Commission of the European Communities

environment and quality of life

REPORTS of the Scientific Committee on Cosmetology

(sixth series)

Report

EUR 11139 EN

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Directorate-General
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FORWARD

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 Directorate-general for the environment, consumer protection and nuclear safety.

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REPORT BY THE SCIENTIFIC COMMITTEE ON COSMETOLOGY CONCERNING CERTAIN PRESERVATIVES

(Opinion expressed on 1. July 1986)

TERMS OF REFERENCE OF THE COMMITTEE

To give its opinion on the use, as preservatives in cosmetic products, of the substances listed in Part 2 of Annex VI to Directive 76/768/EEC (Annex 1).

CONCLUSION

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Preservatives whose use in cosmetic products cannot be evaluated

- n° 53 : Benzethonium chloride (INN)
- n° 54 : Benzalkonium chloride (INN), 1-2-Benzisothiazol-3-(2H)-one 1,1 dioxide, its bromide and saccharinate

BACKGROUND

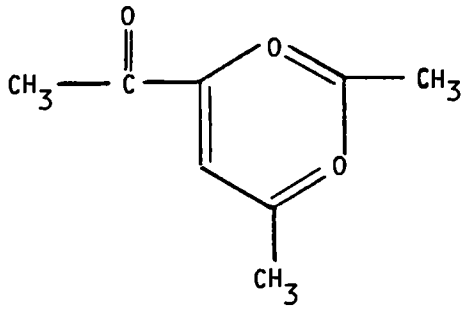
1. In accordance with Article 5 of Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products, as last amended by Directive 86/199/EEC, Member States must allow the marketing of cosmetic products containing the preservatives listed in Part 2 of Annex VI, within the limits and under the conditions referred to, until 31 December 1985.

2. On 1 January 1986 these preservatives will have to be :
   - either definitively permitted;
   - or definitively prohibited;
   - or maintained for a specified period;
   - or deleted from all the Annexes.

3. The Committee is therefore requested to express an opinion on the use in cosmetic products of the preservatives listed in Part 2 of Annex VI of Directive 76/768/EEC (Annex 1).

DISCUSSION

N° 1 2,6-dimethyl-1,3-dioxan-4-yl acetate (Dimethoxane)



$C_8H_{14}O_4$ CAS N° 828-00-2
MW : 174.20

Miscible with water and organic solvents.

Dimethoxane is used in cosmetics at dose levels of 0.2%.
The purity of the compound is not known.
The substance possesses low acute toxicity upon oral, dermal and inhalation exposure.
No sensitization could be detected in human volunteers and guinea pigs.
10% Dimethoxane is well tolerated in the rabbit eye. Higher concentrations induce, after a single application, moderate irritation of the conjunctivae.
Repeated application of a 10% solution was required to induce slight irritation of the guinea pig skin.
1% did not cause iritation in humans.

In a 13-week toxicity study dimethoxane was subcutaneously administered to dogs at dose levels up to 1.0 mg/kg/day. No effects were noted.

In a 90-day subcutaneous rat study the substance was administered at dose levels of 3,10, and 30 mg/kg/day; no clear effects were noted. It would have been preferable to have had a cutaneous study as well; and the top dose level chosen should have been high enough to give a definite effect.

A study in male rats lasting 88 weeks showed that oral administration at a concentration of 1% in drinking water gave rise to the formation of malignant tumours, especially hepatomas.

Studies into the metabolism, mutagenicity and reproduction are lacking.

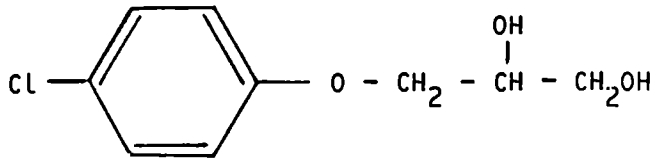
In view of the carcinogenicity in rats, the Committee recommended that the use of dimethoxane in cosmetic products should be suspended, subject to revision in the light of ongoing new N.C.I. studies.

Information : - Data sheet Council of Europe
- IARC - Monograph Vol 15(1977) 177
- Tox Tips June 1981, p. 61.11
- Colipa dossier, January 28, 1983

N° 2 Boric acid

See Report EUR 7297 and Report EUR 8634.

N° 3 Chlorophenesin



$C_9H_{11}ClO_3$ CAS N° 104-29-0
MW : 202.64

Synonyms : - 3(p-chlorophenoxy) propane-1,2-diol
- p-chlorophenyl-glycerol ether

Slightly soluble in water (0.6%), moderately soluble in glycerol (9.5%) and alcohol (15%).

Used in cosmetics up to 0.2%.

LD_{50} values (in mg/kg) are : oral in rats > 1400, in mice 1060, in guinea pigs 820, i.p. in rats 520, in mice 675 and 911; in guinea pigs 425, s.c. in mice 930.

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 soothing 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.

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.

In an oral 13-week study in rats given doses of 50, 100 or 200 mg/kg b.w./day by gavage, no effect on mortality, 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 (no report available).

Chlorphenesin possesses immunosuppressive properties. It is one of the glycerol ethers which are known to cause paralysis and to act as anticonvulsants.

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 behaviour or growth, in haematology or clinical chemistry, and in urine composition (summary report only).

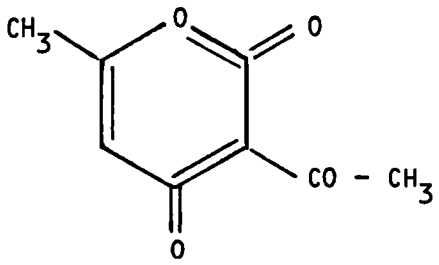
An oral dose of the labeled compound given to rats was rapidly absorbed and reached a peak concentration in the blood in 30 min. The half life in serum was 140 min. More than half of an oral dose was excreted in the urine in 4 hr, partly as the unchanged compound. Four metabolites have been identified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

Although sub-chronic oral studies have been conducted the target organ and a no effect level cannot be established. There is no information on mutagenicity or teratogenicity. The Committee requests further details about those tests which have been incompletely reported, and information on possible immuno-suppressant action at doses used in cosmetics.

No opinion can be expressed because of a lack of data.

Information : Colipa dossier, September 1983

N° 4 3-Acetyl-6-methylpyran-2,4(3H)-dione (Dehydroacetic acid) and its salts



$C_8H_8O_4$ CAS N° 520-45-6

MW : 168.15

Insoluble in water, soluble in organic solvents; the sodium salt is soluble in water.

Dehydroacetic acid and its sodium salts are used in cosmetics up to a level of 0.6%.

These compounds show moderate acute toxicity. The LD_{50} of the sodium salt for the dog is probably under 400 mg/kg b.w. irrespective of the route of administration.

They are neither primary irritants nor sensitizers in rabbits. These observations were confirmed in man by patch testing using occlusion.

Repeated oral administration of 0.3 g/kg b.w. to rats and monkeys caused decreased body weight, ataxia, convulsions and death.

Long-term studies involving administration of 0.1% of the acid in the diet of rats demonstrated no toxic phenomena.

In dogs 60 mg/kg b.w./day caused an uncompensated metabolic acidosis, but 50 mg/kg b.w./day did not.

Reproduction data were not available.
Mutagenicity tests were negative.

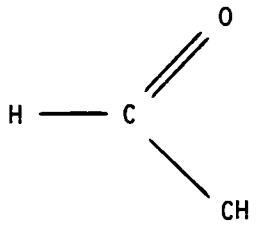
It is rapidly and apparently completely absorbed, following oral administration to man, monkey, dog and rat. It appears that 80% of dehydroacetic acid is metabolised in the body; urinay excretion accounts for less than 20%.

These compounds are detected in the plasma for as long as 2-3 weeks after the cessation of administration.

The use of dehydroacetic acid as a preservative in cosmetic products can be allowed owing to the absence of carcinogenicity and systemic toxicity in animals. Nevertheless the Committee wishes to obtain information on reproduction data upon oral administration.

Information : - Data sheet Council of Europe
- Colipa Partial Submission I, September 1983

N° 5 Formic acid



CH_2O_2 CAS N° 64-18-6
MW : 46.02

Miscible with water, ether and glycerol.

Formic acid is used in cosmetic products at a maximum level of 0.5%.

It has a moderate acute oral toxicity. LD_{50} : 700-1200 mg/kg b.w. in rats and mice.

Formic acid is a severe irritant of the skin and eyes in animals and accidents have been reported in man due to its caustic action on the skin, the eyes and the mucous membranes. No data were submitted concerning any irritant properties at the concentration used in cosmetics.

A sub-chronic inhalation study in rats and guinea pigs has shown that formic acid affects the liver and the kidneys. Marginal effect level : 5 mg/m^3.

In a 5-generation reproduction study with rats 0.2 - 1% calcium formate did not show embryotoxicity or teratogenicity.

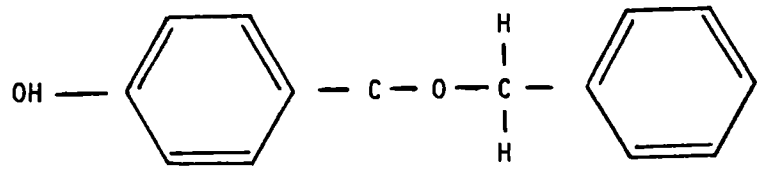
Its sodium salt did not prove mutagenic in two bacterial in vitro tests.

The formate ion is a normal intermediary of human metabolism; 3 to 14% of an administered dose is found in the urine, the proportions varying with the species.

The formate ion is only sligthly toxic and in spite of the caustic action of formic acid on the skin and the mucous membranes, the Committee sees no objection to maintaining the use of this substance in a concentration of 0.5%.

Information : - WHO 1974 Food Add. Series N° 5
- Z. Ernährungswiss. 9(1969)332
- Data Sheet Council of Europe

N° 6 4-Hydroxybenzoic acid benzyl ester



$C_{14}H_{12}O_3$
MW 228.25

CAS N° 99-96-7

Synonym : Benzyl-paraben

Poorly soluble in water, soluble in propylene glycol, highly soluble in ethanol.

Used in cosmetics in combination with other paraben esters up to 0.1% (as acid).

The oral LD_{50} in mice was $>$ 10 g/kg, in rats $>$ 5 g/kg.

From a skin irritation test in rabbits with 500 mg undiluted substance (applied on a gauze patch moistened with isotonic NaCl solution for 4 hours) it was concluded that the substance is not an irritant. A second test in rabbits with 500 mg applied for 4 hours did not produce irritation.

An eye irritation test in rabbits with 0.1 g of the test substance was negative.

Patch tests have been conducted in different countries with thousands of eczema patients using a mixture of benzyl, butyl, propyl, ethyl and methyl paraben, 3% of each, total 15% in vaseline. The incidence of sensitized patients varied from c. 1.0% to c. 4.0%, though in one year in a Canadian hospital the incidence was 6.7% in 405 patients.
Among 2000 patients tested in a hospital in one year, 4 were found sensitive to parabens. These 4 were tested with each of the 5 paraben esters and 2 were found to react to benzyl paraben.

Short-term (19-day) oral administration of 1 g/day to guinea pigs did not produce adverse effects (only a summary report is available).

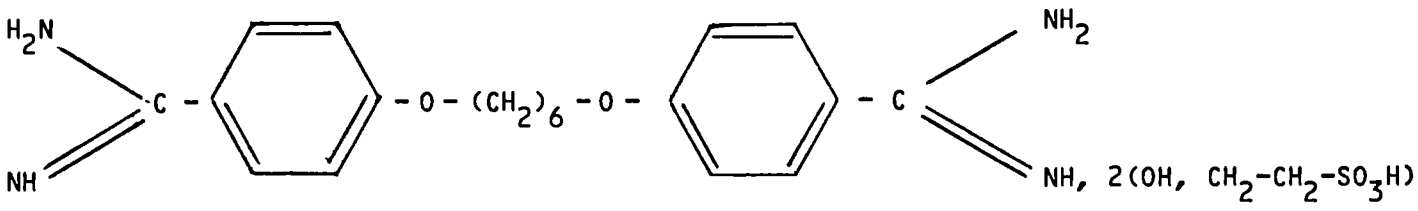
Short-term (5-day) oral administration of 2 g/day to 1 male and 1 female human subjects did not induce toxic effects or clinical abnormalities (summary report only).

An adequate short-term oral test and information on genotoxicity is needed to evaluate this compound. Depending on the results, information on dermal absorption may be required.

No opinion can be expressed because of a lack of data.

Information : Colipa dossier, Submission I, September 14, 1984
" II, September 7, 1984

N° 7 Hexamidine (INN) and its salts (including isethionate (INN) and 4-hydroxybenzoate)



$C_{20}H_{26}N_4O_2$
MW : 354.45

CAS N° 3811-75-4

Synonym : 1,6-di(4-amidinophenoxy)-n-hexane and its salts including di-isethionate and di(p-hydroxybenzoate)

Soluble in water. Insoluble in organic solvents.

Hexamidine is used in cosmetics at a maximum dose level of 0.1%.

The acute toxicity of hexamidine administered by the cutaneous route is very low in rats. In rabbits no dermal LD_{50} value was determined. The oral LD_{50} in rats, mice and rabbits is 500 - 750 mg/kg b.w.

In a concentration of 0.1%, this substance has only a very slight irritant effect on the skin and eye (slight reversible opacity of the cornea). Hexamidine is not a photosensitizing compound.

A subacute (28-day) dermal toxicity study in rabbits showed that solutions up to 2% were only very slightly irritant and revealed no systemic toxicity. No sensitisation was observed in guinea pig studies.

A 90-day dermal study in rabbits revealed no systemic toxicity at dose levels up to 4 ml/kg b.w. of a 0.4% aqueous solution (16 mg/kg b.w.). This presupposes very low absorption by the skin and/or test doses which are too low to give effects.

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

The Committee is of the opinion that the use of hexamidine as a preservative in cosmetics at a concentration of 0.1% may be continued for the present. It wishes to obtain data on :

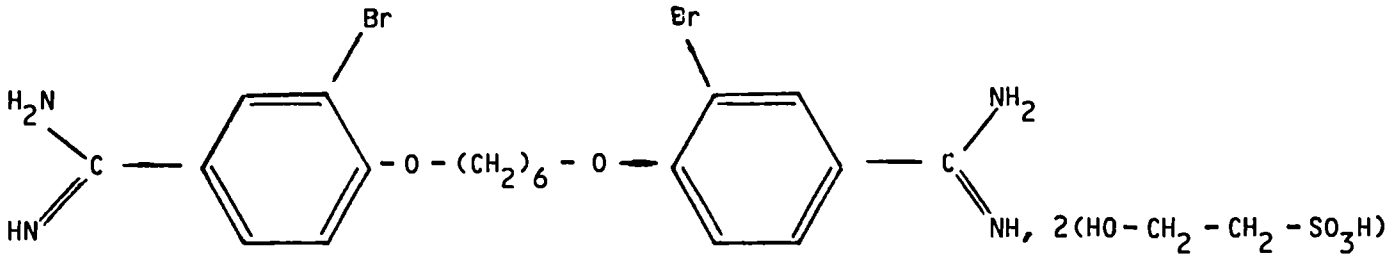
1. a 90-day study using the oral route to establish the no-effect level;
2. mutagenicity studies;
3. if there is substantial dermal absorption, a teratogenicity study.

Thereafter the Committee will reevaluate the toxicity of hexamidine and consider if a study on long-term toxicity including carcinogenicity will be necessary.

Information : - Data sheet Council of Europe
- Data sheet National Institute of Public Health, the Netherlands, based on Colipa file
- Additional submission from Colipa, 27 September 1982

N° 8 3,3'Dibromo-4,4'-hexamethylene-dioxydibenzamidine

(Dibromohexamidine) and its salts (including isethionate)



$C_{24}H_{36}Br_2N_4O_{10}S_2$
MW : 764.2

Synonym : 1,6-di(4-amidino-2-bromophenoxy)-n-hexane and its salts including isethionate

Soluble in water, alcohol, glycerine. Insoluble in benzene.

Dibromohexamidine isethionate is used in cosmetics at a maximum level of 0.1%.

This substance proved to be only slightly toxic in acute toxicity studies in the case of the oral and cutaneous routes (LD_{50} > 4000 mg/kg) but highly toxic in the case of the intraperitoneal route (LD_{50} 71 mg/kg).

It is slightly irritant to the skin of rats and rabbits under severe conditions of skin abrasion (0.5 g/rabbit). Reddened conjunctivae were observed after instillation of 0.25% in the eyes of rabbits. No sensitization reaction has been observed.

A sub-chronic (90-day) oral study in rats with the isethionate salt administered by gavage as suspension in 1% aqueous methylcellulose at levels of 0, 4, 20 and 100 mg test substance/kg/day did not reveal treatment-related effects other than increased salivation following dosing in all treatment groups and increased water consumption in the top-dose group.

In a 90-day dermal toxicity study in rabbits the maximum dose was only 2.5 mg/kg b.w.. The micro-abscesses in the lungs observed in 2/5 of the rabbits of this group were not considered significant.

