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

CONTENTS

32nd meeting of the SCC - November 6-7, 1986

REPORTS OF THE SCIENTIFIC COMMITTEE ON COSMETOLOGY

32d reunion

November 6 -7 1986

SUMMARY

ANTIOXIDANT AGENTS

		Class
Ascorbic acid and its salts	A01	A
Ascorbyl stearate and palmitate	A02	B
Isoascorbic acid and its salts	A04	D

HAIR DYES

2,4 diaminoanisole	A6	D
2,5 diaminoanisole	A34	D

SCIENTIFIC COMMITTEE ON COSMETOLOGY

List of participants

32nd meeting - 6/7 November 1986

Present

Mr DE GROOT
Mr GOULDING
Mr HILDEBRANDT
Mr LOPRIENO
Mr SCHOU
Mr O'MAHONY
Mr STUTTGEN
Mr AGACHE
Mrs DONY
Mrs KNAAP
Mrs ENJOLRAS

Apologies for absence

Mr MUSCARDIN
Mrs MACKIE

Commission

Mrs MASSE
Mr COLLIN)
Mr GONTIER) (DG XI/B/1)

REPORT FROM THE SCIENTIFIC COMMITTEE ON COSMETOLGY

CONCERNING CERTAIN ANTIOXIDANTS

THE COMMITTEE'S MANDATE

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

- AO1 L. ASCORBIC ACID
- AO2 L. ASCORBYL PALMITATE
  STEARATE
- AO4 D. ISOASCORBIC ACID

at the maximal concentration of 2%

(1) L. A S C O R B I C A C I D XI/496/87

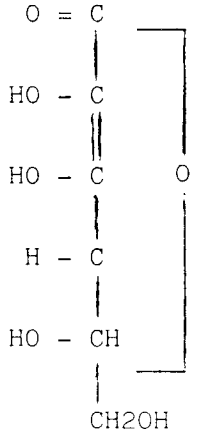
* Formula and synonyms

Colipa AO1

CAS - RN 50-81-7

L. ascorbic acid (8 CI,9 CI)

3 - ceto - L - gulofuranolactone



C6H8O2

MW : 176,12

* Characteristics

Soluble in water (10% W/V)
Slightly soluble in ethanol
Insoluble in oils and fats

* Use

Used in cosmetic products in concentrations not exceeding 2%. It has an antioxidant action by blocking the free radicals by UV radiations and prevents the formation of nitrosamines; its antinitrosating properties seem to correlate with the speed of oxidation indehydroascorbic acid.

* Recapitulation of the studies of toxicity

LD 50 varies from + 200 mg/kg to + 5 gr/kg depending on the animal tested and the route of administration.

Metabolism

Ascorbic acid is rapidly excreted and metabolized. In rats, after intraperitoneal injection of 1.5 to 5.9 mg of 14C-labelled ascorbic acid,19 to 29 % is converted to CO2 and 0,4 % excreted asoxalate within 24 hours.

In man,oxalate formation and urinary excretion represent about half the quantities ingested,the rest being excreted as CO2.

ORAL TOXICITY

In the short-term no effects were observed in rats by oral administration of 6.5 g/kg/bw for 10 weeks but 27.3 g/kg/bw/day caused 77 % mortality in four weeks.

In man,administered at a doe of 1 000 mg/day for three months it caused no toxic effects ; at a dose of 6 000 mg/day for 400 days nausea,vomiting,diarrhoea,headaches,fatigue,skin rashes and disturbed sleep were observedIn small doses it had a diuretic effect.

CUTANEOUS AND MUCOUS TOLERANCE

Ascorbic acid is well tolerated by the skin and mucous membranes and is not sensitizing.

In an eye irritation test (modified Draize) on rabbits, 0.1 ml of a 30 % aqueus solution instilled without rise-off produced slight to well defined redness,returning to normal in 72 hours. The cornea and iris were unaffected.

In a skin irritation test the application of 0.5 ml of a 30% aqueous solution with occlusion to the intact and abraded skin of a rabbit's shaved back produced barely perceptible erythema but no oedema.

In a Maurer optimization test on female guinea pigs,after intradermal injection with and without Freund's Complete Adjuvant,no evidence was observed of sensitization by intradermal injection of 0.1 ml of a 0,1 % physiological saline,or by occluded epicutaneous application of 0.05 ml/cm2 of a 10% dispersion in petroleum ether.

MUTAGENICITY

For bacteria ascorbic acid is not intrinsically mutagenic to Salmonella typhimurium (TA 100) if deionized water is used or if EDTA is added to tap water for the preparation of the incubation medium.But a mutagenic and cytotoxic effect is observed through generation of H202,notably in the presence of Cu++.
In an Ames test on strains TA 92, TA 1535,TA 100, TA 1537,

TA 94, TA 98 and TA 2637,ascorbic acid in solution in a phosphate buffer with the addition of an S9 fraction,did not produce a significant increase in the number of revertants, at the maximum non-cytotoxic dose of 5 mg/plate.

In vitro on mammal cells,ascorbic acid produces chromosome breaking and chromosome exchange in the ovary cells of Chinese hamsters,mutagenic doses being also toxic doses; the peroxide radicals are involved in the process.It produces a dose-dependent increase in sister-chromatid exchanges (SCES) in Chinese hamster ovary (CHO) cells and in human lymphocytes at concentrations from 10^{-4} to 10^{-2} M.

It reduces the rate of DNA synthesis in HeLa cells at the effective dose of 2.5 - 10^{-3} M ,showing no results typical of products producing DNA damage.

In a test on Chinese hamster fibroblasts,it did not produce any chromosome aberration,in a physiological solution at 0.3 mg/ml,but at non-toxic concentrations,with or without Cu^{2+},it produced a relatively high frequency of chromosome aberration in human fibroblasts.

A deactivating effect of the viruses depending on the presence of oxygen has been observed; the target molecule is DNA.

In vivo,a test via an intrahepatic intermediate host,after i.v. injection of S. typhimurium TA 100,in guinea pigs,did not indicate any genotoxic activity even at very high concentrations (5 000 mg/kg/bw/day).In contrast to the in vitro results,no sister-chromatid exchanges (SCEs) were observed in the spinal cord of the Chinese hamster for doses of 200 to 10 000 mg/kg/bw administered orally and intraperitoneally.

In Drosophila melanogaster,ascorbic acid induces lethal sex-linked recessive mutations.

The results of various systems of tests on bacteria and mammal cells show that ascorbic acid can have a comutagenic or anti-mutagenic effect.

CARCINOGENICITY

Ascorbic acid is not carcinogenic in long-term tests on animals. A two-year oral administration study on male and female rats with daily doses of 0, 1 000, 1 500 and 2 000 mg/kg/bw had no effect on haematology,urinary chemistry,blood enzyme activity or liver and kidney function,and caused no microscopic or macroscopic organ lesions

A bioassay of carcinogenicity by administration in the diet to male and female rats and mice of doses of25 000 and 50 000 ppm for 103 weeks indicated that ascorbic acid was neither toxic nor carcinogenic. Furthermore,a dose-related decline of certain spontaneous lesions in older female rats was observed and at the higher dose the number of male survivors treated (mice and rats) was greater thanthe number of survivors monitored.

It can inhibit or promote experimental carcinogenicity.In particular it reduces the incidence of skin tumours caused by chemical carcinomas from UV radiation.

TERATOGENICITY AND REPRODUCTION TOXICITY

Ascorbic acid is neither foetotoxic nor teratogenic.

In a reproduction study on three generations of guinea pigs (by administrationof 1.5 - 4.0 and 100 mg/kg/bw) an increase in the number of young was observed at 100 mg/kg/bw . The tissues of the newborn babies of mothers exposed to 1.5 and 4.0 mg/kg/bw were less satured than the mothers' tissues. There was no difference as regards the babies' size and viability.

There was no toxic effect on the mothers, no foetotoxic or teratogenic effects, nor adverse effects on post-partum development in a study involving oral administration of 250, 500 and 1 000 mg/kg/bw/day to mice,and 150 ,250 and 1 000 mg/kg/bw/day to rats,from day 6 to day 15 of gestation,and thereafter from days 0 to 21 after birth.

Similarly,there were no adverse effects on the mother or foetus,nor any increases in soft-tissue or skeletal malformations, after administration of 5.2 to 520 mg/kg of ascorbic acid,by oral intubation to pregnant CD-1 strain female albino mice from day 6-15 of gestation.

In a similar study on Wistar rats the incidence of complete closure of of the skull was greatest in the high-dose foetuses.

Ascorbic acid used in vitro on mouse embryo (limb bud) cell cultures has no measurable effect on the inhibition of proteoglycane synthesis, the extracellular matrix or the synthesis of cellular DNA.

C O N C L U S I O N

L(+) ascorbic acid is minimally toxic; it is well tolerated by the skin and mucous membranes and is not sensitizing. It is quickly metabolized and excreted. Its cutaneous absorption is not known. It has a mutagenic power in certain systems and is antimutagenic in other test systems; it may interact with DNA, inhibits cell transformation, causes mutations in the fruit fly and chromosome damage in mammal cells, but is inactive on bacteria and in vivo tests. It is not carcnogenic for animals, and can inhibit or promote experimental carcinogenesis.

It has no effect on reproduction, and is neither foetotoxic nor teratogenic.

In cosmetics, it can prevent the formation of free radicals by peroxidation, and can inhibit nitrosamine formation.

In 1981 JEFCA changed the ADI, which had previously been set at 0-15 mg/kg/bw, to "not specified". The daily dose for human consumption recommended by the FDA is 45 mg/day for adults.

Classification A

References : "Unpublished Company data"

(2) L. ASCORBYL PALMITATE AND MONOSTEARATE

XI/496/87

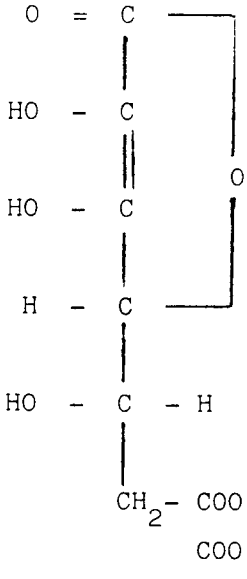
* Formula and synonyms

Colipa A02

CAS - RN 137 - 66 - 6

CAS - RN 25395 - 66 - 8

CAS - RN 51222 - 59 - 4



C22 H38 07

MW : 414,5

CH_2- COO - CH_2 - $(CH_2)_{13}$ - CH_3 (palmitate)

COO - CH_2 - $(CH_2)_{15}$ - CH_3 (stearate)

* Use

Ascorbyl palmitate and stearate are used in cosmetic products at concentrations not exceeding 2 %.

Antioxidant action : At level of 0.5 %, ascorbyl palmitate is a very efficacious antioxidant for vegetable oils with high level in onsaturated fat acids,used in cosmetic W/O emulsions.

Bleaching action : Esters would be able to reduce melanine In Japan it is used at levels of 0.1 to 4 % in cosmetic preparations to inhibit the formation of melanine.

* Recapitulation of the studies of toxicity

LD50 oral (mice) : 2 gr/kg/bw

(rats) : > 5 gr/kg/bw

(for a 33.3 % suspension of monopalmitate or a 15 % suspension of dipalmitate)

LD50 dermal (guinea pigs) : > 3 gr/kg/bw

ORAL TOXICITY

In a study on rats involving administration of 2 and 5 % ascorbyl palmitate in the diet for nine months, growth was significantly retarded at the 5 % dose and two rats of the tenhad bladder stones and hyperplasis of the bladder epithelium. One rat had an inflamed kidney. With the 2 % diet growth was slightly retarded but there was no differencein terms of mortality or histopathology.

No adverse effects were observed after administering in the diet 100, 200, 500, 1 000 and 3 000 mg/kg/bw of L-ascorbyl stearate.

In a two year study of rats fed on heat treated lard containing 2 or 5 % ascorbyl palmitate (equivalent to 424 and 1060 mg/kg/bw or 0.05 and 0.25 % of the total diet) growth rate decreased at the higher dose and two of eight animals had oxalate stones after nine months of treatment.

No effects were observed at the 2% dose (estimated NEL at 125 mg/kg/bw). Sine food-grade palmitic acid contains large quantities of stearic acid, it can be assumed that the ascorbyl palmitate used in this study contained stearic acid.

CUTANEOUS AND MUCOUS TOLERANCE

The monopalmitate and dipalmitate are well tolerated by the skin and mucous membranes.

In a skin irritation test (modified Draize),application of a 10 % aqueous solution of monopalmitate and full-strength dipalmitate with occlusion for 24 hours to the intact shaved skin of albino rabbits was non-irritating.

In an eye irritation test (modified Draize) on rabbits,instillation of 0.1 ml of a 10% aqeous solution of monopalmitate was non-irritating. The instillation of 0.1 ml of full-strength dipalmitate was minimally irritating.

After application of ^{14}C-labelled ascorbyl palmitate to the skin of guinea pigs affected by scurvy, ascorbic acid levels in the skin, liver, kidneys and blood were found to be 4 to 8 times higher than in the control.

C O N C L U S I O N

Ascorbyl palmitate is minimally toxic. It is well tolerated by the skin and mucous membranes. A study of which no details are available indicates that it does penetrate through the skin.The no-effect dose for rats is evaluated at 125 mg/kg/bw, the kidney and bladder being the target organs in the case of long-term administration.Mutagenicity and sensitization data are unavailable.

JEFCA has established an ADI of 0-125 mg/kg/bw for the stearate or the palmitate or the sum of both ascorbyl esters.

Classification : B Information required : - details of skin absorption
- sensitization
- mutagenicity in at least two test systems (reversion on bacteria and chromosome aberration)
- information on possible use to inhibit melanine production and anti-nitrosating action

References :

Colipa : "Unpublished Company data"
RTECS
Dr Robinson's data

(3) D. ISOASCORBIC ACID

XI/496/87

* Formula and synonyms

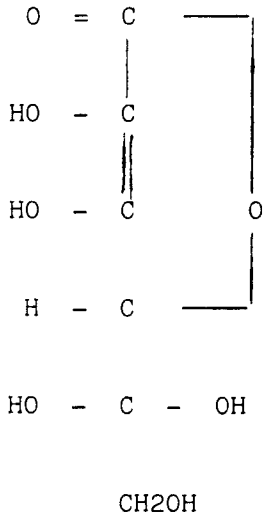
Colipa A04

CAS - RN 89-65-6

D - erythro - hex - 2 - enonic acid, γ lactone (8 CI,9 CI)

Sodium erythorbate (sodium salt)

CAS - RN 7378 - 23 - 6



C6 H8 06

MW : 176,12

* Use

Isoascorbic acid is used in cosmetic products at concentrations not exceeding 2 %.

It is no longer permitted in foodstuffs.

* Recapitulation of the studies of toxicity

ORAL TOXICITY

Isoascorbic acid is absorbed via the gastro-intestinal tract by the same transport system as ascorbic acid and is competitive with it.

When given orally to guinea pigs simultaneously with ascorbic acid, it prevents ascorbate uptake by the guinea pig tissues at doses ± 200 mg/kg ,reducing ascorbate storage and bioavailability up to 50 % in some organs.

It is quickly excreted in the urine but its metabolites are unknown.

In mice fed a diet with 5 % erythorbic acid and 5 % ascorbic acid for two months,increasing dose levels to 10 % for five more months , erythorbic acid was found to replace 45 % of ascorbic acid in the liver and 28-39 % in the brain tissues. The blood ascorbate level was unchanged .

In studies on four humans, a daily intake of 50 mg isoascorbic acid for two weeks produced a deficiency of ascorbic acid and this was followed by 100 mg daily absorption for two weeks.All ascorbic acid concentrations continued to fall during the four-week period and urinary excretion increased considerably. 50-60 % of a 300 mg loading dose of ascorbic acid appeared in the urine. The fate of the retained portion is not known.

No adverse effects were observed when rats were fed 1% in the diet for 36 weeks.

Similarly, no effects are observed regarding growth rate,mortality or histopathology, in rats fed for two years on a 1 % diet, nor in a group of four beagle dogs receiving 1 g/day of isoascorbic acid orally for 240 days and another group receiving 5 g/day for 50 days and later 7.5 g/day for 190 days.

CUTANEOUS AND MUCOUS TOLERANCE

A single application of 2 g/kg of sodium isoscorbate to both the intact and abraded skin of rabbits produced no sign of toxicity during the fourteen -day observation period.

A single instillation of 100 mg of powder into the eyes of albino rabbits with rinse-off after five seconds produced no sign of irritation.Some redness reversible after 24-48 hours was observed in three of the twelve rabbits.

MUTAGENICITY

Erythorbic acid (99.6 % pure) exhibited a relatively weak mutagenic potential in the Ames test, at a dose of 50 mg/plate in respect of the T 100 strain with S_9 mix (222 his^+ revertants) and without activation (144 his^+ revertants).

It was negative in a chromosome aberration test carried out in vitro on a culture of Chinese hamster fibroblasts and in a dominant lethal test.

CARCINOGENICITY

Sodium erythorbate administered in doses of 1.25 and 2.5 % in rats' drinking water for four weeks did not show any carcinogenic potential.There was a slight reduction in certain spontaneous tumours in females treated at 2.5 %.

C O N C L U S I O N

Isoascorbic acid is well tolerated by the skin and mucous membranes.It is neither mutagenic nor carcinogenic.

Acute toxicity is not known and the toxicity data for short-term oral administration are not sufficient to establish a no-effect dose.

The sensitization and skin absorption data are missing.It competes with ascorbic acid (vitamin C) as regards bioavailability.

Classification : D This classification is justified by the competition with ascorbic acid and could be reviewed only if the Committee had data to show the absence of skin irritation

References :

- Colipa "Unpublished Company data"
- Dr Robinson's data
- Abe I
  Exp. Mol. pathol.; vol.41 , iss. 1,1984, p. 35-43
- Fukushima S. et al
  Cancer Letter (Shannon Irel.); vol. 23, iss. 1 ,1984,p.29-37

REPORT 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 is admissible from the health point of view.

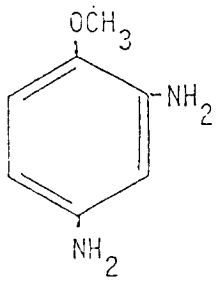
- 2,4 diaminoanisole
- 2,5 diaminoanisole

(1) 2,4 D I A M I N O A N I S O L E A6

* Formula and synonyms

4 - methoxy - m - phenylenediamine CTFA
1 - methoxy - 2 , 4 - diaminobenzene
C.I. : 76050
CAS : 615 - 05 - 4

$C_7 H_{10} N_2 O$ MW : 138,2



* Use

Oxidation hair dye
It is used as a coupler,giving rise to different colours with,f.i. p. phenylenediamine (blue),p. aminophenol (red)

No longer used by big companies ; used only by small companies.

Max.use : 2 % before mixing the solution.

* Recapitulation of the studies of toxicity

LD50 oral (rat) : 460 - 515 mg/kg/bw
i.p (rat) : 372 mg/kg

ORAL TOXICITY
++++++++++++++

- Subacute toxicity : RAT. 2,4 - DAA administered orally at the only one dose level of 23 mg/kg/day for 12 weeks to female rats showed a slight depression of the red and blood cell count and of growth rat.
  RAT Fischer 344 & Mouse B6C3F1. NCI bioassay.5 males and 5 females/ group of both species received in the diet these doses of 2,4-DAA sulphate : 0-0.75-0.125-0.209-0.348 and 0.580 % for 4 weeks followed by a two week observation period.The maximum tolerated doses were 0.5 % for rats and 0.24 % for mice.
- Short-term dermal toxicity : 2,4-DAA containing formulation (2.0 % and 4.0 % in water) tested on shaven intact skin of New Zealand rabbits by topical application produced no toxic effects at 3-7-13 weeks after treatment at the histopathological analyses.

CUTANEOUS AND MUCOUS TOLERANCE
++++++++++++++++++++++++++++++++

DERMAL TOXICITY
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Dermal irritation : the compound applied to intact and abraded skin of rabbits as a 2,5 % (w/v) aqueous solution resulted "mildly irritating".
Eye irritation : the compound applied as a 2,5 % (w/v) aqueous solution on rabbits' eyes resulted non irritating.

Sensitization was tested in Guinea pigs treated with 3 % 2,4-DAA solution containing 2 % Natrosol,2 % Tween 80, 0.05 % Sodium sulphite and 10 % isopropanol(pH = 7) applied 6 days/week for 3 weeks.The results were negative. Another sensitization test on Guinea pig with 2,4-DAA (5-25 % in distilled water) by Magnusson & Kligman's method (reaction evaluated at 24, 48 and 72 h) showed delayed contact hypersensitivity in 1/10 and equivocal reaction in 2/10 animals at the first challenge application.

Dermal absorption. (14-C)-2,4-DAA (4 µg/cm2) of acetone solution applied for 24 h on abdomen of adult males and females rhesus monkey showed that 4.7 % of the dose penetrated in the skin. (14-C)-2, 4-DAA in a formulation applied for 20 min to scalp hair of the Rhesus monkey showed a penetration of ca. 0.02 % (in oxidative mixture) and 0.23 % (without hydrogen peroxide).

(14-C)-2,4-DAA (as ingredient of 3 different formulations) showed an absorption range of 0.26-1.1 % after cutaneous applications on rats evaluated at 24 h.

In hairless Wistar rats a topical application of radiolabelled 2,4-DAA alone or in a complete formulation did not show a difference in the amount penetrated through the skin.

Human studies : 2,4-DAA dihydrochloride (ring-labelled, 4 µg/cm2) applied on the ventral skin surface of the forearm to man for 24 h showed that 3.9 % of the dose penetrated in the skin.

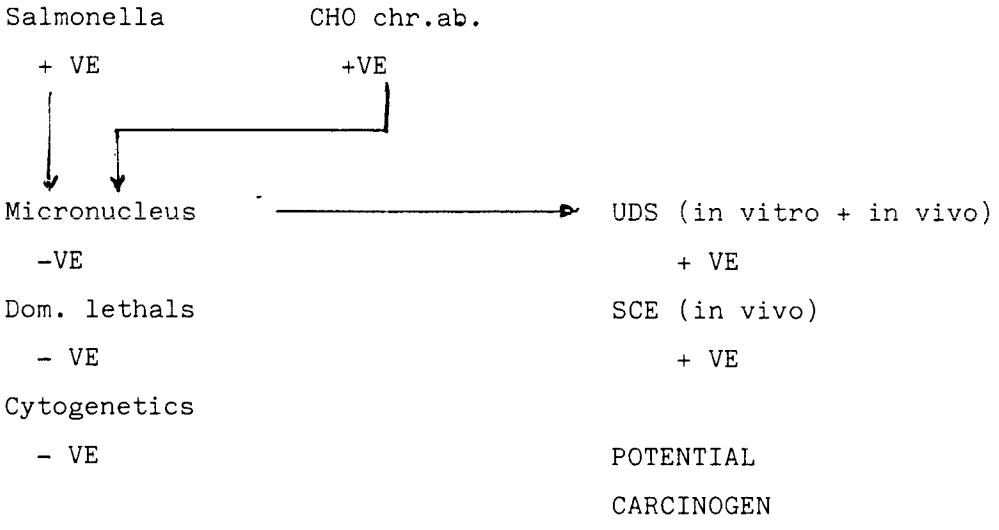
2,4-DAA-14C applied to dry hair of 3 volunteers over a period of 5-8 min. and then left for additional 20 min. before rinsing off,was excreted in the urine at a value of 0,022 % with a t 1/2 of urinary excretion of 18 hrs.

MUTAGENICITY AND GENOTOXICITY

The studies have shown that 2,4-diaminoanisole is mutagenic : (1) on Salmonella (2) on S.pombe; (3) on mouse lymphoma cells; (4) on Chinese hamster V79 (in vitro); (5) on Salmonella/mouse or rat urinary assay (in vivo) ; on D.melanogaster (SLRL) for gene mutation induction ;(6) on Chinese hamster ovary (CHO) cells lines for

chromosome aberration (in vivo); (7) on S.cerevisiae for gene conversion and mitotic recombination; (8) on rat hepatocytes and HeLa cells for the induction of UDS; (9)on human fibroblast for DNA strand breaks and (10) DNA repair in E.coli ; (11) on bone marrow cells of mice (up to 23.3 mg/kg i.p.) for SCE (in vivo) ; and (12) on rat (0.66 nmol/kg i.p.) for DNA/damage alkaline elution assay.
Other mutagenicity studies have shown that the compound is negative on E.coli 343/113; on Salmonella/mouse (up to 3.5 mg) H.M.A. (in vivo); and in morphologically abnormal sperm test on mouse for gene mutation; that thecompound does not induce dominant lethals in rats Charles River treated for 10 weeks with doses of 10-20-40 mg/kg (i.p.) and for 8 weeks with a dose of 20 mg/kg (i.p.); and micronuclea in rats Sprague Dawley treated with 0.5%(w/v) suspension in tragacanth's gum with 0.05 % sodium sulphite (gastric intubation).

Biochemichal studies : 3-H-(ring labelled)-2,4-DAA incubated with purified rat liver nuclei and microsomes in the presence of NADPH led to the formation of radiolabelled product(s) bound covalently to nuclear protein. Pretreatment with ß-Naphthoflavone showed that PB-treatment increased binding (ca. 70 % for both frations).



(according to J.ASHBY,1986)

CARCINOGENICITY

Long-term studies were carried out on mice and rats by a NCI bioassay the compound fed in the diet at 0.12, 0.5 % for rats for 78 weeks followed by 29 weeks observation period and 0.12, 0.24 % for mice for 78 weeks followed by a 19 weeks observation period resulted carcinogenic in both sex and in both species producing follicolar-cell thyroid tumours and malignant tumours of the skin and its associated gland in both sex of rat and C-cell adenoma only in males and, in mice combined incidence of follicular-cell adenomas and carcinomas in female and only fullicular cell adenomas in males.
Various studies, some incomplete and inadequate for the evaluation, performed on rats and mice with formulations containing different level of 2.4-DAA (from 0.33 % to 4.0 %) showed negative results.

TERATOGENICITY

No embriotoxic or teratogenic effects were observed during gestation of Charles River rats treated topically at a dose of 2 mg/kg/bw with formulations containing 2.4-DAA (2 % and 4 % in water)on shaven skin on day 1-4-7-13-16-19. A retarded effect of ossification process (bones of the feet and the cervical and caudal vertebral centre) was observed in mice topically treated twice a week for four weeks before mating and until the 18th of gestation with a formulation containing 2 % of 2.4-DAA and mixed before use 1:1 with hydrogen peroxide equivalent to 0.5 mg/mouse.On the basis of 1 % dermal absorption,the dose producing the teratogenic effects is 5 µg/mouse (200 µg/kg).In a reproductive study on rats treated with 2 % 2.4-DAA containing formulation no negative effects were observed (this study was not available for the present analysis).

C O N C L U S I O N

IARC (1982) classified 2.4-DAA as a chemical with sufficient evidence of carcinogenic potential,The substance showing :

-Sufficient evidence of mutagenicity/genotoxicity in vitro and in some in vivo assays,

-Sufficient evidence of carcinogenicity in two species and in both sexes,

-Some evidence of teratogenicity.

Therefore,the Committee may not allow the use of 2.4-DAA.

Classification : D

Bibliography :

- N. LOPRIENO & G. BONCRISTIANI : A review on toxicology of 2.4 - Diaminoanisole. Pisa, Italy, sept. 1986.
- S C C , 2nd series EUR 8634 (op 1980)

(2) 2,5 D I A M I N O A N I S O L E A34

* Formula and synonyms :

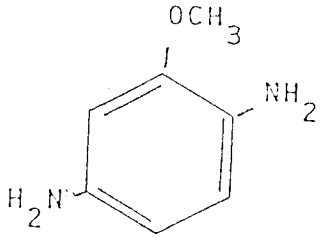
2 - methoxy - p - phenylenediamine

1 - methoxy - 2.5 - diaminobenzene

C.I :

CAS : 5307 - 02 - 8

$C_7H_{10}N_2O$ MW : 138



* Use

- Banned in Italy
- It is used in oxidative and developer hair dyes with couplers, such as m-phenylenediamine, m-aminophneol, m-dihydroxybenzenes (the resulting reactions produce different indo-amines of different colour).

Production and use : 300 kg.

* Recapitulation of the studies of toxicity

LD50 oral (rat) : 60 mg/kg
(mouse) : 34 mg/kg
i p (rat) : 28 mg/kg

O R A L T O X I C I T Y

Short-term oral toxicity : the compound administered as sulfate, 0-1-5-25 mg/kg/day X a minimum of 91 consecutive days by oral gavage to rats showed that 1 mg/kg/day represents the dose with "no-effect level."

A similar study in a dog (0-1-3-9 mg/kg/day/7 day/wks X 91 consecutive days) gave "no-effect level" at 1 mg/kg/day.

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

Dermal irritation studies were carried out on rabbit using two different methods (a & b). The results showed that the compound has resulted "slightly irritating" (dose : 0.5 ml or 0.5 g pasta, IIP = 0.08) in method (a) and "mildly irritating" (dose not reported,IIP = 1.1) in method (b).

Eye irritating : The compound (100 mg as sulfate) applied on rabbits' eye gave in all animals a positive reaction with lesions to conjunctiva (redness : diffuse, crimson red,individual vessels not easily discernible ; chemosis : obvious swelling with partial eversion of lid in two animals swelling with lids about half closed).The grade of ocular reaction was evaluated at 1-2-3-4-7-17 and 21 days.

In another study on rabbit at 1 hour, (1-2-3-4-7 days)reading the compound (= Imexine OAN 0.5 % in water) did not result irritating.

Sensitization was tested in Guinea pig treated with 1 % in paraffin oil of test compound as sulfate using the method of Magnusson and Kligman (reaction evaluated at 24-48-72 h). 3/10 animals showed a temporary skin-sensitization (very slight oedema) and 1/10 a permanent skin sensitization (slight oedema).

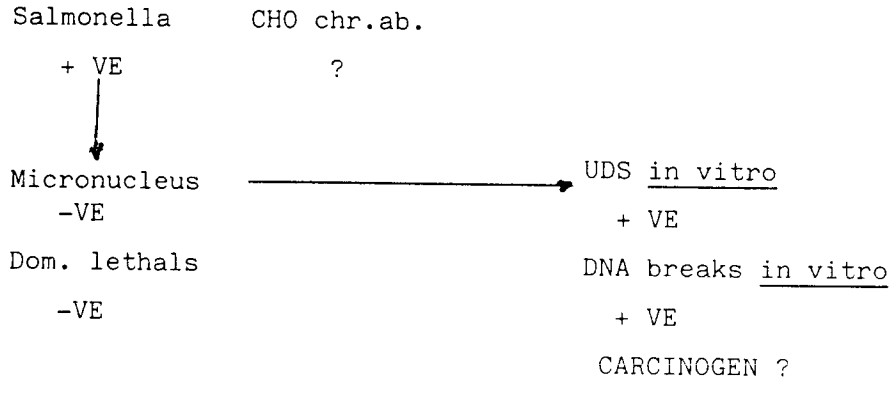
Dermal absorption. (14-C)-2,5-DAA.2HC1 (aqueous solution) applied epicutaneously without hydrogen peroxide on Him : OFA-Sprague Dawley rats at 2 mg (free base)cm2 showed that 1.02 % of the amount applied was absorbed. (14-C)-2,5- DAA.2HCI in commercial preparation (1:1 with 6 % H202) after epicutaneous applications on 9 cm2 of clipped skin areas and 2 mg (free base)/cm2 on unclipped skin)showed an absorption of 0.28 % (clipped skin) and 0.07 % (unclipped).

Short-term dermal toxicity: 2.5-DAA containing formulation (6.0 in water) tested on shaven intact skin of New Zealand rabbits by topical application produced no toxic effects at 3-7-13 weeks after treatment at the histopathological analyses.

Human studies : Skin absorption of a hair dye product with test compound (1.9 %,mixed 1 : 1 with H2O2) applied (27-32 min.) on washed hair of 5 female volunteers was less than 0.102 % of the amount applied, under hypothesis that the distribution of the dye is equal in the whole body. Patch-test performed in human with sensitization to aromatic amino compound (paragroup allergy) revealed a sensibilization for the testcompound similar to p-phenylenediamine.

