions were made to each side : olive oil ; 2%

of the compound in olive oil ; 2% of the compound in ehtanol. The sites were then exposed to irradiation by one of the lamps (320-450 nm) for 30 minutes. Readings were made at 24 and 48 hrs. There was no evidence of photosensitisation. There was no positive control, but the test was nevertheless adjudged to be satisfactory.

Test for percutaneous absorption.

Man. Four volunteers were treated. Areas of 100 cm were delineated on the upper arm. The compound was labelled with 14C and made up in a concentration of 5.02% in an o/w emulsion. About 0.5 g of ointment was applied to the delineated areas. Contact was for 6 hrs, without occlusion. At the end of the experiment, the skin was swabbed clean and also stripped. Urine and faeces were collected for five days. The data given permit the calculation that under the circumstances of the experiment, the flux (J) = 139 $\pm$ 30 ug cm^{-2} H^{-1} and the permeability constant (K_p) = 2.8 $\pm$ 0.6 x 10^{-3} g $cm^{-2}h^{-1}$.

Assuming a maximum exposure for 10 hours a day over 1.8 m^2 of body surface, and a concentration of 6%, the total amount absorbed would amount to approximately 0.6 g/kg bw/day.

MUTAGENICITY

An Ames test was carried out using strains TA 1525, TA 1537, TA 99 and TA 100. Toxicity experiments were not carried out ; in strain TA 100 there was a decrease in revertants with increasing dosage, suggesting some toxicity. Testing was carried out up to 1000 ug/plate in each strain. There was no evidence of mutagenic activity.

A culture of Chinese hamster ovary cells was used to test for the production of chromosomal aberrations in vitro. Without activation, doses up to 80 ug/ml were used, and with activation, doses up to 25 ug/ml. There was a highly significant increase in aberrations in the preparation with activation in a dose of 25 ug/ml.
A standard micronucleus test was carried out in the mouse. There was no evidence of genotoxicity.

CONCLUSION

Acute oral toxicity is low. Tests for primary irritation of the skin and for irritation of the skin on repeated application showed only slight effects at 6%, but this is the use concentration. Tests for phototxicity and photo-sensitivity were negative, although a positive control was not used for the latter.
A 90-day oral test in the rat showed elevated plasma lipids in female rats at doses of 40 and 20 mg/kg bw/day, although this was not significant at the latter dose. The NEL is therefore considered to be 20 mg/kg bw/day.
Percutaneous absorption is marked and was calculated to amount to as much as 0.6 g/kg bw/day.
A test for chromosomal aberration in vitro was positive, the Ames test and a micronucleus test were negative.
There is no sufficient margin of safety for percutaneous absorption.

Classification : D.

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Institut für Pharmakodynamik und Toxikologie der Universität
GRAZ, Pr. KUKOVETZ - July 28, 1987.

4. ETHOXYLATED ETHYL-4-AMINOBENZOATE

EEC 2.2
Colipa S3

CONSULT

Reports of SCC : 40th reunion 11-4-89

CONCLUSION

A reply from Colipa has been received.

a) A test for mucous membrane irritation has in fact been carried out. This was evaluated by the subgroup and found to be satisfactory.

b) The substance "Lusantan 25" is stated to be ethoxylated ethyl-4-aminobenzoate. In the light of this, the Ames test is now regarded as negative.

Confirmation of Classification : C.

REFERENCES
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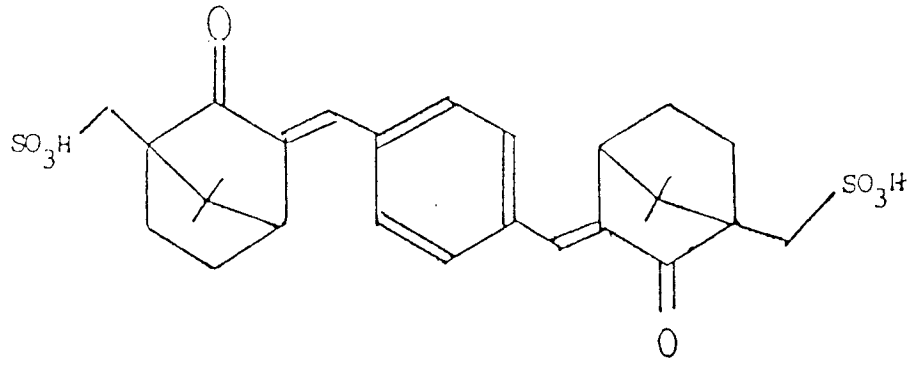
5. MEXORYL SX