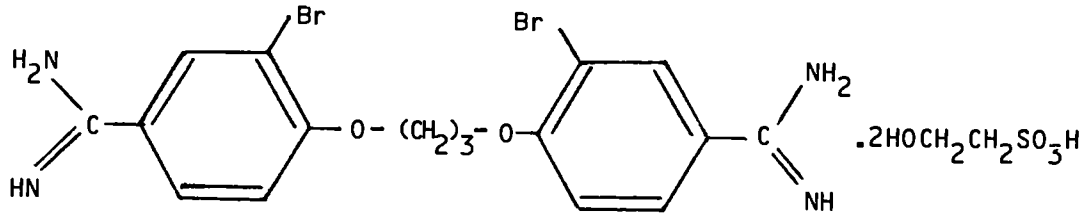
Metabolic studies in rabbits showed only slight intestinal absorption of dibromohexamidine.

An Ames test conducted with the isothionate salt was negative both with and without metabolic activation.

The data available indicate relatively high toxicity, but low absorption from the gut. The Committee wants to obtain information on dermal absorption. If this is considerable a short-term dermal study at appropriate dose levels would be indicated. However, the Committee sees no objection to maintaining the use of this substance as preservative in cosmetic products for the time being at a maximum level of 0.1%.

Information : - Data sheet Council of Europe
- National Institute of Public Health, the Netherlands, based on Colipa file
- Additional submission from Colipa, 27 September 1982

N° 9 Dibromopropamidine (INN) and its salts (including isethionate (INN))



$C_{21}H_{30}Br_2N_4O_{10}S_2$
MW 470.19

Synonym : 1,3-di(4-amidino-2-bromophenoxy)propane di-2-hydroxyethane sulfonate

Soluble in water and ethanol, practically insoluble in ether and chloroform.

Used at levels up to 0.1%.

The oral LD_{50} in mice was > 1000 mg/kg; the i.p. LD_{50} in mice was 78 mg/kg. From a subacute (5-day) oral study in which mice received daily different oral doses, a cumulative LD_{50} of 840 mg/kg/day was calculated.

A skin irritation test in rabbits with an aqueous solution of 0.15% was negative.

An eye irritation test in rabbits with 0.15% in water induced only redness of the conjunctivae in 1/6 animals.

A maximization test in guinea pigs with 1% in distilled water (and using Freund's adjuvant, sodium laurylsulfate, and microscopic examination of the skin) did not provide indications of sensitization.

In a 90-day oral study, rats were treated with 0, 3, 30 and 300 mg/kg b.w./day by gavage. Increased salivation was observed in each treatment group after dosing. In the top-dose group there was occasional pilo-erection during the first 30 days, and one female died. In this group changes were observed in the stomach both grossly and microscopically. The

changes consisted of thickening of the limiting ridge of the stomach accompanied by acanthosis. No changes were observed in body weight, food intake, haematology, blood chemistry, urinalysis or organ weights.

Rabbits treated dermally with 3 mg/kg as a 0.15% aqueous solution each day for 90 days showed lack of hair growth from the 10th week. No other gross changes were observed (no control group).

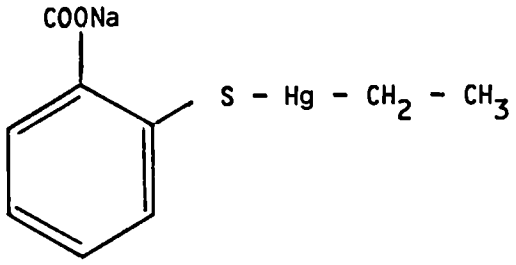
An Ames test with up to 5 ug/plate was negative.

There is no information on reproduction or teratogenicity. The similarity to stilbamidine (used in trypanosomiasis) was noted. The substance may be bound to protein. The effects on the stomach are typical of bromides. The substance is used therapeutically for treating surface infections, e.g. in burns and skin grafting. Information is needed on dermal absorption. Genotoxicity tests required are in vitro gene mutation and chromosomal aberration in mammalian cells. The necessity for further tests would be considered when the above information was available.

No opinion can be expressed because of lack of data.

Information :
- Data sheet provided by National Institute of Public Health, the Netherlands (based on Colipa dossier 14th July 1979)
- Colipa dossier, Submission II, April 1984
- Colipa dossier, Submission III, September 1984

N° 10 Thiomersal (INN)



$C_9H_9HgO_2S\ Na$ CAS N° 54-64-8
MW : 404.84

Synonyms : - [(o-carboxyphenol)-thio]ethylmercury sodium salt
- sodium ethyl mercurithiosalicylate
- Merthiolate
- Thimerosal

Soluble in water and ethyl alcohol. Insoluble in ether and benzene. The use of sodium ethyl mercurithiosalicylate is restricted to eye make-up and eye make-up remover at a maximum concentration of 0.007% (expressed as Hg).

The LD_{50} values vary from 15 to 100 mg/kg, according to the route of administration and the animal species used. Up to 500 mg has been repeatedly given intravenously to man with no ill-effects.

No quantitative data are available concerning skin irritation in animals or man.

Eye irritation did not occur in rabbits treated daily for seven days with 2 drops of 1.0% aqueous solutions. Nevertheless it is recognized that eye irritation in man may be produced by contact lenses sterilised with merthiolate.

Various epidemiological studies in man have shown that the frequency of contact allergy due to merthiolate is 2 to 7%. Sensitization may occur when either the epidermal or the intradermal route is used, and cases of cross-sensitization are observed with a few organic mercury derivatives.

A subacute toxicity study carried out with dogs, involving intravenous injection of merthiolate in doses of 2 mg/kg b.w. every three days for 36 days, showed no pathological changes.

A carcinogenicity study in rats with twice-weekly subcutaneous administration of 0.03, 0.1, 0.3 and 1 mg/kg b.w. of merthiolate for one year and terminated after 18 months revealed no systemic carcinogenicity. Nevertheless, a high incidence of bronchopneumonia related to the injected dose and numerous indurations at the injection site were observed. This study is of limited value as regards the prediction of possible carcinogenicity.

An Ames test with up to 1 mg/plate both with and without metabolic activation was negative. However, in a DNA repair test by the E.coli DNA polymerase A-assay, genetic toxicity was observed when the assay was conducted in the light but not when conducted in the dark.

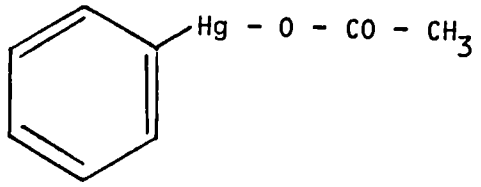
In a teratogenicity study, groups of 10 pregnant rats received daily i.p. injections of 1 ml of either 0.2 or 2.0% thiomersal solution from day 6 through 18 of pregnancy. Although deaths, abortion and resorption of foetuses were increased, malformations did not occur.
Treatment of seven pregnant rabbits with 2 drops of 2% thiomersal in 0.9% saline in both eyes from day 6 - 18 of pregnancy (8 times on day six and 4 times daily thereafter) failed to induce congenital malformations in the foetuses. A relatively high level of Hg was found in the blood and in tissues after application to the eye.

The Committee noted that
1. The data available did not permit a full toxicological assessment.
2. Absorption of thiomersal through the mucous membrane of the eye takes place.
3. There is evidence of accumulation of mercury in the tissues.

On the basis of toxicological information and the desirability to restrict the use of Hg containing compounds, thiomersal must be banned for general use. However, in the absence of effective alternative substances to prevent eye infections, its inclusion in eye make up preparations and eye make up removers can be permitted for the time being, where no satisfactory alternative exists, at a maximum concentration of 0.007% expressed as Hg.

Information : - Data sheets of the Council of Europe
- Clinical Toxicology 4, 185-204 (1971)
- Am. J. Ophthalmology 78, 98-105 (1974)
- Data sheet of the Institut d'Hygiène et d'Epidémiologie, Brussels
- Colipa submission I, Nov. 1983

N° 11 Phenylmercuric salts (including borate)



$C_8H_8HgO_2$ CAS N° 102-98-7
MW : 336,74 (62-38-4)

Sparingly soluble in water. Soluble in alcohol, benzene and acetone. The use of phenylmercuric acetate (PMA) is restricted to eye make-up and eye make-up remover at a maximum concentration of 0.007% (expressed as Hg).

The oral LD_{50} varies from 22 to 60 mg/kg according to the species of animal used. In mice values of 8, 18 and 37 mg/kg are reported for respectively the intraperitoneal, intravenous and subcutaneous LD_{50}.

When given in a dose of 50 µg, PMA severely irritates the rabbit eye.

In man, it can be considered a severe skin irritant when given in a dose of 100 µg. At a concentration of 0.1% and above it is an irritant and a sensitizer. Sensitization does not occur at 0.001%, but a dose-related increase in incidence of sensitized subjects occurred at higher concentrations. In a commercial make-up product, 0.003% was not sensitizing in a maximization test in guinea pigs.

It is a poor skin penetrant; at concentrations of 1% to 0.06% the penetration through stripped human stratum corneum was about 1 ng/cm^2/hr. Nevertheless mercury levels above normal have been reported in urine, blood or hair of chronic users of mercurial bleach creams. In a few cases the urinary levels of mercury were in the range of values reported for mercury intoxication.

PMA is metabolized in vivo, releasing Hg^{++} which is partly excreted by the kidneys upon administration by different routes to various animal species. Accumulation of mercury was found in several tissues (kidneys, central nervous system, liver, muscle, hair).

In various subacute and subchronic oral studies in rats, dose-related changes in enzyme activity were found in the liver and blood. Brain enzymes showed changes which were related to the length of exposure. Inhalation exposure of rats to 0.012 mg/m^3 of PMA caused changes in the blood picture, which were irreversible.

In a chronic oral rat study with dietary levels of 0.1 - 160 ppm only the highest dose caused mortality. Growth depression occurred with 10 ppm and more, kidney damage with 0.5 ppm (as Hg) and above. The no-toxic effect level was $<$ 0.1 ppm because tissue storage of mercury was found even at this low feeding level.

PMA proved to be genotoxic for Bacillus subtilis and to produce chromosomal aberrations in human leucocytes.

It is embryotoxic and teratogenic in hamsters, rats and rabbits when administered by gastric intubation from the 5th to the 12th day of gestation in doses ranging from 1/6 to 1/2 of the LD_{50}.
Teratogenic effects were also observed in hamsters as a result of intraperitoneal injection of a 7500 µg/kg dose on the 8th day of gestation. When 600 µg/day was administered to hens subcutaneously, in the course of a study lasting several months, gradual inhibition of egg-laying was observed. After 20 days, approximately 30% of the daily injected dose was detected in the yolk.

In man, epidemiological studies revealed cases of irritative and allergic dermatitis.
The chronic intoxication syndrome due to organic mercury compounds is characterized by insidious onset, long-continued clinical sequelae and a guarded prognosis as to complete recovery, and is confined to the CNS, in particular the visuosensory cortex and the cerebellum. Ingestion of this substance during pregnancy can have highly detrimental effects on the foetal CNS.

The joint FAO/WHO Expert Committee on Pesticides did not establish an ADI because of the very low no toxic effect level and because it considered any increase in the use of mercury as undesirable.

Because the toxicity of this compound will be determined primarily by the mercury it contains, it is assumed that the other salts have similar toxic properties.

This substance is highly toxic, mutagenic and teratogenic, and must be banned for general use. However, in the absence of effective alternative substances to prevent eye infections, its inclusion in eye make-up preparations and eye make-up removers can be permitted for the time being, where no satisfactory alternative exists at a maximum concentration of 0.007% expressed as Hg.

Information :
- TOXLINE
- Data sheet of the Council of Europe
- Data sheet of the Institut d'Hygiène et d'Epidémiologie, Brussels
- WHO/Food add./67.32 p. 188
- Marzulli and Brown, J. Soc. Cosmet. Chem. 23 (1972) 875-886
- Colipa submission I, Sept. 1983

N° 12 Esters of sorbic acid

$CH_3CH = CHCH = CHCOOH$

$C_6H_8O_2$ CAS N° 110-44-1
MW : 112.12

Synonyms : - 2,4-Hexadienoic acid
- Sorbic acid
- Sorbistat

The esters cannot be evaluated because of lack of information. But the esters are no longer used as preservatives in cosmetic products.

Information : - data sheet Council of Europe
- data sheet of the Free University of Brussels

N° 13 Undec-10-enoic acids : salts, esters, the amide, the mono- and bis(2-hydroxethyl) amides and their sulphosuccinates

$$CH_2 = CH - (CH_2)_8 - CO_2H$$

$C_{11}H_{20}O_2$ CAS N° 112-28-9
MW : 184.28

Synonyms : - 10-undecenoic acid
- undecylenic acid

Practically insoluble in water. Soluble in alcohols, chloroform and ether.

Used in cosmetics at dose levels of 0.2%.

The acute oral toxicity (LD_{50}) varied from 2.5 to 10 g/kg in rats, and from 2.3 to 8.5 g/kg in mice. Mice treated orally with 0.14 to 0.29 g showed hyperirritability. Upon necropsy, engorgement of the stomach and the small intestine, accompanied by petechial haemorrhages were observed. With intraperitoneal administration, the LD_{50} was 0.96 g/kg in mice; with dermal application in rabbits, it exceeded 5 g/kg.

When applied as such to the intact or abraded skin of rabbits for 24 hours under occlusion, undecenoic acid proved to be extremely irritating. A concentration of 30% in ethanol was mildly irritating. The substance was well tolerated by human skin in an open epidermal test at concentrations of up to 60% for 21 days and in a test under occlusion at 4% for 48 hours.

No allergic sensitization reactions were observed in a maximization test performed with a 4% solution in vaseline on 25 human volunteers.

In a sub acute (eight weeks) oral toxicity test in rats, weight loss followed by a gradual recovery was observed with a 2.5% concentration of undecenoic acid in the diet.

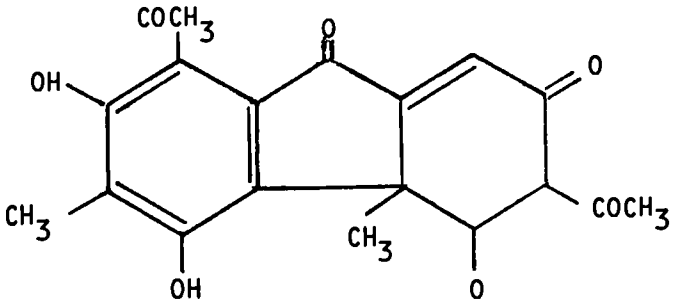
A subchronic (six months) oral toxicity study in rats revealed no sign of toxicity at concentrations of 0.1, 0.2 and 0.4 g/kg b.w./day.
In the same study, no teratogenic effect was noted when the same dosages were given over nine months.

The Committee wishes to obtain information concerning mutagenicity. There is doubt concerning the use and the efficacy of this substance in cosmetics.

NB : The above assessment applies to undecenoic acid only.

Information : - Opdyke, Food Cosmet.Toxicol. 16 (suppl 1) 883-884 (1978)
- BLAISE

N° 14 2,6-Diacetyl-1,2,3,9b-tetrahydro-7,9-dihydroxy-8,9b-dimethyldibenzolfuran-1,3-dione (usnic acid) and its salts (including the copper salt)



$C_{18}H_{16}O_7$ CAS N° 125-46-2
MW : 344.32

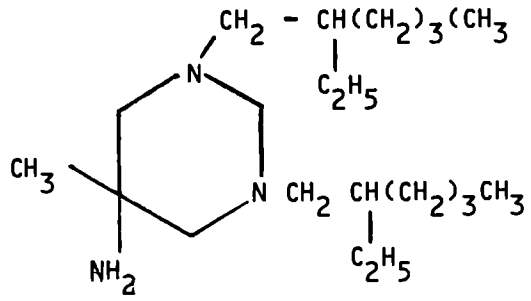
Very sparingly soluble in water, acetone and ethyl alcohol.
Soluble in furfural and fats.
Usnic acid is used in cosmetics at dose levels of 0.2%. It appears that it is seldom used in cosmetic products because of its insolubility. It is probably used in the form of double salts of copper and triethanolamine. The oral, subcutaneous, and intraperitoneal LD_{50} values in mice are 515, 700 and 47,3 mg/kg b.w. respectively. The intravenous LD_{50} for mice, rats and dogs was 25,30, 30 and 40 mg/kg b.w. respectively.

No teratogenic studies have been reported. Freese et al. (1979), regard it as a suspect teratogen based on its physico-chemical properties and its inhibitory effects on growth of human and bacterial cells in vitro.

No opinion can be expressed because of a lack of data. But this substance is no longer used as preservative in cosmetic products.

Information : - Data sheet of the Council of Europe
- Freese etal., Teratology 20(1979) 413-440

N° 15 Hexetidine (INN)



$C_{21}H_{45}N_3$
MW : 339.60

CAS N° 141-94-6

Synonyms : - 5-Amino-1,3-di-(2-ethylhexyl)-hexahydro-5-methylpyrimidine
- Sterisol

A viscous oil with a density of 0.87 g/ml.
Practically insoluble in water, easily soluble in ethanol, acetone and other organic solvents.

Used up to 0.2% in cosmetics.

LD_{50} values are : oral in rats 1.43 g/kg; intraperitoneal in mice 0.030 - 0.085 g/kg; dermal in rats 1.86 ml/kg, and in rabbits 4.0 ml/kg.
Intravenous administration of 5 mg/kg was lethal for cats.

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.

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 consumption were seen in the top dose group. No treatment-related changes were found upon microscopic examination (Only summary report is available).