MUTAGENICITY AND GENOTOXICITY

The compound has produced positive results in Salmonella in the presence of different metabolic activation systems. The compound resulted positive in vitro for genotoxicity on primary rat hepatocytes for the induction of UDS and in human skin fibroblast for the induction of single strand breaks : in both studies purity was not reported. Other mutagenicity studies have shown that the compound is negative for gene mutation on Bacteriophage TD4/E.coli test system, in a spot test on Neurospora crassa, in one study on Salmonella typhimurium and in mouse lymphoma cell lines in the absence of metabolic activation system. The compound did not induce chromosome aberration in vivo by micronucleus test on bone marrow cells on rat (5 mg/kg in 1 % MC by gastric intubation in 2 equal doses separated of 24 hours) and in two different dominant lethal studies on rat : (a) 20 mg/kg i.p. for 8 weeks and (b) until to 25 mg/kg/day for 10 weeks (30 mg/kg/day for first 2 weeks). Induction of mitotic gene conversion on Saccharomyces cererisiae using different experimental procedures was negative.



CARCINOGENICITY

Long-term study was carried out with a formulation containing test compound (6% in water, as sulfate) by dermal application on mouse (0.05 ml/w. for ca. 2 years) : no biologically significant difference was observed between treated and controls groups.

TERATOGENICITY

No biologically significant difference in the mean number of corpora lutea, implantation site and live fetuses, and sex ratio and no significant changes for soft-tissue or skeletal anomalies were seen on rat treated topically at a dose of 2 ml/kg b.w. with a formulation containing 2.5-diaminoanisole (6 % in water)on shaven skin on day 1-4-7-10-13-16-19 of gestation. Study inadequate (treatments every 3 days : only one dose tested.

C O N C L U S I O N

The compound has very high acute toxicity compared to many other hair dyes and a NEL for sistemic toxicity of 1 mg/kg/day.
Due to the inadequacy of long-term carcinogenicity study available, the evidence of carcinogenicity could be assessed.
The teratogenicity study is inadequate.
Short-term studies on mutagenicity/genotoxicity indicate that the compound reacts with DNA in vitro.
The request made by the SCC in 1980 was not followed by any more detailed study.
The human absorption has been calculated to be around 25-30 µg/kg,a value close to the NEL in the animal toxicity.

Classification : D

Bibliography : - N. LOPRIENO & G. BONCRISTIANI : A review on toxicology of 2.5-Diaminoanisole. Pisa,Italy sept.1986
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CONTENTS

33rd meeting of the SCC - February 2, 1987

33rd MEETING OF SCC

20 FEBRUARY 1987

Members of the Committee

Professor A.P. DE GROOT
Doctor R. GOULDING
Professor A. HILDEBRANDT
Professor N. LOPRIENO
Professor J. SCHOU
Professor D.P. O'MAHONY
Professor G. STUETTGEN
Professor P. AGACHE
Professor L. MUSCARDIN
Professor J. DONY
Doctor O. ENJOLRAS
Doctor A. KNAAP was excused

REPORTS OF THE SCIENTIFIC COMMITTEE ON COSMETOLOGY

Opinions expressed on February 2 1987

33rd reunion

SUMMARY

ANTIOXIDANT AGENTS

BHA	A05	EEC N° 320	C
BHT	A06	321	D
DAHQ	A011		C
DBHQ	A012		C

PRESERVATIVE AGENTS

Suttocide	A	P84	C
Decominol		P87	C

ANTI-DANDRUFF AGENT

ONADINE MSD		P50	EEC N° 41	D

Complementary opinions on hydroxy-8-quinoleine

REPORT BY THE SCIENTIFIC COMMITTEE ON COSMETOLOGY

ON THE

USE OF CERTAIN ANTIOXIDANT AGENTS IN COSMETIC PRODUCTS

(opinion expressed on 20 February 1987)

THE COMMITTEE'S MANDATE

To give its opinion on the question whether the use in cosmetic products of certain antioxidant agents is admissable from the health point of view.

- BHA
- BHT

} in a maximal use concentration of 1%

- TAHQ
- TBHA

} in a concentration of 0,2%

(1) B H A : BUTYLATED HYDROXYANISOLE

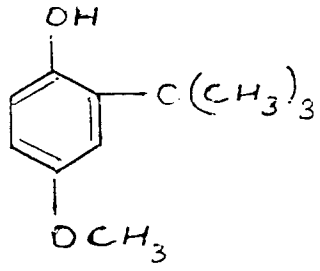
-Formula and synonyms

EEC n° 320

Colipa A05

C11 H16 02

MW : 180,2

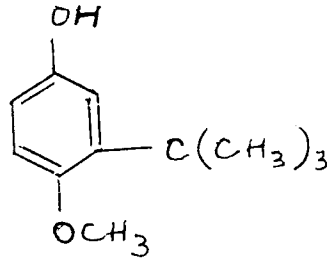


3-BHA : approx 90%

3 - tert-butyl-p-hydroxyanisole

phenol,2 -(1,1 dimethylethyl) - 4 -methoxy

CAS - RN 121 - 00 - 6



2 BHA : approx 8% MW 180.2

2 - tert-butyl-p-hydroxyanisole

phenol,3 -(1,1 dimethylethyl) - 4 - methoxy

The commercial product is a mixture of two isomers (3-BHA and 2-BHA) with the 3-isomer being the major component

- Physical characteristics

White or slightly yellow,waxy solid ; odour aromatic

Insoluble in water

Slightly soluble in fats and oils

Soluble in alcohol,propylene glycol,chloroform and ether

- Use

As an antioxidant in cosmetics at a concentration not exceeding 1 %

(eye make-up,lipstick,skin-care prepar. and perfumes

BHA protects the lipids from peroxydation and acts synergistically with BHT and propyl gallate and is often used in combination with these a.o. for greater efficacy

- Cosmetic quality

Purity : $\geq$ 98,5 of a mixture of 3-BHA (approx. 90 %) and 2-BHA (approx. 8 %)

Impurities :		
	4-hydroxyanisole	$\leq$ 0,5 %
	1-t-butyl-2,5-dimethoxybenzene	$\leq$ 0,5 %
	2,5-di-t-hydroxyanisole	$\leq$ 0,2 %
	hydroquinone dimethyl ether	$\leq$ 0,1 %
	sulphated ash	$\leq$ 0,01 %
	lead	$\leq$ 20 ppm

- Other qualities

  - It offers moderate protection to the skin from UV radiation
  - It inhibits the formation of nitrosamines in an acid pH
  - It shows an antimicrobial activity towards gram + bacteria and fungal organisms.

- Recapitulation of the studies of toxicity

LD50 : oral (mice) = 1,10 - 2 gr/ kg bw

(rats) = 2,10 > 5 gr/ kg bw

(comes from studies before 1960)

LD50 : dermal (rabbit) = 2,10 > 5 g r/ kg bw

(formulation with 0,1 % BHD)

ORAL TOXICITY

A daily gavage at a dose of 1 gr. in olive oil for four to seven days is lethal to rabbits.Adrenal changes and an increase in Na + and K + is observed.

In rats 0,5 gr/kg bw by gavage for 6 days has a transient depressive action on organic acid transport.

Studies on animals and humans show that BHA is absorbed via the gastro-intestinal tract and metabolized.

In humans,BHA is conjugated with glucuronic acid in the liver.

Urinary excretion products consist mainly of glucuronides with small quantities of sulphates and free BHA. No dealkylation or hydroxylation products are detected.The time required to excrete the dose admini - strated varies from 23 to 50 hours.

Although solubility in lipids favours storage in tissues,studies on pigs,rats and dogs and a pharmacokinetic study on rabbits show that the quantities stored are limited because of the metabolism and the rapid excretion.

A recent kinetic study on humans indicated the formation of tertbutyl hydroquinone by metabolic conversion. This product has generally been identified as a decomposition product resulting from UV exposure.

In a large number of short- and long-term studies by oral administration to rats,rabbits,guinea pigs,monkeys,dogs and chickens,the most frequently noted effect was an increase in liver weight and changes in the endoplasmic reticulum; these changes were not generally accompanied by persistent hepatoxicity.The lowest effect level is 25 mg/kg/bw/day for mice and 50 mg/kg/bw/day for monkeys.

In a recent series of studies there was a proliferation of the epithelium of the forestomach,which can be linked to the forestomach tumours induced by BHA in rats.

Three studies on rats involving administration in the diet at concentrations up to 2% for 9 and 27 days, 1, 2 and 4 weeks and 13 weeks showed that the effects on the forestomach - proliferation of the epithelium,increased mitotic rate,hyperplasia,hyperkeratosis and inflammatory changes - increased with the duration of exposure; after the treatment was stopped the lesions regressed slowly but not completely,and the degree of reversibility was inversely proportional to the prior duration of exposure.The no-effect dose is evaluated at 0,25 mg/kg/bw/day.

These observations are confirmed in other species having a forestomach such as the more susceptible hamster,in which papillomas appear after 24 weeks on a 1% BHA diet.
Animal species having no forestomach : daily oral intubation of 1 000 mg/kg/bw of BHA has no effect on the stomach or oesophagus of the guinea pig ; administration of 0,25 , 10,5 and 1% in the diet of beagle dogs for six months has no effect on the stomach,oesophagus, duodenum or liver.Histopathological and histometric examinations showed no changes in the mucous membranes of the stomach,oesophagus or duodenum.The liver weight increased at each dose but caused no histopathological changes.It produces a greater proportion of oesophaglal basal layer cells in mitosis in monkeys after 85 days at a dose of 250 mg/kg/bw/day ; these changes are not accompanied by histological changes and the dose of 125 mg/kg/bw/day has no effect.

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL ROUTE TOXICITY

In special short-term studies on dermal effects,application of BHA (up to 20 %) in lanolin to the ears of guinea pigs for six weeks caused ultrastructural morphological lesions of the epidermis,with destruction of the superficial connective tissue and collagen break-up, BHA causes slight to moderate irritation of the skin of albino rabbits when applied to the shaved,intact or abraded skin with occlusion in preparations containing 0,1 to 0,2 %.
It causes slight to very slight irritation to the eyes of albino rabbits after single or repeated instillation of formulations containing 0,1 and 0,2 %.

In humans

In a number of tests to assess irritation potential,sensitization and photosensitization,

- performed on 10 to 353 human subjects.
- with cosmetic preparations containing concentrations of 0,01 to 0,2 % BHA,
- by simple application with occlusion,
- by repeated application with occlusion followed by a challenge application according to various protocols including modified Draize,Philips and lauryl sulphate maximization,

- by repeated applications with and without occlusion followed by a challenge application and exposure to UV radiation at 360 nm or 280 to 370 nm, according to various protocols including Draize-Shelansky,Schwarz-Peck and Shelansky-Shelansky.

BHA is generally found to be minimally to slightly irritant,non-sensitizing and non-photosensitizing.

Nevertheless,individual cases of contact dermatitis after applications of formulations containing 0,005 and 0,052 % BHA and a variety of allergic reactions following the ingestion of products containing BHA are reported in the literature.

The North American Contact Dermatitis Group estimates a 2 % incidence of skin sensitization among 548 subjects exposed to 2% BHA. In 3/112 patients treated for contact dermatis responding positively to a 2 % BHA patch test,the biopsy and control patch tests revealed a lymphocyte allergy,the patients having been sensitized after repeated local application.

A recent study over a two-year period showed that 7/1 096 patients suffering from facial eczema were allergic to BHA (1% patch test)and five also reacted to TBHQ (1% patch test).

MUTAGENICITY AND CARCINOGENICITY

BHA has exhibited neither mutagenic properties nor genotoxic effects in a number of *in vitro* and *in vivo* test systems.

On bacteria,it was negative in two tests on *Salmonella typhurium* (TA 98, 100 and TA 535, 1537 and 1538) and on *S. cerevisiae* (D 4) with or without activation.

On mammal cells,it produced no chromosome aberration on cultures of human embryo lung cells exposed for 24 hours at a concentration of 2, 20 and 200 ug/ml in isopropyl alcohol,nor on Chinese hamster fibroblasts at a concentration of 10-4 M in ethanol.

Similarly, *in vivo*, after oral administration to rats at doses of 15,150 and 1 500 mg/kg, it does not cause significant aberrations of the chromosomes of the spinal cord and it was not mutagenic in a lethal dominant test. No sex-linked recessive lethal increases were observed in *D. melanogaster* fed on BHA.

After administration at doses of 15, 150 and 1 500 mg/kg to mice, no mutations were induced in an intermediate-host test, on *S. typh*. Conversely, in a similar test on *S. cerivisiae*, it increased mitotic recombinations at all doses.

BHA can have an antimutagenic effect as shown by the results obtained in various test systems on bacteria and mammal cells.

Short-term studies on mice using subcutaneous and intraperitoneal injection show no evidence of carcinogenicity, nor do long-term studies by topical application of 0,1 to 1.0 mg BHA in acetone once a week for 323 to 509 days, or 1 mg in acetone twice a week for 30 weeks.

Long-term oral studies on rats and dogs at doses up to 0,5 % in the diet were negative, but it should be noted that none of these studies is acceptable in terms of standard protocols.

A recent study in Japan, in which BHA was administrated in concentrations of 0,5 and 2,0 % in the diet showed that BHA was *carcinogenic for rats' stomachs* ; it induces a significant increase in the incidence of papillomas and carcinomas of squamous cells in both sexes. The incidence of hyperplasia of the forestomach associated with neoplasic changes is dose-related.

BHA can inhibit or promote experimental carcinogenesis. It inhibits the carcinogenic power of benzopyrene and 7,12-dimethylbenzanthracine on mouse skin.

TERATOGENICITY AND EMBRYOTOXICITY

No embryotoxic or teratogenic effects were observed in a series of teratogenicity studies on mice,rats and rabbits at doses of 60 to 1 000 mg/kg/bw/day.

However,mice exposed to BHA at 5 000 ppm via their mothers' diets during gestation and lactation and then directly up to the age of six weeks showed behavioural changes:reduced sleep,learning speed and orientation reflexes.

In another study on mice,by administration in the diet of 0,5%/kg during gestation,the 30-day-old mice exhibited reduced exploratory reflexes, body weight and cerebral cholinesterase.

In a postnatal study,on rats fed with 1 250,2 500 or 5 000 ppm BHA in the diet for two weeks before mating, and during gestation and lactation,an increase relative to the mortality dose was observed up to the age of six weeks,but not at the age of three months.Administration in the diet of BHA at 50,200 and 400 mg/kg/bw to pigs (Danish landrace SPF) from mating to the 110th day of gestation had no effect on reproduction,nor any teratogenic effects.

However,a reduction in weight gain was observed at the highest dose and a dose-related increase in liver and thyroid weight; histological changes appearing in the thyroid particularly at the highest dose could indicate a reduction in thyroid activity.

CONCLUSION

JEFCA has set an ADI of 0,5 mg/kg/bw of BHA,BHT or the sum of both.

Owing to the ADI value,the absence of information on skin absorption and sensitization problems which may be linked to concentration,the Committee cannot accept use at 1% in cosmetics and wonders about the actual concentration in use.

Classification : C

Information required : - skin absorption
- actual concentration in use

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  Journal of the American College of Toxicology vol3 N°5 1984
  N.L.M'S National Interactive Retrieval Service Medlars II

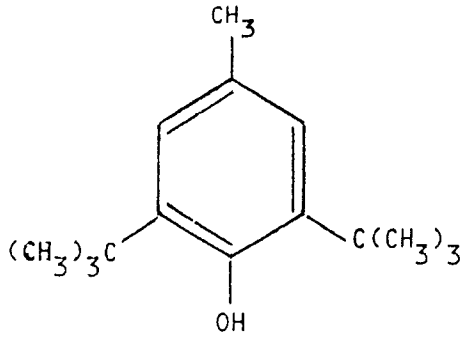
(2) B. H. T. BUTYLATED HYDROXYTOLUENE

* Formula and synonyms

- Colipa AO 6
- EEC n°32
- CAS-RN 128-37-0

- Phenol,2,6-bis (1,4-dimethylethyl)-4-methyl (9 C1)
  BHT

- $C_{15}H_{24}O$



- MW : 220,34

* Characteristics

White,crystalline or flaked solid; odour characteristic
Insoluble in water
Soluble in fats,alcohol,ether and other organic solvents
In a study of the influence of pH and light on the stability of antioxidants,BHT was stable regardless of light or pH except at high pH ($\geqslant$ 9).

* Use in cosmetics

As an antioxidant at a concentration not exceeding 1% (often 0,025 - 0,3 %)

* Other qualities

- it inhibits the formation of nitrosamines (in a concentration of 0,05 - 0,2 %) in creams and shampoos that are preserved with bronopol (0,01 - 0?05).

- it offers protection to the skin from UV radiations in a (0,5 % in diet or topical 1 %) phototoxicity study on hairless mice (LO,WB,BLACK,HS 1973)
- it inactivates certain viruses (Herpes simplex) because of its solubility in fats and its action on the cell membrane
- it reduces the ulcerations caused by adriamycine (in rats).

* Recapitulation of the studies of toxicity

LD_{50}	mouse	(oral)	836-2000 mg/kg/bw
	rat	(oral)	1700-2450 mg/kg/bw
	cat	(oral)	940-2100 mg/kg/bw
	rabbit	(oral)	2100-3200 mg/kg/bw
	guinea pig	(oral)	10700 mg/kg/bw
		(intraperitoneal)	190 2270 mg/kg/bw

ORAL TOXICITY

- BHT given orally is rapidly absorbed from the intestine.The highest tissue levels are found in adipose tissue but accumulation in fat does not seem to occur with repeated administration in rats or mice, and the half-life in liver and fat is 7-10 days in the rat.Samples of human subcutaneous adipose tissue taken from UK adults,consuming an estimated 1 mg of BHT daily,contained 0.01-0.49 ppm; samples from USA adults, consuming an estimated 2 mg of BHT daily,contained 0.34-3.19 ppm.
  The principal metabolism is metabolic oxidation in liver and lungs. By metabolic conversion in human,the studies indicated the formation of 5-carboxy - 7 -(1-carboxy - 1 -methylethyl) 3,3 -dimethyl - 2 - hydroxy 2,3 -dihydrofuran (glucuronide) as principal product.
  With one oral dose 40 mg/kg/bw BHT C^{14} (in humans) 50 % of radio-activity is excreted in urine between 24 hours.We have a slow excretion (25 %) for 10 days.

Long-term and chronic oral toxicity

Systemic effects

- Effect on the liver

Studies (on rats) showed a relation between BHT exposure and enlargement of the liver. It is a rapide and revertible action.
More recent studies on infant and juvenile monkeys have reported that daily doses of 50 or 500 mg BHT/kg/bw for 4 weeks had no effect on urine or serum chemistry or on haematology.Histological examination of the major organs however did show an effect on the liver.
BHT treated animals showed enlargement of hepatocytes and their nuclei with moderateproliferation of endoplasmic reticulum and prominent lipid droplets. In the high-dose group there was fragmentation of the nucleolus in some hepatocytesJuveniles also showed enzyme changes.

In rats with a 60 day-diet at the level of 250 mg/kg and in rats and mice with a 6 day-diet at 0,5 % ,high levels of P-450 cyt were observed.
BHT in the diet of SD rats resulted in a marked decrease in the NADPH-cytochrome P-450 reductase activity of isolated liver microsomal preparations. This effect was not observed when BHT was added in vitro to liver microsomes. An increase in GSH-8 transferase was observed in liver of rats fed 0.4 % BHT in the diet. Dietary BHT is also reported to have an effect on the carboxylation process in the conversion of rat liver microsomal protein to prothrombin. It has also been suggested that phenolic antioxidants such as BHT may protect from carcinogens by lowering cyclic guanosine monophosphate levels.
BHT intreferes with the normal lipid metabolism in the liver.
According to the treated animal kind (rat or monkey) increasing of serum cholesterol level or reduction of total cholesterol levels are found . That could show a difference in metabolism betweem rodents and monkeys.

- Haematology

In haematology studies BHT is reported to have caused extensive internal and external haemorrhaging,in association with hypoprothrombinaemia

leading to death in some,but not all strains of laboratory rats,mice and guinea pigs. The minimum dietary level of BHT to cause such effects was 170 ppm; with continuous feeding,deaths occurred within 40 days at dietary levels of 6900 ppm and above. These effects were not seen in hamsters,dogs,rabbits or quail. Further recent study into the mechanism of BHT induced haemorrhage has indicated that a number of factors may be involved,including reduction in vitamin K and plasma zinc levels,reduced activity of clotting factors,alterations in platelet function and vascular permeability.

- Effect on the thyroid

Increases in numbers of thyroid follicles, thyroid weight and thyroid iodine uptake have been observed in rats given 500 ppm BHT in the diet for up to 90 days, but this was not accompanied by any change in thyroid hormone levels in the blood and long-term studies on rats maintained on diets containing up to 10.000 ppm (1%) have not shown any adverse effects on the thyroid.

- Effect on the lungs

3 or 5 days after a i.p. 0.004 to 2?5 gr/kg injection in the mice, enlargement of the lungs and disorganization of the cell components are found. These changes are linked with a lung weight and a DNA synthesis increasing similar effects are observed with an oral administration.
BHT is metabolized in the lungs and recent studies could show that quinones of similar metabolic products proceeding from microsomal oxidation of toxic phenols have a part in the adverse effects on the lungs.
Adverse effects on adrenal glands,kidneys,heart cells,small intestine contractions and the growth rate are also observed.

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL ROUTE TOXICITY

The possible skin lightening properties of BHT have been investigated by patch testing and normal usage on black-skinned volunteers.A single application of 0.5 or 3 % BHT in a cream base was applied to the

back (intact skin) of 50 black factory workers,with occlusion for 48 hours.Slight erythema and flaking of the skin was observed in 9/50 cases.When 3% BHT in the same cream was applied twice daily over 7 weeks two volunteers suffered adverse reactions - one mild erythema and folliculitis in the test area after 5 weeks and the other erythema and skin flaking after 6 weeks. In no case was there any evidence of skin lightening.

In a maximisation test on human volunteers , 1.0 ml of BHT 2% in pet was applied for 5 alternate days for 48 hour periods, with occlusion to the forearm (pretested with sodium lauryl sulphate(SLS) for 24 hours before initial patch). After 14 days a challenge dose of 0.4 ml test material,at a concentration of 2% pet,was applied to the right side of the back,for 48 hours,with occlusion,preceded by 30 mm application of 5% aqueous SLS to the left side. Relatively little irritation was produced by SLS,and no significant reaction at the non SLS treated sites in the 28 subjects tested.

In control patch testing of 112 patients with BHT 2% pet and BHA 2% pet ,two patients had positive reactions to both and one to BHA only and one to BHT only.A patch test positive to BHT 2% pet was suspected to have caused eczema of the hands for 6 months and of the feet and lips for 3 months in a 24 year old female. When the patient was almost free of symptoms 5% alcoholic solutions of BHA and BHT were swabbed,each on one finger. After 15 minutes both of the treated areas itched, and shortly after an urticarial reaction developed. The symptoms subsided in about 1 hour. The results indicated that BHT and/or BHA produce allergic and not irritant reactions.

In a modified Draize test on 5 guinea pigs each animal was given a total of 10 intracutaneous injections over a 2 week period.The first injection was 0.04 mg BHT in 0.05 ml 10% aqueous ethanol, the second to tenth injections 0.08 mg BHT in 0.1 ml 10% alcohol and a final injection of 0.04 mg BHT. It is reported that signs of local irritation and necrosis were observed. All animals gained weight and appeared healthy. When one animal was given a sensitizing dose of 0.08 mg BHT in 0.1 ml 10% aq alcohol followed by a challenge dose of 0.16 mg in 0.2 ml 10% aqueous alcohol two weeks later,no signs of anaphylaxis were observed.

In an acute dermal study , a dose of 5g/kg to 10 rabbits, produced slight redness in 6 animals, moderate redness in 4 , slight oedema in 3 , and moderate oedema in 3. Recovery data are unavailable.

In a dermal toxicity study,a semi permanent hair dye formulation used as is and containing 0.25% BHT was applied to the abraded skin of 12 rabbits (six male,six female) twice weekly for 13 weeks. The dose used was 1 mg/kg of the 1:1 oxidation mixture. The rabbits were kept in a restrained position for one hour following each application,then shampooed,rinsed,dried and returned to their cages. Body weight gain, relative organ weights,clinical chemistry and results of urinalysis of test animals were comparable with controls. No dye discolouration of the urine was seen at any time during the test. No gross or microscopic lesions were observed.

MUTAGENICITY

The majority of mutagenicity tests for BHT have proved negative. Testsfor bacterial mutation, both in plate assays (concentrations of up to 1000 g/plate), with and without activation,and in a host mediated assay in the mouse (oral doses 30 mg/kg, 0.9 g/kg or 1.4 g/kg) have all been negative. Tests for induction of sex-linked recessive lethal mutations, X-chromosome loss, and translocations in Drosophila also proved negative. However conflicting results have been reported for gene mutation and chromosome damage in mammalian cells in vitro and in dominant lethal assays.

When human embryonic lung (WI-38) cells were analysed at anaphase following exposure to 2.5,25 or 250 μg/ml of BHT a sharp increase in the number of abnormal anaphase figures was observed,particularly evident at the highest dose level tested. Data are unavailable and the significance of the assay is unclear. Chromosome aberrations in metaphase human lymphocyte cultures exposed to 5-40 μg/ml BHT for the final 24 hours of a 72 hours culture have also been reported. The abnormalities described as "fragmented" or "prophase-like" were seen at all dose levels and no clear dose response was obtained.

A more recent study has reported negative results using hamster cells.

All four in vivo studies in bone marrow were reported to be negative. Administration of BHT in the diet for 21 months had no effect on chromosome aberrations in regenerating mouse liver.

A dominant lethal effect has been detected in the first mating week in groups of rats fed 1333 ppm and 4000 ppm BHT in the diet for 10 weeks. Other dominant studies are available which showed negative results. (It is understood that the positive result might be regarded as a questionable response.All currently available dominant lethal studies are to be assessed by the relevant expert Committee within the UK.)

CARCINOGENICITY

According to the studies,contradictory effects are observed in the incidence of lung tumours.

In carcinogenicity studies carried out to date,administration of BHT at range 1000-7500 ppm in the diet to mice for 16 months or more was associated with a dose-related increased incidence of lung tumours, slightly increased incidence of stomach tumours and decrease in reticulum cell sarcomas, compared with controls. The incidence of hepatic cysts was also increased but liver tumours were not seen in either treated or control mice. In two recently conducted studies on rats maintained on diets containing in one case 2500 or 10000 ppm and the other 3000 or 6000 ppm BHT for 104 weeks, no evidence of carcinogenicity was found. However a recent 2-generation long-term carcinogenicity study in Denmark has shown a significant enhanced development of hepatocellular adenomas and carcinomas in rats of both sexes dosed with BHT at concentrations of 0,25,100 or 500 mg/kg/bw.
A dose related response was observed in male F_1-rats.At weaning treated F_1-rats had exhibited a dose related reduced body weight which persisted throughout the study. This was most pronounced in male F_1-rats.

The study does not discriminatebetween an initiating or promoting property of BHT. In addition it differed from other studies in that exposure to BHT commenced in utero thus extending the period of BHT exposure to 141-144 weeks. It has been reported that BHT can cross

the placental barrier and be excreted in rat milk. Additionally none of the tumours found in the Danish study were seen before 111 weeks, the termination point in the other two studies. In the Danish study it should also be noted that survival of the controls was markedly poorer (33%) than that of the top-dose group (83%). It has also been suggested that the lowered body weight of rats dosed with BHT should not be regarded as a "toxic" effect but rather an adaptive response due to possible hyperactivity of the thyroid (see earlier report).

Single i.p. injections of 400-500 mg/kg BHT induced acute lung toxicity in the mouse.Testing of structural analogues to BHT and metabolic studies suggest enhancement of lung tumour yield by chemical carcinogens in susceptible species of mice.

TERATOGENICITY

Effects on reproduction and teratogenic properties are not clear. Recent studies show that BHT goes through the placenta and is found in the milk of rats.
Reproduction studies in a variety of species including non-human primates indicated a no-effect-level of 1000 ppm BHT in the diet or 50 mg/kg/bw. Effects reported in these studies included depression of growth rate of parents and offspring in a two-generation study on rats fed 3000 ppm BHT in the diet, but not at 300 or 1000 ppm, intrauterine deaths associated with maternal toxicity in rabbits given 3-320 mg/kg/day by gavage during foetal development,and developmental delays,reduced pup numbers and pup weight 12 days after birth in mice fed 5000 ppm BHT in the diet but not at 1000 ppm.

Rhesus monkeys maintained on a diet containing BHA and BHT,providing an intake equivalent to 50 mg/kg/bw/dayfor each antioxidant were bred for 1 year and continued on the diet for a second year. Clinical and behavioural observations on infants up to 2 years of age showed no abnormal findings.

In a teratology study on rats, a dose of 2 mg/kg of a semi-permanent hair dye formulation used as it was applied topically to the abraded

skin of groups of 20 mated CR CD female rats on days 1, 4, 7, 10, 13, 16 and 19 of gestation. Except for changes in the colour of the skin and hair at the site of dye application no signs of toxicity were seen throughout the study. Body weight gains were similar in test and control animals. No significant differences in the mean number of corpora lutea,implantation sites and live foetuses were noted.

CONCLUSION

In spite of a lot of studies ,obtained results cannot clearly estimate the usual risk of BHT.In consideration of data showing that BHT could have a teratogenic effect on behaviour and growth, the current ADI of 0,5 mg/kg/bw needs supplementary data to be admitted again. BHT is more toxic than BHA and a usual concentration of 1% in cosmetic products could be cause of a too high systemic exposure level.

Classification : D

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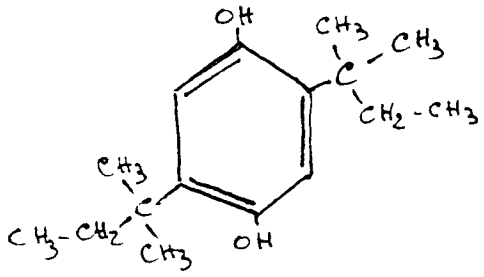
(3) D. A. H. Q. DITERTIARY AMYL HYDROQUINONE

* Formula and synonyms

DAHQ = 2,5 - ditertiary amyl hydroquinone is the aromatic phenol that conforms to the formula



Other names : - 1,4 - Benzenediol 2,5 bis (1,1 - Dimethylpropyl)
- 2,5 - bis (1,1 Dimethylpropyl) 1,4 -Benzenediol
- 2,5 Di - t - Amylhydroquinone
- 2,5 Di - t - Pentylhydroquinone
- Diamylhydroquinone (DAH) CTFA
- Santovar A (Monsanto) (Trade name)
- Colipa n° A011

* Physical characteristics

A fine free-flowing buff-coloured powder
Odourless
Soluble in ethanol,acetone,ether
Insoluble in water

* Use

a) As an antioxidant in cosmetic foundation creams,cuticle softener,cream rouges,at a level not exceeding 0,2 %.
b) As an antioxidant for uncured rubber and used in adhesive films for their adhesive tape application.

* Recapitulation of the studies of toxicity

ORAL TOXICITY

- Acute : LD50 (rats) = 2 gr/kg/bw

- Long term-study

In a long term-study 115 male and 60 female rats were fed diets containing 0, 0.0125, 0.025, 0.05, 0.1 and 0.2% DAH. Body weights,food intake and general appearance were observed weekly for about 200 days then half the males of each group were autopsied and all females receiving a commercial laboratory diet. The remainder were autopsied at 500 days. Body weights of animals both at 200 and at 500 days were similar to those of controls.Weightsof organs for animals autopsied after 200 days did not change with increase in concentration. The gross appearance of these tissues and those from animals autopsied after 500 days did not differ significantly from that of control animals. Microscopic examination of tissue sections revealed no lesions which could be attributed to the test materials.

CUTANEOUS AND MUCOUS TOLERANCE

In an eye irritation test, 0.1 ml undiluted test material (ie 0.2%) in a make up foundation applied to the eyes of six albino rabbits with no rinse off caused slight conjunctivitis in all animals. Full recovery occurred in 48 hours. The cornea and iris membranes were unaffected.

In a skin irritation test, a total of 0.5 g of undiluted test material in a make up foundation,was applied to the intact skin of rabbits four times daily for seven days,without occlusion. No evidence of irritation was found.

When 0.2 ml of make up foundation containing 0.2% 2.5 ditertiary amyl hydroquinone was applied to the intact skin of 12 individuals, with occlusion,once daily for 21 days,no irritation occurred.