. FORMULA AND SYNONYMES

Colipa S71

3,3'-(1,4-phenylenedimethylidyne) bis (7,7-dimethyl-2-oxo- bicyclo(2,2,1)heptane-1-methanesulfonic acid) and its salts



$C_{28}H_{34}O_8S_2$ MW/ 562.7

The material consists of two molecules of camphorsulphonate united by 1,4-divirylbenzene

. CHARACTERISTICS

Freely soluble in water, giving an acidive solution.
In use, it is expected to be neutralised and used as a salt.
Absorption maximum 345 nm.

Test for photostability.

A thin layer of a 4% solution of a.i. was exposed to simulated solar radiation of an intensity substantially greater than which would be found in the south of France, or Corsica. It was found that there was a rapid, though relatively small, fall in the first few minutes : after his, the loss was no more than about 1.5% in 1 hour.

. USE

Proposed for use as a sunscreen agent at levels up to 10% expressed as acid.

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral (rat) : > 1835 mg/kg

The experiment was repeated 3 times, using the a.i. neutralised with triethanolamine
potassium hydroxide
sodium hydroxide

In each case we found

LD50 : > 2092 mg/kg (expressed as acid)

LD50 dermal (rat) : > 1367 mg/kg

ORAL TOXICITY

Subchronic toxicity.

Rat. Oral. A 13 week study was carried out in groups of 10 male and 10 female SD animals. The a.i. was administered by gavage 7 days a week at doses of 0, 100, 300 and 1000 mg/kg bw/day. There were few signs of abnormality during the study. The rate of weight gain was slightly less in the mid and high dose groups. At autopsy, the absolute and relative weights of the thyroid were reduced at the middle and top dose in males only ; the effect was not dose related, and no histological change was found in the gland. The study seems to have been a well conducted one; the NEL is judged to be 1000 mg/kg bw/day.

Test for capacity to affect the production of thyroid hormones.
Rat. Twenty male Wistar animals were used, 10 test and 10 control. The a.i. was neutralised with TEA and given by gavage in a dose of 305 mg/kg bw/day (expressed as acid) daily for 21 consecutive days.
Control animals received distilled water. No abnormalities were observed during the experiment. At the end of the experiment the serum was assayed for its content of thyroxine. There were no significant difference between the groups.

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

Test for capacity to cause irritation of mucous membranes.
Rabbit. Groups of 6 male NZW animals were used. Three experiments were carried out, using, respectively, a.i. neutralised with triethanolamine, potassium hydroxide and sodium hydroxide, all at a concentration of 10.5%. Results were similar in all experiments, and the material at this concentration was judged to be very slightly irritant.

Test for capacity to produce primary irritation of the skin.
Rabbit. Three groups of 6 male NZW rabbits were used. Areas of skin were prepared on either flank, one of which was scarified, the other not. The applications were of 0.5 ml of a 10.4% solution of a.i., and occlusion was used for 24 hrs, with readings at 24 and 48 hr. The 3 experiments were identical except that in the first group the a.i. was neutralised with triethanolamine, in the second with potassium hydroxide, and in the third with sodium hydroxide. The results were similar in all experiments : the cumulative irritancy scores were 6, and 9 respectively. The substance under these conditions was deemed to be non-irritant.