Daily dermal application of 50, 100, 200 or 500 mlg.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 urinogenital systems were seen at any treatment level (Summary reports only).

In a sub-chronic (90-day) oral study, rats received 0, 100, 300 or 1000 ppm 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 b.w.).

An Ames test with 5 strains of S.typhimurium and dose levels up to 5 mg/plate was negative.

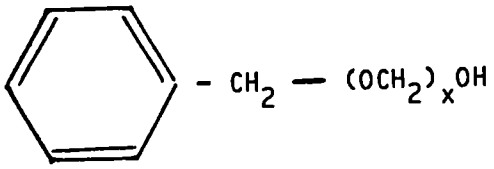
An oral dose of ^{14}C-hexetidine in rats (20 mg/kg) and dogs (10 mg/kg) was rapidly excreted. After 72 hours 60-70% was recovered in the faeces and 20-25% in the urine, while 1-2% was found in the liver and kidneys.

Exact data on dermal absorption are lacking but acute and subacute dermal toxicity studies suggest considerable dermal absorption. There is no information on reproduction or teratogenicity. A negative Ames test is the only information on genotoxicity.
Information on teratogenicity and results of a chromosomal aberration test should be provided.

However, the Committee sees no objection to maintaining the use of this substance as preservative in cosmetic products for the time being, but on the basis of the existing information 0.2% is too high for non rinsed off cosmetics.

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.

N° 16 Benzylformal



$- CH_2 — (OCH_2)_x OH$

x = 1:50 %
x = 2:50 %

Synonym : Preventol D_2

Soluble in organic solvents; solubility in water 25 g/l.

Used up to 0.2% in all types of cosmetics.

The oral LD_{50} in rats was 1700 mg/kg; the i.v. LD_{50} in rats was 153 mg/kg. The animals showed sedation, loss of consciousness, paralysis. The dermal LD_{50} in rats was $>$ 1000 mg/kg.

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.

An Ames test with up to 500 µg/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 250 mg/kg, or 2 x 500 mg/kg with an interval of 24 hours. No increase in the incidence of micronuclei was observed.

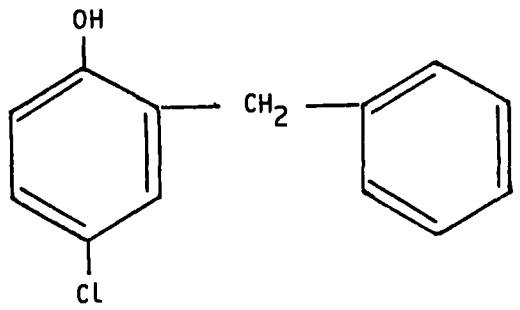
There is no information on short- or long-term oral toxicity, reproduction, teratogenicity or dermal absorption.
The substance liberates formaldehyde. According to Dr Crisp (UK Laboratory Governmental Chemists) this is 0.004% maximum (under test conditions).
In the body, benzylformal may be broken down (initially) to benzyl alcohol and formaldehyde.
Information is required on :

1. Dermal absorption
2. The fate of the substance in the body
3. The effect of the substance and/or its breakdown products in the body, in a short-term, oral toxicity study.

However, the Committee sees no objection to maintaining the use of this substance as preservative in cosmetic products for the time being at a maximum level of 0.2%.

Information : - Data sheet Council of Europe
- Colipa dossier, April 1984

N° 17 chlorofene (INN)



$C_{13}H_{11}ClO$ CAS N° 120-32-1
MW : 218.69

Synonyms : - 2-Benzyl-4-chlorophenol
- Preventol BP
- Santophen 1

Used in cosmetics up to 0.2%.

Insoluble in water, soluble in ethanol, isopropanol, propylene glycol.

Oral LD_{50} values in rats are > 5 g/kg, and 2.8 g/kg.
The dermal LD_{50} in male and female rats was > 2.5 g/kg.

Skin irritation in rabbits was found to be moderate (3.1/8).

An eye irritation test in rabbits showed moderate irritation (37.3/100).

A patch test in humans with a 1% solution, followed by a second patch test with the same solution 10 days later did not reveal signs of sensitizing properties. However, a soap containing 3.125% Santophen was sensitizing.

Short-term dermal toxicity was examined in a detailed study with 4 groups of 5 rabbits/sex treated with 0, 10, 40 and 160 mg/kg b.w./day in polyethylene glycol 400 on 5 days/week. There was no mortality. Changes in behaviour, growth rate, or food intake were not observed. In the two higher dose groups the treated skin showed erythema, oedema, acanthosis, keratosis, subepidermal round cell infiltration and hyperplasia of sebaceous glands.

The skin changes were reversible. There were no changes in haematology. The top-dose group showed slight liver changes consisting of decreases in weight, in activity of alkaline phosphatase and in glycogen content of hepatocytes.

A teratogenicity study was conducted with groups of 24 pregnant rabbits, treated by gavage (with Santophen 1) at dose levels of 40, 80 and 160 mg/kg/day on days 7-19 of gestation. Mortality in the high-dose group (10/24) was high, which resulted in only 7 litters available for examination. Weight loss occurred in each of the test groups during the treatment period. Implantation data, weight, length and ossification of foetuses were not affected by treatment. Litters of the test groups contained more males than females. External-, and skeletal malformations were significantly increased only in the mid-dose group. At 160 mg/kg maternal toxicity occurred. The authors concluded that there were no indications of embryotoxicity or teratogenicity.

A second teratogenicity study was conducted with groups of 25 mated female rats, treated daily (with Preventol BP Technical) by gavage at dose levels of 0, 15, 75 and 375 mg/kg b.w./day.
In the dop-dose group there was mortality (3/24), reduced food intake and body weight gain of the dams, and decreased weight of the foetuses. An increased incidence of skeletal abnormalities in the top-dose group was attributed to the retarded development in this group. These findings indicate maternal toxicity with 375 mg/kg and a no-effect level of 75 mg/kg.

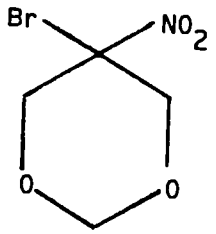
Preliminary mutagenicity data from the U.S. National Toxicology Program (NTP) did not indicate mutagenic properties, either in the Ames test at dose levels up to 100 µg/plate, or in a chromosomal aberration test and a sister chromatid exchange test with CHO-cells at concentrations between 16 µg and 0.05 µg/ml.

The available studies indicate that the substance is of low or moderate systemic toxicity. Irritative and sensitizing properties have been observed and need to be further examined at use levels. Moreover information should be provided on the purity of the technical product and on the possible level of contamination with TCDD.

However, the Committee sees no objection to maintaining the use of this substance as preservative in cosmetic products for the time being at a maximum level of 0.2%.

Information : - Colipa dossier, Submission I, October 1985
- Data sheet Mme Dorlet 20-022 F. 139

N° 18 5-Bromo-5-nitro-1,3-dioxane



$C_4H_6BrNO_4$ CAS N° 30007-47-7
MW : 212.02

Synonym : Bronidox

Poorly soluble in water; soluble in ethanol and propylene glycol.

Bronidox is authorized in rinsed off cosmetics up to a level of 0.1%.

Oral LD_{50} values are 455 mg/kg in the rat and 590 mg/kg in the mouse. Intraperitoneally in the mouse the figure is 31.5 mg/kg. On rabbit skin, 500 mg/kg in olive oil caused death in 24 hours. 100 mg/kg caused severe skin irritation.

A skin irritation test in rabbits with 0.5% in olive oil was negative. 0.1% was negative in hairless mice, but with 0.5% irritation and necrosis occurred. In rabbits 0.5% produced desquamation.

In an eye irritation test in rabbits 0.1% and 0.5% caused irritation, which was severe at the higher concentration, but the effects were reversible. 0.05% was non-irritant. Daily application of 50 µl of a 0.05% solution to the eye of rabbits for 10 days without rinsing did not induce any deleterious effect.

Tests in guinea pigs showed no evidence of sensitization, but the studies were unsatisfactory in some respects. There was no evidence of photosensitization in a test in hairless mice treated with concentrations varying from 0.05 to 0.5%.

Patch testing in man with 0.25% daily with occlusion for 21 days showed that this concentration became progressively more irritant after about 6-8 applications. Slight changes at 0.1% were of dubious significance.

In man, use of 0.1% in shampoos or foam baths for 6 weeks (at least weekly, but not more often than daily) elicited one report (in 118 subjects) of slight irritation of dubious significance. 0.05% in a cream formulation applied daily for 21 days caused no irritation.

In the rat, a six-week study by gavage was carried out with 0, 10, 50 and 100 mg/kg/day; at the end of the period of dosing, the animals were observed for a further 9 weeks. No abnormalities were observed up to 50 mg/kg.

In a 14 week study in rats, 0, 10, 50 and 100 mg/kg/day were given by gavage on 5 days/week. After seven weeks, the dose in the top-dose group was increased to 200 mg/kg, but this part of the experiment was terminated after some of this group died. At the low dose some minor changes were found in the gastric mucosa, perhaps related to the mode of administration. At higher doses, eosinophilic and round cell infiltration in the heart was found, and at the top dose hypoxic myocardial changes were found as well. In the 10 and 50 mg/kg group there was slight growth depression and decreased kidney function.

The compound has been shown to be well-absorbed from the gut and the peritoneal cavity in animals. When applied to the skin, absorption is considerable in rats, rabbits and man (10-40%). Three metabolites have been identified.

In an oral teratogenicity study, dose levels of 0, 5, 15 and 45 mg/kg were administered to groups of 30 rats. All treated groups showed clinical signs of intoxication and enlarged livers. One of the dams in the mid dose group, and four in the high dose group died. Foetuses did not show signs of teratogenic properties of the compound.

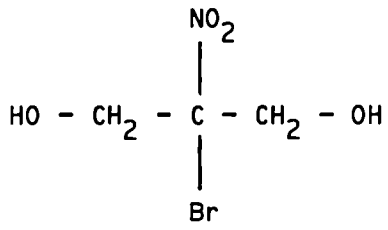
The Ames test was inconclusive. The micronucleus test was negative.

In view of its structure, it is likely that this compound could give rise to nitrosamine formation under certain conditions. The no-toxic effect level is less than 5 mg/kg. Percutaneous penetration is considerable in animals and man. Its continued use may be justified only, if a no effect level is firmly established, and if a gene mutation test turns out to be negative.

However, the Committee sees no objection to maintaining the use of this substance as preservative in cosmetic products for the time being but only for rinsed off products and not more than 0.1%.

Information : - Colipa dossier. Submission I Oct. 15, 1980
Submission II, January 28, 1983
Submission III, September 26, 1984
- Data sheet National Institute of Public Health (the Netherlands), June 1981
- J. Soc. Cosmet. Chem 28, (1977) 427
- Data sheet Council of Europe

N° 19 Bronopol (INN)



$C_3H_6BrNO_4$ CAS N° 52-51-7
MW : 169.97

Synonym : 2-bromo-2-nitropropane-1,3-diol

Soluble in water and ethanol, 50%. Poorly soluble in non-polar solvents. Bronopol is used at dose levels up to 0.2%.
The acute toxicity is moderate to high; LD_{50} in rats and mice (mg/kg) was about 300-350 (oral), 200 (subcutaneous, mouse), 20-30 intraperitoneally, 60-160 dermally (in acetone, mouse).
In the dog by gavage, all doses in excess of 40 mg/kg caused gastric irritation and pyrexia; one of two dogs died at 250 mg/kg.

In the rabbit, a 10% aqueous solution was highly irritating to the eyes. Up to 0.5% was not irritant.
In the guinea-pig, 0.1% to the shaved skin was non-irritant; 1% caused slight irritation.
Irritation of the skin was found at concentrations varying from 0.5% to 2% depending on the solvents.
In dermal tests in man, the threshold of irritation varied from 0.5% to 2% in different experiments. In 149 eczematous patients, a 24 hr patch test with 0.25% showed 4 positive reactions.
In the guinea-pig, the substance was found not to be a sensitizing agent.
In man, 2% was found not to be sensitizing.

In the rabbit, a 9-day study showed gastric irritation and pulmonary effects (perhaps due to aspiration) after various doses by gavage; 3.3 mg/kg/day was well tolerated.

In a 13-week study, up to 20 mg/kg/day were given to dogs by gavage. The probable NEL was 4 mg/kg/day; higher doses induced increased relative weights of liver and spleen, and some leukopenia.

In a 14 days intubation study in the rat, 4 of 5 animals died at 300 mg/kg/day; 100 mg/kg/day was tolerated. In a six week study, rats were treated with 80 (later 300) and 160 mg/kg/day in drinking water. At both levels there was an increased relative kidney weight; 2 M died at 300 mg/kg/day. In a 12 week study in the rat, 100 and 1000 ppm was given in the diet. No abnormalities were found. In a 13 week study, also in the rat, groups of 40 animals were given 20, 80 and 160 mg/kg/day by gavage. There were severe symptoms and mortality at the top dose; at 20 mg/kg one animal showed transitory respiratory distress, but apart from this no abnormalities were noted at this dose.

In a two-year rat study, groups of 60 M and 60 F received 10, 40 and 160 mg/kg/day in drinking water. All treatment groups showed a reduction in water intake, attributed to unpalatability. The top dose group showed clear signs of toxicity including mortality. There was a dose related increase in the incidence of squamous metaplasia of the salivary glands. No significant changes at the low dose by comparison with the controls.

Repeated dermal applications of 90% acetone solutions at various concentrations were made to the shaven skin of mice for 4 weeks. The top dose animals (4% and 2%) were sacrificed after 1 week in view of severe toxic effects; concentrations up to 0.5% were well tolerated.
In rats, 0.5% in an aqueous medium with occlusion caused a severe local reaction. A similar study was carried out over 80 weeks, but due to high mortality, full evaluation is not possible.

Using radioactive substance in aqueous solution, rats and rabbits absorb the substance relatively slowly from the skin - about 11% in 24 hrs. Use of acetone as a solvent leads to somewhat more rapid and greater absorption.

Dominant lethal test, host-mediated assay, and Ames test were negative.

Administration of 20 and 40 mg/kg/day to dams from day 15 of pregnancy to the end of lactation had no adverse effect on offspring in the rat. The same dose to male rats for 63 days before mating, and to female rats from 14 days before mating to day 12 of pregnancy, and also to weaning, did not affect the offspring adversely.

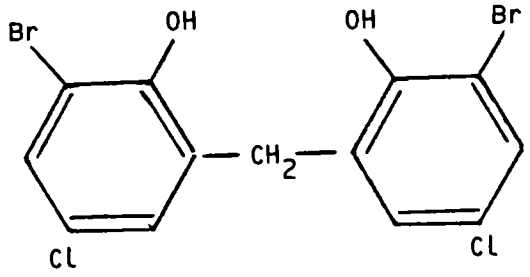
Teratogenicity : tests with up to 100 mg/kg/day orally in rat and rabbit were negative. In a dermal study in rats up to 20 mg/kg/day did not cause any teratogenic effects.

It should be mentioned that Bronopol, as well as other preservatives, may act as a nitrosating agent.

The use of this substance as preservative in cosmetic products at a maximum level of 0.2% can be permitted.

Information : - Data sheet Council of Europe
- Data sheet National Institute of Public Health, the Netherlands, based on Colipa file
- J. environmental Pathology and Toxicology, 4(4) 47-61 (1980)

N° 20 6,6-Dibromo-4,4-dichloro-2,2'-methylene-diphenol (Bromochlorophen)



$C_{13}H_8Br_2Cl_2O_2$ CAS N° 15435-29-7
MW : 426.95

Synonyms : - 3,3'-dibromo-5,5'-dichloro-2,2'-dihydroxyphenylmethane
- Bromophen

Poorly soluble in water (< 0.6%), moderately soluble in ethanol (9.5%).

Used up to 0.1% in non rinsed off products.

Oral LD_{50}-values in rats are 3.7 g/kg and 8.15 g/kg, in mice 1.55 g/kg. The intraperitoneal LD_{50} in rats was 580 mg/kg; the dermal LD_{50} in rats was found to be > 10 g/kg.

In a primary skin irritation study in rabbits, a 50% suspension in water produced very slight erythema in 2/6 animals. The substance was classified "mildly irritating".

A 50% suspension in water was not irritant to the eye of the rabbit.

A guinea pig maximization test with 0.3% for induction, and 3% for the challenge treatment did not reveal any indication of sensitizing properties.

In a phototoxicity test, 5 human subjects were treated once on the forearm skin with 0.05 ml of a 2% concentration in ethanol on two spots, one of which was UV-irradiated. No signs of phototoxicity were observed. After 10 days rest the treatment was repeated with the test substance in paraffin oil. Again 10 days later a third treatment was given using 8% soap solution as a vehicle. There were no signs of photosensitization.

Sub-chronic (13-wk) toxicity was examined in an oral study in rats with dose levels of 0, 250, 500 or 1000 mg/kg/day. All treatment groups showed diarrhoea, caecal enlargement, and increased liver weight. Mortality, growth depression and signs of anaemia occurred in the top-dose group.

In an oral embryotoxicity/teratogenicity study in pregnant rats with dose-levels of 20, 100 and 500 mg/kg, no indications of teratogenicity were observed. In the two higher dose groups, the weight of the foetuses was decreased. Delayed ossification and an increased incidence of lumbar vertebrae were found in the top-dose group.