When a suspension of DAH made up by dissolving 0.1% of crystals in 5 ml of ethanol, adding 0.1 ml of Tween 80 and diluting to 100 ml with 0.9% NaCl solution was applied to the skin of guinea pigs,evaporation of the alcohol left a visible ,crystalline deposit which usually was not irritating.When dissolved in cottonseed oil, an erythema appeared

in 24 hours,disappearing in the next 24 hours. Repeated applications of the oil solutions increased hyperemia , led to papular eruption followed by scab-formation over entire area. When treatment was stopped, lesions gradually disappeared, leaving a normal-looking skin after about a month.Skin sensitization was not found.

CARCINOGENICITY

Tertiary amyl hydroquinone (20-50 µg/ml) is reported to have given significant protection against photodecomposition of benzo(a) pyrene 0.1-0.2 µg/ml irradiated with longwave UV light.

TERATOGENICITY

In a study of the effect of antioxidants on the incidence of foetal resorption in rats as compared with normal animals held on normal diets,2500 mg of 2,5-di-tert-amyl hydroquinone, given as a single dose on day one of gestation, gave a low percentage of resorptions (4.5% from 10 litters with 128 normal foetuses).

MISCELLANEOUS STUDIES

In a study of the effect of lipid antioxidants on normal rat erythrocytes,2,5 ditertiary amyl hydroquinone was non-haemolytic.

2,5-Ditertiary-amyl hydroquinone was found in extracts of several human tissues, including neurological tumours, adult brain and foetal brain. On further investigation it was also found in adhesive tapes used in the hospital laboratory to label glassware, but not in quantities such that would be responsible for contamination of the tissues. One possible source was thought to be an antioxidant preparation used in rubber manufacture.

CONCLUSION

Are required :
- Short-term toxicity test to establish a NEL (no effect level)
- Information on dermal absorption
  potential sensitization
  oral short-term test mutagenicity

(gene on mutation on bacteria and chromosomal aberration in mammalian cells)

Industry should be asked whether there is a need for this substance.

Classification : C

References :

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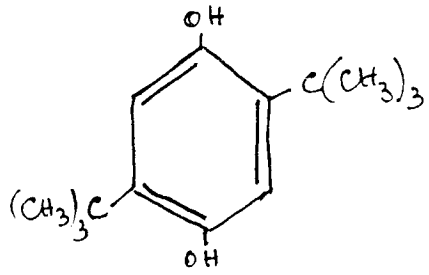
(4) D. B. H. Q. DITERTIARY BUTYL HYDROQUINONE

* Formula and synonyms

DBHQ = 2,5 - Ditertiary butyl hydroquinone is the aromatic compound that conforms to the formula :



Other names : - dibutylhydroquinone
- DBH
- Colipa A012

* Physical characteristics

Soluble in organic solvents,oils and alkaline solutions
Insoluble in water

* Use

As an antioxidant in decorative cosmetics (lipsticks,eyeshadows, coversticks) at a concentration not exceeding 0,2 %.

* Recapitulation of the studies of toxicity

ORAL TOXICITY

Acute toxicity : LD50 (chicken) : 10 g/kg

A 20% solution in ethanol given by gavage to one rat at doses of 500 and 1000 mg/kg caused mild depression. A 30% solution in cottonseed oil given to five rats at 1500 mg dose level produced slight diarrhoea. In each case the effect was attributed to the solvent.

When DBH was given to chickens in the diet at level of 2 gr or more/ kg toxic symptoms were noted.

Short- and long-term studies

Chicken : continued administration of 0,25% in the feed (time not stated) led to nervous symptoms,increased mortality, decreased growth reduced egg production and hatchability.

Oral administration of DBH at the dose of 200 mg/kg to dogs for 2 months and to rats for an undisclosed time produced no evidence of toxic effects.

In a long-term oral study on 115 male and 60 female rats DBH was added with thorough mixing to two diets to give the following concentrations 0, 0.0125, 0.025, 0.05, 0.1 and 0.2%. Body weights, food intake and general appearance were observed weekly for about 200 days. There was no difference between treated and control animals At the end of this time half the male animals and all the females receiving a commercially available laboratory diet were autopsied. Gross appearance of tissues and organ weights were recorded.
No adverse effects were noted. The gross appearance of tissues and those autopsied after 500 days did not differ significantly from that of control animals. Histology studies revealed no lesions which could be attributed to the test material.

CUTANEOUS AND MUCOUS TOLERANCE

In skin irritation tests on rabbits and guinea pigs ,0.1 ml of a 10% solution of DBH in ethanol or cottonseed oil was placed on the skin five times daily, for two weeks. Evaporation of alcohol left a visible,crystalline deposit which usually was not irritating.When dissolved in cottonseed oil erythema appeared within 24 hours. Repeated applications increased hyperemia,led to papular eruption followed by scab-formation over the entire area. When treatment was stopped,the lesions gradually disappeared.Skin appeared normal after 28 days.
It is not clear from the report how many animals were used,the site of application,whether the skin was intact or abraded, with occlusion or not.
In a sensitization test on guinea pigs,a suspension of DBH was made by dissolving 0.1 g of crystals in 5 ml of ethanol, adding 0.1 ml of Tween 80 and diluting to 100 ml with 0.9 NaCl solution.

No evidence of sensitization was observed. Again information is lacking on number of animals,induction route,exposure time and number of applications.

Two reports are available of allergy to DBH when used as an anti-oxidant in cream eyeshadow .Patch testing at 1% in methyl ethyl ketone was positive.The concentration used in the cream was given as 0.52 %.

MISCELLANEOUS INFORMATIONS

Intraperitoneal injection of 200 mg/kg DBH into rats five times weekly for 4 weeks is said to have caused no adverse effects.

In a study on possible depigmentation effect, 0.4% DBH was fed to guinea pigs in the diet for one week, then reduced to 0.2% for a further two weeks. No bleaching of hair occurred. When DBH was given five times daily for 2½ months to a black kitten a partial browning of hair occurred which remained irreversible after the treatment was stopped. During the final six weeks, the daily dose was 100 mg.

CONCLUSION

Are required :
- information on dermal absorption
- adequate data on cutaneous irritation and potential sensitization
- short-term oral toxicity to establish a no effect level (NEL)
- mutagenicity test (gene on mutation on bacteria and chromosomal aberration in mammalian cells.)

Classification : C

References

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REPORT BY THE SCIENTIFIC COMMITTEE ON COSMETOLOGY

ON THE USE OF

SODIUM HYDROXYMETHYLAMINOACETATE AS A PRESERVATIVE AGENT

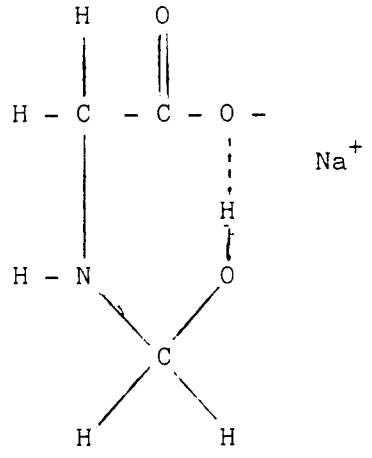
(opinion expressed on 20-2-87)

* The committee's mandate

To give its opinion on the use of sodium hydroxymethylaminoacetate as a preservative agent in a maximal concentration of 0.5% in cosmetic products.

* Formula and synonyms

$C_3H_6NO_3Na$
MW 127,1



Other names:
- sodium hydroxymethyl glycinate
- Suttocide A
- Colipa n° P84

* Characteristics

The compound is strongly alkaline
Highly soluble in water
Soluble in methanol,propylene glycol and glycerin
Insoluble in most organic solvents

* Use

A recently introduced preservative for use in cosmetics at concentrations of 0,05% to 0,5%.

* Recapitulation of the studies of toxicity

ORAL TOXICITY

Acute : LD50 (rat) 1,067 gr/kg/bw and 1,410 gr/kg

In a sub-chronic oral toxicity study, 4 groups of 10 rats/sex received by gavage 0 (control),10, 40 or 160 mg/kg/bw/day as a 2% aqueous solution for 90 days. There were no clinical signs of toxicity or changes in body weight gain, food intake, haematology,clinical chemistry or urine examinations. Gross or microscopic examinations did not reveal any treatment-related changes. It appears, therefore, that the dose-levels were too low to establish the target organ or the type of systemic toxicity induced by this compound. Otherwise, the study was well conducted and reported.
No signs of toxicity established in an oral 90-study at the highest dose level of 160 mg/kg/bw/day appears to indicate a low systemic toxicity. An effective dose and a target organ have therefore not been established.

CUTANEOUS AND MUCOUS TOLERANCE

Dermal LD50 (rabbit) > 2 gr/kg/bw
The undiluted material applied dermally under occlusion caused burned skin probably as a result of the alkaline properties.

Skin irritation tests in rabbits, showed a 5% aqueous solution to be moderately irritating, while a 0.5%solution revealed only slight, transient irritation.
In a repeated dermal application test, guinea pigs received 0.5 ml aqueous dilutions of 50, 7.5, 0.75 and 0.38% under occlusion on days 1, 3 and 6 of a one week period. No signs of oedema or irritation were observed.

Eye irritation tests in rabbits conducted with 100 mg undiluted powder showed moderate irritation when the eye remained unwashed, and mild irritation when the eye was washed after treatment.
A 5% aqueous solution was mildly irritating if not washed out,and not irritating if washing was applied.

Sensitization was examined in guinea pigs, by the Landsteiner test, the maximization test and the Buehler test.In the Landsteiner test,

0.1 ml 0.1% solution in saline was injected intradermally ten times, once every other day. After a 2 weeks rest period, the intradermal challenge injection of 0.05 ml 0.1% solution did not reveal sensitizing potency.
In the maximization test, the induction treatment consisted of 6 intradermal injections of 0.1 ml 5% solution, followed 8 days later, by topical application of 0.3 g moistened powder. On day 22, a topical challenge treatment with a 50 % aqueous dilution produced a positive reaction in 7 out of 10 animals. When the challenge was repeated with 5% and 0.5%, 7 days later, 4/10 respectively 2/10 animals reacted positively. These results indicate sensitizing properties of the test substance.
In the Buehler test, 0.5 ml 0.5% aqueous solution was applied topically 10 times during 3 weeks. After 2 weeks rest, the challenge with the 0.5% solution was negative in each of the ten test animals.

No information on irritation or sensitization in humans was provided. The substance showed slight skin irritation on rabbit skin and sensitizing properties in guinea pigs even at use concentration.

MUTAGENICITY

An Ames test with up to 0.5 mg/plate in 5 strains of S.typhimurium, with and without metabolic activation did not indicate mutagenic properties.

CONCLUSION

The available information shows many gaps which are considered unacceptable, especially for a new substance.
Therefore,evaluation is not possible until the information is available as required in the guidelines.

Are required :
- mucous irritation test
- skin sensitization test in humans
- systemic toxicity (NEL and target organ)
- mutagenicity test (chromosomal aberration in mammalian cells)

Note : The committee noted that the concentration needed for preservation of cosmetics is probably considerably less than 0.5 %.

Classification : C

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Subchronic Oral Toxicity

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Mutagenicity

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REPORT BY THE SCIENTIFIC COMMITTEE ON COSMETOLOGY

ON THE USE OF

3- DECYLOXY- 2-HYDROXY-1-AMINOPROPANE HYDROCHLORIDE AS A PRESERVATIVE

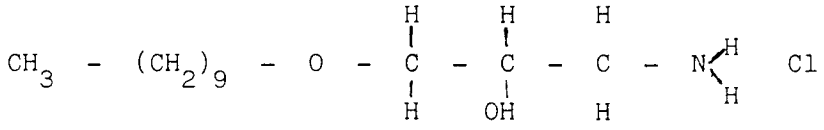
(opinion expressed on 20 February 87)

* The committee's mandate

To give its opinion on the use of 3-decyloxy-2-hydroxy-1-aminopropane as a preservative agent in cosmetics in a maximal concentration of 0,5 %.

* Formula and synonyms

$C_{13}H_{29}NO_2Cl$ MW : 267,5



Other names :
- Decominol
- Ster 4
- PVA 44
- Colipa P 87

* Characteristics

Soluble in water,ethanol,chloroform

Insoluble in hexane

* Use

Proposed for use as a preservative in all types of cosmetics up to 0,5%

* Recapitulation of the studies of toxicity

ORAL TOXICITY

Acute : LD50 (mouse and rat) : 1,2 gr/kg/bw
Damage to gastric mucosa found.

Subacute toxicity

Dog . Oral. Two male beagle dogs were given doses of a.i. in capsules The initial dose was 2.5 mg/kg/bw/ day for 3 days ; the dose was then increased for the next 3 days,and so on.The doses used were : 2.5,10, 40 , 160 and 640 mg/kg/bw/day, over 15 days.Some temporary drowsiness was noted after dosing at the 40 mg/kg level and above, and with some of the doses of 160 mg/kg/bw and all of the doses of 640 mg/kg/bw vomiting occurred. Blood was present in the faeces at the top dose. Some changes in clinical chemistry and haematology do not seem to be significant. At autopsy, ulceration or bleeding points were found in the gastric mucosa. Slight renal changes may have been drug-related.

Subchronic toxicity

Dog. Oral. A 13-week study was carried out in 8 m and 8 f beagles. The a. i. was administered by capsule at doses of 0, 0.2, 1.0 and 10 mgKg/bw/day for 91 consecutive days. No changes in behaviour were noted. The laboratory and autopsy findings were not markedly abnormal ; at the top dose there was a fall in the relative weight of the thyroid gland.

Rat. Oral. A 13-week study in 60 m and 60 f SPF rats was carried out by administering 0, 10, 40 and 160 mg/kg/bw by gavage as a 0.5 % aqueous solution. Behavioural changes were seen early in the experiment at dose levels of 40 mg/kg and above ; at the top-doselevel effects seen were piloerection, decrease in spontaneous activity, and neglect of grooming. Top dose males lost weight after 50 days. Clinical chemistry studies showed uraemia at 40 mg/kg (females only) and 140 mg/kg (both sexes). Tests for occult blood in the faeces were negative. At autopsy,dose related gastric gastric damage was seen macroscopically. An increase in relative liver weight,together with evidence of mesenchymal inflammation was seen at the top dose level.

Thus the NEL in this study was below 10 mg/kg/bw/day,but effects seen at this level were limited to local irritant effects on the gastric mucosa, presumably due to the direct irritant effect of a 0.5 % solution.

CUTANEOUS AND MUCOUS TOLERANCE

Mucous membrane irritation

Rabbit. A 1% solution of a.i. in water was used in a Draize test, with and without rinsing. Irritation was found in all cases, more marked in the eyes not subjected to rinsing. Complete recovery had occurred in a week.

Skin irritation

Rabbit. A 1% aqueous solution with occlusion was mildly irritating. Repeated daily applications of 2% aqueous solution for one month, without occlusion, gave slight irritation in the first week,but none thereafter.

Phototoxicity

Guinea pig. Twenty male animals were tested. A solution of 0.5 % of a.i. in ethanol/water (1:1) was chosen as the maximum dose which was non-irritant. The material was allowed to remain in contact with the skin for 15 minutes before exposure to ultra-violet light.Suitable controls were used. The test was negative.
In the phototoxicity test, 1% of a.i. in ethanol caused some erythema, but this solution diluted with water (1:1) was non-irritant.

Photo-allergenicity

Guinea pig. The animals used in the preceding test were used again. Three sensitizing exposures were carried out, each in the same way as for the phototoxicity tests (supra), at intervals of 3 days. After a 16-day rest, a challenge application and exposure to ultra-violet were carried out. Suitable controls were used. The test was negative.

Sensitization

Guinea pig. A Magnusson-Kligman maximization test in 10 animals was negative when 0.1 ml of 1% solution was used for intradermal induction and a 0.5% solution for the challenge.

MUTAGENICITY

An Ames test was negative up to 50 µg/plate ; 100 µg was strongly inhibitory to the organisms. A gene mutation test in CHO-cells (HGPRT-test) at concentrations up to 0.015 mg/ml was negative. Higher concentrations were not examined because of toxicity to the cells (ref. 18). A micronucleus test in female SD rats treated with one oral dose of 1000 mg/kg was negative.

SYSTEMIC TOXICITY

LD50	intravenously	(mouse)	60 mg/kg/bw
LD50	subcutaneously	(mouse)	2000 mg/kg/bw
		(rat)	4000 mg/kg/bw

Rat. Intravenous. Groups of 10 males received 0, 1.75, 3.5 and 7 mg/kg/bw/day for 15 days into the tail vein. The top dose animals developed necrotic changes in the tail, and thrombosis and fibrosis in the vein, and in them the intended course could not be completed. Abnormalities noted were piloerection and haematuria at all doses ; the haemoglobin and haematocrit values fell markedly in the top dose group. Autopsy showed relatively slight changes. The NEL in this experiment seems to have been $<$1.75 mg/kg/bw/day.

CONCLUSION

The tests show we have a skin and mucous irritating substance (even at the concentration of 1 %). It can also cause hepatic and renal damages in caseof systemic administration.
NEL is probably lower than 10 mg/kg/bw/day (oral administration in rats). Damages of gastric mucosa (dogs and rats) are found.
The oral absorption of the compound appears to be very poor.

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

Classification : C

REPORT BY THE SCIENTIFIC COMMITTEE ON COSMETOLOGY

ON THE USE OF

OMADINE MDS IN SHAMPOOS

* The Committee's mandate

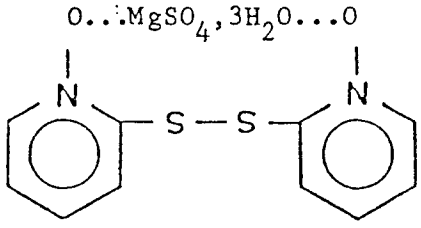
To give its opinion on the use of dithio-2,2'-bispyridine - dioxide 1,1' addition product with magnesium sulphate trihydrate in anti-danduff shampoos at the maximal concentration of 1 %.

* Formula and synonyms

EEC n°41

Colipa P 50

Chemical name : Dithio - 2,2' - bispyridine - dioxide 1,1',with magnesium sulphate trihydrate.



MW : 426,3

Other names : - OMADINE$^{(R)}$ MDS Trade name

- Pyrithion - disulphide magnesium sulphate
- [Bis(2- pyridyl - 1 - OXO) disulphide] magnesium sulphate trihydrate.

* Characteristics

OMADINE MDS is more soluble in water and ethanol than pyrithione itself.

* Use

Used as an anti-danduff agent in shampoos up to 1 %

* Recapitulation of the studies of toxicity

LD50 oral (rats) : 1,1 - 1,35 gr/kg
dermal (rabbits) : 8 gr/kg

ORAL TOXICITY

Acute : In rats single oral doses of 0,125 gr/kg/bw or above were associated with decreased activity, decreased respiration and prostation.
A single oral or intraperitoneal dose of 20 mg/kg in mice did not induce abnormalities in behaviour or growth rate and no microscopic changes were detected in tests or epididymides after 7-21 weeks.

A sub-chronic (90-day) oral study in rats was conducted with 1, 3, 10 and 30 mg/kg/bw/day. Signs of intoxication (abdominal hypotension, kyphosis, and hind leg ataxia) occurred in all treatment groups. No microscopic changes were found in the pancreas but a report on the microscopy of other organs was not available.

In a 6-month oral study in monkeys 5, 15 or 50 mg/kg (4/sex/dose) was administered daily by gavage. Decreased body weight and food intake occurred with 15 and 50 mg/kg. Mortality (isolated cases) occurred at all dose levels, but this was not dose-related and the significance is unclear.

An oral dose administered to rats is excreted mainly in the urine. The compound disappears from the blood in a rapid early phase (c. one hour) followed by a slow phase (20-100 hours depending on the species). The main urinary metabolite is the S-glucuronide of 2-mercapto-pyridine-N-oxide The predominant and persistent metabolite in blood is 2-methylsulfonylpyridine.

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

A skin irritation test in rabbits with 0.5 g (undiluted substance) produced distinct dermal changes. Aqueous solutions up to 5% did not produce skin irritation in humans.

No clear signs of phototoxicity were observed in guinea pigs treated topically with 0.1 ml of up to 5% suspensions in corn oil, followed by UV-A irrediation.

Distinct eye irritation occurred in rabbits with 0.1 ml of a 5% aqueous suspension (score 11/110). Severe eye irritation was induced in monkeys by applying 100 mg of the diluted powder, but 0.1 ml of a 1% aqueous solution was negative.

A sensitization test in guinea pigs using 0.1 ml of a 0.2% aqueous solution in Freunds complete adjuvant injected into the hind foot pad and scapula regions for induction and 0.05 ml of 0.1 and 0.5% aqueous solutions applied topically as the challenge dose produced negative results. Negative results were also obtained when a 10% aqueous solution was applied topically as both the induction and challenge treatment in a Buehler type test.

No photosensitizing properties were observed upon applying 0.1 ml of 1.5% suspension in corn oil and UV-A irradiation.

Dermal absorption has been shown to occur in animals and man.

- In man, 3-15% of a dermal dose was recovered in the urine after application of 4, 12 or 40 $\mu g/cm^2$ in methanol,without occlusion.

- In an in vitro study with abdominal skin of humans, exposure for 1 000 minutes (without rinsing), resulted in recovery of 14% in the horny layer, 0.6% in the epidermis, 1.0% in the corium and 1.0% in the penetration chamber.

- In an in vitro study with human scalp skin exposed for 10 minutes to an ointment containing 1% omadine, the amounts recovered were 1.76% in horny layer, 3.32% in epidermis, 1.32% in corium and 0.07% in penetration chamber.

- When rats were treated with a hair dressing formulation containing 0.1 omadine and applied to the back skin for 4 hours 4.45% of the applied dose was excreted in the urine and 0.31% in the faeces after 24 hours ; at the same time while the treated skin contained 2.55% of the applied dose.

- Dermal absorption in rats, treated with 0.15 ml of shampoos containing 1.5 , I.0 or 0.5% omadine was 0.42-0.66%,calculated from the total amounts detected in urine,faeces and carcass after 48 hours.

The proportion remaining in the treated skin was 4.15-4.76%. Assuming that 50% of the amount in the skin gradually enters the body, the total absorption would be 2.5-3.7% of the amount applied.

MUTAGENICITY

Equivocal results were obtained in a Salmonella assay for gene mutation. Some indication of mutagenic potential was obtained in one study, with an increase in revertants being seen at the highest concentration employed. However this was not confirmed in a repeat experiment. One in vitro test with Chinese hamster lung cells indicated that the compound had the potential to produce chromosome damage but the test was negative when repeated in a modified form. An increase in gene mutation was observed in Chinese hamster ovary cells, but the effects were not dose-related. No dominant lethal properties were observed in tests with mice and rats. The compound did not induce unscheduled DNA synthesis in hepatocytes of rats when tested at concentrations of 0.03 up to 30 ug/ml. This finding indicates absence of DNA damage.

CARCINOGENICITY

In a dermal carcinogenity study, mice were treated with 2 or 20 mg/kg three times/week for 18 months. The top-dose group showed increased mortality in both sexes, liver hypertrophy in males, a relatively high incidence of hepatocellular adenoma and carcinoma, and an increased incidence of malignant lymphoma. The authors considered the toxicological significance of the increased tumour incidences as unclear. Daily treatment of the skin of hairless mice with a 1% solution in 4.8% shampoo base for 20 minutes during 14 days did not affect proliferation of basal cells in the epidermis.

TERATOGENICITY AND REPRODUCTION

In a reproduction study, oral administration of 1.0, 3.0 or 7.5 mg/kg/bw/day to female rats from day 14 before mating through day 21 of the lactation period was associated with high mortality of females in the top-dose group (14/20) and several other signs of intoxication. In the mid-dose group the dams showed decreased numbers of implantations and of viable pups and increased pre-implantation loss. The authors

considered 1.0 mg/kg a no-effect level.

In a peri and post-natal study pregnant female rats were treated orally with 1, 3 or 7.5 mg/kg/bw/day from day 15 of gestation up to day 20 of lactation. Dams of the high-dose group showed mortality and other signs of intoxication. In this group there was a decreased number of viable pups and decreased survival and growth rate of pups during lactation.

Teratogenicity has been examined in several studies.

- In one oral rat study doses of 3, 10, 30 or 100 mg/kg were employed ; maternal toxicity occurred at the two high-dose levels. In addition to these two levels therewas an increased incidence of a major skeletal abnormality consisting of bifurcated ribs.

- In a dermal rat study with dose levels of 1, 10 and 30 mg/kg skin changes occurred in the dams of all groups. Signs of embryotoxicity and growth depression of the dams were seen only in the high-dose group.

- In two dermal studies in swine (with dose levels of 30, I00 and 300 mg/kg in one study and of 10 and 30 mg/kg in the other) signs of maternal toxicity and skeletal anomalies occurred at all levels. The number of sows in each group (4-6) was too small to allow any definite conclusion.

- An oral teratogenicity study in rabbits showed loss of body weight with 5 mg/kg. With 3 and 5 mg/kg post-implantation losses were too high to allow any conclusion concerning teratogenic potential No evidence of teratogenicity was found with 1 mg/kg.

Dermal applications at 5 mg/kg/bw/day were reported to produce foetotoxicity in rabbits (Colipa note of February 1984,no details given) while 30 mg/kg/bw/day dermally produced embryotoxicity in rats and 10 mg/kg/bw/day produced maternal toxicity in swine.The proposed application rate for man is about 2 mg/kg/bw/ application.
The margins between application rates and toxic levels are very small.

CONCLUSION

When evaluating the extensive information on this substance, it appears that toxicity occurs at low levels of exposure and that the no-effect level may well be lower than 1 mg/kg/bw/day.

There is evidence of mutagenic and teratogenic properties.

Dermal absorption is likely to be considerably higher than 0.07 % in humans and 0.03 % in rats.

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).

Classification : D

Information : COLIPA - Submission I : May 11, 1982
- Submission II : February 15, 1984
- Submission III : November, 1984
- Submission IV : June, 1985
- Submission V : September, 1985
- Submission VI : December, 1985
- Submission VII : November, 1986

1. Consumer Product Testing 1979
   Study n° 78437
2. Food and Drug Research Labs 1971 Lab n° 0497
3. " " " " 1971 Report n° 8549
4. Consumer Product Testing 1978 study n° 78107-5
5. Huntingdon Research Center 1973 Project n° 731-340
   Study listed on p. 10 was repeated due to excess amount of
   Omadine MDS on fur post exposure which was ingested during preening
6. Huntingdon Research Center 1973 Project n° 733-370
7. Consumer Product Testing 1978 study n° 78107-1
8. Food & Drug Research Labs 1971 report n° 0734
9. " " " " " " " 0821
10. Industrial Bio-Test 1973 report n° 601-03209
11. International Research & Development Corporation 1982
    report n° 397-039
12. Food & Drug Research Labs 1974 report n° 4074-1274
13. " " " " 1972 " " 8877

14. Consumer Product Testing 1979 report n° 78422

15. International Research and Development Corporation 1982 report n° 397-028

16. " " " " 1982 report n° 397-027

17. Industrial Bio-Test 1974 report n° 651-64101 B

18. " " " 1975 " " 651-04101 D

19. " " " 1976 " " 623-08160

20. International Research & Development Corporation 1981 report n° 397-029

21. Biodynamics 1981 project n° 80-2499

22. " 1977 " " 76-1631

23. International Research & Development Corporation 1980 study n° 397-023

24. Industrial Bio-Test 1976 report n° 622-08049

25. Bioassay systems 1980 report n° 10068

26. " " 1981 " " 10171

27. " " 1980 " " 10250

28. Industrial Bio-Test 1974 report n° 622-05693

29. " " " 1975 " " 622-06511

30. SRI International 1981 report n° LSC-4675 fiscal report - August 21 1981

31. " " 1981 " " LSC-4675 interview report - July 1981

32. Biometric Testing 1976 report n° A-1099

33. Pharmacologist 1974 16,252

34. Reference : Howard Maibach, M.D. 1977 Olin reports, also Wedig, J., Feldman, R., and Maibach, H. 1977. Percutaneous penetration of the magnesium sulfate adduct of dipyrithione in man.
Toxicol. Appl. Pharmacol. 41:1-6

35. Industrial Bio-Test 1975 report n° 632-06372

36. " " " 1975 report n° 632-06541

37. Bioassay systems 1983 report n° 11450

38. A.D. Little, Inc. 1983 report n° C-88661

39. The dermal absorption of Omadine MDS in rats HRC 25.7.84

45. Rougier et al (Subm V)
The measurment of stratem corneum reservoir
A predictive method for in vivo percutaneous absorption studies : influence of appl. time.
J. Invest. dermatol. 84,66 1985

46. Maibach & Wolfran
Percutaneous penetration of hair dyes, Journal Soc. Cosm. Chem. 32,223 1981

47. Report by Pr. Stottgen 3.12.85 (Subm VI)

48. Comparative absorption of pyrithiones (Subm VII)
D. Howes and J. Black
Toxicology 5 (1975) 209-220

- data sjeet Nat. Inst. Publ. Health, the Netherlands 1983
- toxicol. appl. pharmacol. 43 (1978) 373-379

COMPLEMENTARY OPINION ON HYDROXY-8-QUINOLEINE

(expressed on 20 February 1987)

(docs CSC/575/86 - XI/709/85)

Considering the non-effect dose : 75 mg/kgbw in the rat
150 mg/kgbw in the mouse
and the limitation of the use, the Committee can accept the use of hydroxy-8-quinoleine in a concentration of 0.03 % as a stabilizing agent of H_2O_2 in the non-rinsed capillary products.

CONTENTS

34th meeting of SCC - June 4, 1987

35th meeting of SCC - October 13, 1987

REPORTS BY THE SCIENTIFIC COMMITTEE ON COSMETOLOGY

34th REUNION - JUNE, 30th 1987

LIST OF PARTICIPANTS

Present

Mr. AGACHE
Mr. DE GROOT
Mrs. DONY
Mrs. ENJOLRAS
Mr. GOULDING
Mrs. KNAAP
Mr. LOPRIENO
Mr. MUSCARDIN
Mr. O'MAHONY
Mr. SHOU
Mr. STUTTGEN

Apologies for absence

Mr. HILDEBRANDT

Commission

Mrs. MASSE
Mr. COLLIN (expert)
Mr. GONTIER (DG XI/B/1)

SUMMARY

Opinions expressed on June 30th, 1987 concerning the use of :

- PRESERVATIVE AGENTS

1. Chlorhexidine (EEC n° 31)
2. Alkyl Trimethyl Ammonium Bromure (EEC n° 55, P72)
3. Germal II (P79)

- HAIR DYES

2 NPPD (B25)

REPORT BY THE SCIENTIFIC COMMITTEE ON COSMETOLOGY ON THE USE OF 1,4 - DIAMINO - 2 - NITROBENZENE

. THE COMMITEE'S MANDATE

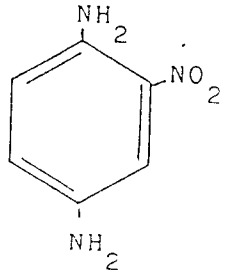
To give its opinion on the question whether the use of 2 NPPD as direct hair dye at the maximal concentration of 1% is admissible from the health point of view.

. FORMULA AND SYNONYMES

CI 76070

CAS Reg. n° 5307 - 14 - 2

COLIPA B25



$C_6 H_7 N_3 O_2$

1,4 - DIAMINO - 2 NITROBENZENE

2 - NITRO - 1,4 - PHENYLENEDIAMINE

2 - NITRO - 1,4 - BENZENEDIAMINE

2 - NITRO - 4 - AMINOANILINE

2 - NITRO - 1,4 - DIAMINOBENZENE

2 - NITRO - p - PHENYLENEDIAMINE (2 NPPD)

o - NITRO - p - PHENYLENEDIAMINE

DIAMINONITROBENZENE

m - NITRO - p - PHENYLENEDIAMINE

o - NITRO - p - PHENYL ENEDIAMINE

. USE

As direct hair dye up to 1%.
It is used as a direct dye or in combination with oxidant dyes.
It produces brown and red shades on the hair.
Production and use : 2500 Kg.

. PRECEDING CONCLUSIONS

SEC, SECOND SERIES, EUR 8634 (1983) P 7.

"In view of the positive carcinogenity findings in animals, at the doses used, the SCC recommends that its use might be discontinued. Nevertheless, this decision could be modified because of the product's low percutaneous resorption and because the carcinogenicity tests by the dermal route were conducted on a mixture of the substance and not with 2 NPPD alone".

17-10-87 : Banned in Italy and Denmark
Recommended for banning in F.R.G.