Rabbit. Three NZW animals were used, and 0.5 ml of a 36.7% solution of a.i. was placed upon an area of clipped skin and maintained under semi-occlusive conditions for 4 hrs. Reading was at 1, 24, 48 and 72 hrs. There was no evidence of primary irritation.

Test for capacity to produce sensitisation.

Guinea pig. The a.i. was neutralised with triethanolamine. A modified Magnusson-Kligman technique was used. Preliminary testing was carried out on 2 male and 2 female animals the definitive test was carried out on 10 male and 10 female animals. There was no evidence of sensitisation.

Test for capacity to produce phototoxicity and photoallergenicity.

Guinea pig. Following preliminary testing, a 10.4% solution of a.i., neutralised with triethanolamine, was used. Three male and 2 female animals were treated with 0.5 ml of the solution followed by occlusion for 90 minutes. These animals were not irradiated, and served as controls. In the test, 10 males and 10 females were similarly treated and thereafter given a minimum erythema dose from 2 lamps which covered the spectrum from 285 to 400 nm. Reading was at 6 and 24 hrs after irradiation. No differences were found between test and control animals.

The test for photoallergenicity was carried out as follows. Induction was carried out by the use of 4 intradermal injections of 50% FCA followed by occlusive patches, as above, on days 4, 7 and 9. In each case exposure to 15 minutes of irradiation as above was given, and then 40 minutes of irradiation with 310-400 nm. Following a rest period of 14 days, a single occluded application, as above, was made to a previously untreated area.

Following removal of the patch, the animal was irradiated for 90 minutes using the longer wavelength lamp only. Reading was at 6, 24 and 48 hrs. There was no evidence of abnormality in any animal.

Test for capacity to produce photocontact allergy.

Man. A cream formulation was used, containing 10% a.i. neutralised with triethanolamine. The cream also contained butyl-p-hydroxybenzoate 0.2% and imidazolidinyl urea 0.2%. Fifteen white females and 10 white males were used, aged from 18 to 50. Following the determination of a minimal erythema dose for each subject, using a xenon arc solar simulator, 10 microlitre/ cm^2 of the cream was applied to the skin of the back and occluded for 24 hrs. After removal of the patch, the ara was exposed to 3 meds. After a 24 hr rest, the procedure was repeated, and thereafter repeated twice a week for 3 weeks. All these induction applications were made at the same site. After a 10 day rest period, the challenge was carried out by making the above application in duplicate on 2 new sites on eihter side of the midline. One patch was removed after 24 hrs, and the other left in place : the area was then exposed to 4 J/cm^2 of UVA. Reading was at 24 and 72 hrs, and was blinded. There were no adverse reactions of any kind.

Test for percutaneous penetration.

Rat. Five male and five female SD rats had a single application of a 36.7% solution of a.i. applied and maintained under semi-occlusive conditions for 24 hrs. The animals were observed for 14 days, and then subjected to gross necropsy. No abnormality was found.

Rat. Eight female hairless SD rats were used. A cup was glued to the skin of the back which covered an area of 1 cm^2. A 5% formulation of the a.i. doubly labelled with 14C, was made up in "Ambre Solaire" cream. An amount of 2 mg of the cream, containing 178 nmoles of a.i., was applied and allowed to remain in contact for 4 hrs. From the data, it is possible to calculate that the permeability coefficient is 3.8 +/- 0.6 x 10^{-6} $gcm^{-2}h^{-1}$. Assuming the permeability constant is the same in rat and man, and an application to the whole body area (1.8 m^2) over a 10 hour day at the maximum concentration, i.e., 10% this would amount to an absorption of about 68.4 mg, i.e. an absorption of 1.14 mg/kg bw/day.

Man. Human skin was obtained in the form of surgical specimens from two negro women. The skin was sectioned by dermatome to obtain samples 300 to 400 um thick. There was sufficient skin to enable three experiments to be carried out with each specimen. A Franz cell was used, and 30 mg of a 5% cream formulation of the a.i. was applied to the skin. Contact time was 24 hrs. The amount of a.i. penetrating was less than the sensitivity of the HPLC method for its estimation (50 ng/ml). The penetration was judged to be less than 0.12 ug/cm^2. The permeability coefficient calculated from these data would be less than 0.1 x 10^{-6} $gmcm^{-2}h^{-1}$.