In a sub-acute inhalation study in rats with a bromophen aerosol, a concentration of 220 mg/m^3 did not induce any detrimental effects when applied 2 hours/day, seven times weekly, for 4 weeks.

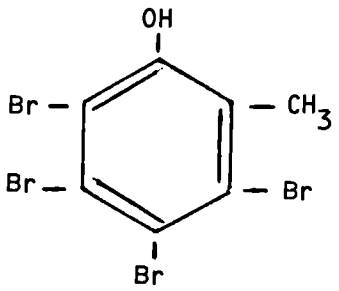
An Ames test with the Salmonella strains and with E. coli up to 10 µg/plate was negative.

A metabolism study with the ^{14}C-labelled compound was conducted in rats by oral, intravenous and dermal application. The substance is poorly metabolized and mainly excreted with the faeces after conjugation in the liver. Only 2% of an oral dose and 4% of an intravenous dose is excreted by the kidneys. Blood plasma contains only a relatively small amount of metabolites. The pattern of metabolites after different routes of administration is very similar. An oral dose is absorbed only slowly and results in a maximum blood level only after 8 hours. A dermal dose of the radiolabelled compound showed 2% absorption after 2 hours and 8 to 10% after 24 hours.

The Committee noted the structural similarity between this compound and hexachlorophene and drew attention to the known deleterious effects of the latter compound at high concentrations to brains of neonates in man and non-human primates. A no effect level in a short-term oral toxicity study should be established. A chromosomal aberration test and an adequate Ames test are required. However, the Committee sees no objection to maintaining the use of this substance as preservative in cosmetic products at a maximum level of 0.1%.

Information : - Colipa dossier, December 1984
- Data sheet Council of Europe

N° 21 Tetrabromo-o-cresol



$C_7H_4Br_4O$ CAS N° 576-55-6
MW : 423.77

Practically insoluble in water; soluble in alcohol, ether and alkali hydroxides.

Used in cosmetics up to 0.3%.

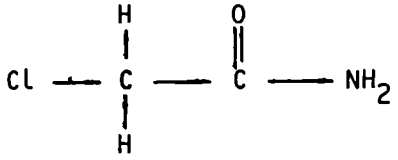
The oral LD_{50} in mice was 0.8 g/kg.

The undiluted substance is irritating to the skin and the mucous membranes. A 2% solution was not irritating to the skin of guinea pigs, nor to the eye of rabbits. Four out of one hundred human volunteers reacted positively to solutions of 0.25 - 2.0%.

No opinion can be expressed because of a lack of data, but the substance is no longer used as preservative in cosmetic products.

Information : Data sheet Council of Europe

N° 22 2-Chloroacetamide



C_2H_4ClNO CAS N° 79-07-2
MW : 93.52

Moderately soluble in water (5%); easily soluble in ethanol. The product manufactured in the EEC is 99.5 - 99.8% pure (impurities are ammonium-chloride 0.1 - 0.2%, and monochloroacetic acid 0.03%).

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

The oral LD_{50} of a 70:30 mixture of chloroacetamide and sodium benzoate was 0.37 g/kg in rats and 0.15 g/kg in mice. The intraperitoneal LD_{50} of chloroacetamide was 50 mg/kg; hydropic degeneration of the liver of rats occurs within 3 hours following an i.p. dose of 75 mg/kg.

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.

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 solution 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.

Single patch testing of 296 eczema patients with 0.2% aqueous chloroacetamide (99% pure) induced skin reactions in 7 patients (2.3%).

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%. Daily patch testing of 25 subjects with 0.1 or 0.2% in formulations was likewise negative.

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 Buehler 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% aqeous 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.

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.

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.
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.
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.

In a 13-wk feeding study in rats with levels providing 12.5 and 50.0 mg/kg b.w./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 weights of testicle and liver, suppression of spermiogenesis and proliferation of Leydig cells. No treatment-related changes occurred with 20 and 100 mg/kg diet.

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

Rats treated with an intraperitoneal dose of 20 mg/kg on day 7 of gestation or on day 11 and day 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).

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.

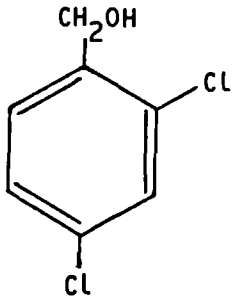
Chloroacetamide has shown considerable sensitizing potency in humans at concentrations in the range of those present in cosmetics. This property might be due to a contaminant in certain batches of the substance.
However the Committee sees no objection to maintaining the use of this substance as a preservative in cosmetic products for the time being at a maximum level of 0.3%.

Information : - Data sheet Council of Europe
- Colipa dossier, Submission I, November 1984
Submission II, June 20, 1985
Submission III, September 13, 1985
Submission IV, February 1986
- Toxicol. appl. Pharmacol. 55 (1980) 273-280
- Dermatologica (Basel) 144 (1972) 108
- Contact Dermatitis 1 (1975) 265
- Contact Dermatitis 7 (1981) 51

N° 23 3,4-Dichlorobenzyl alcohol

Not used as preservative in cosmetic products.

N° 24 2,4-Dichlorobenzyl alcohol



$C_7H_6Cl_2O$ CAS N° 1777-82-8
MW : 177.04

Synonym : Dybenal

Poorly soluble in water (0.2%), soluble in organic solvents.

used at levels up to 0.15%.

The oral LD_{50} in rats was 3 g/kg; in male mice 2.3 g/kg. The subcutaneous LD_{50} in mice was 1.7 g/kg.

Preliminary studies in rabbits showed no eye irritation at 0.08% in aqueous solution, or 0.1% in propylene glycol (PG). Skin irritation studies carried out in a limited number of guinea pigs showed irritation with 5% and above in PG, but not with 1%. In preliminary rabbit studies repeated dermal application of 0.5% caused very slight erythema. On human skin (numbers of subjects not specified) no irritation occurred with 2.5% in a cream formulation on 8 successive days, although a stinging sensation was noticed if damaged skin was treated.

Sensitization in guinea pigs was not induced with 2% in acetone, 0.1% in water or 2.5% in a cream formulation.

A short-term (3-wk) oral study in rats with dose levels of up to 500 mg/kg b.w., and an incomplete, sub-chronic (13-wk) study with 0.36 and 0.72 mg/kg b.w. were negative. A short-term (4-wk) oral study in guinea pigs treated with 1.2 mg/kg bw/day for 4 weeks was likewise negative.

No mutagenic properties were found in tests with 5 strains of S.typhimurium and E. coli.

A teratogenicity study in rabbits treated orally with 20 mg/kg/day was negative.

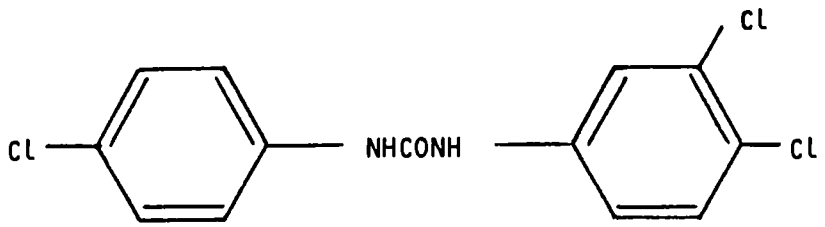
Dermal absorption and excretion in rats was rapid, particularly when formulated in non-polar vehicles. Nearly 90% of the dermal dose applied was excreted in the urine over 96 hours.

It was noted that mutagenicity tests had been made with the substance in a drug, containing other mutagenic agents.
The Committee drew attention to the inadequate doses used in two oral studies and recommended that an oral study and a teratogenicity study be made at doses sufficiently high to demonstrate a possible effect.

However the Committee sees no objection to maintaining the use of this substance as a preservative in cosmetic products for the time being at a maximum level of 0.15%.

Information : Colipa dossiers, Submission I : October 1980
Submission II : June 1982
Submission III : April 1984

N° 25 Triclocarban (INN)



$C_{13}H_9Cl_3N_2O$ CAS N° 101-20-2
MW : 315.59

Synonym : 3,4,4'-Trichlorocarbanilide

Poorly soluble in water, soluble in acetone. Used at levels up to 0.2%.

The oral LD_{50} in rats was $>$ 34.6 g/kg b.w. when given in corn oil and 23.1 g/kg b.w. when given as a 5% soap solution.

No skin- or eye-irritation was observed in rabbits with solutions of 2% in dimethylphthalate, or of 0.2% in soap.

A sensitization test and a photosensitization test in guinea pigs, with 0.05 ml of a 0.1% solution in oil was negative and no cross-sensitization with tetrachlorosalicylanilide was observed.

An oral dose is excreted mainly in the faeces. Metabolism has been extensively studied in man and other species : o - hydroxysulfates and N and N' - glucuronides are formed. Blood levels of TCC and metabolites remained low in rats and humans, even upon rigourous oral or dermal exposure. Absorption through human skin in vitro was very low.

Sub-chronic (90-day) toxicity studies did not reveal toxic effects upon daily oral treatment of monkeys with dose levels up to 300 mg/kg b.w., or upon dermal treatment of rats with dose levels up to 200 mg/kg.

Two year feeding of rats revealed testicular atrophy at dietary levels of 1.0 and 0.3%, but not at 0.1%.

In a reproduction study in rats 250 mg TCC/kg and more caused degenerative testicular changes, while 500 mg/kg and more also induced reduction of implantation sites. No changes were seen at 125 mg/kg.

No teratogenic properties were found upon oral administration of 500 and 1000 mg/kg in rats, and of 30 and 100 mg/kg in rabbits. Dermal treatment of rats and rabbits with 40 and 200 mg/kg was likewise negative.

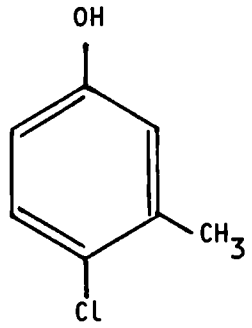
No dominant lethal changes were induced in mice treated intraperitoneally with levels up to 300 mg/kg.

The available information does not suggest that the substance presents a considerable risk to humans. Since, however, trichloroazobenzene and trichloroaniline may be present as contaminants, information is desirable on the purity of the substance and on the identity of impurities.

However the Committee sees no objection to maintaining the use of this substance as a preservative in cosmetic products for the time being.

Information : - Data sheet by National Institute of Public Health, the Netherlands
- British Library automated information service (BLAISE)

N° 26 4-Chloro-m-cresol



C_7H_7ClO CAS N° 59-50-7
MW : 142.59

Synonyms : - 4-Chloro-3-methylphenol
- Chlorocresol

Poorly soluble in water (0.4%), freely soluble in organic solvents.

Use level up to 0.2% in all types of cosmetics except those for contact with mucous membranes.

Oral LD_{50} values for rats are (in mg/kg) : 1830 and 5129 (for males) and 3636 (for females). The s.c. LD_{50} for rats was 400 mg/kg. For mice the s.c. LD_{50} was 360 mg/kg, the i.v. LD_{50} 70 mg/kg.

The substance was irritating to the skin of rabbits when tested at 0.2 - 0.8% in aqueous solution.

In an eye irritation test in rabbits 0.1% was severely irritating; 0.05% produced slight changes.

In an open epicutaneous test (Klecak) in guinea pigs induction with 1, 3, 10 and 30% in a polyethylene glycol (Lutrol) and challenging with diluted and pure substance did not reveal sensitization reactions.
However, when tested in the maximization test 25% was strongly sensitizing and 1% was weakly sensitizing.
In 252 normal human subjects, 10 induction treatments with 5, 10 and 20% and challenging after 2 weeks with 5% in petrolatum did not produce sensitization.

In another test with 31 normal humans no positive reaction was seen after induction and challenging with a 5% concentration.
From a large number of observations in dermatological patients 0.3 - 0.6% showed positive sensitization reactions.
Cases of cross sensitization with chloroxylenol have been reported.

In a short-term (90-day) feeding study in rats with 0, 150, 500 or 1500 ppm in the diet slight growth retardation occurred with 500 and 1500 ppm. Haematology, clinical chemistry, organ weights and gross- and microscopic pathology showed no treatment-related changes. Therefore, 150 ppm was considered a no-toxic effect level.

In a short-term (3 weeks) dermal study 10 rabbits/sex/group received 15 skin applications at levels of 10, 40 and 160 mg/kg/day. Apart from gross and microscopic skin changes, there was no evidence of treatment-related systemic effects (only a summary report is available).

An Ames test with up to 12.5 mg/plate was negative.
In a micronucleus test in mice, 2 oral doses of 200 and 500 mg/kg b.w. given at an interval of 24 hours no changes were detected in the incidence of micronucleated erythrocytes.

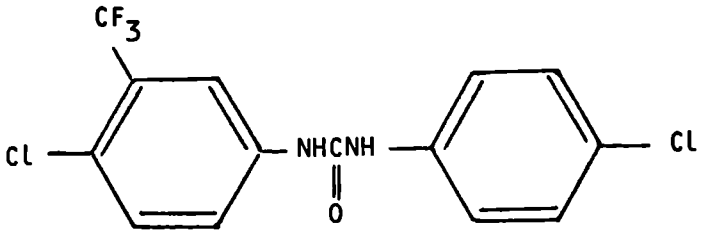
Upon oral administration (300 mg/kg b.w. of rats) the substance is rapidly absorbed and excreted mainly (54 - 95%) in the urine as unchanged compound within 24 hours. Two highly polar urinary metabolites were detected but not identified. No residues were detected in the liver during a 13-week feeding study, but very small amounts (up to 3 µg/g) were found in fatty tissues.

The substance is a severe eye irritant and possesses sensitizing properties in maximization tests in guinea pigs but is not significantly sensitizing in humans. It showed little systemic toxicity upon oral administration. No systemic effects were found when relatively high levels were applied dermally.
The substance is used medicinally in multidose aqueous injection solutions at a level of 0.1% without apparent ill-effects. Mutagenicity tests were negative. Studies on possible effects on reproduction, or teratogenic properties are lacking, but the percutaneous absorption seems to be low.

However, the Committee sees no objection to maintaining the use of this substance as a preservative in cosmetic products for the time being at a maximum level of 0.2%.

Information :
- Data sheet Council of Europe
- NIOSH data
- Colipa dossier, September 1983
- Data sheet National Institute of Public Health, the Netherlands

N° 27 Halocarban (INN)



$C_{14}H_9Cl_2F_3N_2O$ CAS N° 369-77-7
MW : 349.15

Synonyms : - 4,4'-dichloro-3-(trifluormethyl) carbanilide
- Clofucarban
- Irgasan CF3

Insoluble in water, soluble in organic solvents.
Used in cosmetics up to a level of 0.3%.

The acute, oral LD_{50} in rats is $>$ 5 g/kg. The i.v. LD_{50} in rats is 29 mg/kg.

No skin irritation was observed in rabbits, and eye irritation was slight.

No sensitization or local effects were seen in guinea pigs upon repeated, dermal application of 0.1% suspensions.

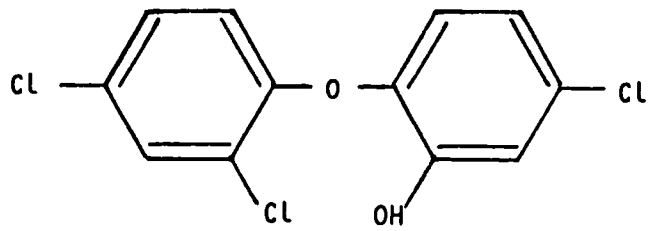
Repeated application (12 weeks, 5 times/week) of 0.1 - 0.5% solutions to the skin of rats did not cause irritation, and no microscopical changes of the internal organs were found.

A metabolic study has been reported : the metabolite found was glucuronide.

On the basis of the above information an evaluation is not possible, but the substance is no longer used as preservative in cosmetic products.

Information : Data sheets Council of Europe

N° 28 Triclosan (INN)



$C_{12}H_7Cl_3O_2$ CAS N° 3380-34-5
MW : 289.55

Synonyms : - 2,4,4'-Trichloro-2'-hydroxy-diphenylether
- Irgasan DP 300

Poorly soluble in water; readily soluble in organic solvents.
Use level : 0.5%.

The acute oral and dermal toxicity is low ($LD_{50} > 4$ g/kg in several species). However, the acute intravenous LD_{50} in rats and mice is 29 and 19 mg/kg respectively. The dermal LD_{50} in rabbits is 9.3 g/kg.

The substance possesses considerable irritating potency for the skin and mucous membranes at concentrations above the use level of 0.5%. Solutions of 0.3 - 0.5% in alcohol have been found to cause dermatitis in man on repeated application.

No sensitizing or photosensitizing properties were observed in guinea pigs or humans.

Absorption through the skin and the intestinal wall is considerabe in several animal species. Up to 10% dermal absorption was found in man. The absorbed substance is excreted partly as glucuronide.

Sub-chronic oral studies revealed liver changes at exposure levels higher than 125 mg/kg in rats and 25 mg/kg in dogs, but no toxic effects were seen in other studies in rats, dogs, rabbits or monkeys at dose levels of respectively 160, 25, 138 and 3 mg/kg b.w.

Upon chronic dermal treatment of mice with c. 0.5 - 1.0 mg/mouse 3 times/week the incidence of certain tumours was slightly higher in test animals than in controls. This finding was not considered significant.