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral (rat) : 1800 - 3080 mg/kg
i.p. (rat) : 348 mg/kg

- Metabolism. 2 - NPPD 14 - C radiolabelled showed at 4 days after dosing to rats by differents route (oral, i.p. or s.c.) that ca. 4% of the dose was retained in the body, mainly in the intestinal tract and lesser in the liver and kidney. The metabolic pattern in urine showed 6 radioactive components for rat and 7 for mouse.

ORAL TOXICITY

Subacute toxicity : RAT Fischer 344 & Mouse B6C3F1. NCI bioassay. 5 males and 5 females/group received in the diet for 4 weeks period, followed by 2 weeks observation period, these doses of 2 - NPPD : 0-315-680-1465-3155-6800 ppm for rats and 0-810-1180-1740-2550-3750-5550-8080-11830 ppm for mice. The maximum tolerated dose was 1100 ppm for males and 2200 ppm for females rats and 4400 ppm for mice.

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

- Dermal irritation : the compound applied as a 2.5% (W/V) preparation (0.5% aqueous gum tragacanth containing 0.05% anhydrous sodium sulphite) resulted non irritant.
- Eye irritation : the compound applied as 2.5% (W/V) suspension (0.5% aqueous gum tragacanth containing 0.05% anhydrous sodium sulphite) on rabbit's eyes resulted "mildly irritating".
- Sensitization : was tested in Guinea pigs treated with 3% 2-NPPD solution containing 2% Natrosol, 2% Tween 80, 0.05% Sodium sulphite and 10% isopropanol (pH = 7) applied 6 days/week for 3 weeks, sensitization evaluated 2 weeks later. The results showed a weak reaction (4/20 animals have an allergic effect).
- Dermal absorption : (14-C) 2-NPPD 14-C (86µg) in ethanol solution (40 µl) showed a high penetration to the clipped skin of rats (males : 11.7 µg/cm2, females 24.6 µg/ml) and mice (31-36 µg/cm2). 6.3 µg/cm2 (4%) of 3-H labelled compound (157 µg/cm2 as a 0.8% (W/V) penetrated in the skin of rats at 48 hours after application of a 50% solution of a semi permanent hair colorant shampoo base for 20 min. before rinsing off, and a similar study 4.6 µg/cm2 (4.8%) of 14-C labelled compound (96 µg/cm2, 0.5%) penetrated in the rat skin after 48 hours after treatment with 50% aqueous shampoo solution for 20 min. and 6.4% after 30 min. (14-C) -2NPPD (4 µg/cm2 on clipped sin for 24 hours) showed a skin penetration of 29.9% (percent of the dose applied) in monkey (ventral surface of forearm) and 17.7% in swine (back site). Dermal absorption in rhesus monkey through the scalp was 0.55% of the applied dose.
- Short term dermal toxicity : 2-NPPD containing formulation (1.1% in water) tested on shaven intact or abraded skin of New Zealand rabbits by topical application produced no toxic effects at 3-7-13 weeks after treatment at the histopathological analyses.
- Human : The cutaneous resorption study of 2-NPPD (20 mM/1 in isopropanol) in vivo on man show that ca. 49% (24 h), ca. 67% (48 h) and 65% (72 h) of the compound penetrates in the skin. In the normal application 0.75% of absorption by scalp has been observed in a period of 30 days.

MUTAGENICITY

Studies have shown that 2-nitro-p-phenylenediamine is mutagenic :

(1) on Salmonella typhimurium in the absence and in the presence of S9mix ± hydrogen peroxide ;

(2) on mouse lymphoma cells for gene mutation induction ;

(3) on two chinese hamster cells lines for chromosome aberration (in vitro) ;

(4) on rat hepatocytes and HeLa cells for the induction of UDS, and DNA repair in E.coli.

The mutagenicity studies have shown that the compound does not induce dominant lethals in rats Charles River treated for 8 weeks with a dose of 20 mg/k (i.p.) and for 10 weeks until to 40 mg/kg (i.p.), micronuclea in rats Sprague Dawley treated with 2 g/kg (gastric intubation), SCE in bonve marrow of chinese hamsters treated i.p. (up to 300 mg/kg) or by gavage (up to 500 mg/kg), mitotic gene conversion in the yeats. The 2-NPPD induced morphological transformation in C3H/10T½CL8 and reduced lymphocite transformation.

CARCINOGENICITY

Long term studies were carried out on mice and rats by a NCI bioassay, the compound fed in the diet at 550-1100 ppm for males and 1100-2200 for females rats for 78 weeks (27 weeks observation period) and, 2200 and 4400 ppm for mice for 78 weeks (12-13 weeks observation period) showed that 2-NPPD resulted positive in the females mice producing a significative increase of hepatocellular neoplasms (adenomas) and no conclusive evidence for the carcinogenicity in males mice and rats were obtained. After the reevaluation of the hystopatology the presence of adenoma was confirmed in one case and it was excluded in a second case (parenchyma cell proliferation). A formulation containing 2-NPPD at 0.015% level tested in A and DBAf mice by repeated topical application in aqueous acetone solution showed lymphoid tumours in both strains, but only in DBAf the incidence was statistically significant. Other studies carried out on mouse and rat (formulation 7401 containing 1.1% of 2-NPPD), and dog (Dye/Base composite containing 0.24% : only summary conclusions, data not available for evaluation) resulted negative.

TERATOGENICITY

2-NPPD (suspensions in sterile distilled water : 0-32-64-128-160-192-224-256 mg/kg/day) administered once a day 1% b.w. (10 ml/kg) by subcutaneous injection on days 6-15 of gestation of female mice (CD-1) showed teratogenic effects and maternal toxicity in a same range doses (160 mg/kg/day and above). The compound showed a significant reduction in average weight gain by the dams during pregnancy (32 and 128-256 mg/kg) and in average fetal weights (128-256 mg/kg/day); it produced a significant increase in the average percent malformed fetuses : principally cleft palate and, also significant fused ribs, white foci in the left ventricle of the fetal heart (160 and above mg/kg) and bilateral open eye (224 mg/kg). The dose without teratogenic effects was 128 mg/kg/day. A retarded effect of ossification process (bones of the feet and the cervical and caudal vertebral centre) was observed in mice topically treated twice a week for four weeks before mating and until the' 18th day of gestation with a formulation containing 1% of 2-NPPD equivalent to 0.5 mg/mouse. No teratogenicity effects were observed with formulations containing the compound on rat (1.1%, 1% or 0.24%) and rabbit (0.24%). In two reproductive studies on rat treated with 0.24% or 1.1% 2-NPPD containing formulation no negative effects were observed. (One study was not available for the present analysis).

CONCLUSION

No other long term carcinogenicity studies have been produced last evaluation of this compound by SCC in 1980. The compound has been found in a main study carcinogenic for females mice only, but the results have been questioned on the basis of other pathological evaluation analysis. No carcinogenic effect was observed in male mice and male and female rats. The compound has been found mutagenic and genotoxic in vitro assay (bacterial and mammalian cells) for different genetic end points. In vivo mutagenicity studies (dominant lethal, micronuclea and SCE in bone marrow cells) have produced negative results. The low scalp penetration in human (0.75% after 30 days) and the low animal toxicity observed may suggest the absence of health risk by this compound.

The evidence for carcinogenicity is inadequate (no conclusive evidence). The evidence for mutagenicity is positive in vitro and negative in vivo. More appropriate genotixicity studies, namely DNA damage in mouse liver, DNA binding in liver cells, may provide evidence more adequate for final evaluation of the toxicological potential of this compound.

The SCC in his plenary meeting of June 30th, 1987 asked for the following information :

1. a UDS test in vivo on hepatic mouse cells to obtained detailes of the reaction potential to DNA ;
2. a short-term study by oral administration to determine the NEL.

CLASSIFICATION : C

BIBLIOGRAPHY

N. LOPRIENO & G. BONCRISTIANI : A review on toxicology of 1,4 - Diamino - 2 - nitrobenzene.
Pisa, Italy, Sept. 1986.

REPORTS OF THE SCIENTIFIC COMMITTEE ON COSMETOLOGY

CONCERNING THE USE OF CERTAIN PRESERVATIVE AGENTS

IN COSMETIC PRODUCTS

THE COMMITTEE'S MANDATE

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

- chlorhexidine at the use level of 0.5 %
- alkyl trimethyl ammonium bromide at the maximal use of 0.1 %
- diazolidinylurea at the maximal use level of 0.5 %

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

(1) CHLORHEXIDINE

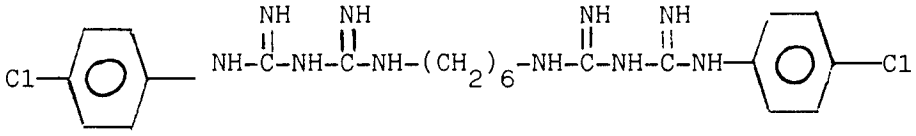
* Formula and synonyms

EEC n°31

Colipa P 24

1,6-di-(N-p-chlorophenyl-N'-diguanido)-n-hexane

(chlorhexidine)



$C_{22}N_{10}Cl_2H_{30}$

* Characteristics

Insoluble in organic solvents and fats

Poorly soluble in water

* Use

As a preservative agent at the use level of 0.5 % active ingredient.

* Recapitulation of the studies of toxicity

The IV LD50 = 25 mg/kg Values are comparable for acetate and gluconate.

ORAL TOXICITY

Acute : The oral LD50 (rats and mice) is relatively high : 2gr/kgbw

A sub-chronic (90-day) oral study in rats with levels up to 0.3 % chlorhexidine gluconate in drinking water revealed infiltration of lymphocytes and vacuolation in several organs, and giant cells in testicles and lymphnodes.Similar changes were seen in a rat study with the acetate .The no-effect level was 50 mg/kgbw.

A chronic oral rat study with the gluconate in the drinking water revealed histiocytes in mesenteric lymphnodes at all dose levels (5, 25 and 50 mg/kg b.w.).

SKIN AND MUCOUS TOLERANCE

DERMAL ROUTE TOXICITY

Eye injury was found by E.M. examination of rabbit eyes treated with aqueous solutions of the gluconate at concentrations of 0.1% and higher.

Despite extensive use in man, sensitization has not been a problem.

A sub-chronic (90-day) dermal study in monkeys, bathed daily with a skin cleanser containing twice the normal level of chlorhexidine gluconate and subsequently rinsed with water, was negative.
After 8 weeks of treatment no

Dermal application of ^{14}C-labelled chlor hexidine as a 5% aqueous dilution or as a 4% handwash formula to human forearm skin, without occlusion, for 3 hours, and measuring labelled material in urine and faeces for up to 10 days, revealed only 0.009% of the dose applied in the faeces, while the urine did not contain measurable levels ($<$0.5 mg/l urine).
Repeated scrubbing the forearm skin of healthy humans with 5 ml of a 4% preparation or 3 minutes, 5 times daily, 5 days a week, for 3 weeks, did not result in detectable levels in the blood.
Hospital staff washing hands usually 5 times each day with 5 ml of a 5% digluconate preparation for one minute, followed by rinsing with water and then repeating the washing procedure for a further 2 minutes, failed to show detectable blood levels ($<$ 0.01 mg/ml) when examined after 3 weeks exposure (Case et al. Chemotherapy 3 (1976 367 - 374); Colipa subm. II, ref.2).
Babies were bathed daily by applying a small quantity (amount not specified) 4% chlorhexidine detergent-based preparation to the head (excluding the face) and washing off with water. The preparation was then applied to the rest of the body. Next, the baby was immersed in water and dried. Blood samples taken at 1 and 4 hours after bathing showed low levels of chlorhexidine (less than 1 µg/ml) in only 15 out

of the 34 babies examined (Cowen et al. Arch. Dis. Children 54 (1979) 379-383 ; Colipa subm. II,ref.4).

In another study, 51 babies were sponged from the neck down with 30 ml of a 1:10 aqueous dilution of a 4% chlorhexidine gluconate solution. Blood samples taken after one hour showed no detectable levels of the test compound (i.e. 0.1 µg.ml) (O'Neill et al. Current Therap Res. 31 (1982) 485-489 ; Colipa subm. II,ref 5).

MUTAGENICITY

Mutagenic properties of chlorhexidine were established in an Ames test. A positive effect was found also in a DNA-repair test with E. coli.

A chromosome aberration test in Chinese hamster ovary cells in vitro with 1, 10 and 100 µg/ml,conducted with and without metabolic activation, was negative.

In a micronucleus test in mice, two intravenous applications of up to 2 x 30 mg/kg (the maximum tolerated dose) did not increase the number of polychromatic erythrocytes containing a micronucleus.

CONCLUSION

The potential dermal exposure of humans to chlorhexidin is close to the lowest level which induced lymphatic histiocytosis in a chronic rat study. Nevertheless the safety margin is probably sufficient, because percutaneous absorption in humans is only minimal.
Mutagenicity tests in microbial systems showed positive results,but a chromosome aberration test and a micronucleus test were negative.

Classification : A

The minimal percutaneous absorption authorizes the classification A though the NEL was below 5 mg/kgbw in the rat.

Information

- Data sheet National Institute of Public Health - The Netherlands
- Toxicol. appl. Pharmacol. 52 (1980) 255-261
- Colipa dossier : - Submission I, January 24, 1984
  - Submission II, February, 1987
- BLAISE and TOXLINE
- Cowen et al. Arch. Dis. Children 54 (1979) 379-383
- O'Neill et al. Current Therap. Res.31 (1982) 485-489
- Case et al. Chemiotherapy 3 (1976) 367-374

SUBM I - January 1984

1. L'OREAL - Laboratories of "Recherche Fondamentale"
   Department of Toxicology
   25 July 1983

SUBM II - February 1987

2. D.E. Case et al 1976
   "Chlorhexidine : Attempts to Detect Percutaneous Absorption in Man" Chemotherapy, Vol. 3, 1976, p. 367-374

3. D.E. Case 1980
   "Chlorhexidine : Attempts to Detect Percutaneous Absorption in Man" Int. Congr. Symp. Ser. - R. Soc. Med. 1979 (publied : 1980) Series n° 23, p. 39-43

4. J. Cowen et al 1979
   "Absorption of Chlorhexidine from the Intact Skin of Newborn Infants"
   Archives of Disease in Childhood, 54, 1979, p. 379-383

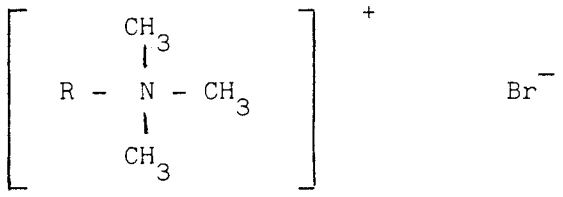
5. J. Oneill et al 1982
   "Percutaneous Absorption Potential of Chlorhexidine in Neonates"
   Current Therapeutic Research, Vol. 31, n° 3,
   March 1982 p. 485-489

6. Noël De Graeve 1985
   "Evaluation of Clastogenic Potential of Chlorhexidine Digluconate Using Chinese Hamster Ovary-Cells"
   U.T.G., Genetic Toxicology Testing Unit - Sart Tilman (Liège) - Belgique - Report 05.07.1985

(2) C. T. A. B.

* Formula

Alkyl (C_{12} - C_{22}) trimehtylammoniumbromide/chloride



R = alkyl MW 364,48

* Characteristics

Soluble in water and alcohols
Insoluble in ether

* Use

As a preservative agent in cosmetics at the use level up to0.1 %.

* Recapitulation of the studies of toxicity

PARENTERIC TOXICITY

LD50 intraperitoneal (rat) : 56 mg/kg bw
(mouse) : 39,8 mg/kg bw
(rabbit) : 125 mg/kg bw
subcutaneous (mouse) : 75-80 mg/kg bw

Single intravenous administration of 4 mg/kg to dogs caused a fall in blood pressure, while 12 mg/kg i.v. in monkeys produced traces of haemoglobin in urine without severe effects.

ORAL TOXICITY

Acute : LD50 (rats) : 1 gr/kg bw

In a sub-acute oral study, groups of 6 mice fed a diet containing 0.1 % or more showed growth depression ; with 0.2 % they died in 35-63 days, while with 0.4 % the survival period was only 9-12 days. Mice fed 0.5 % in the diet died between 3-12 days,showing destruction of mucosal epithelial cells and haemorrhages in the intestinal tract; with 0.05 % marked hypertrophy and hyperplasia of the duodenal and jejunal mucosa occurred after 3 months, but with 0.02 % no changes were found after 6 months.

In a one-year oral study, rats received 0, 10, 20 or 45 mg/kg bw in drinking water. The top-dose group showed reduced weight gain and food conversion, wetting and brown discolouration of the fur over the anterior ventral region and increased caecal weight. Caecal enlargement also occurred with 20 mg/kg.No changes were seen with 10 mg/kg.

Upon oral administration of the ^{14}C-labelled compound to rats, 80 % of the dose was still present in the gastrointestinal tract after 8 hours, and 20 % was excreted in the bile during the first 12 hours. These results indicate slow intestinal absorption. Only small amounts were found in the organs, which disappeared largely within 4 days. Excretion of radioactivity in the faeces and urine amounted to 92 % and 1 % respectively within 3 days. Examination of bile and urine by TLC indicated that some metabolism occurs in the rat. No radioactivity was found in expired CO_2.

SKIN AND MUCOUS TOLERANCE

DERMAL TOXICITY

A primary skin irritation study in rabbits with a 1 % solution did not produce any changes. With 5 % there were mild effects (erythema, oedema, scab formation) lasting less than 48 h.; with 25 % there was mild tomoderate erythema and oedema, while with 50 % thse changes were moderate to severe. Dermal application of 1 % to humans produced reactions in 2.8 % of the treated subjects.

14 cases of hypersensitivity to this substance have been reported when used in treatment of burns. In another study, skin sensitivity was confirmed by patch testing in 46 patients.
Eye irritation occurred in the rabbit eye with concentrations of 0.1% and above.With 0.1 % the changes disappeared in 24 hr.

The percutaneous absorption of the ^{14}C-labelled substance in the rat was 3.15 % from a 3 % aqueous solution which was not rinsed, 0.093 % from a 0.5 % concentration in a hair rinse formulation (rinsed after 5 min.) and 0.59 % from a 1 % aqueous solution (rinsed after 15 min.)

MUTAGENICITY

Mutagenic properties were examined in an Ames test with S.typhimurium strains TA 1535, TA 1537 and TA 1538,both with and without metabolic activation and using up to 500 µg cetyl trimethyl ammonium chloride/ plate. The results did not show any significant increase in the number of revertant colonies.

Another Ames test with 5 strains of S. typhimurium with and without metabolic activation and using up to 625 µg/plate,was likewise negative.

A test with E. coli by the Mohn method in vitro, with up to µg test compound/ml did not reveal any mutagenic potential.

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

TERATOGENICITY

A teratogenicity study in mice, treated once intraperitoneally with 10.5 or 35.0 mg/kg bw on day 8, 10, 12 or 14 of gestation, revealed an increased incidence of cleft palate,and skeletal defects in skull and sternum in both treatment groups, and embryolethality in the high-dose group.

In a teratogenicity study in rats, groups of 20 animals were treated daily by gavage with a substance designated Bromal Lot no.16593 at levels of 8, 25 or 50 mg/kg from day 5 through day 14 of pregnancy. The top dose group showed signs of toxicity both in the dams and in the foetuses, and an increased incidence of unossified bones and of several visceral abnormalities. The authors considered all defects congenital in origin, not indicative of teratogenicity. No increased incidence of abnormalities occurred in the intermediate dose group.

CONCLUSION

This substance has shown considerable systemic toxicity including indications of teratogenicity.
However,absorption through the intestinal wall and through the skin is low.
A chromosomal aberration test on mammalian cells is needed.

Classification : B

(3) DIAZOLIDINYLUREA

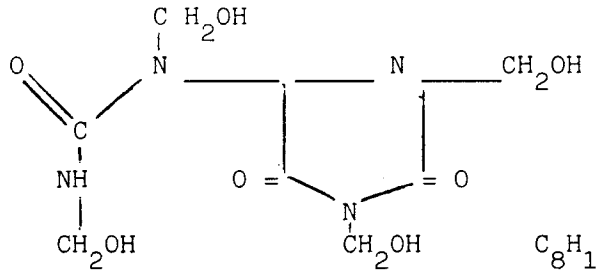
* Formula and synonyms

- N - (hydroxy methyl) - N - (1,3- dihydroxymethyl - 2,5 - dioxo - 4 - imidazolidinyl (- N' - hydroxymethyl) urea.

Germall II

CEE n° 17

Colipa P 79



$C_8H_{14}N_4O_7$

MW 278.23

* Characteristics

Soluble in water

Insoluble in most organic solvents

* Use

Use level as a preservative up to 0.5 %

* Recapitulation of the studies of toxicity

ORAL TOXICITY

Acute : LD50 (mouse) : 3700 mg/kg bw

LD50 (rat) : 2570 mg/kg

A 7-day oral toxicity test was carried out in which the a.i. was given by gavage to groups of 5 rats/sex in doses of 0, 100, 200, 400, 800 and 1600 mg/kgbw/day. There were slight transient behavioural changes at 200 m/kg and more marked and prolonged ones at 400 mg/kg and above.Necropsy showed gastric changesin some animals.

A 29-day oral toxicity test in rats was carried out , in which the a.i. was given by gavage to groups of 5 animals/sex in doses of 0, 100, 300 and 900 mg/kg bw/day. All animals survived. There were transient behavioural changes at 100 mg/kg, and more severe and long-lasting changes at higher doses. There was a reduction in gain of body weight, and a decrease in food intake, in top dose males. The top-dose rats showed gross and microscopic evidence of gastric lesions, and the weight of the adrenals was increased. Similar changes, though less marked, occurred with 300 mg/kg. The no-toxic effect level was stated to be greater than 100 mg/kg bw/day.

In a 90-day oral rat study , the test substance was fed in the diet at levels providing 0, 10, 25 or 100 mg/kg bw. There were no treatment-related changes in growth rate, haematology and clinical chemistry or in the results of the pathological examinations. Therefore,100 mg/kg was considered to be a no-toxic effect level.

The supplementary information received since the previous evaluation confirms an oral no-effect level of 100 mg/kg bw or slight lower.

CUTANEOUS AND MUCOUS TOLERANCE

The dermal LD50 (rabbit) when applied undiluted as a powder is >2000 mg/kg.
Skin irritation tests in rabbits with 0.5 ml of 1.0 % and 5.0% solutions in water were negative.

An eye irritation tst in rabbits with 0.1 ml of 1.0 and 5.0 % aqueous solutions did not induce any changes.
In a sensitization test in guinea pigs by the Landsteiner-Draize method, the induction treatment consisted of 10 intracutaneous injections of 0.1 ml 0.1 % in saline solution.After a 2 weeks rest period, 0.05 ml of a 0.1% solution was given intracutaneously as a challenge. The test was considered negative.

In a maximization test in guinea pigs, induction was performed with the undiluted substance applied topically, and with a 5% solution applied intradermally. The induction areas were pretreated with sodium lauryl sulphate. The response to the challenge treat-

ment,conducted 2 weeks later, suggested the test substance to be a weak sensitizer.

Phototoxicity tests in groups of 9-11 human subjects with 0.2 g subscreen cream or sunscreen lotion containing 0.5% diazolidinyl urea,did not induce signs of phototoxicity,when applied 10 times on a closed patch for 24 hours, followed by UV- or UV-A-irradiation at a distance of 10 cm during 15 minutes. A similar phototoxicity test in 11 subjects with one application of 0.2 g sunscreen lotion containing 0.5% and irridiated with UV-A was likewise negative.

A sensitization study in 110 women was conducted with a sunscreen formulation containing 0.25% of the preservative applied with 10 successive patches under semi-occlusion. The challenge patch with the same formulation,after a 2 week rest, resulted in only one dubious positive reaction. No cross-sensitization was observed in 71 patients (who had shown sensitization to various preservatives and under other chemicals) upon patch testing with 0.5 and 1.0% Germall II in water petrolatum.

In several photosensitization tests, groups of 28-30 human subjects were treated with 0.2 g of 0.5% in a sunscreen cream ,or in a sunscreen lotion 10 times, each time for 24 hours and each treatment followed by UV-, UV-A- or UV-B- irradiation for 15 minutes.After a 10-18 days rest period, the challenge treatment with an occluded patch for 24 hours followed by UV-, UV-A- or UV-B- irradiation did not reveal photoallergic properties, although one slight transient reaction was observed.
A skin cleanser with 0.3% was examined in a prophetic patch test on 104 subjects and in a repeat insult patch test on 52 subjects.
No positive reactions were observed (summary report only).

MUTAGENICITY

The Ames test with dose levelsup to 800 µl/plate repeatedly showed relatively high numbers of revertants. The results were inconclusive. A micronucleus test in the mouse with oral dose levels of 1200, 2000 and 2800 mg/kg was negative.

CONCLUSION

The confirmed NEL of 100 mg/kg bw 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,
- and of a gene mutation in mammalian cells become available.

Classification : B

SCIENTIFIC COMMITTEE ON COSMETOLOGY

List of participants

35th meeting -13 October 1987

Present

Mr. DE GROOT
Mrs. DONY
Mrs. ENJOLRAS
Mr. FIELDER
Mr. GOULDING
Mr. LOPRIENO
Mr. MUSCARDIN
Mr. O'MAHONY
Mr. SCHOU
Mr. STUETTGEM

Apologies for absence

Mr. AGACHE
Mr. GRANADOS JARQUE
Mr. HILDEBRANDT
Mrs. KNAAP
Mr. RAMOS MORGADO

Commission

Mrs. MASSE
Mr. COLLIN (expert)
Mr. GONTIER (DG XI/C/2)

SUMMARY

Opinions expressed on October 13th, 1987 concerning the use of :

- PRESERVATIVE AGENTS

1. HEXETIDINE (EEC n° 15) (P20)
2. BENZYL FORMAL (EEC n° 16) (P21)
3. CHLORACETAMIDE (EEC n° 22)
4. PHENOXY PROPANOL (EEC n° 56) (P54)
5. SUTTOCIDE A (P84)

- COLOURING AGENTS

1. CI 13065
2. CI 42535
3. CI 42555
4. CI 44045
5. CI 73900 (46500)
6. CI 61554
7. ACID RED 195

- HAIR DYES

4 NOPD (B24)

REPORT OF THE SCIENTIFIC COMMITTEE ON COSMETOLOGY

CONCERNING THE USE OF CERTAIN PRESERVATIVE AGENTS

IN COSMETIC PRODUCTS

(opinion expressed on 13 October 1987)

THE COMMITTEE'S MANDATE

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

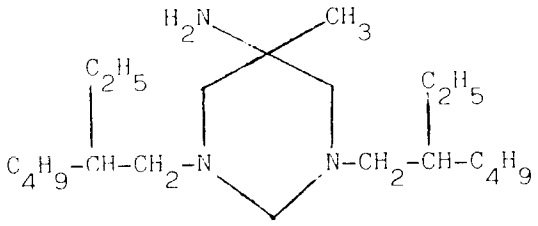
- Hexetidine
- Benzylformal
- 2-Chloroacetamide

- Phenoxypropanol
- Suttocide A

in their usual level is admissible from the health point of view.

(1) HEXETIDINE

* Formula and synonyms



$C_{21}H_{45}N_3$

MW 339.60

CAS Reg.no. 141-94-6

5-amino-1,3-di-(2-ethylhexyl) - hexahydro-5-methylpyrimidine

Other names : Hexetidine
Sterisol
EEC n° 15
Colipa P 20

* Characteristics

A viscous oil with a density of 0,87 g/ml
Practically insoluble in water
Easily soluble in ethanol, acetone and other organic solvents

* Use

Used up to 0.2% as a preservative agent in cosmetics.

* Recapitulation of the studies of toxicity

ORAL TOXICITY

Acute : LD50 (rat) = 1.43 g/kg bw

In a 3-wk oral test in rats with 20, 50, 100 and 200 mg/kg/day, the top dose caused mortality ; with 100 mg/kg there was reduced erythropoiesis. No changes were seen at lower levels.

In a one year study, rats received 0.02, 0.05 or 0.1% in the diet. Growth retardation and decreased food concumption were seen in the top dose group. No treatment-related changes were found upon microscopic examination. (Only summary report is available).

In a sub-chronic (90-day) oral study, rats received 0, 5,15 or 50 mg/kg bw in the diet. There were no deaths or clinical signs of toxicity. Growth depression, decreased intake of food and water,changes in biochemical blood parameters and increased organ-to-body weight ratios for several organs,occurred in the top-dose group.Microscopically, the top dose rats showed signs of systemic lipidosis. No detailed report of the pathological findings was available. It is stated that 300 ppm was a no-effect level (or 27 mg/kg bw).

An oral dose of ^{14}C-hexetidine in rats (20 mg/kg) and dogs (10 mg/kg)was rapidly excreted. After 72 hours 60-70% was recovered in the faeces and 20-25% in the urine, while 1-2% was found in the liver and kidneys.

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL ROUTE TOXICITY

No skin irritation or sensitization was observed in 200 volunteers examined for primary irritation with 1% in an ointment. A repeated insult test in 50 humans with 1% in oil caused mild irritation ;10 and 5% in oil caused strong primary irritation but no sensitization.

Very slight eye irritation was seen in rabbits with 0.1% ointment, moderate irritation with 4% aqueous dispersion, and with the undiluted substance, and severe irritation with 25% ointment and 50 % dispersion.

A 0.1% aqueous solution used twice daily as a mouth wash by 200 volunteers for 3 months did not induce clinical signs, primary irritation or sensitization.

In a study in 327 patients with cervicovaginal infections 0.1% as a gel and 0.5% solution did not induce irritation, sensitization or systemic toxicity.

In a skin sensitization test in guinea pigs, induction with 10 intradermal injections of 0.001, 0.005 and 0.01% in acidic saline,followed by one intradermal injection of 0.001% and topical application of a

25% in absolute ethanol after 14 days, no signs of sensitization occurred. Repeating the challenge treatment after one week, and also after 2 weeks, was likewise negative.

Daily application of 50, 100,200 or 500 mg/kg to rats for 3 weeks (5 days/week) caused mortality in the top-dose group. No changes occurred at lower levels.
Dermal treatment of rabbits with 0.0625 up to 4.0 ml/kg/day for 90 days caused mortality and growth retardation with 0.25 ml/kg and above. No effects on haemopoietic and urogenital systems were seen at any treatment level (Summary reports only).

Dermal LD50 in rats : 1.86 ml/kg bw
in rabbits : 4 ml/kg.

MUTAGENICITY

An Ames test with 5 strains of S.typhimurium and dose levels up to 5 mg/plate was negative. A chromosomal aberration test with CHO-cells in vitro was negative (The exposure concentrations used in the latter test are not clearly indicated in the report ; it is stated in the German text that the highest test concentration was a $6x10^{-5}$ dilution (Colipa subm. I,ref. 1).

TERATOGENICITY

In a teratogenicity study in rats ,groups of 20 females were treated orally with 12.5, 25 or 50 mg hexetidine/kg bw/day by gavage on days 6-15 of pregnancy. Forty control females were treated with the vehicle (corn oil) only. The top-dose induced growth retardation and decreased food intake of the dams. There was no indication of foeto-toxicity, embryolethality or of a teratogenic response, in this well conducted and reported study. (Colipa subm I, ref.2).

PARENTERIC TOXICITY

LD50 (intraperitoneal in mice) : 0.03-0.085 g/kg; intravenous administration of 5 mg/kg was lethal for cats.

CONCLUSION

The substance showed considerable systemic toxicity in animals and comparison of oral and dermal toxicity in rats indicates high dermal absorption.
No teratogenic or mutagenic properties were detected.
The oral NEL in rats was 27 mg/kg bw/day.
Dermal exposure in humans from use in cosmetics will be less than 1 mg/kg.

Classification : A

Information

- Data Sheet Council of Europe
- Environmental Safety Laboratory, Unilever Research, Colworth House, England.
  Dossier Safety Evaluation of Hexetidine. Vol. I and II
  Document : D 83/037, August 1983.
- TOXLINE 1974 - 1984, MEDLARS 1981 - 1984 and RTECS have been searched.
- Colipa submission I, May 1987.