A similar test was carried out with skin from two other negro women. The results were the same.

Rat. Skin from 2 female hairless rats was used in a Franz chamber in the same way as for human skin. The result was the same as with the test using human skin. These <u>in vitro</u> results, when analysed, give a permeability coefficient of not more than 0.8 x 10^{-6} $gm\ cm^{-2}hr^{-1}$, and the amount absorbe in man, over a 10 hr day (as above) would be less than 12.8 mg, or 0.2 mg/kg bw.

MUTAGENICITY

A standard Ames test was carried out. There was no evidence of a mutagenic effect, with or without activation.

A standard test for chromosomal aberration in vitro.
Was carried out using Chinese hamster ovary cells.
There was no evidence of induction of aberration.

Test for photomutagenic activity.
The a.i. was neutralised with TEA and tested for its ability to induce tryptophan-independent revertants in E. coli WP2. Following a range finding study, concentrations from 8 to 5000 ug/plate were tested, with and without exposure to UVA and UVB irradiation. Suitable positive and negative controls were used. There was no evidence of photomutagenicity.
Chinese hamster ovary. A similar experiment using cultured cells was carried out, except that following a pilot experiment the concentrations tested were 1250, 2500 and 5000 ug/ml. Exposure to UVA and UVB was carried out. Suitable positive and negative controls were used. There was no evidence of clastogenic activity.

CONCLUSION

Evaluation. The a.i. at 10.5% is only slightly irritant to the eye, and at 10.4% it is non-irritant to the rabbit skin. The proposed use level is 10%. The data on sensitisation, phototoxicity, photosensitisation and photocontact allergy are regarded as satisfactory.

Inhalation toxicity testing would be required only if it were proposed to market an aerosol formulation. Tests for mutagenicity and photomutagenicity were negative.
Teratogenic testing showed no evidence of abnormality ; however it was carried out in one species only, and the dose used did not cause toxicity.

Classification : A.

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SCC OPINION

BERGASOL SUNTAN PRODUCTS CONTAINING 5-MOP

Background

5-MOP occurs naturally in oil of bergamot (up to 3000 ppm). The use of this oil is at present allowed in suntan products by the Cosmetics Directive, but the SCC have recommended that the maximum level of 5-MOP arising from the presence of natural essences in suntan products should be 1 ppm.

The SCC have now been asked to consider data on a specific range of suntan products marketed by Bergasol and containing 15-60 ppm 5-MOP (as the natural product bergapten). These also contain sunscreens and it is claimed that such formulations behave differently to 5-MOP alone, particularly with regard to the photomutagenicity and photocarcinogenicity which gave rise to the concern with 5-MOP itself. Bergasol have provided a substantial amount of data to support this submission and this is summarised in Appendix I to this report.

The question that the SCC have to consider is difficult and raises points that the Committee does not, in general, have to consider. There is a finely balanced argument regarding health risks and benefits, whereas in general the health risks of cosmetic ingredients are minimal. In the case in question, the active melanogenic ingredient, 5-MOP, clearly gives rise to concern regarding its photomutagenic and photocarcinogenic potential. However, sunlight itself induces skin cancer and the suntan products in question are designed to enhance tanning. More rapid tanning leads to a reduced cumulative dose of UVA and UVB radiation whilst attaining a tan that is protective against subsequent exposure.

There is also the additional problem as to how 'clearance' could be given to specific products under the Cosmetics Directive which is designed to consider either allowed or prohibited ingredients rather than products.

The available data are summarised below:-

Toxicity of 5-MOP itself

The data on 5-MOP itself are reviewed in Appendix II and are summarised below, for background information.