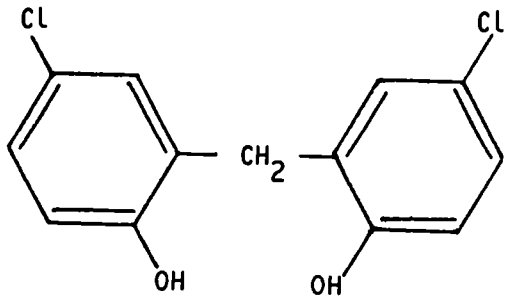
Teratogenicity studies in mice, rats and rabbits with oral dose levels up to 100 mg/kg were negative. This level was negative also in a one generation reproduction study in rats.

No mutagenic properties were detected with 1500 mg/kg in a dominant lethal assay in mice in screening tests for chromosome abnormalities in Chinese hamster cells, or in the in vivo test for somatic mutations (spot test) in mice treated i.p.

The use of this substance as preservative in cosmetic products at a maximum level of 0.3% can be permitted.

Information : - Data sheet National Institute of Public Health, the Netherlands
- TOXLINE (HEEP and IPA)
- Data sheet Council of Europe

N° 29 Dichlorophen (INN)



$C_{13}H_{10}Cl_2O_2$ CAS N° 1215-74-3
MW : 269.12

Synonym : 5,5'-Dichloro-2,2'-dihydroxydiphenylmethane

Poorly soluble in water, readily soluble in ethanol and ether.
Use in cosmetics up to a level of 0.5%.

The acute oral toxicity (g/kg) in rats was 2.69, in mice 1.0, in dogs 2.0 and in guinea pigs 1.25. The intravenous LD_{50} in rats was 17 mg/kg.

Skin irritation in rabbits was mild by applying 0.5 g, but eye irritation was severe with 0.05 mg.

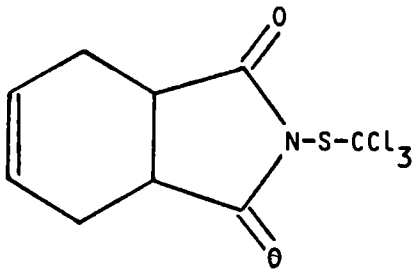
Sensitizing properties have been reported, as well as cross sensitization with hexachlorophene.

Radioactive studies in the rat showed 75% absorption over two days following oral administration, with extensive metabolite conjugation as glucuronide and sulphate.

Evaluation is not possible on the basis of the available information, but the substance is no longer used as preservative in cosmetic products.

Information : - Data sheet Council of Europe
- Registry of Toxic Effects of Chemical Substances 1978
- CBAC

N° 30 N-(Trichloromethylthio)cyclohex-4-one-1,2-dicarboximide (Captan-ISO)



$C_9H_8Cl_3NO_2S$ CAS N° 133-06-2
MW 300.57

Synonyms : - Captan
- Vancide 89

Practically insoluble in water, sparingly soluble (0.2 - 0.3%) in ethanol and other solvents.
The technical grade product (Vancide 89) is used as an agricultural fungicide, the purified product (Vancide 89 RE) as preservative in cosmetics and topical pharmaceuticals.

Used in cosmetics up to 0.06%. Not used for products in contact with mucous membranes.
Oral LD_{50} values for rats are 2.65, 9.0 and 12.5 g/kg; for mice 6.5 and 7.0 g/kg, for rabbits 2.0 g/kg. Intraperitoneal LD_{50} values for rats are between 25 and 100 mg/kg, and for mice 462 mg/kg.

A skin irritation test in rabbits with a formulation (containing captan 0.25 g, propylparaben 0.50 g, distilled water 10.0 g and Tween 20 89.25 g) was negative. Daily application of this mixture to the skin (2 ml/kg) b.w. for 90 days did not induce dermal changes.

In an eye irritation test in rabbits the same formulation was slightly irritating.

A maximization test in guinea pigs with this formulation did not provide evidence of sensitization.

A photosensitization test in guinea pigs with the formulation by the method of Vinson and Borselli was negative.

Sub-acute (21-day) dermal treatment of rabbits with the formulation in an amount of 12 mg/cm^2 over an area of 15 x 20 cm daily on 5 days a week did not adversely affect body weights, food intake, haematology or urine parameters. Mild erythema occurred at the treated skin sites. No pathological changes were observed.

An oral dose is well absorbed from the intestinal tract and rapidly metabolized and eliminated, mainly in the urine. Several degradation products have been identified, e.g. thiophosgene.

Dermal absorption in rats treated with 0.5 or 5 mg of the ^{14}C-labelled compound in aqueous medium varied between 6.4 and 9.0% within 2 - 4 hours after application. There were no significant differences in mean percent absorption between doses.

In a multigeneration study in rats technical captan was fed in the diet at levels providing 0, 25, 100, 250 or 500 mg/kg b.w./day. Pregnancy rate and pup survival were reduced at 250 and 500 mg. Growth depression and reduction of litter size and pup weight occurred at all dose levels. This study did not provide a no-effect level.

A teratogenicity study (conducted with the third litter of the F_1-parent animals of the above reproduction study) failed to reveal any effect on incidence of foetuses or litters with malformations.
Inhalation exposure of mice to 488 mg/m^3 during 4 hr/day, from day 6 - 13 of gestation, did not induce malformed foetuses. Similarly no teratogenicity was observed in mice treated orally with 100 mg/kg b.w./day.
Hamsters treated orally with 50, 100, 200, 300 or 400 mg/kg b.w. showed growth depression at 300 and 400 mg/kg but no teratogenicity or other signs of maternal or embryonic toxicity.
In another oral hamster study with 50, 200 or 400 mg/kg b.w./day of the technical product, the top-dose group showed maternal weight loss, reduced litter size and foetal weight, and an increase in resorptions. With 200 mg/kg maternal weight gain and foetal weight were depressed. A slight increase in a foetal rib abnormality was dose related.

An oral teratogenicity study in rabbits with 6, 12, 25 or 60 mg/kg of the technical substance showed signs of maternal toxicity at 12 mg/kg and above. There were no indications of embryonic toxicity or teratogenicity. In a second rabbit teratogenicity study dose levels of 18.75, 37.5 and 75.0 mg/kg body weight were administered orally by gelatin capsule. No signs of teratogenicity were seen even at dose levels showing maternal and foetal toxicity (I.B.T.-study).
Groups of at least 7 Rhesus monkeys were orally treated with 10, 25 or 75 mg/kg from day 21 - 34 of pregnancy. There were no signs of maternal or foetal toxicity or of teratogenicity (I.B.T.-study).
Pregnant beagle dogs received captan in the diet throughout the gestation period at levels providing 30 or 60 mg/kg b.w. No deleterious or teratogenic effects were observed (I.B.T.-study).

An oral carcinogenicity study was conducted in mice which received 215 mg/kg b.w. daily from an age of 7 day until weaning, and then 560 ppm in the diet for 18 months. No significant increases in tumour incidence was seen (IARC 1983).
A second mouse carcinogenicity study with dose levels of 8000 and 16000 ppm in the diet for 80 weeks showed a small, though dose related and statistically significant increase in duodenal adenocarcinoma (IARC 1983).
A chroçnic feeding study in rats with 4000 and 8000 ppm in the diet (later changed to 2000 and 4000 ppm, and in the final 33 - 34 weeks of the study to diets not containing the test substance) revealed slight increases in adenomas of the thyroid and adrenals which were considered unrelated to treatment (IARC 1983). The results of the latter carcinogenicity studies in mice and rats have been confirmed in other studies with a different strain of rats and mice (Lowy, 1985).

A large number of mutagenicity studies has been performed. The results have been summarized by IARC (1983) as follows :

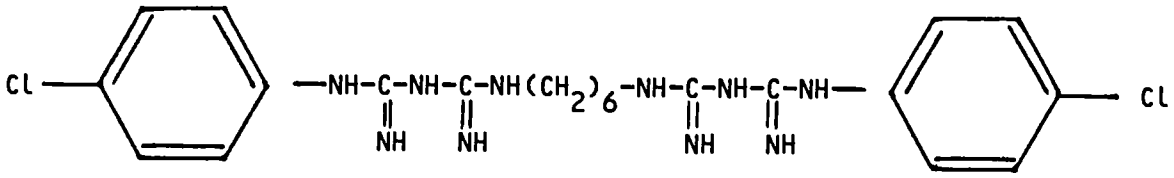
> "Captan was mutagenic to bacteria and yeast. Both positive and negative results were obtained in the host-mediated assay in mice. Weak or negative effects were observed in Drosophila melanogaster. Captan induced chromosomal aberrations, sister chromatid exchange and mutations at several loci, but not unscheduled DNA synthesis in cultured mammalian calls. No increase in micronucleated erythrocytes or chromosomal aberrations was detected in treated mice or rats; positive results obtained in dominant lethal tests in mice and rats were not confirmed by other studies. Thus, there is sufficient

evidence to establish the mutagenicity of captan in cellular systems, but the data were insufficient to establish its mutagenicity in mammals".

The ADI for Captan as a pesticide residue in foods has been set at 0.1 mg/kg b.w./day. The use of the substance in cosmetics, even at the reduced maximum level of 0.06%, could lead to a human exposure, considerably higher than the maximum considered acceptable from its use in agricultural crops. In addition to the possible carcinogenicity this substance was rejected on other toxicological characteristics.

Information : - Colipa dossier, September 1984
- Colipa submission II, September 1985
- FAO/WHO, Pesticide residues in food : 1982 evaluations
- IARC Monograph 30 (1983)
- WHO Pesticide Residues Series No. 3 (1974)
- R. Lowy, C.S.C./477/85, Nov. 1985

N° 31 Chlorhexidine (INN) and its digluconate, diacetate and dihydrochloride



$C_{22}H_{30}Cl_2N_{10}$ CAS N° 55-56-1
MW : 505.48

Synonym : 1,6-di-(N-p-chlorophenyl-N'-diguanide)-n-hexane

Poorly soluble in water, insoluble in organic solvents and fats.
Use level : 0.5%.

The oral LD_{50} in rats and mice is relatively high (c. 2 g/kg), but the i.v. LD_{50} is much lower (c. 25 mg/kg). Values for the acetate and the gluconate were comparable.

Eye injury was found by EM 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) oral study in rats with levels up to 0.3% chlorohexidine 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/kg b.w.

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 chlorhexidine was found in the blood (11 µg/l) which may indicate very low

dermal absorption. Trace amounts were, however, present in some organs and in faeces which might be due to grooming. Microscopy of selected organs revealed no changes attributed to treatment.

In a one-generation reproduction study in rats, 44 mg/kg b.w. (in drinking water as the gluconate) induced growth retardation and increased pup mortality; 5 mg/kg was without effect. No teratogenic potency was observed with dose levels of chlorhexidine (as base) up to 50 mg/kg by gavage, but oral absorption is very poor.

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.)

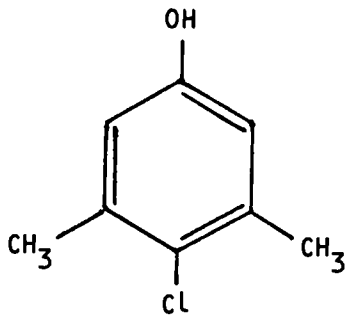
Mutagenic properties of chlorhexidine were established in an Ames test. A positive effect was found also in a DNA-repair test with E. coli. However, in a micronucleus test in mice two intravenous applications of up to 2 x 30 mg/kg (the maximal tolerated dose) did not increase the number of polychromatic erythrocytes containing a micronucleus.

There is only a small margin between potential dermal exposure of humans and the lowest oral exposure that induced lymphatic histiocytosis in the chronic rat study. For this reason information on dermal absorption in man is urgently required.
In view of the positive results obtained in two mutagenicity tests in microbial systems, a chromosomal aberration test on mammalian cells in vitro is requested.

However, the Committee sees no objection to maintaining the use of this substance as a preservative in cosmetic products for the time being at a maximum level of 0.3%.

<u>Information</u> : - Data sheet National Institute of Public Health, the Netherlands
- Toxicol. appl. Pharmacol. 52 (1980) 255-261
- Colipa dossier January 24, 1984
- BLAISE and TOXLINE

N° 32 4-chloro-3,5-xylenol



C_8H_9ClO CAS N° 88-04-0
MW : 156.61

Synonym : p-Chloro-m-xylenol (PCMX)

Poorly soluble in water (0.05%), soluble in ethanol.
Readily soluble in other organic solvents.
Use level : 0.5%.

The oral LD_{50} in rats and mice is c. 3 g/kg. The dermal LD_{50} is 3 g/kg. Recovery has occurred after ingestion of 15 g in man.

Marked eye irritation occurred in rabbits treated with a 30% solution in propylene glycol, and moderate eye irritation was noticed with 2% in a shampoo. A 2% ethanol solution patch-tested in humans neither caused skin irritation nor sensitization.

Short-term (21 day and 90-day) dermal toxicity was examined in rabbits by daily application of 1.0 ml 1.8% or 18% in propylene glycol to the abraded skin for 21 days, or to the intact skin for 90 days. Skin irritation was slight in the low-dose animals and considerably more marked in the high dose animals. In this respect no difference between intact and abraded skin was noticed. There were no treatment-related differences between groups in growth rate, haematology, urine composition or in gross or microscopic pathology.

In a sub-chronic (13-week) oral toxicity study in rats, 0, 1.2, 60 or 120 mg/kg b.w. were applied daily as an aqueous emulsion. Salivation occurred upon dosing. There was transient growth retardation in the mid- and top-dose groups. The top-dose group showed various haematological changes, impaired renal function, and increased weights of the liver and kidneys, not associated with microscopical changes. Increased kidney weights occurred also in the mid-dose group (summary report only).

The same levels as used in the 13-week rat study were administered by gavage in a 13 week dog study. There were no deleterious effects on body weights, haematology, or clinical chemistry. Total reducing substance in the urine was increased at the two high dose levels. Increased liver weights not accompanied by histological changes, occurred in all dose groups (summary report only).

When applied to the skin of humans the substance is absorbed, and shows prolonged circulation in the blood at a low level, of generally < 1 mg/100 ml.

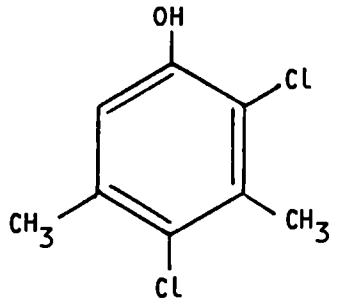
An Ames test with only 1.0 µg/plate was negative.

The summarized reports of the 13-week oral studies in rats and dogs suggest that the no-toxic effect level may be around 1 mg/kg b.w. However, considerably higher doses (18 mg/kg) did not induce overt signs of toxicity upon dermal administration to rabbits for 13 weeks. The Committee wishes to examine the detailed reports of the 13-week oral studies and also to see the results of an Ames test carried out with higher doses.

However, the Committee sees no objection to maintaining the use of this substance as a preservative in cosmetic products for the time being at a maximum level of 0.5%.

Information : - Data sheet Council of Europe
- Colipa dossier, September 1983

N° 33 2,4-Dichloro-3,5-xylenol



$C_8H_8Cl_2O$ CAS N° 133-53-9
MW : 191.06

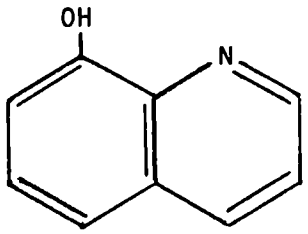
Poorly soluble in water, soluble in organic solvents.
Use in cosmetics up to a level of 0.1%.

The only toxicological information available is an oral LD_{50} in rats (3.94 g/kg) and in mice (6.0 g/kg).

No opinion can be expressed because of a lack of data, but the substance is no longer used as preservative in cosmetic products.

Information : Data sheet Council of Europe.

N° 34 Quinolin-8-ol and its salts



C_9H_7NO CAS N° 148-24-3
MW : 145.15

Synonyms : - 8-Hydroxyquinoline
- Oxychinoline
- Oxine

Hydroxyquinoline is insoluble in water and ether, slightly soluble in other organic solvents; the sulphate dissolves well in water.

Used in cosmetics at dose levels of 0.3%.

LD_{50} values reported are : oral in rats 1200 mg/kg, in guinea pigs $>$ 1.2 g/kg for the free base; i.v. in mice 50 mg/kg, in rabbits 65 mg/kg for the free base; s.c. in mice 25 mg/kg, in rats 200 mg/kg for the sulphate; i.p. in mice 43 mg/kg.

Sensitizing properties have been reported from studies in man.

Oral administration to dogs resulted in rapid intestinal absorption and renal excretion and slow excretion with the bile.

Intravenous treatment of rabbits with a single dose of 8-hydroxyquinoline in amounts of 10 - 50 mg/kg resulted in destruction of the ß-cells in the pancreas and increased blood glucose levels. Pancreatic damage was observed also in several other species upon i.v. administration.

Feeding rats 400 mg/kg b.w. for 16 weeks caused hemosiderosis in the liver and spleen. In another rat feeding study hepatotoxicity and renal toxicity occurred on diets providing 100-250 mg/kg b.w. for 30-40 days.