SUB I - May 1987

1. Test report of the laboratory of Dr. Leimbeck,
   dated April 2, 1987 Bad Kissingen

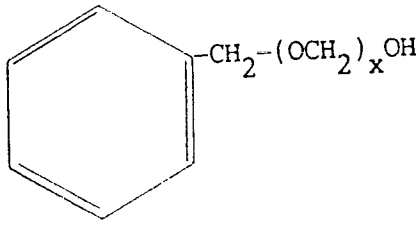
2. W.E. Parish
   "Hexetidine rat teratology study : effects on pregnancy and post-natal development"
   Vol I and II
   Environmental Safety Laboratory Unilever Research - England
   24 July 1986

(2) BENZYLFORMAL

* Formula

EEC n+ 16

Colipa P 21



Benzylformal

Mixture of benzyloxymethanol and benzyloxymethoxymethanol

Preventol

* Characteristics

Soluble in organic solvents

Solubility in water 25 gr/l

* Use

Used up to 0,2% as a preservative agent in all types of cosmetics.

* Recapitulation of the studies of toxicity

LD50 oral (rats) : 1700 mg/kg bw

I.V. (rats) : 153 mg/kg

dermal (rats) : > 1000 mg/kg

(rabbit) : 1429-2000 mg/kg

ORAL TOXICITY

Acute ; LD50 oral (rat) : 1700 mg/kg

There is no information on short- or long-term oral toxicity.

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL ROUTE TOXICITY

A skin irritation test in rabbits with 500 mg undiluted substance applied to the intact skin of the ear for 8 hours induced redness and oedema ; when applied for only 2 hours, slight redness was observed. A 0.2% aqueous solution applied for 24 hours did not induce any changes.

In an eye irritation test in rabbits 50 mg undiluted substance caused erythema and oedema and an opaque cornea.A 0.2% aqueous dilution only produced erythema.

A sensitization test by the Landsteiner-Draize method with 0.1% of the test substance in saline both for the induction and for the challenge treatment did not reveal signs of sensitization.

A sub-chronic (90-day) dermal study was conducted in groups of 10 rabbits/sex which received 0, 1, 4 or 16 mg /kg b.w. daily,5 days/ week. With 4 and 16 mg,the skin showed dose-related changes at the site of application. In the top dose group, growth depression occurred in females and decreased cholesterol values in both sexes. The weight of the pituitary was decreased in males of the intermediate- and high-dose group, however, no treatment related pathological changes were found in the internal organs . One mg/kg was a clear no-effect level in this well conducted dermal study.

MUTAGENICITY

An Ames test with up to 500 g/plate was positive for S.typhimurium TA 100. This result is attributed to the presence of 29.7% formaldehyde in the product. In a micronucleus test, male and female mice received 2 x 500 mg/kg with an interval of 24 hours. No increase in the incidence of micronuclei was observed.

TERATOGENICITY

There is no information on reproduction or teratogenicity.

CONCLUSION

The substance liberates formaldehyde (at a maximum of 0.004% under test conditions, according to D r. Crisp of the U.K. Laboratory of Governmental Chemists).
There is no information on short- or long-term oral toxicity, on reproduction or teratogenicity. A chromosomal aberration test is also lacking. Although studies on dermal absorption are not available high uptake through the skin is suggested by comparing oral and dermal LD50-values. In a sub-chronic dermal study in rabbits, 1 mg/kg bw/day was a no-effect level. One mg/kg bw/day might be also the dermal exposure in humans if 0.2% is used in all types of cosmetics. It is doubtful, therefore, whether continued use of this substance is justified.
Therefore, informations are required on :

- dermal absorption in humans
- a chromosomal aberration test in mammalian cells in vitro
- a short-term oral toxicity study to establish systemic effects

Classification : B

Information

Data Sheet Council of Europe

Sub I - Avril '84

1. Bayer AG, test of Toxicology, report n° 1002
   18.10.1968
2. Study n° TO 004.574
   "90-Tage-Hauttoxizität aus Kaninchen unit Preventol D2"
   von Heyden GmbH, Toxicologie, Werk Regenisburg 13.1.1983
3. Bayer AG, Institut f. Toxikologie, report n° 10.619 5.2.1982
4. " " " " report n° 10.497 12.1.1982

Sub II - Mai '87

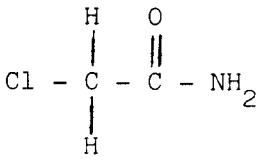
5. Mobay Chem. Corp. Stilwell Kansas (report 67442)

Sub III - Sept '90

6. Bayer AG. Preventol D2 - Subakute toxikologische
   Untersuchunger an Wisar Ratten (Verabreichung mit der
   Mogensonde über 28 bzw. 29 Tage)
   Bencht n° 19293 20.7.90

(3) 2 - CHLOROACETAMIDE

* Formula



C_2H_4ONCl

EEC n° 22

Colipa P 27

* Characteristics

The product manufactured in the EEC is 99.5-99.8% pure (impurities are ammonium-chloride 0.1-0.2% and monochloracetic acid 0.08 %).
Moderately soluble in water (5%)
Easily soluble in ethanol

* Use

Used mainly as a mixture of chloroacetamide and sodiumbenzoate (70/30), in rinsed off products at the level of 0.15%, in non-rinsed off products at the level of 0.3%.

* Recapitulation of the studies of toxicity

LD50 (of a mixture of 70/30 mixture of chloroacetamide and sodium benzoate)

Oral (rats) = 0.37 gr/kg bw
(mice) = 0.15 gr/kg

LD50 (intraperitoneal) = 50 mg/kg.

ORAL TOXICITY

Acute : LD50 (rat) = 0.37 gr/kg (mixture 70/30)
(mice) = 0.15 gr/kg

In a 13-wk feeding study in rats with levels providing 12.5 and 50.0 mg/kg bw/day, marked testicular atrophy occurred at each dose level.

Increased weight of the thyroid was also observed (no further details provided). A recent 90-day oral study in rats was conducted with a 99.7% pure substance at levels of 0,20,100 and 500 mg/kg diet. The top-dose induced growth depression, increased leucocyte counts, reduced weight of testicle and liver, suppression of spermiogenesis and proliferation of Leydig cells. No treatment-related changes occurred with 20 and 100 mg/kg diet (Colipa subm. IV).

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL ROUTE TOXICITY

A 1% solution was not irritating to the skin of guinea pigs.

Single patch testing of 209 dermal patients with 0.5% of the 70/30 mixture (containing 99% pure chloroacetamide, solvent not mentioned) did not result in any skin reaction, even after exposure of several persons to sunlight (ref. 1, subm. II; only summary report).

In another single patch test with 0.1% aqueous solution of a similar mixture in 200 dermal patients, no skin reactions were seen.Repeated application of the same daily for 14 days to 10 healthy persons and to 15 persons with allergy for benzoic acid, p-hydroxybenzoic acid ester, or peruvian balsam,did not induce irritation (ref. 2,subm. II; only summary report).

Single patch testing of 296 eczema patients with 0.2% aqueous chloroacetamide (99% pure) induced skin reactions in 7 patients (2.3%) (ref. 3, subm. II, summary report). Single patch tests with the 70:30 mixture of chloroacetamide (99% pure) and sodium benzoate did not induce irritation in 14 humans treated with 1%, in 10 humans treated with 0.5% or in 102 humans treated with 1% (ref. 10, subm. III). Daily patch testing of 25 subjects with 0.1 or 0.2% in formulations was likewise negative (ref. 10, subm. III).

A 5% aqueous dilution of the 70:30 mixture applied to the rabbit eye was found to be non-irritating.
A single application of a 10% solution/suspension to the rabbit eye induced redness of the conjunctivae in 1/4 animals. Slight redness was observed upon daily application of 1% in an ointment for 12 days.

A similar response ,however, occurred in controls treated with the ointment only.
A 0.2% preparation was well tolerated in the eye of 5 humans.

In a sensitization test by a modified Bhehler method with the mixture of chloroacetamide (99% pure) and Na-benzoate (70:30) guinea pigs received topical induction treatments with 0.5 ml 0.3% in water, once a week, for 3 weeks. After a 14-day rest period, a challenge treatment with 0.3% in water,did not induce any sign of sensitization.
In a second sensitization test, a 1% aqueous solution of the mixture (containing the 99% pure compound) was painted on the skin of guinea pigs nine times every other day. After a 14-day rest period the same solution applied as a challenge treatment did not induce any positive response.
A third test was conducted with 0.1% of the mixture in a skin cleaning formulation, by rubbing it into the intact and scarified skin of guinea pigs in an amount of 0.1 ml, three times weekly for three weeks. This induction treatment caused erythema and oedema. Since the challenge treatment with 0.1 ml, 0.1% solution after 14 days did not produce more severe changes, the test substance was not considered a sensitizer.
A fourth test conducted in a similar way,however,with a 1.0% (instead of a 0.1%) solution, also induced erythema and oedema. These findings were not considered the result of sensitizing properties.
In a fifth test, guinea pigs were treated daily for 4 weeks with 1% and 3% of the pure compound in an aqueous mixture. The challenge treatment with 0.2% after 10 days rest did not produce sensitization (ref.3; subm. II; summary only).
In a maximization test in guinea pigs with the 99% pure compound (using for induction a 9% aqueous concentration intradermally and a concentration of 9% in vaseline topically) a challenge treatment with 3, 1 and 0.3% in distilled water given after 14 days rest did not produce sensitization (ref. 11 ; subm. III).

A sensitization study was conducted in 147 humans by topical application of a 0.5% aqueous solution of chloroacetamide of unknown purity, on every other day for 3 weeks. The challenge, also with 0.5% after

14 days, consisting of two consecutive patch applications for 48 hours each (on the same site, but away from the induction site) resulted in a high incidence of sensitized subjects, viz. 19/33 females and 28/114 males (ref. 4, subm. II).
In another study in humans with 1.25% in a cream base used both for induction and challenge treatment, 35/205 subjects showed a positive response (ref. 5, subm. II).
Positive reactions were obtained with 2/18 humans treated with 0.18% in a cream. A positive result was obtained also with 0.18% in water. In another patch test on 200 humans with 0.18% in a cream no positive reactions occurred (ref. 6, subm. II).
Tests with 1832 patients of a dermatological clinic showed 30 clearly positive reactions (1.6%) (Colipa sumb. V : May 1987).

Dermal treatment of rats with 12.5 and 50.0 mg/kg bw for 13 weeks did not induce fross or microscopical changes (no further details provided).

MUTAGENICITY

Mutagenicity tests were conducted with the 70:30 mixture of chloroacetamide and Na-benzoate. An Ames test with up to 1000 µg/plate was negative.
Chinese hamsters treated with up to 50 mg/kg intraperitoneally did not show chromosomal aberrations or an increased incidence of micronucleated erythrocytes.
A dominant lethal test in male mice treated intraperitoneally with 114 and 123 mg/kg was likewise negative.

TERATOGENICITY

Rats treated with an intraperitoneal dose of 20 mg/kg on day 7 of gestation,or on days 11 and 12 showed no signs of toxicity to the mothers or the foetuses (no further details were provided).

Single administration (route ?) of 50 mg/kg to pregnant rats did not affect foetal development (no further details were provided).

CONCLUSION

Chloroacetamide has shown considerable sensitizing potency in humans at concentrations in the range of those present in cosmetics.
This property might be due to a contaminant in certain batches of the substance.

The Committee agrees with provisional acceptance if information is provided on the results of ongoing surveillance.

Classification : A + mention "contains chloroacetamide"
(annexe VI n° 41)
Maximum level use : 0.3%

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   Universitäts-Klinik und Poliklinik für Hautkrankheiten
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   Abteilung für Allergologie
   Hamburg 18.12.1984

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   Contact Dermatitis 3, 19-26 1977

5. Marzulli, Maibach 1973 - Antimicrobials :
   experimental contact sensitization in man
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   Dermatologica 142. 191-192

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   Allergie aux conservateurs
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9. Prof. Dr. Gloxhuber, HENKEL & Cie GmbH, Düsseldorf 1966
Copy of Expert report
page 2

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Berlin 27.7.1985 Ref. 10

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mod. Method of B. Magnusson and A.M. Kligman Ref. 11

12. Drei Monate Fütterung an der Wistar-Ratte
September 9 1985
Unpublished Company data

4. PHENOXYPROPANOL

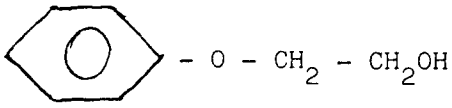
. Formula and Synonymes

EEC n° 56

COLIPA P54

1 - phenoxy propane - 2 - ol : chemical name

phenoxy propanol = propylene phemoxetol



$C_8H_{10}O_2$

MW 138,17

. CHARACTERISTICS

Soluble in water (1%)

Mixable with alcohol, ether and chloroform

. USE

Used only in rinsed-off cosmetics.

- up to 1% as a perservative
- up to 2% for other purposes

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral (rat) : 2554 - 2720 mg/kg/bw

ORAL TOXICITY

In a 2-wk oral test in rats with 0, 200, 500 or 1250 mg/kg/day by gavage, the top-dose was lethal. The two lower dose levels induced clinical signs and gross pathological changes.

A 2-wk oral test in rabbits with 0, 100 and 500 mg/kg/day showed paralysis and death in the top-dose group. No effect were noted with 100 mg/kg.

In a 4-wk oral study in rats with 0, 40, 120 and 400 mg/kg/day, the top-dose group showed clinical signs including sedation and abnormal gait, but no pathological changes. No abnormalities were seen with 120 mg/kg.

In a 4-wk oral test in rabbits with 0, 50, 100 and 200 mg/kg/day, the top-dose induced a general decline of condition, and increased liver weights. With 100 mg/kg growth rate and food intake were reduced. No change occurred with 50 mg/kg.

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

A skin irritation test in rabbits with the undiluted substance produced only minimal irritation.

In an eye irritation test in rabbits the undiluted substance elicited moderate to marked reactions of cornea, conjunctivae and iris. With 5% and 2% aqueous dilutions there were no eye reactions attributable to the substance.

A maximization test in Guinea pigs with 0.5% in water for induction and 75% aqueous preparation for the challenge did not produce evidence of sensitization.

Dermal exposure of rabbits to 0, 20, 50 or 1000 mg/kg/day for 90 consecutive days induced transient signs of toxicity only in the top-dose group (peripheral hypothermia, paralysis). Gross and microscopic examination were negative. (In this study the stratum corneum was removed at the site of treatment to enhance penetration).

In a subsequent 90-day study in rabbits with several exposure levels of 50 up to 1000 mg/kg/day, similar clinical signs of toxicity (including decreased motor activity, immobility, paralysis and hypothermia) were seen with 400, 500 and 1000 mg/kg. The signs which occurred shortly after the daily dosing, became noticeable at the end of the first week of treatment, decreased in frequency and intensity as the treatment progressed, and were no longer apparent after week 6. The "no-toxic effect level" was 300 mg/kg. Local skin changes, largely confined to exfoliation, occurred in all treatment groups (Colipa submission IV, ref. 24).

A dermal dose of 2 ml of a 2% solution of the radioactively labelled compound, caused a maximum blood level in rabbits of 4-5 ug/ml. An average of 54% of the applied radioactivity was excreted in the urine and an additional 23% was excreted in the faeces. Two radioactive compounds (a glucuronic acid conjugate and the aglycone) were identified in the urine, and an additional metabolite was extracted from the faeces. Absorption of the labelled compound through the skin of hairless mice amounted up to 73% if applied in 0.5% aqueous solution (Subm. IV, ref. 21). Rabbit skin was found to be twice as permeable for the compound as abdominal human skin (ref. 22). The penetration through grafted abdominal human skin in vivo was 70% when applied unrinsed in a liquid soap, but only 9% if rinsed off shortly after application (ref. 23).

MUTAGENICITY

An Ames test with 4 strains of S. typhimurium, using up to 5 mg/plate, did not reveal mutagenic activity (ref. 26). A chromosomal abberration test in human lymphocytes in vitro using up to 0.8 mg/ml culture medium showed no clastogenic activity (ref. 27).

TERATOGENICITY

In teratogenicity studies, rats and rabbits were treated orally during pregnancy with dose levels of 0, 80, 160 or 320 mg/kg/day for rats and 0, 25, 50 or 100 mg/kg/day for rabbits. In neither of these two studies were there adverse effects on foetal development.

CONCLUSION

The substance has shown considerable toxicity upon oral administration to animals, and dermal absorption is high if not rinsed off. No mutagenic or teratogenic properties were detected. Oral and dermal exposure levels above 200 mg/kg body weight/day in rats and rabbits were found to effect the central nervous system. No-effect levels varied between 50 and 300 mg/kg in different studies in rats and rabbits. If used in rinsed-off cosmetics only, the margin of safety is considered sufficient.

CLASSIFICATION A.

SECTION II - REFERENCES

1. Official US protocol - US Fedral Register 191.12
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2. Official French method - Journal Officiel de la République Française
   Arrêté du 5 Avril 1971 (J.O. du 9.2.73)
   complété par l'Arrêté du 17 Janvier 1973 (J.O. du 5.6.73)
   complété par l'Arrêté du 16 Avril 1973 (J.O. du 5.6.73)

3. Irritants de Référence - Interprétation de l'irritation oculaire provoqueée par des substances étalon.

4. VICK International Ltd Report N° 73/UK 18/072 -
   Life Science Research - Stock Essex CH4 - 9 PE - 2nd October '73
   Propylene Phenoxetol. Irritance towards Rabbit Skin and
   Eye Mucosa, and Acute Oral Toxicity to Rats
   Life Science Research (LSR) October 1973

5. HUNTINGDON Research Center - Report 3398/10 D/75 -
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6. HUNTINGDON Research Center - Report 3399/11 D/74 -
   22 November 1974 - R. Davies - Irritant effects of Propylene phenoxetol on Rabbit eye and mucosa

7. HUNTINGDON Research Center - Report 3559/D 9/74 -
   6 January 1975 - R. E. Davies - Acute toxicity to rats of Propylene phenoxetol

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9. VICK International Report 76/VNL 16/018 - Propylene phenoxetol in Topexane Base : Toxicity in repeated Oral administration to Rats for 14 days
(Range-finding test) LSR January 1976

10. VICK International Report 76/VNL 17/023 - Propylene phenoxetol in Toxpexane Base : Toxicity in repeated Oral administration to Rats for 4 weeks
Life Science Research - Stock Essex LSR February 1976

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Life Science Research - Stock Essex - LSR January 1976

13. VICK International Report 76/VNL 21/020 - Propylene phenoxetol : Toxicity to Rabbits by Dermal application for 13 weeks
Life Science Research - Stock Essex - LSR February 1976

14. VICK International Report 76/VNL 15/067 - The absorption of Propylene phenoxetol through intact and abraded rabbit skin
Life Science Research - Stock Essex - LSR April 1976

15. VICK International Report 76/VNL 22/052 - Propylene phenoxetol : Effects upon Pregnancy in the Rats
Life Science Research - Stock Essex - LSR March 1976

This second submission on P 54 consists of :

- Ref. 17 : A safety assessment of P 54 as used in rinse-off cosmetic products (doc. 85/270)
- Ref. 18 : CUNLIFFE W.J., Experiment to determine the amount of Topexane left on the face after washing
  Internal. Report May 27 1977
- Ref. 19 : Protocol AMES-Test - Life Science Research, September 10 1985
- Ref. 20 : Protocol Lymphocyte Cytogenetic Study -
  Life Science Research, September 11 1985

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28. Ashby J. The prospects for a simplified and internationally harmonised approach to the detection of possible human carcinogens and mutagens. Mutagenisis 1, 3-16 (1986)

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Report 76/VNL 15/067

* See submission I, February 1980

5. SUTTOCIDE A (P84)

(opinion expressed on February 20th, 1987, 33th reunion)
Conclusion : classification C.

New informations : Colipa Subm. II (8/87)

Micronucleus test in mice

The results of MNT indicate that Suttocide A did not induce a statistically significant increase in micronucleated PCEs or change in the PCE/NCE ratio at doses of 750, 1250 and 1750 mg/kg of body weight administered in single oral doses with sacrifice times of 30, 48 and 72 hours. (Inconsideration of Suttocide A as a 50% aqueous solution, the actual calculated doses were 375, 625 and 875 mg/kg). These findings are based upon the absence of a significant increase in the incidence of micronucleated PCE per 1000 PCEs in the Suttocide A treated animals as compared with those treated only with the vehicle control. Therefore, Suttocide A is considered negative under the experimental conditions of this protocol.

This new data doesn't justify a modification of opinion.

CLASSIFICATION C.

REFERENCE :

1. Heddle, J.A., M. Hite, B. Kirkhart, K. Mavournin, J.T. MacGregor, G.W. Newell and M.F. Salamone. Induction of Micronuclei as a Measure of Genotoxicity. A report of the U.S. Environmental Protection Agency Gene-Tox Program, 1983, Volume 123, pages 61-118.

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REPORTS OF THE SCIENTIFIC COMMITTEE ON COSMETOLOGY ON THE USE OF CERTAIN COLOURING AGENTS IN COSMETIC PRODUCTS

. THE COMMITTEE'S MANDATE

To give its opinion on the question whether the use of certain colouring agents in cosmetics is admissable from the health point of view.

Consult Reports of See seventh series EUR 11303
for preceding informations and opinions.

CI	13065	P. 23 - 25	
CI	42535	P. 90 - 91	
CI	42555	P. 92 - 93	
CI	44045	P. 97 - 98	
CI	61554	P. 117 - 118	
CI	73900	P. 103 - 104	(CI 46500)
ACID RED	195	P. 138	

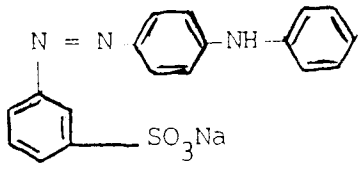
1. CI 13065

. FORMULA AND SYNONYMES

CAS Reg. N° 587-98-4

Acid Yellow 36
Ext. D. and C. Yellow N°1
C. Ext Gelb 10
Metanil Yellow

m(p-anilinophenylazo) benzene sodium sulfonate



$C_{18}H_{15}N_3O_3S,Na$

Mw 376.41

. USE

Used at levels up to 0.5% in non rinsed off products and up to 0.1% in rinsed off products.

. PRECEDING CONCLUSIONS (1st of July 1986)

The Committee saw no objection to maintaining the use of this colouring agent for the time being in rinsed off products. No opinion could be expressed for non rinsed off products.

Required informations :

- a clear NEL by oral administration
- a sensitization test
- a chromosomal aberration test

. NEW INFORMATIONS

In vivo studies in mice given orally 2mg/kg/day for 30 days, or i.p. a single dose of 50 mg/kg showed strong clastogenic activity. Positive in vitro tests for chromosomal aberrations had already been reported in 1978. An in vivo test in mice given a single i.p. dose varying from 2.5-200 mg/kg bw showed dose-related increases of sister chromatid exchanges in bone marrow.

CONCLUSIONS

The substance possessed considerable systemic toxicity and may adversely affect spermatogenesis. A no-effect level has not been established, but it is less than 25 mg/kg bw/day. The substance was found to induce chromosomal aberrations and sister chromatid exchanges both in vivo and in vitro. The secondary amine group of the molecule may lead to formation of nitrosamines which pass the skin. In view of the above properties and the gaps in the available information, e.g. on sensitization, the continued use of this colourant in cosmetics is not considered justified.

CLASSIFICATION D

REFERENCES

Colipa Subm. III , June 1987.

- Giri et al. J. Fd Sci. Technol. (India) 21 (1984)
- Giri et al. Cancer Letters 30 (1986a) 315-320
- Giri et al. Cancer Letters 31 (1986a) 299-303

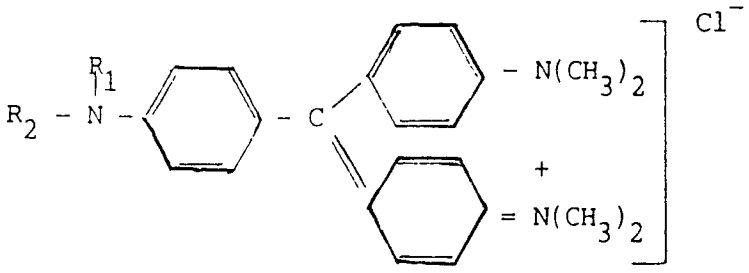
2. CI 42535

. FORMULA AND SYNONYMES

CAS Reg. N° 8004-87-3

- Basic Violet 1
- Methyl Violet

bis-4,4' - dimethylamino - 4'' - methylamino - triphenyl - carbenium chloride



R_1, R_2=H / R_1=H, R_2=CH_3 / R_1, R_2=CH_3

$C_{23}H_{26}N_3$ / $C_{24}H_{28}N_3$ / $C_{25}H_{30}N_3$ $^+$ Cl^-

MW 344 358 372

This colour is called Methyl Violet or Gentian Violet but it is actually a poorly defined combination of violet rosanilins comprising a series of dyes including Crystal Violet (Mut. Res. 98 (1982) p. 157).

. USE

Use level up to 0.1% in rinsed off and non rinsed off cosmetics, and up to 2.5% in hair setting lotions.

. PRECEDING CONCLUSION (1st of July 1986)

Because this dye is not clearly defined, and the results of the genotoxicity test depend on the source of the sample an evaluation is not possible. An opinion can not be given until information is available on the level of CI 42555 in CI 42535.

CONCLUSION

This colourant is chemically not well defined, which might explain why the results of genotoxicity tests depend on the source of the sample. One sample was found to contain 5% CI 42555 a related colourant with genotoxic and carcinogenic properties.

CLASSIFICATION D

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4'-(N-Dimethyl)-Amino-4"-(N-Dimethyl)-Amino-N-Methylfuchsoniumchloride (NBL)
29.10.1976

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Irritant Effects of Substanz n° 11
4'-(N-Dimethyl)-Amino-4"-(N-Dimethyl)-Amino-N-Methylfuchsoniumchloride (NBL)
on Rabbit Eye Mucosa
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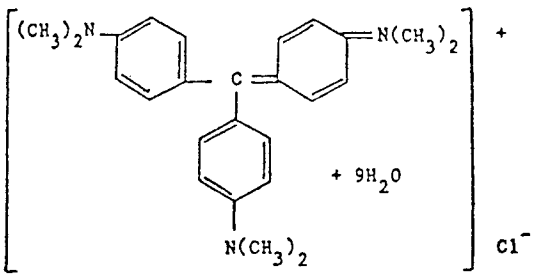
Department of Radiation Genetics and Chemical Mutagenesis, University of Leiden, THE NETHERLANDS
Report 9 November 1988

3. CI 42555 see /284/87

. FORMULA AND SYNONYMES

CAS Reg. N° 548-62-9

- Basic Violet 3
- C - Ext. Violet 6
- Crystal Violet
- Gentian Violet



$C_{25}H_{30}N_3.Cl$

MW 408

This dye is the major component of CI 42535 which is a mixture of dyes.

. USE

Used up to 0.1% in rinsed off and non rinsed off cosmetics and up to 0.5% in hair colourants. Industry proposed to restrict its use to hair colourants up to 0.2% (Colipa Subm II, June 1987).

. PRECEDING CONCLUSIONS (1st of July 1986)

Although Gentian Violet is used extensively in medical practice, the Committee came to the conclusion that this substance should not be used as a colourant in cosmetics. However, the Committee noted that Gentian Violet has therapeutic uses. Industry was to be asked to define the extent to which the toxic compound was present in the mixtures.

. NEW INFORMATIONS

A chronic toxicity and carcinogenicity study in mice with dietary levels of 0, 100, 300 and 600 ppm (equivalent with approximately 0, 13, 36 and 68 mg/kg/day) resulted in dose-related increase in mortality, in hepatocellular carcinoma in both sexes, and in reticulum cell sarcomas in uterus, vagina, ovaries and bladder of females after 2 years. These effects were noticeable even in the low-dose group. The authors calculated the virtual safe dose (VSD) to be 1 ppb for males and 2 ppb for females (equivalent with 0.0001 and 0.0002 mg/kg bw/ day respectively) (Littlefield et al, 1985). This chronic mouse study was initiated by NTP. Oral carcinogenicity and reproduction studies in rats which also formed part of this project, have not yet been noticed in the literature.

The metabolism of the ^{14}C-labelled dye was examined after oral administration by gavage of a single dose to rats and of multiple doses to rats and mice on 7 days. After a single dose, tissue residues were maximal at 4 hr in liver, kidney, muscle and gonads ; in fat, a maximum was reached after 24 hrs. The highest residue occurred in fatty tissue. Urinary excretion of the label was 1.6-2.2% in rats and 6-8% in mice (McDonald et al. 1984a).

Gentian Violet is metabolized to leucogentian violet by the intestinal microflora of man, rat and chicken, and by several genera of anaerobic bacteria (McDonald and Cerniglia, 1984b).

In vitro metabolism studies showed demethylation of the compound by liver microsome preparations from mice, rats, hamster, guinea pig and chicken. The pattern of demethylated metabolites was comparable among the species with little difference between the sexes (McDonald et al. 1984c).

Dermal absorption, examined in vitro after 30 minutes contact time, was 0.03% through hairless rat skin, and not detectable through human epidermis (Colipa Subm. II, ref. 28).

CONCLUSIONS

This substance is highly toxic. Oral dose levels as low as 2.5 mg/kg bw/day in rats, and 0.5 mg/kg bw/day in rabbits produced signs of maternal toxicity in teratogenicity studies. Mutagenic properties have been well established. In an oral mouse study the substance was found to be carcinogenic. There are indications of teratogenicity in animals and humans. Although gentian violet is used in medical practice the Committee had insufficient arguments to maintain the substance in cosmetics, even if used only at 0.2% in hair colourants.

CLASSIFICATION D

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4. CI 44045

. FORMULA AND SYNONYMES

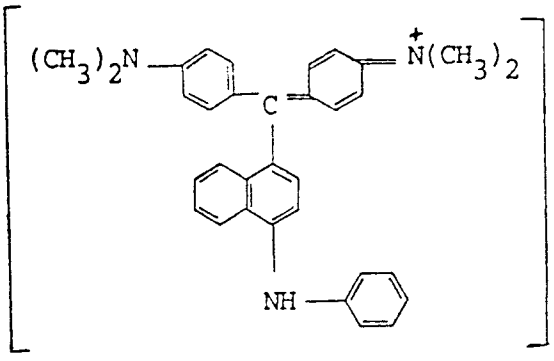
CAS Reg. N° 2580-56-5

Basic Blue 26

L - Ext. Blau 2

C - WR Blau 8

Bis - 4,4' - dimethylaminophenyl - 4'' - phenylaminonaphthyl - carbenium chloride



Cl^-

MW 506

$C_{33}H_{32}H_3,Cl$

. USE

Use level 0,1% in rinsed off and non rinced off cosmetics, and up to 0,5% in hair dye formulations.

. PRECEDING CONCLUSIONS

No opinion could be expressed because a lack of data.

Were required : - an adequate short-term oral toxicity study
- an Ames test
- a chromosomal aberration test

. NEW INFORMATIONS

In a short-term (13-wk) oral toxicity study 10 rats/sex were treated by gavage with 10 mg/kg b.w./day on 5 days/week. Ten rats/sex served as control. No treatment-related changes were observed at this low exposure level, which was only c. 1% of the LD_{50}. (Colipa subm. III,ref. 7).

An Ames test with up to 1 mg/plate was negative.
A chromosomal aberration study in CHO-cells in vitro up to 1.5 ug/ml culture medium was also negative. (Colipa subm. III, ref. 8 and 9).

CONCLUSION

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

Classification : C.

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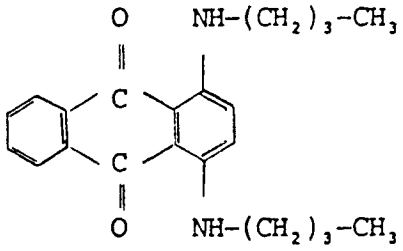
5. CI 61554

. FORMULA AND SYNONYMES

CAS Reg. N° 12769-17-4

Solvent Blue 35

C - Ext. Blau 12



. USE

Before used at level up to 0,1% in rinsed off and now rinsed off products. Actual use level up to 50 ppm only for hair care products.

. PRECEDING CONCLUSIONS (1st of July 1986)

In view of the high systemic toxicity and the absence of a NEL the Committee recommends not to use this colouring for cosmetics.

CONCLUSION

After 5 years no further information had been provided, the Committee confirmed its opinion in October '87.

Classification : D.

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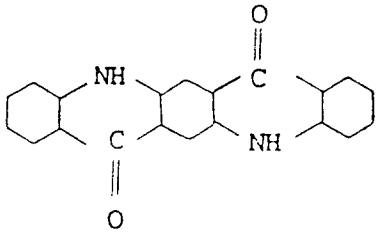
6. CI 73900 (Formerly 46500)

. FORMULA AND SYSONYMES

CAS Reg. N° 1047-16-1

Pigment Violet 19



$C_{20}H_{12}N_2O_2$

MW 238

. USE

Use level 0,4% in rinsed off products and 0,003% in non rinsed off products.