5-MOP itself is well absorbed orally and may be absorbed through the skin. It is widely distributed in tissue, extensively metabolised, and rapidly excreted in the urine. It has very low oral toxicity, but is strongly phototoxic, producing marked skin reactions after exposure to UV radiation. Only limited data are available on sub-acute toxicity. The combination of 5-MOP plus UV radiation is

clearly mutagenic, much information being available on the interaction of psoralens with DNA in the presence of UVA. Linear psoralens such as 5-MOP are bifunctional, forming DNA adducts that react further producing DNA cross-linking. 5-MOP plus UVA has been shown to produce positive results in mutagenicity studies in bacteria, yeasts and mammalian cells, and this combination is also clastogenic. Several studies have been carried out to investigate the skin carcinogenicity of 5-MOP plus UVA in animal bioassays. Such treatment has clearly been shown to produce skin cancer in mice. There are no adequate data available to assess the carcinogenicity of 5-MOP in the absence of UV radiation, nor are any adequate data available in man. However the combination of 8-MOP plus UVA (PUVA treatment) is clearly carcinogenic in man, and is included in the IARC Group I Carcinogens (ie known human carcinogens).

BERGASOL PRODUCTS; data submitted by Bergasol

Comprehensive data on both the toxicity of these products and the health benefits are given in Appendix I. These are summarised below.

Studies in animals and human volunteers showed no significant skin irritation or sensitization, nor any significant photo-irritancy or photo-toxicity. Furthermore the data provided by Professor Agache indicates that the absorption of 5-MOP from such formulations is minimal (less than 1%), although greater amounts of 5-MOP were present in the stratum corneum itself.

The crux of the problem is however the potential photomutagenicity and photocarcinogenicity with respect to the induction of skin cancer and this is considered in some detail below:-

Several studies have been carried out to investigate the mutagenicity of Bergasol preparations using Salmonella typhimurium TA102, but interpretation of these are difficult since somewhat conflicting results were obtained, possibly due to differences in the amount of material diffusing from the oil emulsion formulations through the agar medium. Some studies indicated a reduction in the number of revertants but positive results were obtained with commercial preparations and mutagenic potential cannot be ruled out from these studies. In addition it was pertinent that data from limited studies to investigate the mutagenicity of 'blister fluid' obtained from skin preparations of human volunteers treated dermally with a Bergasol preparation suggested that this had some photomutagenic potential when tested using the yeast strain Saccharomyces cerevisiae D7 (studies by Professor Dubertret). However, in view of the very limited nature of these studies (no dose response, no repeat of the results in an independent experiment) no firm conclusions can be drawn.

Long term bioassays for skin cancer in mice have given conflicting results. Limited data from an early study showed

no protection using a UV filter (ethyl hexyl paramethoxy cinnamate). More extensive studies using a Bergasol formulation containing 5-MOP plus 2 UV filters (2 ethylhexyl, 4 methoxy cinnamate and 1,7, 7-trimethyl, 3-benzylidene-bicyclo (2,2,1) - 2 heptanone) revealed substantial protection against skin tumour induction in albino mice, but only during the treatment period (45 weeks). Animals exposed to SSR after treatment with 5-MOP plus sunscreens then went on to develop tumours sooner than those treated with sunscreen alone. In another study in pigmented mice of the same strain, much less protection was seen, but it was argued that these mice were not an appropriate model for human pigmentation due to the absence of epidermal melanin. It is thus difficult to assess the significance of these studies or to identify the mechanisms involved. It is not possible to assess the increased risk of skin cancer in those using formulations containing 5-MOP plus UV filter. The data do not allow concerns regarding this aspect to be discounted.

More recent studies have been reported using an 'accelerated' in vivo model for skin tumourigenicity based on hairless mice treated with UV radiation (with or without bergapten containing 50 ppm 5-MOP) as 'initiator' for 4 months together with croton oil, as promoter, for the first 2 months. At the end of the 4 month period a similar incidence of tumours was seen following treatment with UV radiation and bergapten (50 ppm 5-MOP) as UV radiation alone, ca 60% incidence of skin papillomas. A marked reduction of the incidence of skin tumours was seen when sunscreens were incorporated in the treatment formulation (20% incidence) but the most impressive results were obtained by the additional incorporation of antioxidants such as BHT or β carotene or by the addition of an inhibitor of polyamine (putrescene) synthesis.