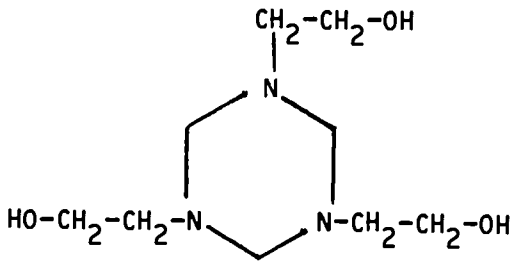
In 1977 IARC examined several carcinogenicity studies in mice and rats conducted by oral, subcutaneous and intravaginal administration, and in mice by skin application and bladder implantation. These studies were considered of limited value for various reasons and no evaluation could be made of the carcinogenicity of this substance. Meanwhile further longterm studies were conducted and reported recently by NTP (1985). Groups of 50 rats/sex and of 50 mice/sex were fed diets containing 0, 1500 and 3000 ppm of the test substance. The top-dose groups showed relatively low body weights. No significant differences in mortality occurred between groups in either species. Extensive gross and microsopic pathology did not reveal treatment-related changes and there was no evidence of carcinogenic properties. Haematological and clinical examinations were not conducted.

The substance showed mutagenic properties in the Ames test. A test on induction of aneuploidy in Neurospora crassa was equivocal. Chromosome aberrations were observed in root tips of Vicia faba, and in bone marrow cells of mice after i.p. injection of 40 mg/kg b.w. Equivocal or inconclusive results were obtained in a variety of other short-term tests such as a Drosophila test, a micronucleus test in mice and a chromosome aberration test in human leucocytes in vitro.

No information is available on skin or eye irritation, on dermal toxicity or on dermal absorption. Information on reproductive effects or teratogenicity is also absent. The substance seems to be relatively harmless because 1500 ppm in the diet of rats (75 mg/kg b.w.) and in the diet of mice (150 mg/kg b.w.) was a no effect level. These substances are no longer used as preservatives in cosmetic products.

Information :
- Data sheet National Institute of Public Health, the Netherlands
- IARC Monograph 13 (1977) 101-112
- NTP Technical Report series No. 276, April 1985

N° 35 1,3,5-Tris(2-hydroxyethyl)hexa-hydro-1,3,5-triasine



$C_9H_{21}N_3O_3$ CAS N° 4719-04-4
MW : 219.28

Synonyms : - Tri-(ß-hydroxyethyl)-hexahydrotriazine
- Grotan BK

Easily soluble in water; soluble in ethanol, acetone, chloroform.

Used in cosmetics at dose levels of 0.3%.

The oral LD_{50} was 0.37 ml/kg in mice. For rats oral LD_{50} values of 0.46 ml/kg, 0.58 ml/kg, and 316 mg/kg are reported. The acute dermal LD_{50} was c. 0.5 ml/kg in the rabbit.

The undiluted substance induced severe erythema and slight oedema of the skin of rabbits. Slight erythema and oedema were seen also with aqueous dilutions of 1.0 and 0.1%. No local effects were seen, however, in patch tests with 80 humans receiving 0.05 ml of concentrations varying from 0.05 to 1.0%.

Severe eye irritation occurred in rabbits with 0.1 ml of the undiluted substance. No deleterious effects were, however, observed if the eye was washed out 2, or 4 seconds after instillation of the test substance.

No sensitization was observed in guinea pigs treated with 0.043 mg/day during the induction period and with 5 mg as the challenge dose.

Single dermal treatment of rabbits with 1 or 2 ml undiluted substance/kg body weight for 24 hours killed the animals. With 0.5 ml/kg damage of liver and lungs was observed. In a subsequent study in which rabbits received a single dermal treatment of 7 ml/kg of 0.1% or 1.0% dilution of the test substance either in water, in paraffin oil or in emulsions of oil in water or of water in oil, there was no mortality and no systemic effects were seen.

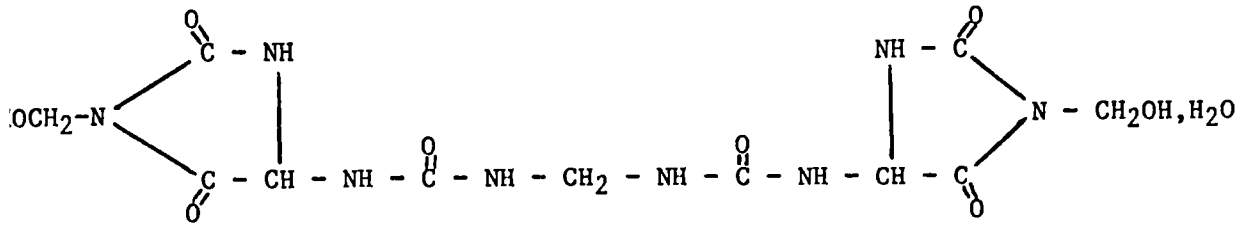
In a 90-day study in rats with various feeding levels up to 5400 ppm, high mortality occurred in all groups, but no significant treatment-related changes were observed. The study showed several deficiencies.

No indications of mutagenic properties were obtained in a micronucleus test in rats treated by gavage, by dermal administration and by subcutaneous injection with various dose levels up to 960 mg/kg per 2 administrations.

This substance shows considerable toxicity, irritant properties and dermal absorption. On the basis of available information evaluation is not possible.
The Committee wishes to obtain additional data on short-term oral toxicity, and on eye irritation with dilutions below 1%. Information on teratogenicity is also requested.

Information : - Data Sheet National Institute of Public Health, the Netherlands
- Colipa dossier, Partial submission (1), September 1983

N° 36 3,3'-Bis(1-hydroxymethyl-2,5-dioxoimidazolidin-4-yl)-1,1'-methylenediurea ("Imidazolidinyl urea")



$C_{11}H_{16}N_8O_8$
MW : 406

CAS N° 39236-46-9

Synonyms : - Germall 115
- Eurill K 200

Soluble in water, propylene glycol, glycerine
Used in cosmetics at dose levels of 0.1 - 0.6%.

The acute toxicity of the substance is low : the oral LD_{50} in rats and mice is $>$ 5 g/kg, the i.v. LD_{50} in rats and mice is c. 0.8 g/kg, the acute dermal toxicity in rabbits and guinea pigs $>$ 2 g/kg.

Skin irritation tests with aqueous solutions of 5% in rabbits, and 10% in humans were negative. No phototoxicity was observed with solutions up to 5% in guinea pigs.

Intracutaneous injections of 0.1% saline solutions in guinea pigs did not provoke sensitization. Tests in man with concentrations up to 10% and with as many as 200 subjects provided little or no evidence of sensitization. No photoallergic sensitivity was observed in women after using formulations with 0.5% of the test substance daily for 4 weeks.

Eye irritation was not observed in rabbits with aqueous solutions up to 20%. In the form of a fine white powder the substance produced mild transient conjunctival irritation.

Sub-acute (25-day) oral treatment of rats with 0.5% in drinking water produced no evidence of toxicity.
Sub-acute dermal treatment of rabbits (6 h/day, 5 days/week, for 3 weeks) with dose levels up to 200 mg/kg/day of the undiluted substance only resulted in slight irritation. There was no effect on growth, haematology, urinalysis or gross pathology.
Sub-chronic (90-day) dermal treatment of rabbits with 200 mg/kg/day of the compound as a 10% aqueous solution over 10% of the total body surface produced local skin changes (acanthosis, hyperkeratosis, and intradermal infiltration of inflammatory cells). There were no abnormalities in haematology, blood biochemistry, urinalysis, organ weights, or gross and microscopic pathology.
Sub-chronic (90-day) feeding of the test substance in the diet of rats at levels providing 0, 6, 28, 130 or 600 mg/kg b.w./day, induced growth retardation of males in the 28, 130 and 600 mg/kg groups. Haematology, biochemistry, urinalysis and pathology revealed no toxicity. Similar results were obtained in a 90-day rat study with 0.5, 1.0 or 2.0% in the drinking water, but growth depression occurred in the top-dose group, which ingested 1300 mg/kg/day.

A teratogenicity study in mice treated by gavage with 0, 30, 95 or 300 mg/kg/day from day 6-15 of gestation increased the number of resorptions and/or fetal deaths but the number of abnormalities in either soft or skeletal tissue was not affected.

An Ames test with up to 0.5 mg/plate with and without metabolic activation was negative (no detailed report is available).

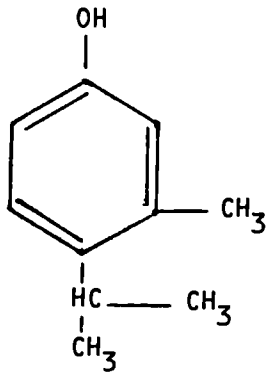
The available information suggests the substance to be of relatively low toxicity. Details of the Ames test carried out are required and a chromosome aberration test in mammalien cells in vitro.

However, the Committee sees no objection to maintaining the use of this substance as a preservative in cosmetic products for the time being at a maximum level of 0.6%.

Information : - Final report of the safety assessment of imidazolidinyl urea. J.Environm. Pathol. Toxicol. 4 (4) 1980, 133-146
- Data sheet National Institute of Public Health, the Netherlands, March 1981

- Colipa dossier, Submission I : 18.1.1981
- Colipa dossier, Submission II, March 1984

N° 37 4-Isopropyl-m-cresol



$C_{10}H_{14}O$ CAS N° 3228-02-2
MW : 150

Synonyms : - 3-Methyl-4-(1-methylethyl)-phenol
- 4-isopropyl-3-methylphenol
- O-cymen-5-ol

Slightly soluble in water, and freely soluble in most organic solvents. Used in all types of cosmetics at dose levels up to 0.1%.

Oral LD_{50} values in mice are $>$ 2.2, 2.25 and 1.1 g/kg, the i.p. LD_{50} in mice was 0.47 g/kg.

Skin irritation in rabbits with 5% in polyethylene glycol was minimal, and with 1% in vaseline absent. In guinea pigs skin irritation with 10% in ethanol was minimal. No skin irritation was seen in a human patch test with 0.5% in polyethylene glycol, or with 1% in vaseline.

An eye irritation test in rabbits with 0.1% in propylene glycol produced slight effects only during the first few hours after treatment. With 1% in vaseline only very slight redness of the conjunctivae occurred. The eye irritating potential is very low.

In a maximization test, guinea pigs received induction treatments with 20% in acetone. Upon the challenge treatment with 0.1, 1.0, 5.0 and 10% in acetone the incidence of positive reactions was 0/10, 0/10, 1/10 and 3/10 respectively. It was concluded that the sensitizing potential was very low. A maximization test in 27 healthy humans with 5 induction exposures to a 1%

concentration and a challenge treatment with 0.1% in both vaseline and cream bases (after treating the test sites with 5% sodium lauryl sulphate) did not produce any positive reaction.

Repeated intraperitoneal injections of mice with up to 300 mg/kg/day in an oil/water emulsion did not induce mortality. Higher levels caused asphyxia and death.

Feeding the substance in the diet of mice at dose levels up to 80 mg/animal for 30 days caused intoxication and mortality with 50 mg/animal and more. No toxicity was noted with 30 mg/animal or less ($\leq$ 1200 mg/kg b.w./day) (only a summary report is available).

A 90-day feeding study was conducted in mice with 1, 10, 100 or 200 mg/100 g (it is not mentioned whether the levels are expressed per 100 g diet or per 100 g body weight). The two highest levels caused growth retardation. It is stated that there were no changes in survival, haematology, biochemistry, urine composition, or in gross or microscopic pathology (Summary report only).

No mutagenic properties were observed in an Ames test, or in a test with E.coli at levels of up to 800 µg/plate.

In a 6-month rat study with test groups of 4 animals, daily administration of 0.05, 01, 0.5 or 1.0 g/kg by gavage, was without effect (Summary report only).

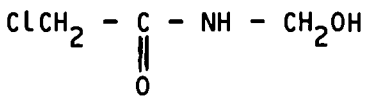
No detailed reports are available of the feeding studies, which were conducted around 1955 in Japan.

A well-conducted, short-term, oral study in rats is desirable, as well as further information on mutagenicity. Information on percutaneous absorption is also sought. In the light of these data the necessity, if any, for further studies can be determined.

However, the Committee sees no objection to maintaining the use of this substance as a preservative in cosmetic products for the time being at a maximum level of 0.1%.

Information : - Data sheet Council of Europe
- Colipa dossier, September 1983
- Colipa dossier, April 1985

N° 38 2-Chloro-N-(hydroxymethyl) acetamide



$C_3H_6ClNO_2$ CAS N° 2832-19-1
MW : 123.54

Synonyms : - N-Methylolchloroacetamide
- Grotan HD2
- Parmesol P 35

A clear colourless liquid. It is used in concentrations up to 0.4%.

LD_{50} : oral : Mouse, 360 mg/kg
Rat, 1.28 ml/kg (as 10% v/v aqueous solution)
dermal : Rabbit, 2.8 - 5.5 ml/kg as 10% aqueous v/v solution with occlusion for 24 hrs. Mortality occurred in three out of four rabbits treated with 5.5 ml/kg, but no deaths occurred with 2.8 mg/kg. Post-mortem gross and histological examination showed dose-related changes in skin only; liver, kidney and heart were normal.

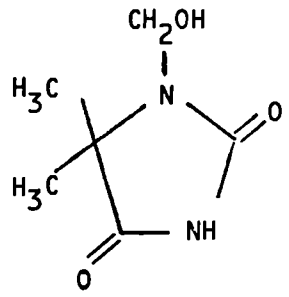
Patch tests in man were carried out on 50 subjects with various formulations containing 0.2% of the substance; these were negative. In another patch test, with occlusion, carried out in 100 subjects, 0.15% gave no reaction in 99; one subject showed a mild reaction. In a similar study, also on 100 subjects, the substance in concentrations of 0.08, 0.12, 0.05 (sic) and 0.1% had no adverse effect.

It is noted that 2-chloroacetamide is a relatively powerful skin sensitiser. Some of the N-substituted congeners are irritant to the skin and are absorbed by the percutaneous route. Systemically they have been noted to be toxic to the liver and kidney. They also exhibit central nervous system toxicity.

No opinion can be expressed because of a lack of data, but this substance is no longer used as preservative in cosmetic products.

Information : Data sheet Council of Europe

N° 39 1-Hydroxymethyl-5,5'-dimethyl-hydantoin



$C_6H_{10}N_2O_3$ CAS N° 28453-33-0
MW : 158.16

Synonym : Monomethylol-dimethyl-hydantoin

This compound liberates formol at pH 6, which acts as preservative. On heating, the formol combines with the parent compound to form a polymer which has properties suitable for use as a hair lacquer.

No data on animal experiments have been presented.

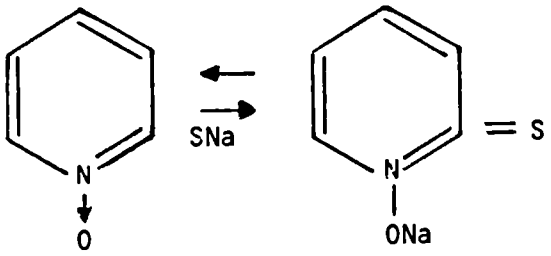
In the data provided, it has been assumed that the toxicity of the compound is due only to formol. Formol is stated to be very irritant, and a potent sensitising agent. It is a potential pulmonary carcinogen.

Sensitisation occurs in about 5% of human subjects when exposed to formalin, an aqueous solution of 37% formol. In subjects already sensitised to formol, irritation is found with solutions of formalin of 0.01% to 1.0% (equivalent to an aqueous solution of formol of 0.0037% to 0.37%, or 37 µg/ml to 3.7 mg/ml).

No opinion can be expressed because of a lack of data, but this substance is no longer used as preservative in cosmetic products.

Information : Data sheet, Council of Europe

N° 40 Pyrithion sodium (INM)



$C_5H_5NOS.NA$ CAS N° 3811-73-2
MW : 127.18

Synonyms : - Pyridine-1-oxide-2-thiol sodium salt
- Sodium-pyridinethione
- Sodium-omadine

Soluble in water and methanol.
Used in cosmetics at dose levels of 0.5%.
The LD_{50} (in mg/kg) is : oral in rats 660 - 900, in mice 870 - 1000; i.v. in mice 335; dermal in rabbits 300 in one study, and 2500 in another.

Moderate erythema of the skin occurred in rabbits with 25 mg/kg applied as a 40% paste in CMC. Death may occur with 100 mg/kg. In humans 500 mg of a 0.5% ointment was not irritating.

Slight eye irritation was seen in rabbits treated with aqueous concentrations of 0.2% and higher.

Photosensitization tests with 2.0% aqueous solutions were positive in guinea pigs but not in humans.

Sub-acute, oral studies have been conducted in several species. In rats toxic effects occurred in a 4-week study with 200 mg/kg b.w./day. In a 4-week oral dog study eye damage and complete blindness were observed with 10 - 20 mg/kg b.w./day. No eye damage occurred in monkeys treated orally with 25 mg/kg b.w./day for 5 days. With 20 ppm in the diet of chickens paralysis of the legs developed, while with 10 ppm there was only some growth depression.

Sub-acute dermal studies revealed intoxication in rabbits with 100 mg/kg/day for 9 days. Severe toxic effects occurred in rabbits with 300 mg/kg b.w./day for 14 days. In the monkey, nineteen daily applications of 0.75 ml of a 2.5% solution in PEG 300 (19 mg/monkey) produced effects only at the treated sites.