. PRECEDING CONCLUSIONS

The Committee saw no objection to maintaining the use of this colouring agent is cosmetic products for the time being. Information on possible sensitizing properties, and a detailed report on the short-term oral study and a chromosome aberration test were required.

CONCLUSION

In the recent submission, it is stated that studies with this colourant are in progres, including 28-days oral toxicity, in vivo mutagenicity and bio-availability.

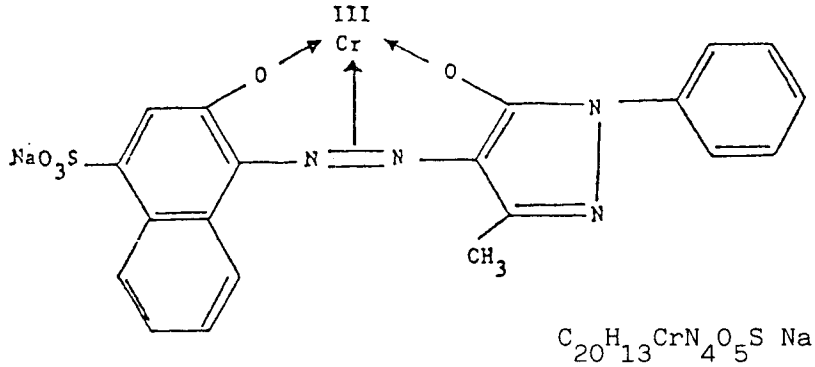
Classification : B.

7. CI ACID RED 195

. FORMULA AND SYNONYMES

CAS Reg. N° 12220-24-5

4 - (2' - oxo - 4' - sulphonaphthylazo) - 1 - phenyl - 3 - methyl - 5 - oxo - pyrazole, chromiumcomplex (1/1) monosodiumsalt



MW 496

$C_{20}H_{13}CrN_4O_5S\ Na$

. USE

Used up to 0.03% in rinsed off cosmetics and up to 0.02% in non rinsed off products.

. PRECEDING CONCLUSIONS

Information was required on sensitization, on genotoxicity and on short-term oral toxicity. The Committee had also required information on the incidence of deletorious effect (Stevens - Johnson syndrome) in patients using pyrazole containing drugs which are also exposed to cosmetics containing this colourant.

. NEW INFORMATIONS

ORAL TOXICITY

A sub-acute (28-day) oral study was conducted in groups of rats/sex, treated with 0, 250, 500 or 1000 mg/kg bw/day by gavage. All test groups of males showed lower body weights than did the controls, but there was no relationship with the dose. In females, albumin levels were higher-, and globulin levels were lower in all test groups than in controls, but no dose-related response was noticeable. Liver weights of females were decreased in the intermediate-, and high-dose group. These changes were not accompanied by microscopical abnormalities (Colipa subm. III, ref. 11).

CUTANEOUS AND MUCOUS TOLERANCE

Sensitization potency was examined by a modified maximization test in guinea pigs which first received one intradermal injection of 0.1 ml 50% Freund's complete adjuvans in saline on day one. Then they were treated topically 7 times in 15 days with 0.5 g aqueous mixture with 18.5% a.i. under occlusion for 48-72 hrs. After a 12 day rest period the topical challenge treatment with 0.5 g of test product containing 37% a.i. induced a slight response in 2/20 animals. Therefore the colourant was classified as a week sensitizer (Colipa subm. III, ref. 9).

A dermal test with the colourant in 20 users of pyrazolon drugs (4 of which were allergic to Metamizol and 5 to Propyphenazon) did not induce skin reactions. These results do not support the possibility of cross reactions caused by the colourant in users of pyrazolon drugs (Summary report only, in Colipa subm. III, ref. 10).

MUTAGENICITY

An Ames test with up to 5 mg/plate was negative.
A chromosomal aberration test was conducted in CHO - cells with concentrations up to 0.8 mg/ml. In the absence of the metabolic activation system there was an increase only in gaps at the two high concentrations. It was concluded that the test substance did not induce any dose dependent increase in chromosomal aberrations (Colipa sumb. III, ref. 12 and 13).

CONCLUSION

Although the target organ and a no effect level have not been clearly established, there was little if any toxicity upon very high oral exposure of rats. Because, in addition, the use levels in cosmetics are relatively low, the use of this colourant seems to be justified, if sensitization is not a problem.

Classification : A.

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Consultant Dermatologist 20.12.83

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Georg - August - Universität GOTTINGEN

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"Mutagenic Evaluation of the Dye CI Acid Red 195 in Ames Salmonella typhimurium/Microsome Plate Test"
Division Recherche Fondamentale - Département de Mutagénèse - L'OREAL - Aulnay - sous - bois - FRANCE
Report 31.12.1986

13. A.T. NATARAJAN & F.H. SOBELS (1987)
"Evaluation of Compound CI Acid Red 195 in the Chromosome Aberration with Chinese Hamster Ovary Cells (in vitro)"
Department of Radiation Genetics and Chemical Mutagenesis - University of Leiden - THE NETHERLANDS
Report 27 April 1987

REPORT BY THE SCIENTIFIC COMMITTEE ON COSMETOLOGY

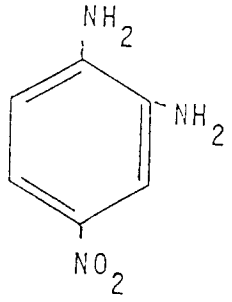
ON THE USE OF 1,2-DIAMINO-4-NITROBENZENE

. THE COMMITTEE'S MANDATE

To give its opinion on the question whether the use of 4. NOPD at the maximal level of 3,5% is admissible from the health point of view.

. FORMULA AND SYNONYMES

CI 76020
CAS Reg. 99-56-9
Colipa B24



1,2-DIAMINO-4-NITROBENZENE

4-NITRO-o-PHENYLENEDIAMINE (4-NOPD)
2-AMINO-4-NITROANILINE
4-NITRO-1,2-DIAMINOBENZENE
4-NITRO-1,2-PHENYLENEDIAMINE
p-NITRO-o-PHENYLENEDIAMINE

Mol.w. 153.1

$C_6H_7N_3O_2$

. USE

It is used in direct or semi-permanent hair colouring products in combination with oxidant dyes at the maximal use concentration of 3,5% and at normal use concentration of 0,6%. It produces brown, red and blonde shades on the hairs without any chemical reaction.

. PRECEDING CONCLUSIONS

SEE, SECOND SERIES, EUR 8634 (1983)

"In view of the absence of conclusive carcinogenic effects in animals. The SCC saw no reasons for prohibiting 4 NOPD at present but wished to obtain additional information concerning percutaneous resorption and the repetition of more realistic carcinogenicity tests and in the meantime it could accept its continuing use on a provisional basis. The implementation of this recommendation will be reviewed each year". (Hair dye which was temporarily acceptable for use in cosmetic products until 31 december 1985 : EUR 8634, p.l. 1980).

17-10-86 : Banned in Italy and Denmark ; recommended for banning in F.R.G.

. RECAPITULATION OF THE STUDIES ON TOXICITY

LD50 oral (rat) : 2100-3720 mg/kg
(NIOSH : 681 mg/kg)
i.p. (rat) 1600 mg/kg

ORAL TOXICITY

Subacute toxicity : RAT Fisher 344 & Mouse B6C3F1. NCI bioassay. 5 males and 5 females/group received in the diet for 7 weeks period these doses of 4-NOPD : 0 (2 groups) - 681 - 1000 - 1430 - 2160 - 3150 - 4600 - 6800 and 10000 ppm for rats and 0 (2 groups) - 1470 - 2160 - 3150 - 4600 - 6800 - 10000 - 14700 and 21500 for mice. The maximum tolerated dose was 750 ppm for rats and 7500 ppm for mice.

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

Dermal irritation : the compound applied to intact and abraded skin of rabbit as a 2.5% (w/v) preparation resulted non irritating.

Eye irritation : the compound as a 2.5% (w/v) suspension on rabbit's eyes caused a mild conjunctival inflammation and did not persist for more than 24 hours.

Sensitization was tested in Guinea pigs treated with 3% of 4-NOPD solution containing 2% Natrosol, 2% Tween 80, 0.05% Sodium sulphite and 10% isopropanol (pH = 7) applied 6 days/week for 3 weeks, sensitization was evaluated 2 weeks later. The results showed a relatively strong reaction (18/20 animals have an allergic effect).

Dermal absorption : 4-NOPD (120 µg/cm2 as a 0.6% (w/v), 200 ul as 3-H labelled hair dye solution) was applied on 10 cm2 of the skin in a 50% solution of a semi-permanent hair colourant shampoo base for 20 min. before rinsing off. Absorption was evaluated from the levels of 3-H present in the excreta and carcasses of the animals 48 hours after application. The results showed that 2.2 µg/cm2 (1.83%) of 4-NOPD apparently penetrated in the skin.

Short term dermal toxicity : 4-NOPD containing formulation (0.25% in water) tested on shaven intact skin of New Zealand by topical applications produced no toxic effects at 3-7-13 weeks after treatment at the histopathological analyses.

Human : In a repeated insult patch test in human (previous sensitized to p-phenylenediamine) with the compound no positive reactions were observed.

MUTAGENICITY

Studies have shown that 4-NOPD is mutagenic *in vitro* :

(1) on *B.subtilis* ;

(2) on Salmonella, in several experiments and in different experimental conditions ;

(3) on *E.coli* ;

(4) on Mouse lympoma test ; and *in vivo* :

(5) on Salmonella (urinary assay on rats) ;

(6) on *Drosophila* (X-recessive lethals and visible mutation tests systems by microinjection to adult and in SLRL test system by oral administration for 3 days) for gene mutation induction ;

(7) on 3 different cells line of chinese hamster (fibroblast line CHL, prostat gland and A(T1) CI-3 cell line) for chromosome aberrations *in vitro* ;

(8) on rat (until to 100 mg/kg/day i.p. × 10 wks.) by dominat lethal test (weak response) *in vivo* ;

(9) on *B.subtilis* (rec-assay) ;

(10) on *E.coli* by umu-test and differential killing tests system ;

(11) on *S.cerevisiae* for the induction of mitotic recombination ;

(12) on CHO cells and in mammalian cells culture (SCE in vitro).

Other mutagenicity studies have shown that the compound is negative for the induction of gene mutation *in vitro* in *A.nidulans* for forward mutation in two genetic markers ; in *S.cerevisiae* for the induction of reversions at three genetic loci ; and in *N.crassa* in ad-3 reversion mutations system ; and negative *in vivo* on *Drosophila melanogaster* in a minute loci and SLRL tests by fed for 21 hours.
The compound did not induce chromosome aberrations *in vivo* on rats (5000 mg/kg, g.i.) and mice (300 mg/kg, i.p.) by micronucleus test on bone marrow and dominant lethal test on rat (20 mg/kg, i.p. for 8 weeks). The compound has resulted negative for genotoxicity by DNA repair test on HeLa human cell line, and in E.coli for the induction of SOS function on primary rat hepatocytes culture *in vitro*, and *in vivo* on primary rat hepatocytes culture *in vitro*, and *in vivo* for the induction of SCE in bone marrow and in intestinal epithelium of Chinese hamster cells.

CARCINOGENICITY

Studies were carried out on mice and rat by a NCI bioassay, the compound fed in the diet at 750 and 375 ppm for rats for 103 weeks and 7500 and 3750 ppm for mice for 102 weeks showed no significant evidence of carcinogenicity. A formulation containing 4-NOPD at 0.6% level tested in A and DBAf mice by repeated topical application in aqueous acetone solution showed lymphoid tumours in both strains, but only in DBAf the incidence was statistically significant ; this report is incomplete and the experimental protocol is not adequate. Other studies carried out on mousse and rat (formulation 7403 containing 0.25% of 4-NOPD) and dog (Dye/Base composite containing 0.16% of 4-NOPD) have produced negative results.

CONCLUSION

- Negative evidence of carcinogenicity on two species (mice & rats).
- Sufficient evidence for mutagenicity in vitro, but negative in vivo mutagenicity and DNA damage.
- Some evidence of systemic toxicity on rat.
- Negative evidence of teratogenicity with doses lower than 128 mg/kg/day on rabbits.

The SCC in his plenary meeting of October 13th, 1987 requested a short-term oral toxicity study to determining the NEL.

Classification : B.

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Pisa, Italy, sept. 1986.

CONTENTS

2nd ADDITIONAL OPINION OF THE SCIENTIFIC COMMITTEE ON COSMETOLOGY RELATING TO THE USE OF BENZOYL PEROXIDE

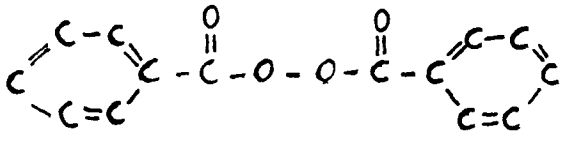
(Opinion delivered on 19.1.1988)

The Committee has examinded the new data sent in by the Member States which are opposed to the Commission's proposal to regulate the use of benzoyl peroxide in cosmetics in accordance with the opinion delivered on 22.11.1983 and 17.12.1984.

For preceding informations, consult :
- Reports of SCC, fourth series, EUR 10305, P9, P23.

. FORMULA AND SYNONYMES

Benzoyl peroxide
Dibenzoyl peroxide
Benzoyl superoxide
Benoxyl (T.N.)
Oxy - 5
Cas reg n° 94-36-0



. USE

Used in anti-acne formulations.

In the medical practice BP use-concentrations in anti-acne preparations are 5 or 10%. (37) (38)
The Scientific Committee on Cosmetology (SCC) of the EC in 1983 has approved of a use concentration of 3% in cosmetic formulations. (20)
In a recent meeting of the SCC a proposed use concentration of 2% is mentioned. (33)

. RECAPITULATION OF ADDITIONAL INFORMATIONS

- Skin irritation after application of BP formulations has been demonstrated to occur in humans at concentrations of 2.5% and higher. At lower concentrations the data are scarce. In a limited study in humans absence of skin irritation at 1.2% BP was found but in rabbits concentrations as low as 0.1% were skin riitating.
- The available data on phototoxicity are too limited to allow drawing conclusions on this point.
- In controlled human studies high sensitisation rates have been found for 5 or 10% BP formulations. These results are not refuted by the mostly anecdotal reports on low sensitisation rates after clinical use. Almost all sensitisation data refer to BP use concentrations $\geq$ 5%. Controlled sensitisation studies in humans with the currently proposed use conentrations of 2 or 3% are lacking.
- The available carcinogenicity studies were of limited design. Given the inadequacies in study duration, number of dose levels tested and application frequency, the results of the studies, while not showing a carcinogenic effect by BP, cannot be considered conclusive evidence for the absence of such an effect.

- In tests in hamsters for tumour promotion by BP after chemical initiation the result was positive. In the sensitive mouse strain Sencar BP clearly exerts a tumour promotive effect after chemical initiation, an effect for the induction of which relative low and infrequent exposure suffice. Other mouse strains appear less sensitive.

  The Limited design of all available tumour promotion/cocarcinogenicity studies (in all cases duration $\leq$ 60 weeks) reduces the value of the negative results obtained in some of the studies. Thus, the absence of an effect in the studies with UV-treatment - (such studies are particularly relevant with regard to anti-acne use of BP) - cannot be considered as conclusive evidence for the absence of an effect by BP when UV-radiation is used as initiator or cocarcinogen. A study of adequate design could resolve this issue.

- On the basis of the available evidence BP cannot be qualified as a mutagenic agent. The relevance of the (positive result in) in vitro studies for DNA-damage/strand breaks or SCE's for possible mutagenicity or carcinogenicity is unclear.

CONCLUSION

The overall toxicological profile of benzoyl peroxide is incompatible with the level of human exposure resulting from cosmetic products.
In particular, the Committee cannot accept the risk of the widespread utilisation of a substance which promotes skin tumours in a low dose, whether the reservations on concentration, formulation, scope and labelling. The Committee recommends the banning of the use of benzoyl peroxide in cosmetic products.

Classification : D.

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REPORTS BY THE SCIENTIFIC COMMITTEE ON COSMETOLOGY

37th REUNION

APRIL 12, 1988

LIST OF PARTICIPANTS

Present

Mr. AGACHE
Mr. DE GROOT
Mrs. DONY
Mrs. ENJOLRAS
Mr. FIELDER
Mr. GOULDING
Mr. KAPOULAS
Mr. LOPRIENO
Mr. O'MAHONY
Mr. RAMOS MORGADO
Mr. SHOU
Mr. STUTTGEN

Absent

Mr. GRANADOS JARQUE
Mrs. KNAAP
Mr. MUSCARDIN

Commission

Mrs. MASSE
Mr. GONTIER (DG XI/C/2)
Mr. COLLIN (expert)

SUMMARY

Opinions expressed on April 12th, 1988 concerning the use of

- HAIR DYES (XI/827/87 - XI/151/88)

1. 2 AMINO 4 NITROPHENOL (B26)
2. 2 AMINO 5 NITROPHENOL (B27)
3. ORTHO - AMINOPHENOL (A14)
4. META - AMINOPHENOL (A15)

- PRESERVATIVE AGENTS (XI/204/88 - XI/189/88)

1. CHLORPHENESIN (EEC n° 3 P4)
2. BENZETHONIUM CHLORIDE (EEC n° 53 P70)
3. BENZALKONIUM CHLORIDE (EEC n° 54 P71)
4. CHLORQUINADOL (P76)

- U.V. FILTERS

1. GLYCERYL PARAAMINOBENZOATE (EEC n° 2.4)
2. ETHYL HEXYL - 4 - METHOXYCINNAMATE (EEC n° 2.13)
3. TERT - BUTYL - 4 - METHOXYDIBENZOYLNETHANE (EEC n° 2.31)

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 :
- 2 - amino - 4 - nitrophenol
- 2 - amino - 5 - nitrophenol
- ortho - aminophenol
- meta - aminophenol

is admissible from the health point of view.

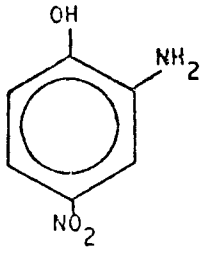
1. 2-AMINO-4-NITROPHENOL

1. FORMULA AND SYNONYMES

CI 76530

CAS Reg. 99-57-0

Colipa B26



$C_6H_6N_2O_3$ MW:154,1

2 - amino - nitrophenol

2 - amino - 4 - nitrophenol

1 - hydroxy - 2 - amino - 4 - nitrobenzene

. PRECEDING CONCLUSIONS

From See, Second Series, EUR 8634 p.12

"Insufficient data for carcinogenic assessment ; additional information concerning carcinogenicity in animals after dermal application of adequate doses".

. NEW INFORMATIONS

- Cancerogenicity ; The compound (98%) was tested by NCI for the study of carcinogenicity on Fischer 344 rat and B6C3F1 mice by oral gavage administrations of 0 - 125 - 250 mg/kg for 2 years (5-d/wk). The results showed evidence of carcinogenicity in male rats (renal cortical (tubular cells) adenoma (vehicle : 0/50 ; 125 mg/kg ; 1/48 (2.1%) ; 250 mg/kg 3/50 (6%). Nonneoplastics effects were observed in male and female rats (chronic nephropaty and pigmentation of the small and large intestines). No evidence of carcinogenicity in female rats and male and female mice.

- Mutagenicity and genotoxicity ; A pure preparation of the compound resulted positive for gene mutation in vitro by reverse mutations on Salmonella typhimurium (4 studies), Sordaria brevicollis and on mouse lymphoma L5178Y/TK+/- assay ; positive for chromosome aberrations in vitro on CHO and CHL cells system ; negative for chromosome aberrations in vivo by micronucleus test on rat at a dose of 5000 mg/kg and dominant lethal on rat at dose of 20 mg/kg (inadequate studies) ; and positive for genotoxicity by DNA-repair liquid test on E.COLI and for the ability to induce sister chromatid exchange in CHO cells in vitro.

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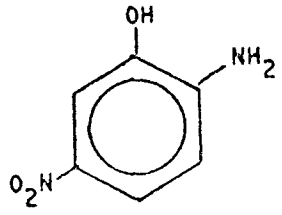
2. 2-AMINO-5-NITROPHENOL

. FORMULA AND SYNONYMES

CI 76535

CAS Reg. 121-88-0

Colipa B27



$C_6H_6N_2O$

MW : 154,1

2 - amino - 5 - nitrophenol

1 - hydroxy - 2 - amino - 5 - nitrobenzene

. PRECEDING CONCLUSIONS

From see, second series, EUR 8634 p13

"Insufficient data available for a conclusive toxicological assessment ; additional data are required for the assessment of its potential for carcinogenicity in animals after dermal applications".

. NEW INFORMATIONS

- Carcinogenicity : The compound was tested by NCI for the study of carcinogenicity on Fisher 344 rat and on B6C3F1 mice. 2-Amino-5-nitrophenol (98% purity) has been administered in corn oil by oral gavage, 5-day per week, at dose levels of 0 - 100 - 200 mg/kg (5ml/kg) to groups of 50 male and 50 female mice, and 0 - 400 - 800 mg/kg (10 ml/kg) to 50 males and 50 female mice for 2-years. The results showed evidence of carcinogenicity in male rats : pancreatic acinar cell adenomas (vehicle : 1/50 ; 100 mg/kg : 10/50 ($P < 0.002$) ; 200 mg/kg : 3/49). Nonneoplastic effects were observed either in male and female rats. In male and female mice : inflammation and pigmentation of the large intestine. No evidence of carcinogenicity were observed in female rats and female and male mice.

- Mutagenicity and genotoxicity. A pure preparation of the compound resulted positive for gene mutation in vitro by reverse mutations on Salmonella typhimurium (6 studies/1 study negative, but this latter was inadequate because performed only in the absence of metabolic activation), and by forward mutations on mouse lymphoma L5178Y/TK+/-assay ; positive for chromosome aberrations in vitro on two different cells line of chinese hamster (CHO and CHL) ; negative for chromosome aberrations in vivo by micronucleus test on rat at a dose of 3000 mg/kg and dominant lethal on rat at dose of 20 mg/kg (inadequate studies) ; positive for genotoxicity by DNA repair liquid test on E.coli and for the ability to induce sister chromatid exchange in CHO cells in vitro.

CONCLUSION

Classification : D.

BIBLIOGRAPHY

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Tech. Rep. Ser. N° 334, March 4th, 1987/

3. o-AMINOPHENOL

. FORMULA AND SYNONYMES

CI 76520
CAS Reg. 95-55-6
Colipa A14
o - aminophenol
1 - hydroxy -2- aminobenzene
2 - aminophenol
2 - hydroxy - aniline
o - hydroxy - aniline

MW : 109,129

. USE

Oxidative hair dye
Max. use : up to 1%
0,5% in combination with H_2O_2

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral (rat) : 1052 - 1058 mg/kg

ORAL TOXICITY

Rat : 150 mg/kg : no nephrotoxicity and no hepatotoxicity.

- Short term oral : o-AP administered oral to rat (50 mg/kg in 1% propylene glycole x 3 months) showed slightly toxic effects : bronco-pulmonary injuries at anatomopatological analyses in several rat and slight and isolated increase in alcaline phosphatase. No mortality occured.

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

- Dermal irritation : it resulted slightly irritating (0.1 ml, 1% in propylene glycole) on rabbit.
- Eye irritation : it resulted not irritating (0.5 ml, 1% in propylene glycole) on rabbit.
- Sensitization : it showed negative results on guinea pigs (0.5 g 3 times/wk X 3 wks and at the start of 4 wk ; challenge reaction on day 36 with 0.5 g).
- Human-sensitization : A sensitized man with dermatitis related to tires and other rubber products, IPPD and PPD positive, showed at patch-test focal flare after 1 wk, in the site of application of o-A.P(27 aromatic amines tested).
- Dermal absorption : In a formulation (0.8%) mixed 1:1 with H_2O_2 (0.32% of o-AP, i.e. 587 nmol or 64 ug) applied on the back of female Hairless rat (1 cm2) for 30 min. showed these absorption values : 4.55% with human hair ; 4.66% with m-AP (0.8%) and human hair ; 6.62% with m-AP (0.8%).
- Short term dermal : o-AP containing formulation (0.3%, mixed 1:1 H_2O_2) showed negative results after dermal topical application (1 ml/kg, 2 time/wk. X 13 wks) on shaven or not shaven skin of rabbit.

MUTAGENICITY

The compound tested for gene mutation resulted positive in vitro on Salmonella (2 studies) and in vivo on mice with Sperm-Head abnormality ; positive for chromosome aberrations using micronucleus test on mouse (1 study) ; and positive for genotoxicity in vitro on human fibroblasts and lymphocytes using SCE analyses and on E.coli for DNA damage.

The compound resulted also negative for gene mutation in vitro on Salmonella (3 studies) and S.pombe ; negative for chromosome aberrations in vitro on CHO cells and in vivo by chromosome aberrations and micronucleus test on mice ; and negative for genotoxicity on CHO cells in vitro and hamster chinese (bone marrow) in vivo for induction of SCE.

CARCINOGENICITY

Long term studies was carried out with a hair dye composite containing test compound (0.3%, 1:1 with H_2O_2) by dermal topical applications on mice (0.05 ml/wk. ^{X} 2y) and rats (0.2 ml increased by 0.1 ml to 0.5 ml 2 appl/wk, ^{X}2y - to Fo generations from the time of weaning to the weaning of young (F1 generation) : no biologically significant difference was observed between treated and control groups.

TERATOGENICITY AND REPRODUCTION

The compound resulted teratogenic without maternal toxicity on Syrian golden hamster (100-150-200 mg/kg i.p. in acified isotone saline on the morning of day 8 of gestation (8 days after the evening breeding) showing at day 13 of gestation several malformations in the fetuses : neural tube defects (exencephaly, enocephale and spina bifida), eye defects, limb defect, rib defects, tail defects and umbilical hernia. The compound containing formulation (0.3% in water, 1:1 with H_2O_2) tested on rats (2 mg/kg bw day to the shaven skin on day 1-4-7-10-13-16-19 of gestation) showed negative results, but the report resulted incomplete, because no detail on histopatology and body weight were presented.

Embryotoxicity : the compound showed moderate acute embryoletality (LD50) by HET test : 0.91 mg/egg at day 1 (ca. 20 ppm) and 0.75 at day 5 (ca. 15 ppm), with a no-effects of 0.2 mg/egg (1 d.) and 0.1 mg/egg (5 d.).

Reproduction : the compound containing formulation (0.3%, with 6% H_2O_2) showed negative results in a reproduction study on raty by dermal topical applications (0.5 ml/appl. start to 0.2 ml/appl. increased of 0.1 ml/appl/week) twice weekly through growth, mating, gestation and lactation to the weaning of the F1b, F2b and F3c litters of the respective generation.

MISCELLANEOUS INFORMATIONS

Protein binding : o-Aminophenol after oxidation with ferricyanide formed adducts with proteins.

Blochemical studies in vitro : o-Aminophenol had no effects on mitochondrial respiration and phosphorilation with different oxidative substrate, only slightly reduction of the P/O ratio at the highest doses ($>$0.5 mM). It had no effects on mitochondrial integrity structure, only a physiological swelling increase of mitochondria observed.

Effects on hemoglobin : in Vitro. The compound rapidly produced ferrihemoglobin formation on both human hemoglobin and washed red cells in vitro, more rapidly in washed red cells. The oxidized o-Aminophenol, by the activated oxygen, preferred phenoxazone condesation at high dose ($<$100 uM) and binding with hemoglobin at low dose (0-80 uM) ; it is oxidized by the activated oxygen in oxyhemoglobin. Glutathlone diminished the covalent binding of o-Aminophenol to hemoglobin and pre-treatment of hemoglobin with p-chloromercuribenzolc acid (block the reactive groups SH) reduced to 1/5 the amount observed with native hemoglobin. The fetal hemoglobin is more susceptible than adult hemoglobin for methemoglobin formation, it has been reported that this result was also obtained at different values of pH in Vivo. The dose of 50 mg/kg bw in aqueous by i.p. injections on 2 adult Japanese quail showed a peak of 25% of methemoglobin after 10 min. (0.4 umol/ml blood) no undetectable after 30 min.

CONCLUSIONS

The SCC requires additional data for defining the no effect level in an oral subacute toxicity study (28 days), the potential for the induction of UDS or DNA binding in vivo and conclusive data for teratogenicity.

Classification : C.

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LONG TERM TOXICITY/CARCINOGENICITY STUDY

Method : Bladder implantation testing
Species : Albino mice
Number of animals : 37 (treatment group)
55 (control group)
Method of administration : Implantation of cholesterol pellet containing the test colorant in the bladder of the mice
Dose level : 12.5 %
Total duration : 40 weeks
Negative control : Pellets of Cholesterol alone
Results : The implantation of Cholesterol pellet cuased 5 carcinomas in 55 mice (9.0 %) while the pellet containing o-Aminophenol caused 2 carcinomas in 37 mice (5.4 %) and no benign tumors
Conclusion : o-Aminophenol is considered inactive

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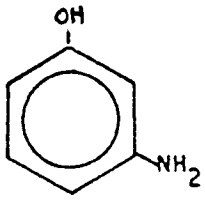
4. m-AMINOPHENOL

. FORMULA AND SYNONYMES

CI 76545
CAS Reg. 591-27-5
Colipa A15
m-Aminophenol
1-hydroxy-3-aminobenzene
3-amino-phenol
1-3-amino-phenol
m-hydroxy-aniline



MW : 109,29

. USE

oxidative hair dye
max. use : 2%
1% in combination with H_2O_2

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral (rat) : 812 - 1660 mg/kg
(mouse) : 330 mg/kg

ORAL TOXICITY

The compound did not show nephrotoxicity on Fisher 344 rat orally treated with 1.37 mmol/kg in 0.5 mM HCl in isotone saline.

Short term oral : m-Aminophenol administered by oral intubation to rats (20 males and 20 females) for 12 weeks (5 times/wk) showed that the dose of 50 mg/kg does not represent a toxic cumulative dose.

CUTANEOUS AND NUCOUS TOLERANCE

DERMAL TOXICITY

Skin irritation : The compound (2.5% (w/v) in 0.5% aqueous gum tragacanth with 0.05% sodium sulfite resulted mildly irritating (reading at 24 and 72 h) on abraded and intact skin of rabbit (1/3 animals at 72 h with very slight oedema).

Eye irritation : 2.5% (w/v) in 0.5% aqueous gum tragacanth with 0.05% sodium sulfite, instilled into one eyes of 3 rabbit resulted non irritating ; only minimal conjunctival irritation was observed 1 hour after instillation in all 3 rabbits.

Sensitization : m-Aminophenol (3% in water with 2% Natrosol, 2% Tween 80 and 0.05% sodium sulfite) showed no allergic reaction in guinea pig by open epicutaneous method. In another study on guinea pigs it has been reported that m-Aminophenol (0.1 ml, dose not reported) showed no sensitized animals after challenge reaction.

Human : m-Aminophenol resulted negative in a patch test on a sensitized man (positive to IPPD and PPD) and it was unable to produce cross-reactions.

Dermal absorption : m-Aminophenol HCl (ring ^{14}C, radiochemical purity 98%) administered dermally (2mg/kg = 0.3 ml of an 8% aqueous solution on the shaved skin (9 cm^2) for 30 min. before shampooing and rinsing), subcutaneously or orally (37.5 mg/kg bw, 1 ml of 1% aqueous solution) to OFA Sprague Dawley rats showed that after dermal

study 0.41% (B.1 µg/cm2) penetrated in the skin ; 89-95% of the dose absorbed was revealed in the urine after 24 hours and reached the maximum level in the organs after 35 min. in subcutaneous and oral administration treatment it was mainly excreted in the urine. The labelled compound (ring [14C]) containing formulations (1%, 2% and 3%, 1:1 with 6% H_2O_2) applied on the shaven skin of OFA Sprague-Dawley rats for 30 min. before shampooing and rinsing showed these values of dermal absorption : 0.14% (1% of m-AP) ; 0.16% (2% m-AP) ; 0.15% (3% m-AP) ; and 0.053% (2% m-AP) when skin was not shaven. After rinsing 92-96% of the radioactivity was found in the rinsing water.

Short term dermal : The compound containing formulations (0.04%-0.7%), mixed 1:1 with H_2O_2 by topical application for 13 wks (twice weekly) on abraded and intact skin of rabbit showed negative results.