Benefits of incorporation of bergapten (5-MOP)

Considering the protective effect of 5-MOP in sunscreens, data has clearly established that the inclusion of 5-MOP enhances pigmentation. In addition the series of studies by Dr Young showed that the 5-MOP induced tan protected against UV radiation induced DNA damage in humans as measured by unscheduled DNA synthesis (UDS) in individuals of skin type I to V. It was particularly noteworthy that the most sensitive population for UV induced skin cancer (ie types I and II) became like types III and IV in this regard.

However the mechanisms underlining the effects seen are not known and it is important to note that UDS is an indirect measure of damage, and a decrease may reflect a reduction in repair. The situation is complex and it is clear that there is no direct proportionality between UDS and SSR induced DNA damage. The extent to which 5-MOP induced cross-linking is detected is unknown. It is premature to draw any conclusions from this work. No meaningful assessment can be made of the relative risk of induction of a tan by 5-MOP plus SSR compared to SSR alone.

Conclusions

In summary, consideration of the health risks of products containing 5-MOP gives rise to concern. 5-MOP itself plus solar simulated radiation (SSR) is clearly mutagenic, and produces an increased incidence of skin cancer compared by SSR alone. Data provided on the Bergasol products containing 5-MOP plus UV filters gives a less clear-cut picture, but does not allow concerns about this aspect to be completely discarded.

Concerning the benefits, 5-MOP is clearly efficacious at inducing a tan. The data provided on the protective effect of this tan on subsequent DNA damage induced by SSR, as measured by Unscheduled DNA Synthesis, are difficult to interpret, in view of the lack of knowledge regarding the mechanisms involved. It is not possible to draw any meaningful conclusions regarding the relative risk of inducing a tan by SSR plus 5-MOP plus sunscreens, as compared to SSR sunscreens alone.

Much of the information provided is of research interest, but was not believed to be directly relevant to the Bergasol products on the market. For example the studies of Khettab et al relating to the incorporation of antioxidants with sunscreens. Such combinations were shown to offer complete protection against the development of skin tumours in the 4 month initiation/promotion model in hairless mice.

The Committee were concerned about procedures whereby specific cosmetic products could be considered acceptable. In this instance it was argued that certain products were less harmful than would be expected from the 5-MOP content alone, because of the protective effect of other substances present eg sunscreens, but this would entail a need to consider not only the concentration of the component of concern (5-MOP) but also the presence of other specific compounds at specified levels. [This would, in effect, result in the need for clearance of specific products]. The question of balancing health risks versus health benefits had been raised. The Committee felt that it was questionable whether it was appropriate to consider such arguments for cosmetics; it is a requirement that cosmetic products are not harmful.

On the available data the Committee felt that it was not possible to conclude that sunscreen products containing 15-60 ppm or more 5-MOP were without risk of producing harm due to their potential for photomutagenic and photocarcinogenic effects. It was the opinion of the Committee that the maximum amount of 5-MOP in all sun tan products should be 1 ppm.

European Communities – Commission

EUR 14208 – Opinion of the Scientific Committee on Cosmetology (11/86 – 10/90) – Commission Decision 78/45/EEC of 19 December 1977 concerning the institution of a Scientific Committee on Cosmetology (OJ L 13, 17.1.1978, p. 24)

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The Scientific Committee on Cosmetology was set up by Commission Decision 78/45/EEC on 19 December 1977 (OJ L 13, 17.1.1978, p. 24) in order to provide the Commission with informed opinions on any scientific and technical problems arising in connection with cosmetic products, and in particular on the substances used in their manufacture, on their composition and on the conditions for their use.

The members of the Committee are independent scientists, highly qualified in the fields of medicine, toxicology, biology, chemistry or other similar disciplines.