Dermal absorption is considerable under various conditions and in different species. Abrasion of the skin enhanced absorption in rabbits. In rats the absorbed substance is largely metabolized to pyridine-N-oxide-2-sulfonic acid.

Semi-chronic (15-week) oral studies in rats, dogs and monkeys with 50, 100 and 300 mg/kg b.w. induced mortality and changes in the liver and kidneys with 100 and 200 mg/kg.

In reproduction studies, reduced litter size occurred when males were treated orally with 50 mg/kg/day for a few weeks. When the same level was applied to females for 3 weeks before mating and during pregnancy, embryonic resorption was increased.

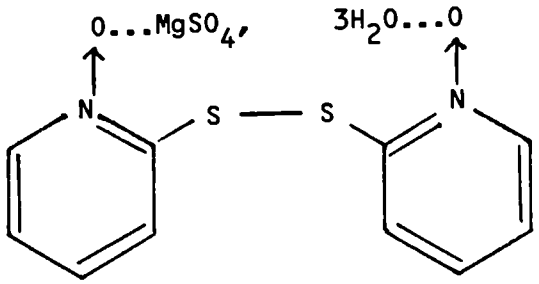
In teratogenicity studies in rats 50 mg/kg/day, orally, caused increased embryonic resorptions. With lower dose levels embryonic survival was not affected, but a slightly increased incidence of rib deformities was seen with 7.5 mg/kg. The probable no-effect level was 3 mg/kg.

Na-omadine is toxic and is rapidly absorbed through the skin. Blindness was oberseved in dogs treated orally with 10 - 20 mg/kg/day for 4 weeks. The substance is embryotoxic and teratogenic. The no-effect level has not been firmly established, but in rats it is lower than 50 mg/kg b.w. and probably even lower than 5 mg/kg b.w.

This substance is no longer used as a preservative in cosmetic products.

Information : Data sheet National Institute of Public health, the Netherlands

N° 41 2,2'-Dithiobis(pyridine-1-oxide), addition product with magnesium sulphate trihydrate



CAS N° 43143-11-9

MW : 426.3

Synonyms : - Pyrithion-disulphide + magnesium sulphate
- Omadine MDS

The adduct is better soluble in water and ethanol than pyrithione.

Used in rinse-off cosmetics up to 0.2%, but up to 1.0% rinse-off hair care products for other purposes than preservation.

The oral LD_{50} values in rats varied from 1.1 to 1.35 g/kg. The dermal LD_{50} in rabbits was 8 g/kg.
A single oral or intraperitoneal dose of 20 mg/kg b.w. of mice did not induce abnormalities in behaviour or growth rate and no microscopic changes were detected in testicles and epididymides after 7 - 21 weeks.
A single oral dose of 0.125, 0.250, 0.50 or 1.00 g/kg b.w. of rats was associated with decreased activity, decreased respiration and prostration in all dose groups, and 3/5 rats died in the top-dose group.

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 irradiation.

Distinct eye irritation occurred in rabbits with 0.1 ml of a 50% aqueous suspension (score 11/110). Severe eye irritation was induced in monkeys by applying 100 mg of the undiluted powder, but 0.1 ml of a 1% aqueous solution was negative.

A sensitization test in guinea pigs with 0.1 ml of a 0.2% aqueous solution applied in the induction period and 0.05 ml of 0.1 and 0.5% aqueous solution as the challenge dose was negative. Negative results were obtained also when a 10% aqueous solution was applied in the induction period and as the challenge treatment.
No photosensitizing properties were observed upon applying 0.1 ml of a 5% suspension in corn oil and UV-A irradiation.

A sub-chronic (90-day) oral study in rats was conducted with 1, 3, 10 and 30 mg/kg b.w./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 was administered daily by gavage. Decreased body weight and food intake occurred with 15 and 50 mg/kg. The significance of mortality and changes in organ weights which occurred in all treatment groups cannot be evaluated as a result of the small number of animals (4/sex/group).

In a reproduction study, oral administration of 1.0, 3.0 or 7.5 mg/kg b.w./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 postnatal study pregnant female rats were treated orally with 1, 3 or 7.5 mg/kg b.w./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 with 3, 10, 30 and 100 mg/kg maternal toxicity occurred at the two high-dose levels. In addition at these two levels there was 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, 100 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 member 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.

Mutagenic properties were observed in an Ames test when concentrations were used above the threshold of toxicity. At lower concentrations (1-100 µg/plate) the test was negative. One in vitro test with Chinese hamster lung cells indicated mutagenic properties, but the latter test was negative when repeated in a modified form. Increases in mutation frequency were obtained also with 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 µg/ml. This finding indicates absence of DNA damage.

In a dermal carcinogenicity 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.

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-methyl-sulfonylpyridine.

Dermal absorption occurs in animals and man.

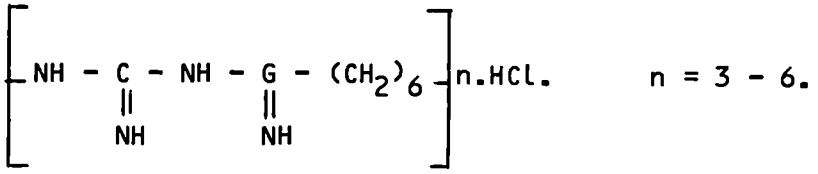
- In man, 3 - 15% of a dermal dose was recovered in the urine, when 4, 12 and 40 $\mu g/cm^2$ were applied in methanol, without occlusion.
- In an in vitro study with abdominal skin of humans, exposure for 1000 minutes without rinsing, the amounts recovered were 14% in the horny layer, 0.6% in the epidermis, 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%, the amounts recovered were 1.76% in horny layer, 3.32% in epidermis, 1.32% in corium and 0.07% in penetration chamber
- In rats dermal absorption was $< 1\%$ by applying shampoo solutions (0.15 ml/rat) containing 0.5, 1.0 or 1.5%.
- Rats treated with a hair dressing formulation, containing 0.1% and applied to the back skin for 4 hours showed 4.45% of the dose applied in the urine and 0.31% in the faeces while the treated skin contained 2.55% at 48 hours after application.

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 b.w./day. There is evidence of mutagenic and teratogenic properties.
Dermal absorption is likely to be considerably higher than 0.07%.
Use levels up to 0.5% in cosmetics could result in dermal exposure of 1 mg/kg in humans. If 10% dermal absorption occurs the margin of safety is probably only a factor of 10. Industry proposed to decrease the use level as a preservative in rinsed off products to 0.2% and requested to allow up to 1.0% for other uses;

However the Committee sees no objection to maintaining the use of this substance as a preservative in cosmetic products for the time being at a maximum level of 0.2% in rinsed off products.

Information : - Colipa dossiers - 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
- Data sheet Nat. Inst. Publ. Health, the Netherlands, 1983
- Toxicol. appl. Pharmacol. 43 ((1978) 373-379)

N° 42 Poly(1-hexamethylenebiguanide hydrochloride)



$(C_8N_4H_{16})_n(HCl)_n$

Highly soluble in water and ethanol, poorly soluble in non-polar solvents. Commercial products contain c. 20% active ingredient.

Used at levels up to 0.3%.

Oral LD_{50} values of formulations vary from about 135 to $>$ 1000 mg/kg for rats; an i.p. LD_{50} value of 2.5 - 25 mg/kg was reported also for rats. These values are expressed as active ingredient.

A skin irritation test in rats treated on 3 alternate days with a 25% PHMB solution produced severe effects. Similarly, treating rats on 6 days with 10% also caused severe irritation, but no effects were seen with 5% or less.

Eye irritation in rabbits with a 25% solution was severe, but only slight inflammation occurred if the eye was washed after 5 seconds.
No irritation was seen with a 5% solution.

Moderate sensitization occurred in a maximization test in guinea pigs upon induction and challenge treatments with a 0.2% solution. A similar result was obtained in a Buehler test in guinea pigs after induction and a challenge treatment with a 2% solution.
In a photosensitization test in 13 human volunteers treated with 0.4 ml 5% solution, 3 days a week for 4 weeks, and exposed to the sun for one hour, five volunteers showed slight signs of a positive effect.

Daily oral treatment by gavage for less than 3 weeks caused mortality in mice with 50 mg/kg, and in rats with initially 500 mg/kg and subsequently 125 mg/kg.

In a 90-day study, rats fed 1250 ppm PHMB in the diet showed increased iron pigmentation and Kupffer cells in the liver. No changes occurred with 625 ppm in the diet (30 mg/kg b.w./day).

Dogs fed 500, 1500 or 4500 ppm in the diet showed growth depression and decreased food intake at the top-dose, and liver damage at the top-dose and intermediate dose. No changes occurred in the low-dose group (12.5 mg/kg b.w./day).

Inhalation exposure of rats to 0.55 mg/m^3 and above caused mortality in a 3-week study. No significant changes were found at 0.005 mg/m^3.

A chronic feeding study with 0, 100, 200 and 1000 ppm PHMB in the diet was conducted in mice, which had been exposed already in utero. The top-dose caused decreases in growth rate, food intake and food efficiency, and liver enlargement. No toxicologically important changes were found with 200 ppm (20 mg/kg b.w./day). There was no evidence of carcinogenicity.

In a 2-year rat study dietary levels of 200, 1000 and 2000 ppm PHMB were used. Body weight and food intake showed dose-related decreases. At the two higher levels there were dose-related increases in the weight of the spleen and adrenals and some histiocytic conglomerates in the mesenteric lymph nodes. No evidence of carcinogenicity was found. The no-effect level was 200 ppm or 10 mg/kg b.w./day.

An 80-week dermal study was conducted in mice with 0, 0.6, 6.0 or 30 mg PHMB/mouse/day, applied as an ethanolic solution on 5 days a week. The two higher dose groups showed growth depression and skin changes. In addition, the top-dose group showed exophthalmos, hepatitis and a relatively high incidence of liver tumours. The no-toxic effect level was 0.6 mg/mouse (12 mg/kg b.w./day).

No evidence of mutagenicity was found in an Ames test, or in in vitro tests with hamster kidney fibroblasts, mouse lymphoma cells, or human lymphocytes.

There were no adverse effects on reproduction with feeding levels up to 1000 ppm PHMB in the diet of mice, or up to 1300 ppm in the diet of rats.

A teratogenicity study in rats treated throughout gestation with 200, 1000 or 2000 ppm in the diet showed an increased incidence of foetuses with extra ribs only in the high-dose group. In a similar study, mice treated with 10, 20 or 40 mg/kg on days 6 - 15 of gestation showed changes of the skull in the two higher dose groups. The lowest dose was considered a no-effect level. No indication of teratogenicity were found in rabbits treated with dose levels up to 32 mg PHMB/kg b.w./day.

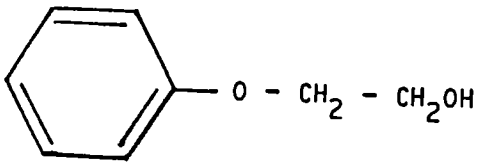
In a metabolism study the radio-labeled compound was administered to rats by gavage in an amount of 20 mg/kg. More than 90% was excreted in the faeces and c. 5% in the urine within 48 hours. Very little was found in expired air. Metabolic change did not seem to occur.

The substance has been extensively examined. Experience in use over 16 years has produced no evidence of sensitization in man. The substance is heat-stable and non-volatile, so that inhalation exposure is unlikely to be a problem.

The use of this substance as preservative in cosmetic products can be permitted.

Information : - Data sheets national Institute of Public Health, the Netherlands
- Reports ICI

N° 43 2-phenoxyethanol



$C_8H_{10}O_2$ CAS N° 122.99.6
MW : 138.17

Poorly soluble in water (2%); miscible with acetone, ethanol, glycerine; soluble in fats.

Use level : up to 1.0%.

The acute toxicity studies indicate that in the rat phenoxyethanol is less toxic upon percutaneous administration (13 ml/kg b.w.) than upon oral administration (1,3 ml/kg b.w.). At lethal doses it affects the central nervous system.

Intramuscular administration (of aqueous solutions of 0.4% and above, 1 ml/kg b.w.) induced necrosis at the injection site in rats and rabbits.

A 2% aqueous solution is mildly irritating on the skin and well tolerated by the mucous membranes of the eyes of rabbits.

The substance is neither irritating nor sensitizing in guinea pigs or in man.

A 90-day oral toxicity test in the rat showed that the no-effect level was 80 mg/kg/day. The target organs at high levels were the liver, kidneys, lungs, testicles, thyroids and blood.

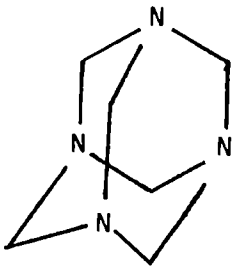
The available information suggests that phenoxyethanol is relatively harmless. It is necessary, however, to obtain information on :
- genotoxicity

- teratogenicity
- the degree of purity; in particular the presence of any ethylene oxide residue resulting from the manufacturing process.

The Committee sees no objection to maintaining the use of this substance as preservative in cosmetic products.

Information : Data sheet National Institute of Public Health, the Netherlands

N° 44 Hexamethylenetetramine (Methenamine)



$C_6H_{12}N_4$ CAS N° 100-97-0
MW : 140.19

Synonym : 1,3,5,7-tetra azatricyclo(3-3-1-1)-decane

Easily soluble in water, moderately soluble in alcohol and chloroform.

Use level in cosmetics up to 0.2%.

The subcutaneous LD_{50} in rats was 200 mg/kg; an intravenous LD_{50} for rats of 9200 mg/kg has been reported.

A skin irritation test in rabbits with a 0.2% aqueous solution produced slight erythema in 2/6 rabbits. The solution was classified slightly irritating. However, daily local application of 2 ml of the same dilution on 5 days per week for 6 weeks did not induce gross or microscopic changes of the skin;

An eye irritation test in rabbits with 0.1 ml of a 0.2% aqueous solution did not induce any abnormalities.

The same low concentration of 0.2% used both for the induction treatment and for the challenge treatment, did not provoke signs of sensitization in a maximization test in guinea pigs.

In a 90-day oral study, rats were treated by gavage with 0.2 g or 0.4 g/rat/day administered as a 40% aqueous solution. There were no abnormalities in behaviour or weight gain. A yellow discolouration of the fur was observed.

Administration of 0.4 g/rat/day by gavage to one group of 15 rats/sex for nearly one year did not cause growth depression, increased mortality or gross abnormalities of organs. The yellow discolouration of the fur was noticed already after a few days of treatment and slowly increased in intensity. It disappeared after a withdrawal period of 2-3 months.

In a long-term study 16 males and 16 females, 2-months old rats werd fed 0.16% in the diet until their natural death. Body weights, and muscular activity showed no significant differences with the controls. Mean life span was slightly decreased. Relative weights of the 4 organs weighed were comparable. Post mortem examinations revealed no treatment-related changes, apart from yellow coloured hair in a few test rats.
In one mating cycle conducted with the test and control rats average litter size of the test rats was lower than in controls, though not significantly lower. Health, body weights and relative weights of organs of descendents showed no significant differences between test rats and controls.

A carcinogenicity study was conducted in rats given 1% in the drinking water for 104 weeks. Then, treatment was discontinued and the rats were observed until week 156. One group was given 5% in the drinking water for only 2 weeks because of high mortality. Treated rats showed yellow colouration of the fur which is attributed to a reaction between formaldehyde, present in contaminating urine, and kynurenine, a normal constituent of rat hair. Treated rats showed no increased incidence of tumours.

Three strains of mice received 1% in the drinking water for 60 weeks. One of these strains received also 0.5% for 60 weeks, or 5% for 30 weeks. The animals were observed for 100 weeks. Slight reduction in growth rate and survival was observed with 5%. There were no indications of carcinogenicity.

In a subcutaneous injection study, infant mice and rats were given 5 injections of 5 g test substance/kg b.w. on alternate days. Then the animals were observed throughout their remaining life-span. No evidence of carcinogenicity was found.

In a reproduction study in dogs the substance was fed in the diet at levels of 600 or 1250 ppm on day 4-56 after mating. Treatment did not affect pregnancy rate, weight gain of the pregnant animals, length of gestation or litter size. In the high-dose group there was a slightly increased

percentage of still-born pups and a slightly impaired weight gain and survival during the lactation period. Behaviour, appearance, motility and muscular co-ordination were normal. This study did not reveal teratogenic properties.

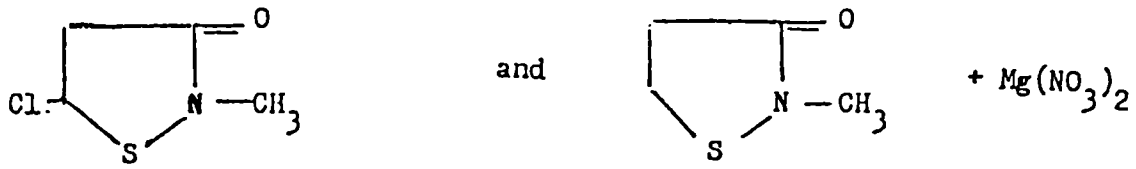
Rats fed 0, 400, 800 or 1600 ppm in the diet for 2 years were mated at the age of 20, 28 and 35 weeks. There were no effects on growth rate, survival, reproduction and viability of offspring.