MUTAGENICITY

Studies have been demonstrates that m-Aminophenol does not induce gene mutation in Salmonella, E.coli, S.pombe and S.cerevisiae in vitro and in vivo by Sperm-head abnormality on Mouse and SLRL on Drosophila ; chromosome aberrations in vitro on CHO cells and in vivo on bone marrow cells on mouse by micronuleus test on mouse and rat ; genotoxicity effects in vitro on E.coli (DNA Repair by plate-test), S.cerevisiae (mitotic gene-conversion) and V79 (SCE) genotoxicity effects in vivo on mouse (SCE, s.c. treatment) lymphocytes (SSB), human fibroblasts (SCE) and S.cerevisiae.
Positive results were obtained in one study on Salmonella TA1538 with co-factors for gene mutation in vitro and for genotoxicity in vitro on mouse (SCE, i.p. treatment) ; the compound is resulted positive for ability to produce aneuploid products of meiosis in Neurospora. Formulations containing m-Aminophenol resulted negative ; (a) for heritable translocation test on rat (1%),

(b) chromosome aberrations and SCE in human volunteers (dyed the hair : 3-6% H_2O_2 solutions, lymphocytes analysis).

CARCINOGENICITY

Dermal topical application : Four oxidative formulations (7403, 7406, P-25, P-26, mixed 1:1 with 6% H_2O_2) containing 0.7%, 0.09% and 0.04% m-Aminophenol tested on Swiss Webster mouse by dermal-topical application (0.05 ml/cm2 X 21-23 months) showed negative results. m-AP containing in two formulations (0.7%, 1:1 with H_2O_2) tested on Charles River rat (Fo generation) from the time of weaning to the weaning of their young (F1a generation) by dermal topical applications (0.2 ml increased by 0.1 ml to 0.5 ml, 2 times/week X 2 years) showed negative results.
m-AP in other two formulations (0.09% nad 0.02%) tested with the same previous treatment schedule on rats showed hyperkeratosis and/or acanthosis of stomach mucosa in several males and females and hepatocellular hypertrophy/hyperplasia or hyperplastic/hyper-trofic nodules in livers of several rats, especially males treated with formula at 0.09% m-AP level (possible compound related).

TERATOGENICITY AND REPRODUCTION

Reproduction : In a multireproduction study on rat with formula-tions (0.02%-0.9%) applied dermally (0.5 ml/rat) twice week for through, growth, mating, gestation and lactation to weaning at the F1b, F2b and F3c litters of respective generations given negative results.

Teratogenicity study performed on Syrian golden hamster by i.p. injection (100-150-200 mg/kg in 10% DMSO aqueous solution) on the morning of day 8 of gestation (8 days after the evening bree-ding) showed (females killed at 13 days of gestation) that the compound induced some malformation (type not reported) at 150 mg/kg (6/84=7.1% malformed fetuses) not statistically significant,

without maternal toxicity, m-AP (0-0,10-0,25-1.00% in the diet) administered to female rats for at least 90 consecutive days showed at the dose of 1% maternal toxicity (some effects also at 0.25%) without teratogenicity or embryotoxicity effects.
m-AP containing formulations (0.04%-0.7%, 1:1 with H_2O_2) were applied (2 mg/kg/day) to the shaven skin on rat on day 1-4-7-10-13-16-19 of gestation, or mouse (0.7% ; 0.05 ml/mouse 2 times week for 4 weeks prior to mating through day 18 of gestation), or rabbit (0.7% ; 2 ml/kg 2 times week from 4 weeks prior to mating through 30 day of gestation).
In rat only significant reduction of the mean live fetal weight was noted. In mouse a retarding effect of ossification process (bones of the feet and the cervical and caudal vertebral center) and slightly lower fetal weights. In rabbit no signs of maternal toxicity ; only focal alopecia until to the last 3rd of gestation) and a reduction of fetal survival as possible embryotoxic effects were observed. These results are not significant for teratogenicity potency.

MISCELLANEOUS INFORMATIONS

Hemoglobin effects : m-Aminohenol did not cause metahemoglobin formation in either fetal hemoglobin or adult hemoglobin at differents walues of pH (6.35-7.20).

Immunosuppressive : THe compound (2.5 mg, 4 times with 1/4 maximal tolerated dose, sc. inject.) resulted negative for immunosuppressive action on mice.

CONCLUSION

Classification : A.

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IN VIVO ASSAY

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Cosmital Sa - MARLY - SWITZERLAND
Report n° 9396/46 - 14 July 1982

REPORT OF THE SCIENTIFIC COMMITTEE ON COSMETOLOGY ON THE USE OF CERTAIN PRESERVATIVE AGENTS IN COSMETIC PRODUCTS

. THE COMMITTEE'S MANDATE

To give its opinion on the question whether the use of certain preservative agents in cosmetic products is admissable from the health point of view.

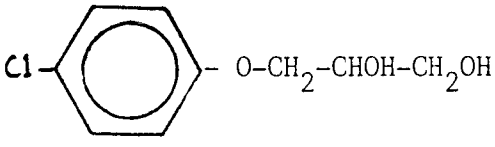
1. CHLORPHENESIN CSC/204/88

. FORMULA AND SYNONYMES

EEC N°3
Colipa P4
CAS N° 104-29-0
3-(p-chlorophenoxy) propane -1,2 - diol (chem.name)
chlorphenesin
p-chlorophenyl-glycerol ether

$C_9H_{11}CLO_3$

MW : 202,64



. CHARACTERISTICS

Slightly soluble in water (0.6%)
Moderately soluble in glycerol (9.5%) and alcohol (15%)

. USE

As a preservative in cosmetics at a concentration up to 0.2%.

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral	(rat)	:	1400 mg/kg
	(mouse)	:	1060 mg/kg
	(guinea pig)	:	820 mg/kg
i.p.	(rat)	:	520 mg/kg
	(mouse)	:	675 - 911 mg/kg
	(guinea pig)	:	425 mg/kg
S.C.	(mouse)	:	930 mg/kg

ORAL TOXICITY

An oral dose of the labelled compound given to rats was rapidly absorbed and reached a peak concentration in the blood in 30 min. The half life in serum was 140 min. More than half of an oral dose was excreted in the urine in 4 hr, partly as the unchanged compound. Four metabolites have been identified : 3-p-chlorophenoxylactic acid, p-chlorophenoxyacetic acid, a conjugate of chlorophenol, and a conjugate of chlorphenesin.

In an oral 13-week study in rats given doses of 50, 100 or 200 mg/kg bw/day by gavage, no effect on growth rate or food intake was observed. Examination of vaginal smears provided no evidence of interference with oestrus. No gross changes were observed at autopsy (a detailed report is not available).

CUTANEOUS AND MUCOUS TOLERANCE

A skin irritation test in rabbits was negative (no details).
In repeated insult patch tests with 18 humans, application of 0.05 ml of 0.2% in hand cream, skin lotion and skin smoothing milk on 5 successive days was negative, or produced slight erythema in some cases.

An eye irritation test in rabbits with 1% in glycerine, did not provoke corneal irritation.

MUTAGENICITY

No evidence of mutagenic potential was obtained in a well-conducted Ames test with up to 0.5 mg/plate. Mutagenicity was examined also by the CHO/HGPRT locus bioassay.
Treatment of the cells in vitro with up to 1.5 mg/ml did not demonstrate mutagenic potential (Colipa subm. III).

MISCELLANEOUS DATA

Dogs given 75 or 150 mg/kg/day, (route not specified) 5 days a week for 18 weeks, did not show any significant changes in behavior or growth, in haematology or clinical chemistry, and in urine composition (summary report only).

Chlorphenesin may affect the immune system : both stimulating and inhibiting properties have been reported (Colipa subm. II). Lymphocyte function in vitro was found to be suppressed by 20-50 µg/ml culture medium (Colipa subm. II, ref. 19). Tumour inhibiting properties have also been reported. It is one of the glycerol ethers which are known to cause paralysis and to act as anticonvulsants. In a repeated intramuscular injection test in mice, with 0.5 ml of a 0.6% aqueous solution daily for 40 days there were no observable effects on growth or on the state of the organs.

CONCLUSION

ALthough short-term studies have been conducted, insufficient information on these studies is available.

Are 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.

2. BENZETHONIUM CHLORIDE SCC/204/88

. FORMULATIONS AND SYNONYMES

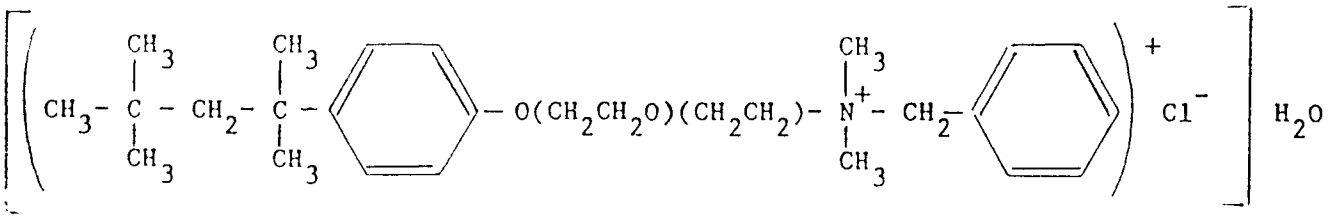
EEC N° 15

Colipa P70

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

Hyamine 1622

phenerol chloride



$C_{27}H_{42}NO_2CL$

. CHARACTERISTICS

Soluble in water, alcohols and other organic solvents.

. USE

Used in cosmetics as a preservative agent at level of 0.1%.

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral (rat) : 420 mg/kg

i.p. " : 33 mg/kg

i.v. " : 19 mg/kg

Intranasal administration of 0.06 ml of a solution of 0.25% or more was lethal to rats.

ORAL TOXICITY

In a 28-day feeding study, rats received diets with 0, 20, 100, 500 or 2500 ppm, providing intake levels of 0, 1.7, 8, 40 or 200 mg/kg bw/day. The changes in the top-dose group included growth retardation, caecum enlargement, signs of liver damage and decreased serum levels of inorganic phosphorus in males. The latter finding was the only effect considered treatment-related in males fed 500 ppm. The diet with 100 ppm (8 mg/kg bw/day) was a clear no-effect level (Colipa subm. III, January 1988).

In a one year study, groups of 3 dogs were fed 0, 5, 100 and 500 ppm in the diet. No changes were observed in growth rate, haematology or in gross - or microscopic pathology.

A two year study has been conducted with groups of 5 rats/sex, fed diets containing 0, 50, 200, 1000, 2500 and 5000 ppm. The top dose induced mortality. With 2500 and 5000 ppm testicular atrophy and caecal enlargement occurred. With 1000 ppm there was only caecal enlargement.

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

Various dilutions applied to the eye of rabbits produced barely perceptible irritation at concentrations of 0.01 and 0.03%.

Skin irritation in rabbits did not occur when 2 ml of a 0.1% dilution were applied daily 5 days a week for 4 weeks. In humans, 0.1 ml of a 5% aqueous solution applied under patches for 48 hours, was irritating (C.I.R. 1985).

A sensitization test in humans with 0.12% in formulations applied to the skin under closed patches was negative (C.I.R. 1985).

Upon sub-acute, dermal application of 2 ml 0.1% solution to the skin of rabbits daily, 5 days/week for 4 weeks no systemic effects were observed (summary report).

Sub-chronic (13-wk) dermal studies in rats and mice are being conducted by the NTP.

Dermal absorption was examined by applying 1.0 ml of a 10% aqueous solution of the ^{14}C-labelled compound under occluded patches to the skin of two rabbits on 4 consecutive days. One rabbit had the skin abraded. Blood samples taken on each day, showed an average concentration of 0.2 ppm, which corresponds to 0.003% of the amount applied. No mention is made of analyses in urine, faeces or carcasses (ref. 3, sumbm. II).

MUTAGENICITY

An Ames test with up to 100nmoles/plate was negative. Another Ames test with up to 7500 µg Hyamine 1622/plate was likewise negative. It was stated that in an in vitro assay with CHO-cells no evidence was found of sister chromatid exchange or chromosomal aberrations but a report is not available (Colipa subm. III, January 1988).

CARCINOGENICITY

Several subcutaneous injection studies have been conducted in rats and mice. In one study in rats, a dose-related increase in the incidence of granulomatous reactions (mainly fibrosarcomas) occurred at the injection site (Cosmetic Ingredient Review 1985). It is not clear wheter this result is an indication of carcinogenic properties of the test substance.

TERATOGENICITY

An oral teratogenicity study in rabbits with 1, 3 and 10 mg/kg/day revealed signs of maternal toxicity with 3 and 10 mg, increased mortality of mothers and pups with 10 mg, and an increased incidence of supernumerary ribs with 3 and 10 mg. The latter finding was attributed to stress (ref. 4, subm. I).

In a second teratogenicity study in rabbits with oral dosing of 1.125, 3.558 and 35.576 mg/kg/day, the high dose induced maternal and foetal mortality. A dose-related increase in foetal resorptions occurred in all treatment groups although the change was statistically significant only in the high-dose group. The mid-dose was not clearly without effect (ref. 8, subm. I).

In a teratogenicity study in rats with oral dosing of 1.125, 3.558 and 35.576 mg/kg/day the high-dose group showed decreased maternal body weight and an increased number of smaller pups. An increased variation in ossification occurred in all treated groups. Skeletal malformation was increased in the high-dose group. Slight hydrocephalus was seen in one pup of the mid-dose group and in 5 pups (in 2 litters) of the high-dose group (ref. 6, subm. I).

A second oral rat teratogenicity study with 0.059, 1.125, 3.558 and 35.576 mg/kg showed lower maternal body weights, increased variation of skeletal ossification and increased incidence of skeletal malformations (wavy ribs) in the top-dose group only. The latter finding was considered to be within the limits for historical controls (ref. 7, subm. I).

Fertility and reproductive performance were examined in rats treated orally with 1.125, 3.558 and 35.576 mg/kg/day prior to and during mating and during the gestation and lactation period. The high-dose produced growth depression, increased irritability, respiratory signs in the parents and decreased viability and decreased body weight of pups at birth. Fertility and general reproductive performance were not affected (ref. 5, subm. I).

Peri - and postnatal effects were examined in rats dosed orally with 1.125, 3.558 and 35.576 mg/kg/day from day 15 of gestation through day 20 of lactation. A slight decrease in foetal viability occurred in all dose groups and in postnatal survival in the mid- and top-dose group (ref. 9, subm. I).

Maternal and foetal absorption of the ^{14}C- labelled compound was examined in pregnant rats treated orally with 1.125 and 3.558 mg/kg/day on days 6 through 15 of gestation. Average blood levels in the two groups were 1.5 and 0.97 ng/g respectively. In urine, the maximum levels were 52 and 149 ng/ml after a single oral dose. Virtually all radioactivity was recovered in the maternal faeces and carcass. Results of foetal analyses varied between not-detectable and 6.8 ng/g foetus (ref. 4, subm. II).

MISCELLANEOUS DATA

Concentrations as low as 0.002% inhibited the motility of the isolated ileum of rats and rabbits. Blood pressure measurements in the dog indicated nearly complete blockage of sympathetic ganglions at an i.v. dose of 2 mg/kg.

CONCLUSIONS

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 to have adequate information on dermal absorption.

Classification : C.

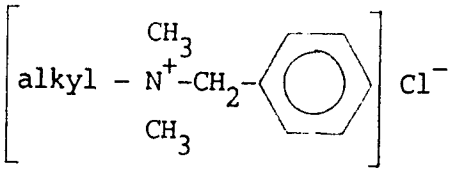
3. BENZALKONIUM CHLORIDE SCC/204/87-88

. FORMULA AND SYNONYMES

EEC n° 54

Colipa P71

Alkyl C_8-C_{18}dimethylbenzylammoniumchloride, -bromide and -saccharinate



dodécyl : $C_{21}H_{38}N.Cl$

. CHARACTERISTICS

Soluble in water and alcohols.

Poorly soluble in hydrocarbons, oils and fats.

. USE

Used as a preservative at levels of 0.25%

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD 50 oral (rats and mice) : 0,5 - 1,0 gr/kg bw (obtained for commercial products with different alkyl groups)

i.v. (mice) : 12,8 - 26 mg/kg intranasal administration of 0,06 ml of a 0,125% solution was lethal for rats.

ORAL TOXICITY

Sub-chronic (13-wk) oral studies in rats revealed toxicity and mortality at dose levels of 25 mg/kg bw and above. With 25 mg/kg bw, administered to dogs daily for 52 weeks, mortality and gastrointestinal damage was observed.

Further, sub-chronic (90-day) oral studies in rats and dogs, conducted in 1968, became available recently (Colipa subm. IV, ref. 20 and 21). In both studies, dose levels of 5, 12.5 and 25 mg/kg bw/day were administered ; in the rat study by stomach tube in the dog study by capsule. No changes attributed to treatment were observed. Because the studies showed several deficiencies, the results do not justify to establish a no-effect level.

In a 2 year rat study, 0.5% in the diet (250 mg/kg bw) caused high mortality and pathological changes in the gastrointestinal tract. Microscopical changes of the intestinal tract were seen also in a 2-year study with a second commercial product at dose levels of 25 and 12.5 mg/kg, and in a 2-year study with a third product at a dose level of 30 mg/kg bw. Dogs given 50 mg/kg bw/day by gavage (at a concentration of 5%) showed changes in the intestinal tract after one year.

The distribution of the compound was studied after oral, rectal and intramuscular administration of the 10-fold lethal dose to rabbits, dogs and cats. Most of the dose remained at the application site. After oral and rectal administration, small amounts were detected in blood and liver. Upon rectal administration a small amount was found also in the kidneys (ref. 16, subm. II).

CUTANEOUS AND NUCOUS TOLERANCE

DERMAL TOXICITY

Skin irritation tests in rabbits with 0.1% solutions, and in humans with 1.0% solutions were negative. With extended contact period in the rabbit, or repeated application in humans these concentrations produced distinct irritation. In rabbits, repeated application 0.3% induced only mild erythema.

Eye irritation in rabbits may occur upon a single application of 0.01% solution and above and upon repeated application of 0.004%. Concentrations of 0.01% and above caused eye irritation in guinea pigs when applied repeatedly on the same day. Single treatment of human eyes with 0.1%, or daily treatment with 0.03 - 0.04% caused irritation.

Soft contact lenses disinfected daily with 0.0025% benzalkonium-chloride + 0.01% EDTA induced severe irritation when brought into contact with the rabbit eye for 6 hr/day.

A sensitization test in 100 male and 100 female volunteers with 0.1%, applied daily for 5 days, followed by a challenge treatment with 1% after 3 weeks, was negative. In the literature only a few cases of sensitization in humans have been reported. Short-term oral administration to several animal species in the diet or the drinking water containing concentrations of 0.02% or more induced toxic effects.

A dermal 90-day study was conducted in rats with a formulation containing 1% stearyldimethylbenzylammoniumchloride and 0.2% benzalkoniumchloride 50%. Once daily, 5 days/week for 13 weeks the rats received topically 2.4 ml/kg (2.4 mg benzalkoniumchloride/kg). It is stated that no significant local or systemic effects occurred. However, the report is confusing and incomplete (ref. 7, subm. II).

Dermal life-time studies in mice and rabbits, treated topically with 0.02 ml of 8.5 or 17.0% solutions twice weekly showed local skin damage in both species, but no skin tumours.

Skin penetration tests in vitro with pieces of human skin were conducted in aqueous solutions of 0.005M to 0.1M benzalkoniumchloride (i.e. 0.17 to 3.4%). No penetration into the dermis was detected when the solution was unbuffered or acid. Measurable penetration occurred when the epidermal barrier was damaged or with intact skin in solutions of pH 11 (ref. 13, subm. II).

No penetration was found in vitro with skin from hairless rats exposed to 2.5% ^{14}C-dimethylbenzylammoniumchloride for 4.5 hours (ref. 14, subm. II).

In a similar in vitro test with human epidermis the mean penetration was 1.47% of the dose applied (ref. 15, subm. II).

MUTAGENICITY

A Ames test with S.typhimurium His G 46-uvr B exposed to 10-100 µg/plate was negative.

A micronucleus test in mice treated i.p. with 20 mg/kg bw, twice, with an interval of 24 hours did not reveal increased numbers of micronuclei (ref. 11, subm. II).

The substance was found to induce repairable DNA damage in the E.coli DNA polymerase A assay, but no mutagenic properties were observed (ref. 10, subm. II). No forward mutations were induced in Schizosaccharomyces pombe P_1 with or without metabolic activation (ref. 18, subm. III).

A chromosome aberration test with CHO-cells in vitro was negative (ref. 19, subm. III).

TERATOGENICITY

In an oral teratogenicity study, groups of 15 pregnant rabbits were treated by gavage with 0, 10, 30 or 100 mg/kg/day (in aqueous solu- of 0.5, 1.5 and 5.0% respectively) from day 7 through day 19 of gestation. All rabbits of the high dose group died. The intermediate dose caused maternal and embryotoxicity. Signs of maternal toxicity occurred also in the low-dose group. There were no indications of teratogenic properties. (Colipa subm. I, ref. 1).

A dermal teratogenicity study was conducted in rats treated topically with 0.5 ml aqueous solutions of 1.6, 3.3 and 6.6%, (estimated to be about 30, 60 and 120 mg/kg) once daily from day 6 to day 15 of pregnancy. No embryopathic effects were observed (ref. 12, subm. II).

CONCLUSION

Benzalkoniumchloride possesses considerable irritative properties for the eye and the gastrointestinal tract, and was highly toxic under certain conditions of acute exposure. In short-, and long-term studies changes have been observed in rats and rabbits with oral doses of 12.5 mg/kg/day, while mortality occurred in dogs and rats with 25 mg/kg/day. The substances is used in cosmetics for preservation and for other purposes. From the estimated maximal daily use of cosmetics and the levels used in the different types of product, a potential daily exposure to 5 mg/kg bw/day is calcu- lated. A no-effect level for systemic toxicity has not been firmly established, and dermal absorption under practical use conditions is not known.

In view of the low safety margin it is essential therefore that detailed information be provided on skin absorption, and an in vivo study in rabbits (using radiolabelled material, single dose, back skin) is requested. It was also noted that 0.1% produced marked eye irritation, and the use of significantly higher concentrations in cosmetic areas where some eye contact was likely to occur, was of concern.

Classification : C.

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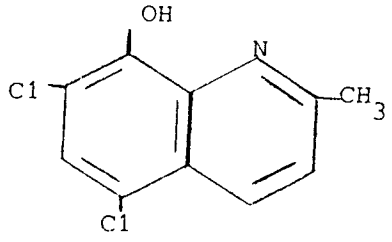
4. CHLORQUINALDOL SCC/204/88

. FORMULA AND SYNONYMES

Colipa P86

Sterosan

5,7 - dichloro - 2 - methylchinolin - 8 - ol



$C_{10}H_7Cl_2NO$

MW 228.08

. CHARACTERISTICS

Moderately soluble in organic solvents (chloroform acetone, ether)
Practically insoluble in water.

. USE

Used as a preservative in cosmetics up to 0.05% (also used at 3% in ointments and creams in medical and veterinary drugs as antiseptic and fungistatie).

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral (rats) : 2,9 gr/kg
(mice) : 0,583 gr/kg
(dogs) : $\succ$ 1 gr/kg

LD50 subent (mice) : 2,9 - 3,1 gr/kg
(rats) : $>$ 4,0 gr/kg
(dogs) : $>$ 0,5 gr/kg

i.p. (mice) : 0,185 gr/kg
(rats) : 1,39 gr/kg

ORAL TOXICITY

A short-term (28-day) oral study with 0, 7 and 21 mg/kg bw/day administered in maize oil by gavage to groups of 7 rats/sex did not reveal any changes which were attributed to treatment. Both the design of the study and the report showed deficiencies (Colipa subm. II, ref. 43 ; summary report only).

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

An eye irritation test in rabbits with 0.1 ml cleansing milk containing 0.02, 0.1 or 0.5% did not reveal clearly more eye changes than did the cosmetic without the test compound (ref. 22).

The substance is known to possess sensitizing potency for humans. In a study with 5558 patients, 2.4% were positive to a mixture of Vioform + Sterosan (ref. 36). Cross sensitization of Sterosan with other halogenated hydroxyquinolines has been observed (ref. 39).

Repeated dermal application of 0.05 ml of a 3% cream to the intact and scarified skin of hairless mice, daily for 28 days, only induced some reddening in 2/10 mice on a few days, while thickening of the skin was seen in 1/10 mice (ref. 21). More sever dermal reactions occurred in 90-day topical application studies in rabbits and dogs (see below, ref. 12-15).

The sub-chronic (90-day) dermal toxicity was examined in groups of 5 rabbits/sex by applying 0.7 g/kg bw of an ointment containing 0, 1, 3 or 10% of the test substance each day for 13 weeks (or 0, 7, 21 or 70 mg/kg bw/day). In the top-dose group all animals died or were killed when moribund in the first 2 weeks. In the top-, and mid-dose group there were haematological changes, and blood was found in urine.

Increases in blood urea-N, absence of spermiogenesis, and microscopical renal changes occurred in all treated groups (ref. 12, 14). In this study, oral uptake was not fully excluded, but in a repeat study with the 10% ointment under occlusion similar changes were found though less severe (ref. 13).

A 90-day dermal study with the 0, 1, 3 and 10% ointments (or 0, 7, 21 and 70 mg/kg bw/day) was also conducted in groups of 2 beagle dogs/sex treated with 7.0 g/dog on 7 days/week.
In all treated groups the dogs showed dermal changes at the application sites, enhanced blood sedimentation rate and changes in white blood cell counts. In addition the 3 and 10% groups showed weight loss, increased numbers of leucocytes, increased activity of some blood enzymes and microscopical liver changes (ref. 15).

Dermal absorption studies with the $[^{14}C]$-labelled compound in guinea pigs in vivo showed that 96-98% of the dose, applied in various formulations, could still be removed from the skin after a 7-hr contact period (ref. 23). In humans treated with a cream, 96% was found not to have penetrated after 8 hrs (ref. 23).

In vitro tests with intact skin from guinea pigs and from nude mice showed more penetration (20-30%) especially when the compound was applied in ethanol (40 and 50% respectively into skin of guinea pigs and nude mice) (ref. 23).

In a study in humans (exposed to Sterosan in ointments and creams), up to 19.5% of a dermal dose was recovered in the urine, while after oral administration of an equal dose (30 mg) the mean urinary excretion (mainly in the form of the glucuronide) was 67.6% (ref. 40, Dermatologica 159 (1979) 239-244.

MUTAGENICITY

An Ames test with up to 100 µg/plate was negative, but 5 ug and above already caused growth depression or complete inhibition of the bacteria (ref. 18).

In the mammalian cell culture HGPRT system with Chinese hamster ovary cell line V79 exposed to up to 15 µg/ml, no mutagenic activity was detected. The highest concentration tested was distinctly toxic (ref.19).

A micronucleus test in mice given once intraperitoneally 37.5, 75 or 150 mg/kg bw, with bone marrow counts after 24, 48 and 72 hours did not reveal an increase in micronucleated cells (ref. 20).

Embryotoxicity studies were conducted in rats and rabbits by dermal application of 0.5, 5.0, 50.0 or 250.0 mg/kg bw, in a 10% containing ointment under occlusion for 5 hours daily on days 7-16 of pregnancy in rats and on days 7-19 of pregnancy in rabbits.

Apart from local irritation of the skin in the highest dose group of rats and in the two higher dose groups of rabbits, there were no other signs of intolerance. The reproduction data were not influenced by treatment. In the rat study several abnormalities of the skeleton were more frequent in the treatment groups than in controls, but it is stated that the incidence were in the normal range (ref. 16).

The two higher dose groups in the rabbit study showed growth retardation which was attributed to the severe local skin damage. The higher incidence of a few bone abnormalities in the treated groups were not considered to represent embryotoxic or teratogenic effects (ref. 17).

CONCLUSION

The substance shows sensitizing potency in humans. Results of dermal absorption studies vary widely. In humans, 19.5% of a dermal dose was recovered in the urine. In short-term studies in rabbits and dogs, dermal application of 21 mg/kg bw/day induced haematological effects, and microscopical changes in kidneys and liver have been reported. Slight systemic changes were seen also upon dermal treatment with 7 mg/kg.

However, no changes were observed upon short-term oral treatment of rats with 21 mg/kg bw/day.

Interpretation of these studies is difficult in view of the apparent inconsistencies between species, but a no-effect level of below 7 mg/kg must be assumed on the present, together with good absorption through the skin.

The safety factors arising from cosmetic use, making such assumptions, were relatively low, and it was felt that more data, from an adequate 90-day study, using the oral route and a sensitive species (preferably the dog), were needed to obtain more definite information on a NEL.

Classification : C.

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Guidelines for testing chemicals 407 ;
Himberg 3-7-1987

REPORTS OF 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 certain U.V filters in cosmetics is admissible from the health point of view.

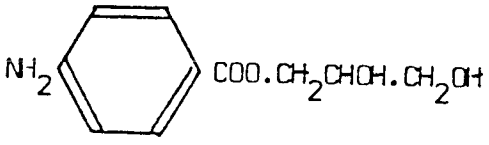
- Glyceryl paraaminobenzoate up to 5%
- 2 - Ethylhexyl - 4 - methoxycinnamate up to 10%
- 4 - tert - butyl - 4' - methoxydibenzoylmethane up to 5%

1. GLYCERYL PARAAMINOBENZOATE

SCC/414/88

. FORMULA AND SYNONYMES

EEC N° 2.4
Colipa S6
Glyceryl - 1 - (4-aminobenzoate)
Glyceryl PABA
"ESCALOL 106"



$C_{10}H_{13}O_4N$

MW = 211,2

. CHARACTERISTICS AND COSMETIC QUALITY

Creamy-white powder.
Insoluble in water and mineral oils.
Soluble in ethanol and propylene glycol.
Powder 80-90% pure, containing as impurities glyceryl di-para-amino-benzoate and traces of p-aminobenzoic acid and glyceryl-tri-paraamino-benzoate.
Stated to be "free of benzocaine".

In a test for purity of samples of glyceryl-PABA, 2 from one manufacturer in 1983 showed substantial amounts of benzocaine present. On the other hand, there was a smaller concentration of benzocaine in another sample (from a different manufacturer) in 1978, and in 1983, a sample from the latter source showed no benzocaine (limit of detection, 0.05%). It is understood, however, that analyses of this sunscreen agent in the EC has frequently shown evidence of the presence of benzocaine.

. USE

Up to 5%

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral (rat) :> 8 gr/kg

ORAL TOXICITY

A marketed lotion containing 3% a.i. and 3% amyl p-dimethylaminobenzoate was non-toxic to rats and mice in a single oral dose of 50 ml/kg (=1.5 g/kg of a.i.).

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

Irritation of the mucous membrane.

Rabbit : Twelve animals were treated with 2 commercial solutions, applied to one or other eye. Each lotion contained 3.15% a.i. and 3.15% amyl p-dimethyl-aminobenzoate. Half the animals had rinsing after 2 mins. Slight conjunctivitis subsided in 1 hr in the rinsed eyes and in 24 hrs in the unrinsed eyes.

In 8 rabbits 0.5 ml of a 5% solution of a.i. in propylene glycol was instilled into the eye. Washing was carried out in 5 animals. No detectable response was found in any animal.

Irritation of the skin

Rabbit : A 5% solution of a.i. in propylene glycol was applied to intact and abraded skin of 6 rabbits under occlusion for 24 hrs.
No irritant response was found.

Two commercially available lotions were tested. Each contained 3.15% a.i. and 3.15% amyl p-dimethylaminobenzoate. Six animals received 0.5 ml of one preparation, the remaining 6 received 0.5 ml of the other. Applications were made to both intact and abraded skin, and the sites in 3/6 animals in each group were occluded. Contact time was 24 hrs, and readings were made up to 72 hrs. There was slight initial erythema in all animals which quickly cleared.

A similar study which used 0.2 ml instead of 0.5 ml had the same results.

Man : Fifty human volunteers received 15 applications, each of 24 hrs duration, with occlusion. This strength of the solution is not given. An interval of 24 hrs was allowed between successive applications and 48 hrs at weekends. Following a 2 weeks rest, the test material was applied to the same sites in the same way. The substance was judged not to be an irritant or a sensitiser.

Sensitisation

Guinea-pig : Ten animals were induced with 5% a.i. in propylene glycol under occlusion. A challenge with the same material did not produce sensitisation.

Man : (See also above, under irritation). In 25 m, a product containing 3% a.i. and 3% amyl p-dimethyl-aminobenzoate was tested. Following 24 hr pretreatment with 5% sodium lauryl sulphate under occlusion, the product was applied to the forearms under occlusion for 5 alternate 48-hr periods. After a 10-day rest period, new sites were pretreated with 10% sodium lauryl sulphate for 1 hr under occlusion. Challenge patches were then applied for 48 hrs under occlusion. Sensitisation was not found.

Photosensitivity and phototoxicity

Man : A mixture of 3% a.i. and 3% amyl p-dimethyl-aminobenzoate was tested in 35 volunteers. 0.2 ml of test material was applied to normal and stripped skin on the back (probably for 24 hrs with occlusion). Each site was then exposed to 6 MEDs of xenon radiation filtered through window glass. Observations were made 1, 3 and 24 hrs after irradiation. No abnormality was found.

Fifteen women and 25 men had the above mixture applied daily to the face and upper trunk, after which the subjects were exposed to 3 MEDs. Four women and 8 men complained of very mild itching around the eyes, but there were no visible signs of reaction in any subject.

Thirty-five volunteers (same as in first paragraph, above) had 0.2 ml of the test material applied to an area of 25 cm^2 of stripped skin on the back, which was then exposed to 3 MEDs from a xenon tube and occluded (times not specified). This procedure was repeated 5 times at intervals of 48 hrs. Ten days later 0.2 ml was applied both to stripped and normal skin followed by exposure to 3 MEDs of xenon tube radiation. After occlusion, probably for 24 hrs, the sites were inspected at 24, 48 and 72 hrs. No evidence of photocontact allergy was found.

Twenty patients known to be sensitive to benzocaine were patch tested with 5% benzocaine with occlusion and simultaneously with two commercial sunscreen preparations under the same conditions. It had previously been established by HPLC that one of these preparations contained 0.3% free benzocaine (arising as a contaminant in the manufacturing process) and the other 0.001%. All patients reacted positively to the benzocaine patch ; 11/20 to the preparation containing 0.3% benzocaine.

In a number of clinical studies, patients with allergic reactions to glyceryl-PABA were studied. One author found that the compound was a common sensitiser and that there was invariably cross-reaction with benzocaine. There was also cross-sensitivity with aniline and paraphenylenediamine. The a.i. was also capable of acting as a photosensitising agent. In another report, one patient was shown to have both photosensitisation and contact allergy to the compound.

In another study, 2 patients with sensitivity to the a.i. were identified among 23 patients complaining of skin disorders following exposure to the sun. Of these, 1 was found to have a photosensitivity and the other a contact allergy. In both cases the sensitivity was to PABA but not to benzocaine. Three patients with benzocaine sensitivity however, did not show a cross-reaction by glyceryl-PABA.

Subacute toxicity

Rat : Eight animals had applied 0.2 ml of the commercial lotion mentioned above daily to the skin for 32 days. No abnormality was found.

Rabbit : Over a period of 20 days, 20% of a.i. was applied to intact and abraded skin of rabbits (numbers not specified) at levels of 1, 2 and 4 g/kg bw daily. Control animals received vehicle only. No abnormal systemic or topical signs were found.

Subchronic toxicity

Rat : Groups of 10 m and 10 f were treated with 0, 55.5, 277 and 555 mg/kg bw/day in the form of a solution of 18.5% a.i. in propylene glycol (vehicle only for controls). These doses were selected to correspond with similar tests carried out with "Escalol" (another aminobenzoate) and also on the basis of work which showed that the average

human female used about 4 mg/kg bw/day of the a.i. ; thus the doses were considerably higher (about 14, 69 and 239 times use level). Treatment was carried out daily by application to shaved skin, approximately 15% of body surface, 5 days a week for 13 weeks. Various haematological and biochemical tests were carried out in weeks 7 and 13. All survivors were subjected to necropsy. A selection of tissues was weighed, and histological examination carried out if abnormal weight or appearance was noted.

All animals survived. No abnormal changes in body weight or behaviour were noted. High dose males showed slight skin irritation, otherwise no skin abnormality was found. High dose females showed a significant increase in relative and absolute weights of the spleen, but the histological and haematological pictures were normal in these animals. There were slight changes in the blood picture in high dose animals, but no definite trend was present, and the changes were slight in absolute terms. There were no significant abnormalities in urine. Biochemical examination of the blood showed no definite abnormalities. NEL 277 mg/kg bw/day.

CONCLUSION

. The Committee felt that there were several gaps in the data provided.

. There were no reports of tests on oral toxicity
mutagenicity or
carcinogenicity

. The concentrations used in testing for irritation and sensitisation were low in relation to the proposed use level.

. The purity of the compound and its freedom from benzocaine should be rigorously established.

. It was important to know definitely whether it was absorbed from the skin.

. Teratogenic activity would be necessary.

Classification : C.

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First Version	:	August	1983.
Revised	:	November	1983.
Revised	:	August	1987.
Revised	:	March	1988.

2. 2-ETHYLHEXYL-4-METHOXYCINNAMATE SCC/414/88

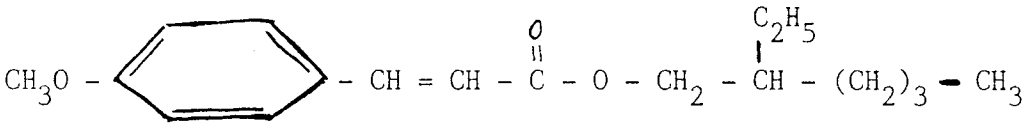
. FORMULA AND SYNONYMES

EEC N° 2.13

Colipa S28

Neo Heliopan AV MW = 290

Parsol MEX



. CHARACTERISTICS

Odorless, pale yellow, slightly oily liquid.

Insoluble in water.

Miscible in alcohols, propylene glycol, mono-myristate and various oils (ref. 1).

. USE LEVELS

Maximum concentration authorized (council Directive) : 10%

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50 oral (mouse) : > 8 gr/kg (ref. 1, 2, 6)

(rat) : ≥ 20 ml/kg (ref. 3)

ORAL TOXICITY

Subacute toxicity

Rat : Three week oral study. Groups of 5 m and 5 f animals were given 0, 0.3, 0.9, and 2.7 ml/kg/bw/day by gavage for 3 weeks. All animals of the top dose groups exhibited loss of body weight and a reduced relative and absolute weight of the thymus.

Male rats showed a decrease in absolute weight of the left kidney and female rats showed a decrease in the absolute weight of the heart.
At the 2 lower doses the only significant alteration observed was an increased absolute weight of the pituitary gland in male rats receiving the lowest dose. As the number of animals was low, the investigators considered this not to be biologically relevant.
N.E.L. 0.9ml/kg bw/day.

Subchronic Toxicity

Rat : Thirteen week oral study. Four groups of 12 m and 12 f SPF rats received the compound in the diet at levels of 0, 200, 450 and 1,000 ml/kg bw/day. During the experiment the usual clinical observations were carried out, as well as extensive haematological and biochemical studies. Full gross necropsy was carried out on all survivors.
Histological investigations were carried out in half the animals of the control and top dose groups. The organs studied included the heart, lungs, liver, stomach, kidneys, spleen, thyroid and retina.
In the remaining animals histological examination of the liver only was carried out. Six control animals and 6 top dose animals were allowed to recover over 5 weeks, and then examined.

The results of the experiment showed no dose-related mortality. The kidney weights of top dose animals were increased, but were normal again in the animals allowed a recovery period ; the increase was attributed to a physiological response to an increased excretion load.
There was a dimunition of glycogen in the liver, and a slight increase in iron in the Kupfer cells, in the high dose animals. Two of these also showed minimal centrilobular necrosis of the liver with some infiltration ; similar less marked findings were attributed to infection.
High dose females had increased GLDH which reversed during the recovery period. The N.E.L. was probably 450 mg/kg bw/day. (ref. 10).

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

Mucous membrane irritation

Rabbit : 4 animals/group.
Instillation of 0.1 ccm of a test preparation (concentration unknown) into the conjunctival sac. No further treatment in one group, subsequent washing out in another.
No signs of inflammation (ref. 5).

Rabbit : Draize test with 0.1 ml of the undiluted test liquid.
The chemical was considered as "practically non-irritating to the eye". (ref. 1, 2, 6).

Skin irritation

Guinea pig, 20 animals.

Epicutaneous application of the undiluted chemical twice daily for 16 d. No signs of irritation (ref. 5).

Man, 60 subjects, 20 of them with sensitive skin.
Occlusive patches containing the undiluted test liquid were applied for 24 h. No irritation immediately after exposition nor after 24 and 48 h of observation (ref. 5).

27 men and 22 women.
Patch test using an unspecified concentration. No positive results after 24 and 48 h (ref. 1).

Man, 50 subjects, about one third of them with extremely sensitive skin. The product was very well tolerated on the skin (ref. 1, 2, 6).

Man, 54 subjects.
Draize repeated insult patch test of a 7.5% dilution in petrolatum. No irritation (ref. 1, 8).

Man, 53 subjects.
Draize repeated insult patch test at a concentration of 2%. No irritation (ref. 7).

Sensitization

Guinea pig, 20 animals.
Induction : epicutaneous application of the undiluted chemical twice daily for 16 d. Interval without treatment : 3 d. Challenge : one application daily for 3 d. No sensitization (ref. 5).

Guinea pig, 4 animals/group.
One group received 0.05 ml of the undiluted test liquid by intracutaneous injection on 5 subsequent days. In another group 0.025 ml of a 50% acetone solution were applied topically daily for 3 weeks to 2 cm^2 areas on the shaved sides.
No sensitization by either topical or intradermal route (ref. 1).

Man, 53 subjects.
Draize repeated insult patch test carried out at a concentration of 2%. No sensitization (ref. 7).

Man, 54 subjects.
Induction : a formulation containing 7.5% of test liquid diluted in petrolatum was applied 11 times for 48 h under occlusive conditions. Interval without treatment : 14 d. Challenge : application of a single dose. No reaction (ref. 8).

Phototoxicity and Photosensitization

Man, 10 subjects.
Application of patches for 24 h and subsequent UV-radiation with a suberythema dose. No signs of phototoxicity (ref. 5).
Tests which "showed that the product did not provoke photosensitization" (ref. 1, 6). No details available.

Dermal Toxicity

Rat : 13 week dermal study. Four groups of 10 m and 10 f SD rats were treated by application of various concentrations of a.i. in light mineral oil. The doses were : 0, 55.5, 277 and 555 mg/kg bw/day applied to shaved skin 5 days a week for 13 weeks. (The doses are believed to be maximally 135 times the amount used daily by the average consumer). Various laboratory and clinical tests were carried out during the experiment. All animals showed a slight scaliness at the site of application, which may have been a reaction to the vehicle. Body weight gain was greatest at the low dose. Haematology showed no significant change. SAP was elevated in high dose animals but the investigators did not believe the change to be significant. The relative liver weight in high dose animals was elevated, but microscopic appearances were normal. The n.e.l. was put at 555 mg/kg bw/day, but may be 227 mg/kg bw/day in view of the liver findings (ref. 11).

Permeation of skin

In vitro tests.
Rat : Naked rat skin : This was set up in a chamber experiment. Most of the material was found in the stripped skin ; there was less in the stratum corneum, and least in the chamber. Approximately the chamber contained : after 6 hr 1.13% ; after 16 hr, 11.4% ; and at 24 hr 17.9%. The figures for the horny layers and the stripped layers combined were, respectively, 31.4%, 44.4%, and 45.7% (percentages of applied doses). Solutions of 3% and 20% of a.i. gave similar results.

Another set of experiments was carried out in which varying amounts of "Parsol 1789" were added to the a.i. in the formulation. There seemed to be no effect on the absorption of the a.i. (ref. 14).

Pig :

A similar experiment using mini-pig skin was carried out in which "Parsol 1789" was used as well as the a.i. Using 3 sorts of formulations, about 3% of a.i. was found in the chamber in 6 hr. Using the concentrations proposed for commercial use (i.e., 7.5% of "Parsol 1789" and 2% of a.i.) about 2.2% was found in the chamber. It is calculated that the total absorption for 75 kg consumer would be about 70 mg - about 0.9 mg/kg bw. (ref. 15).

Man :

A test on human abdominal skin in a chamber has been carried out. With 7.5% a.i. about 0.03% is found in the chamber in 1 hr., 0.26% in 6 hr., and 2.0% in 18hr. Varying mixtures of a.i. and "Parsol 1789" were investigated (ref. 16).

In vivo tests :

Man : Eight healthy volunteers had small amounts of radioactive a.i. applied to the interscapular region. One group of 4 had the material applied under a watch glass ; the other 4 had it applied on gauze, with occlusion in one case. Tests for a.i. absorption were negative except for about 0.2% in the urine. The concentrations used seem not to be stated (ref. 17).

MUTAGENICITY

Salmonella mutagenesis assays were performed on strains TA-98, TA-100, TA-1535, TA-1537, and TA-1538 in the presence and absence of S9 mix. Positive results with strain TA-1538 without metabolic activation. This may have been a batch effect. The chemical also increased the frequency of sex-linked recessive lethals in Drosophila melanogaster (ref. 9).

Ames test : From another laboratory, a very weak positive was found with strain TA-1538 without activation, at 10 µl/plate ; it was not found in 2 replicates, nor in a second Ames test (ref. 18).

Chinese hamster V 79 cells. There was a very slight increase in mutant colonies with dose (ref. 19).

Test for mutagenesis and possible crossing over in S. cerevisiae : negative (ref. 20).

In vitro human lymphocyte test - negative (ref. 21).

Balb/c 3T3 cell transformation : negative (ref. 22).

Unscheduled DNA synthesis : negative (ref. 23).

Drosophila mutagenicity studies (feeding, larvae and adults) : negative (ref. 24).

Mouse micronucleus test : no effect up to 5000 mg (ref. 25).

Drosophila : somatic mutation and combination test using wing structure : negative (ref. 26).

CARCINOGENICITY

Test for inhibition of UV-induced tumours in hairless mice.

The technique involved exposure of hairless mice to repeated doses of UV simulating the solar energy spectrum. After a rest period, 3 applications a week were made to an area of skin of 12-o-tetradecanoyl phorbol-13-acetate (at first at 10 g/ml, but later at 2 g/ml as the higher concentration was irritating). Suitable controls were used. The test group was protected by 50% a.i., and 7.5% gave an effect equivalent to reducing the insolation four-fold. It had been suggested that the a.i. could itself be a promoter, but the authors found no evidence for this (ref. 25).

TERATOGENICITY

Rabbit : Groups of 20 f rabbits were mated and given 0, 80, 200 and 500 mg/kg bw/day by gavage during the period of organogenesis. Except for a slight reduction of maternal and foetal weight in the top dose, there was no evidence of embryotoxicity or of teratogenesis. The N.E.L. was put at 200 mg/kg bw/day (ref. 12).

Rat : Following a pilot study, groups at 36 rats were mated and treated with 0, 250, 500 and 1000 mg/kg bw/day of a suspension of a.i., probably by gavage, from day 6-14 of gestation. Owing to an error, the preparation of the control foetuses led to their destruction, so this part of the test was repeated under identical condition Subgroups of each dose group were allowed to litter normally and rear the offspring. No abnormalities were observed, except that the percentage of resorptions in the high dose group was elevated by comparison with the other groups. The investigator reports, however that this relatively high rate is the usual one with this strain of rat in this laboratory, and he attributes the difference to an unusually low level of resorption in the other groups. The N.E.L. may be at 1000 mg/kg bw/day (ref. 13).

CONCLUSION

The data presented suggest that the compound is not an irritant or a sensitiser ; however, the tests for sensitising capacity in man were carried out at levels below the proposed maximum use level. There is no carcinogenicity study, but tests for gene mutation and chromosomal aberration were very probably negative. The Committee would like to see a test for percutaneous absorption in man carried out under realistic conditions, and the results expressed if possible as an absorption coefficient rather than as a percentage of the applied dose. The evidence presented, however, does suggest that substantial absorption from the skin might be expected.

Classification : C.

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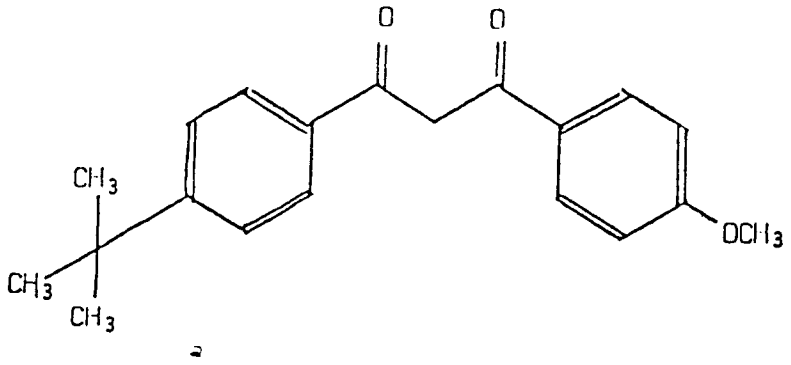
3. 4-TERT-BUTYL-4'-METHOXYDIBENZOYLMETHANE SCC/414/88

. FORMULA AND SYNONYMES

EEC N° 2.31
Colipa S66
Parsol 1789



$C_{20}H_{22}O_3$

MW 310.4

. CHARACTERISTICS

Crystalline substance.
Insoluble in water.
Soluble in carbitol 15%, chloroform 15%, acetone 5%, ethanol 2%.

. USE

Use level up to 5%.

. RECAPITULATION OF THE STUDIES OF TOXICITY

LD50	oral	(rat)	:	16 gr/kg bw
	oral and ip	(mouse)	:	8 gr/kg
	dermal	(rat)	:	1 gr/kg bw ref.(1-2-3).

ORAL TOXICITY

Suchronic Toxicity

Rat : Thirteen-week study. Four groups of 12 m and 12 f animals were given the a.i. in the diet in amounts equivalent to 0, 200, 450 and 1000 mg/kg bw/day.

There were no treatment-related deaths. Food consumption was reduced in the intermediate and top dose groups. There was a fall in the red cell count in females at the intermediate and top dose levels. The plasma protein levels were somewhat higher in all dosed animals, but this did not seem to be dose-related.

At necropsy, the relative liver weights of males were increased at the top dose, and the absolute and relative liver weights of females were increased in both the intermediate and top dose animals. One top dose animal showed damage to the germinal epithelium of the testis.

Supplementary groups of 6 rats were treated with the top dose and then allowed a 4-week recovery period. At sacrifice, the liver weights of these rats were similar to those of control rats.

If the increase in liver weight is accepted as an effect, the NEL is 200 mg/kg bw/day ; otherwise it may be 450 mg/kg bw/day. (4).

CUTANEOUS AND MUCOUS TOLERANCE

DERMAL TOXICITY

Irritation of mucous membranes

Rabbit : A standard Draize test was carried out, using a.i. dissolved in diethyl phthalate, without rinsing. There was no adverse effect up to the limit of solubility, 20% (6).

Irritation of the skin

Rabbit : Five groups, each comprising 10 m and 10 f, were studied. In each group, 5 animals of each sex had the skin of the application site abraded. The amounts applied were 30, 60 and 360 mg/kg bw/day, with occlusion for 6 hrs, for 21 consecutive days. Some irritation was found in the vehicle control animals. There was a dose-related erythema in treated animals. Abrasion of the skin did not affect the findings. No treatment-related changes were found in body weight, food or water consumption, or in haematological or biochemical tests. No abnormality was found on histoligical examination, except at the sites of application (7).

Rabbit : [Two ?] groups of 6 animals were used. The a.i. was dissolved in ethanol/2-phenylethanol (50/50) in a concentration of 10% ; 0.5 ml was applied to an abraded and a non-abraded site on the skin of each animal for 4 hrs with occlusion. A very slight irritant effect was found, and was thought to be due to the vehicle. No individual data are given, and the report is somewhat difficult to interpret (8).

Tests for sensitisation

Man : Repeated insult patch test.
Eleven male and 49 female subjects were recruited ; 8 failed to complete the study. About 0.2 ml of a 10% solution was applied under occlusion for 24 hrs on 10 occasions, with rest intervals of 24 or 48 hrs. On completion of this course, a 10-day rest period was allowed and then challenge applications were made to the original site and to a new site. No adverse reaction was observed (9).

Tests for capacity to produce sensitisation

Guinea-pig : Freund's complete adjuvant test.
Two groups of 8-10 animals were used. Induction was by 3 intradermal injections, on days 0, 4 and 9, of 50% [?] emulsion of the a.i. in Freund's complete adjuvant.

Animals of the control were injected with Freund's adjuvant only. On days 21 and 35 a challenge was made by epicutaneous application of 0.025 ml of a.i. (solvent not specified) at the minimal irritant concentration and 3 lower concentrations (not specified in the report). There was no evidence of sensitisation (10).

Guinea-pig : Magnusson-Kligman maximisation test. Groups of 20-25 animals were used as test and control groups respectively. Induction was by intracutaneous injection of 0.1 ml of 5% a.i. in Freund's complete adjuvant, 5% in saline and adjuvant alone. This was followed 7 days later, by an epicutaneous application of a 20% suspension of a.i. with occlusion for 2 days. The challenge was carried out on day 21 ; 20% and 6% were applied for 24 hours. There was no evidence of sensitisation (11).

Guinea-pig : Open epicutaneous test. The experimental group consisted of 20 animals and the control group of 10. The account given is obscure, but it would appear that the maximum non-irritating concentration and the minimum irritating concentration were first determined. Solutions of 20% and 6% were applied to one flank for 21 days. Challenge was made at 21 days and 35 days by application of a 10% solution to the opposite flank. There was no evidence of sensitisation (12).

Test for potential to cause phototoxicity

Guinea-pig : Ten animals were used. Five sites were delineated on each flank. To each of 4 sites, 20% of a.i. in acetone was applied with 2% DMSO as a maximising agent. An alcoholic solution of 0.1% 8-methoxypsoralen was used on the fifth site as a positive control. Thirty minutes later, one flank was exposed to non-erythemogenic UV-A radiation at 20 J/cm^2 . Sites were scored 4, 24 and 48 hrs after application. There was no evidence of phototoxicity (13).

Test for potential to cause photo-allergenic effects

Guinea-pig : Groups of 10 animals were used. In the 2 test groups, induction was carried out as follows :

(a) 4 injections of 0.1 ml of Freund's complete adjuvant were made in the neck to delineate a square.

(b) applications of 0.1 ml of a.i. in acetone were made over 8 cm^2 in this area, and 30 minutes later UVA irradiation at 10 J/cm^2 was applied.

(c) Procedure (b) was repeated 5 times over the subsequent 2 weeks.

(d) On days 21 and 35, a challenge was applied to both flanks using 0.025 ml of solution over 2 cm^2.

The left flank of each animal was then irradiated as above ; reading was at 24 and 48 hrs. In the first experimental group, the induction concentration was 10% and the challenge concentration was also 10% in the second experimental group, the concentrations were 1% and 10% respectively. A negative control group received no a.i. by way of induction, and for the positive control 3% tetrachlorosalicylanide replaced the a.i. There was no evidence of photo-allergenicity (14).

Test for potential to cause photosensitisation

Man : Twenty-five volunteers were used. The a.i. was incorporated into petrolatum at 2%, to which 2% DMSO was added as a maximising agent. A minimum erythema dose for each subject was determined by finding the time taken to produce erythema using UVA + UVB, 285-400 nm.

Induction was then carried out by application of the a.i. to two areas of the skin of the back, with occlusion. One of these was a test area, the other an irritancy control. The patches were removed after 24 hrs. After a further 24 hrs, the test area was exposed to 3 minimal erythema doses. This entire procedure was carried out 6 times, beginning on days 1, 4, 8, 11, 15 and 18. Challenge was carried out about 10 days after the completion of induction. The test material was applied to two fresh sites and occluded for 24 hrs. Radiation was then applied using 10 J/cm^2. UVA, 320-400 nm. A radiation-only site was used as a control. There was no evidence of any adverse reaction (15).

Subacute Toxicity

Rat : Dermal, Four-week study. Four groups, each of 5 m and 5 f animals were used. Dose levels and conditions were : control (abraded skin) ; 120 mg/kg bw/day (abraded skin) ; 200 mg/kg bw/day (abraded skin) ; 230 mg/kg bw/day (intact skin). Exposure was for 5 hrs a day, with occlusion, followed by rinsing. There was some skin irritation in all groups. Some changes in haematological findings occurred, but they do not appear to be significant. No significant changes were found in biochemical values. Necropsy was grossly normal, and histological examinations revealed no abnormality, either in internal organs or the skin (5).

Tests for percutaneous absorption

Rat : Isolated naked rat skin in vitro. A test was carried out using a chamber in the usual way. The a.i. was labelled with ^{14}C, and 180 $\mu g/cm^2$ was applied to the skin as a 1.5% solution in acetone or deltyl (proposed use level 5%). Radioactivity was measured in the residue on the skin, in the stratum corneum prepared by stripping, in the remainder of the skin and in the chamber fluid. The durations of the experiments were 1 and 6 hrs. Levels of radioactivity were expressed as percentages of the applied radioactivity. The results were as follows :

(a) Acetone solution : 1 hr, stratum corneum 11.5%, remaining skin 4.2%, chamber fluid, nil (total 15.7%). 6 hr : 10.1%, 10.8% and nil (total, 20.9%).

(b) Detyl solution : 1 hr, 4.1%, 5.0% and nil (total 9.1%) ; 6 hr: 4.0%, 7.3% and nil (total 11.3%).
Thus there was somewhat less absorption from the deltyl solution. The number of experiments carried out is not clear, but probably it was one at each dose and time interval (22).

In another similar experiment, the a.i. was dissolved in carbitol at a concentration of 1.5%, and amounts of 120, 360 and 1200 ug/cm^2 were applied to the skin with exposures for 1, 6, 16 and 24 hrs.

There was only trivial penetration, at most, to the chamber.
The totals found in the strippings + skin were :

At 120 µg/cm^2:	1 hr 6.7% ;	6hr 16.7% ;	16 hr 27.7% ;	24 hr 44.3%.
At 360 µg/cm^2:	7.8% ;	17.5% ;	39.8% ;	41.7%.
At 1200 µg/cm^2:	4.6% ;	13.8% ;	25.8% ;	33.9%.

Thus, although the amounts in the skin increase with time, they do not seem to be influenced by concentration in the range studied (23).

A similar set of experiments was carried out, except that in this case they were designed to see whether the incorporation of both the a.i. and "Parsol MCX" in the vehicle made any difference to the absorption of either substance. The solvent was carbitol. Readings were at 1 and 6 hrs. The results, as far as the a.i. was concerned, were broadly similar to those of the preceding experiments (24).

Pig : Minipig skin in vitro.
It seems probable that the method used was similar to that for the rat skin, but this is not stated. The contact time was 6 hrs. The aim was to study the effects of various formulations, and to see whethe "Parsol MCX" affected absorption of the a.i., or vice versa. It is possible to extract those data which refer to the permeation of the a.i. only. A single concentration (2%) was used, in 3 different vehicle - o/w lotion, - o/w cream, and - w/o cream. The application was of 120 µg/cm^2 . The results showed that the total amounts found in the skin (stratum corneum + remaining skin) were as follows, for the respective formulations : 2.6%, 3.7% and 2.9%. (These were similar in the presence of 7.5% of "Parsol MCX"). The authors calculate that these values, extrapolated to man under reasonable use conditions, would suggest that 129 µg/kg bw/day would be absorbed into the skin (25).

Man : Skin permeation in vitro.
Abdominal cadaver skin was used in a chamber experiment. The a.i. was labelled with ^{14}C and formulated as 2% in a w/o cream. The cream was applied at a dose of 2.5 mg/cm^2 (= 50 µg a.i./cm) and left for 1, 6 and 18 hrs. After stripping, the remainder of the skin was cut horizontally to give layers which consisted of epidermis, upper corium, lower corium, and subcutaneous fat respectively.

Radioactivity was measured in the strippings, the various skin layers, and the chamber fluid. The total amounts in the skin, with the preparation containing the a.i. only, are given for comparison with the animal data summarised above. They were : 1 hr, 4.51%; 6 hr, 7.03% ; 16 hr, 15.96%. Activity was found in the fat after the longest exposure only, and then in an amount of 0.10%. No activity was found in the chamber fluid at any time. In general, activity decreased as the distance form the surface of the skin increased ; and activity increased in all layers as exposure became longer. The amounts reaching the deepest layers were very small - e.g., even at 18 hrs the percentage of radioactivity found in the lower corium was 0.34% (26).

Rat : in vivo.
A 1% solution of ^{14}C labelled a.i. dissolved in carbitol was used. This was applied to the rat skin at a dose of 120 $\mu g/cm^2$. Whether occlusion was used is not mentioned. After 6 hrs, the skin was cleaned and the radioactivity in the washings estimated. The animals were killed, and the treated area of skin excised. The amounts of radioactivity found in the strateum corneum and in the deeper layers were estimated, and were found to be 1.4% and 2.3% respectively.
(Summary report only ; no experimental details. Origin of report not stated).

Man : in vivo.
This study was designed to test the effect of "Parsol MCX" on the absorption of a.i. In a first experiment, ^{14}C-labelled a.i. was applied to the backs of 4 healthy volunteers, 1 with occlusion and 3 without. The application was of 200 μl of a 10% solution in carbitol, spread over 10 cm^2 (= 2 mg/cm^2 a.i.). Exposure was for 8 hrs. From the subject with occlusion, the percentage of radioactivity recovered from the strippings was 0.48, and from the urine 0.08. In the absence of occlusion, the figures were 0.17 and 0.013, respectively (mean of 3 subjects). In a second experiment, the same procedure was repeated, except that unlabelled "Parsol MCX" was added to the a.i.

In this case, the respective recoveries were 0.32 and 0.04 (occluded) and 0.56 and 0.03 (mean of 3 experiments without occlusion). No radioactivity was found in the blood or faeces in any case (27).

MUTAGENICITY

An Ames test was carried out with up to 500 µg a.i. dissolved in DMSO. The test was negative with and without activation (16).

A test was carried out using Saccharomyces cerevisiae with up to 125 µg of a.i./ml. There was no evidence of mutagenic effects (17).

Tests were carried out on V79 chinese hamster lung cells to see whether the a.i. induced mutation at the HGPRT locus. Concentrations higher than 20 µg/ml difficulties because of solvent toxicity (methanol). In addition, the a.i. was cytotoxic in the absence of activation. For this reason, 20 µg/ml was the highest concentration tested. There was no evidence of mutagenic effect (18).

Mouse : Micronucleus test.
Doses of 1000, 2000 and 5000 mg/kg bw were given 30 and 6 hrs before sacrifice. The test was negative (19).

TERATOGENICITY

Rat : In a preliminary test, it was found that 1000 mg a.i./kg bw/day did not induce any maternal abnormalities, although there was some increase in resorptions. This was chosen as the top dose. In all, 4 groups of 36 animals were used. The dosing rates were 0, 250, 500 and 1000 mg/kg bw/day by gavage from days 7 to 16 of pregnancy. The numbers pregnant in each group were, respectively, 33, 35, 31 and 34. At day 21, the animals were divided into approximate half-groups ; one half was selected for sacrifice and the other to continue to delivery and rearing.

The only suspicious finding was a litter with a few [3 ?] unilateral eye defects at the top dose. Since this was confined to one litter it was not considered to be compound-related. All other groups showed no important changes. The rearing subgroup was normal. There was no compound-related maternal toxicity (20).

Rabbit : Four groups of animals were used - control, 80, 200 and 500 mg/kg bw/day (owing to an error, these target doses were not quite achieved). The a.i. was given by gavage from day 7-19 of pregnancy. The numbers pregnant in each group were, respectively, 17, 19, 17 and 19. There were some maternal deaths, probably not compound-related. In the low dose group, there was a high incidence of resorptions ; the reason for this is not clear. At sacrifice, there appeared to be a high incidence of abnormalities in the medulla oblongata region, the nature of which is not very obvious from the report. Probably however, the changes are not significant in that they do not appear to be compound-related. The report, in general, is not easy to interpret (21).

CONCLUSION

The Committee felt that the results of the mutagenicity studies might render a long-term carcinogenicity study unnecessary. It would be preferable if the data on absorption in man could be expressed in terms of an absorption coefficient rather than as a percentage of the dose applied.

The teratological study in the rabbit has many anomalous findings, and is difficult to evaluate.

Details of the absorption spectrum of the compound should be supplied.

As a matter of general principle, the Committee queried whether photomutagenicity studies should be carried out on this and other ultraviolet absorbing compounds.

Classification : C.

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