From a considerable number of studies, both short-term and long-term, it appears that the substance possesses a low degree of toxicity. Even 1% in the drinking water of rats and mice was well tolerated in long-term studies. No teratogenic, or carcinogenic properties have been reported. An ADI of 0.15 mg/kg b.w. was established by JECFA. However, no information on mutagenic properties is available, and interaction with nitrite at pH 1-3 might yield nitrosamines. The compound has been known to have been used as an urinary antiseptic in man with no adverse effects.

The use of this substance as preservative in cosmetic products can be permitted.

Information : - Colipa dossier, Submission I, September 1983
- Data sheet Council of Europe
- JECFA WHO Food Additive Series No. 5 (1974)

N° 45 Mixture of 5-Chloro-2-methyl-isothiazol-3(2H)one and 2-methylisothiazol-3(2H)-one with magnesium chloride and magnesium nitrate



CAS N° 26172-55-4

MW : 148.5 MW : 114.0

Synonyms : - Methylchloroisothiazolinone and methylthiazolinone
- Kathon CG

The ratio of chloromethyl to methyl is 3:1.
Trade names : "Kathon" at various strengths, and forms, and with different stabilisers. "Kathon CG" is cosmetic grade and is complexed with magnesium nitrate. It contains 1.5% total isothiazolones.

Soluble in water, methanol, and ethanol, pH 2-4.

Use level 0.05 to 0.15% (0.00075% to 0.00225% a.i., or 7.5 to 22.5 ppm a.i.) in all types of cosmetics.

Oral LD_{50} values are 50 mg/kg in rats and 52 mg/kg in male mice. The dermal LD_{50} in rabbits was $>$ 75 mg/kg. The inhalation LC_{50} for rats was 650 mg/m^3 for a vapour and 200 - 300 mg/m^3 for an aerosol.

In skin irritation tests in rabbits, aqueous solutions of 0.056%, 0.28% and 0.56% of a.i. (or 560, 2800, or 5600 ppm) were respectively non-irritating, moderately irritating and severely irritating. In guinea pigs 1.5% was irritating. In man (modified Lauman-Maibach test) 0.02% was slightly irritating and 2.8% was severely irritating.

In a maximization test, guinea pigs received induction treatment with 0.0056% a.i. The challenge treatment with the same concentration after 2 weeks did not induce sensitization reactions (IBL-study).

A similar test using 0.0015% for injection and 0.3% on a patch as induction treatment showed positive reactions in 10/20 animals upon the challenge treatment by injection with 0.03%. A second challenge with 0.015% one week later was uniformly negative.

A modified Buehler test in guinea pigs, using either 1.0% of the methyl compound or 0.2% of the mixture on covered patches for induction, showed positive effects in all animals when challenged with 0.2% Kathon, and in 3/20 animals when 0.02% was used. Similar results were obtained with 1.6% of the methyl compound but not with lower concentrations. Only weak cross sensitization was observed between the product and the chlorocompound.

In a repeated, occlusive insult patch test in 18 humans, 0.3 ml 0.0025% was applied for 24 hours, every 48 hours, 9 times. After a 2-week rest, the challenge dose (the same as the induction dose) induced irritation in one subject and sensitization in one subject.
The sensitizing properties of the substance have been confirmed in several tests in humans under various conditions. In a threshold test with 9 sensitized persons (verified with 100 ppm) one was found positive with 25 ppm, five with 50 ppm and three not until 100 ppm.
In an international patch testing programme using high concentrations (100-300 ppm), 30 positive reactions were found in 7307 subjects tested (0.41%).

In a photosensitization test, 0.15% was applied to the guinea pig skin on a patch, 4 times a week for 2 weeks, and half the animals were subjected to UV-irradiation. Ten to 14 days after the test insult exposure the animals were challenged with 0.3% a.i. No evidence of phototoxicity or photosensitization was found (IBL-study).

Sub-acute (2-wk) oral studies with up to 224 ppm a.i. in the diet of rats, and up to 1550 ppm a.i. in the diet of dogs did not reveal any abnormalities. A 4-wk oral rabbit study with 0, 0.4, 1.3, 3.9 and 13.2 mg/kg b.w. by gavage, 5 days/week, revealed gastro-intestinal irritation with haemorrhages at the two high dose levels and mortality (16/20) at the top dose. The no-effect level was 0.4 mg/kg b.w.

In a sub-chronic (90-day) study with groups of 15 rats/sex, fed diets with the test compound at levels providing 0, 3, 10 and 30 mg/kg b.w., no treatment-related changes were observed.

In another 90-day rat study in which 25, 75 or 225 ppm a.i. was administered in the drinking water (c. 2, 6, and 18 mg/kg b.w.), the top dose group showed decreased total protein content of blood serum and enlarged livers and kidneys. The no-effect level was considered to be 75 ppm (or c. 6 mg/kg b.w.).
A 90-day study in dogs with 0, 84, 280 or 840 ppm a.i. in the diet (c. 2.1, 7.0 or 21 mg/kg b.w.) did not reveal any abnormalities.

Inhalation of 30 µg/l air by rats, 6 hrs/day on 5 days/week, induced mortality, respiratory distress, and weight loss. No changes were seen with 10 and 3 µg/l air.
A 13-wk inhalation study in rats with 0, 0.19, 1.62 and 6.33 µg/l did not produce mortality. The no-effect level was considered to be 0.19 µg/l.

In a sub-acute (3-wk) dermal study, groups of 10 rabbits/sex were treated with 0, 5.6 or 28 mg/kg b.w., 5 times/week. Microscopic examination of the skin, liver and kidneys was negative.
A 13-wk dermal study with groups of 12 rabbits treated with 100, 200 and 400 ppm a.i., on 5 days a week showed mortality in the test groups (which was not considered treatment-related), and local irritation in the top-dose group.

Dermal absorption is considerable in rats. Upon a single application of ^{14}C-"Kathon CG" about 50% seemed to be absorbed. Another dermal rat study indicated 40% absorption. At low exposure levels a greater propertion seems to be held by the skin.
When 0.2 ml 2000 ppm of a.i. ^{14}C-labelled, was applied to the skin of rats only once, some radioactivity was found in the testicle even at day 28.
Multiple dermal treatment of rats with 0.2 ml, 500 or 1000 ppm labelled a.i. daily for 4 days resulted in 26% excretion in 24 hours.
I.v. administration of the ^{14}C-labelled compound in a single dose of 0.8 mg/kg b.w. of rats, was followed by fairly uniform distribution in the body, except that the blood levels were much higher than the plasma levels. The half life times (in hours) were : plasma 38 : whole blood 300, liver 98, kidney 90, testicles 120.

Effects on reproduction were studied by mating 9 or 10 male and female rats from each group at the end of one of the oral 90-day studies. No evidence of an adverse effect was noted upon observing the offspring.

Teratogenic properties were examined in groups of 25 female rats dosed orally with 1.4, 4.3 and 14 mg a.i./kg b.w./day, from day 6 - 14 of pregnancy. No evidence of teratogenicity was found.
Groups of 15 female rabbits were dosed orally with 0.23, 0.60 and 2.0 mg a.i./kg b.w./day during pregnancy. There was some embryotoxicity but no evidence of teratogenicity.

Mutagenic properties have been extensively examined.
In the Ames test, mutagenic potency was found with TA 100 in the absence of metabolic activation. Positive results were obtained also in the mouse lymphoma test in vitro. However, negative results were obtained in a gene mutation test in Drosophila, in a chromosomal aberration test in vitro, and in chromosomal aberration tests in vivo in mice and rats treated orally with 15 mg/kg. A micronucleus test in mice treated orally with 3, 9, and 30 mg/kg, and another micronucleus test in mice treated intraperitoneally with compounds 1 and 2 at 250 mg/kg, were all negative. Negative results were also obtained in a mitotic gene conversion test in yeast, an UDS-test in rat hepatocytes and a cell transformation test with $C_3H_{10}T$ 1/2 cells. No binding of the compound with cell DNA could be established in spite of the positive results in certain tests.

In a long-term, dermal carcinogenicity study in mice, groups of 40 males were skin painted, 3 times weekly, with 0.01 mg a.i. (or 0.2 mg/kg b.w.). Two test animals developed a tumour at the site of application. Two control mice treated with water also developed a tumour, but not at the application site.

With 30 mg/kg b.w. administered in the diet of rats for 3 months, no changes were observed, although the oral LD_{50} in the rat was only 50 mg/kg b.w. The no-effect level in different oral studies varied widely and indicated less toxicity when the compound was administered in the diet than when given by gavage or in drinking water. Information should, therefore, be provided on the stability of the compound under test conditions involving incorporation in the diet.

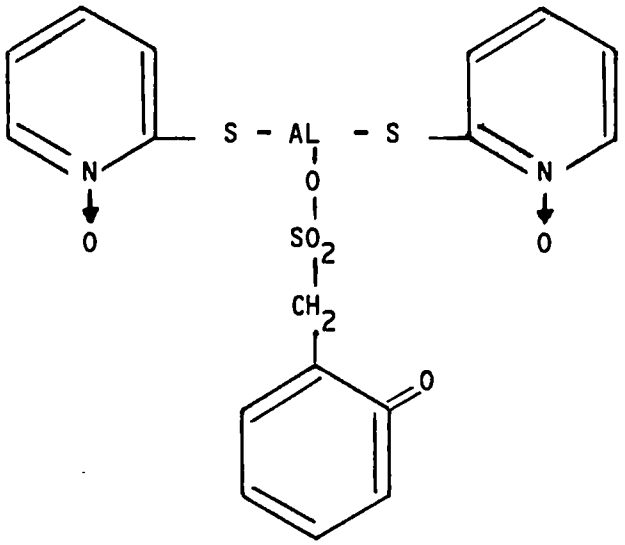
The Committee sees no objection to maintaining the use of this compound as preservative in cosmetic products.

Information : - Colipa dossiers
- Data sheet Bundesgesundheitsamt, Berlin
- Evaluation Dr N. Loprieno, January 1984

N° 46 Pyridin-2-ol-1-oxide

No opinion can be expressed because of a lack of data. But, this substance is no longer used as preservative in cosmetic products.

N° 47 Pyrithione aluminium camsilate (INNM)



$C_{20}H_{23}N_2O_6S_3Al$
MW : 510

Synonym : Bis(N-oxopyridyl-2-thio)aluminium camphosulphonate

Poorly soluble (0.2%) in water and ethanol.

Used at levels up to 0.2%.

The oral LD_{50} in rats was 2.5 g/kg, in mice 1.9 g/kg.

In a primary skin irritation test in rabbits with 0.5 ml of a 0.2% aqueous solution the substance was not irritating. Daily skin application of 2.5 ml of the 0.2% aqueous solutions for 30 days seemed to be well tolerated by rabbits but insufficient details of the results were reported.

No eye irritation occurred in rabbits by applying 0.1 ml of a 0.2% aqueous solution.

Guinea pigs treated in the induction period with aqueous solutions stated to contain 1% of the test substance and challenged with aqueous concentrations of 0.2 and 0.5% did not show any skin reaction;

In sub-chronic (13-week) oral studies, mice and rats received 7.5 and 20 mg/kg/day, 5 days/week, by stomach tube. In mice growth depression and increased activity and agressiveness occurred in both treatment groups. No gross or microscopic changes were reported.

In rats there was also growth depression, increased activity and agressiveness. Blood biochemistry showed increased contents of urea, lipids, triglycerides, and cholesterol, which were, however, considered by the authors to be in the normal range. Some organs showed changes in weight, but no gross or microscopic abnormalities were reported. The reports on these studies show several deficiencies.

A micronucleus test in mice treated twice intraperitoneally with dose levels between 300 and 500 mg/kg failed to reveal mutagenic activity.

Information on dermal absorption or on systemic toxicity upon dermal application is not available.

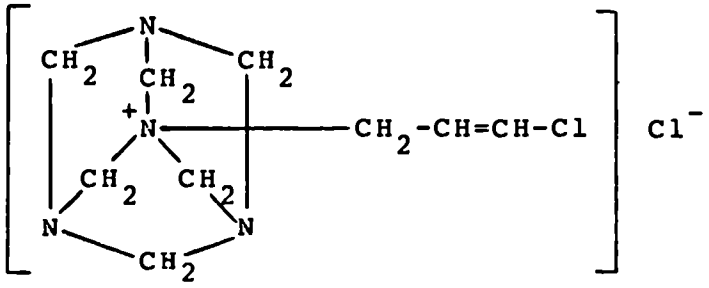
The oral 90-day studies suggest that the no-effect level for mice and rats is lower than 7.5 mg/kg. Because information on dermal absorption is not available, it is possible that the use of up to 0.2% in cosmetics is too high. In order to complete evaluation of this preservative information on dermal absorption is required; if this is substantial teratogenic tests should also be carried out. The mutagenic test already done is insufficient.

No opinion can be expressed because a lack of data. The closely related compound Na-pyrithione shows considerable dermal absorption and is markedly toxic. The Committee believes it to be important that dermal studies (absorption and penetration) be carried out under comparable conditions in all pyrithiones listed in Annex IV. These compounds are known to be teratogenic and mutagenic and have a very high toxicity.

Pyrithione aluminium camsilate is no longer used as preservative in cosmetic products.

Information : Colipa dossier

N° 48 Methanamine 3-chloroallylochloride (INNM)



$C_9H_{16}N_4Cl_2$ CAS N° 4080-31-3
MW : 251

Synonyms : - 1-(3-chloroallyl)-3,5,7-triaza-1-azonia adamantane chloride (cis isomer)
- Dowicil 200

Highly soluble in water, soluble in propylene glycol and ethanol.

Used up to 0.2% in rinsed off and non rinsed off products.

Oral LD_{50} values for rats of 2.6 g/kg and 1.5 g/kg have been reported. The oral LD_{50} for female rabbits was 78.5 mg/kg.
Dermal LD_{50} values for rabbits were 2.8 g/kg, 1.2 g/kg, 0.9 g/kg and 0.6 g/kg.

In a skin irritation test in rabbits with 0.5 g undiluted substance slight to moderate erythema and moderate oedema were seen (score 1.2).

Eye irritation tests in rabbits with 0.1 g undiluted substance induced slight to moderate redness.

In a sensitization test in guinea pigs (modified Maguire method) four applications of 0.1 ml 10% solution in 8 days, and a challenge after 14 days of rest, induced a positive response in 1 out of 10 animals;
The compound was not considered to be a sensitizer.

A photosensitization test was conducted with groups of 25 male and 25 female volunteers by applying 1% in water or 0.1 to 0.75% in formulations, 25 times, and irradiation for 30 seconds. The challenge dose, 18 days after induction, did not elicit any positive response.

A sub-acute (30-day) dermal study in rabbits with 0, 25, 50 and 100 mg/kg showed growth depression with 100 mg, decreased liver weights with 50 and 100 mg, and dose-related dermal changes.
In a sub-chronic (90-day) dermal rabbit study 1.04, 10.5 and 31.3 mg/kg was applied daily, 5 days a week. No dermal or systemic effects were observed.

In a teratogenicity study in rats with 100, 200 or 400 mg/kg b.w./day applied by gavage all dose levels induced maternal and foetal toxicity. In a second oral rat study with 5, 25 and 75 mg/kg, the two highest dose levels induced maternal toxicity, decreases in the weight and in the degree of ossification of the foetuses, and an increased incidence of microphthalmia. With 5 mg/kg there was neither maternal nor foetal toxicity.
A tentative teratogenicity study was conducted in rabbits with oral doses of 3, 10 or 20 mg/kg. The highest dose group showed maternal toxicity but no embryolethality or detrimental effects on reproductive parameters.
A dermal teratogenicity study was conducted in rats with dose levels of 250 and 500 mg/kg b.w. in a 50% aqueous solution. The reproductive parameters were not affected and there were no signs of fetotoxic or teratogenic properties.

The metabolism was examined in rats by single administration of aqueous solutions of the ^{14}C-labelled compound orally or dermally in amounts of 5 or 75 mg/kg, and by intravenous administration of 5 mg/kg. Dermal absorption during 48 hrs was 1.5 to 2.0% of the applied dose at both dose levels. The percentage absorbed dermally was not dependent on the applied dose or concentration. Oral absorption was nearly complete.
Following oral or i.v. administration, the compound was extensively metabolised and rapidly excreted, partly (30%) as CO_2 in expired air. The urine contained 4 - 5 metabolites which have not been identified. The half life was c. 21 hrs for the 5 mg/kg dose and c. 29 hrs for the 75 mg dose. The pattern of urinary metabolites after dermal application was not the same as after oral dosing.

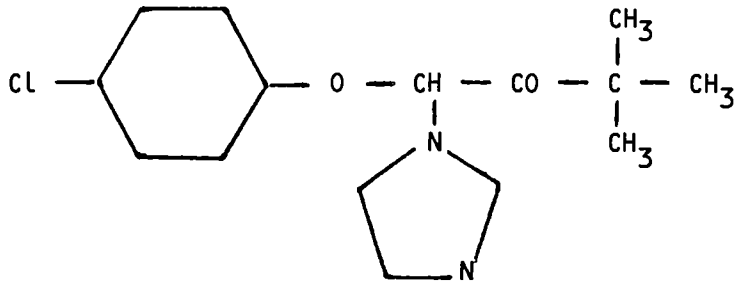
An Ames test with up to 500 µg/plate was negative. A test for unscheduled DNA synthesis in hepatocytes with up to 0.2 mol/l was also negative.

The available data indicate that the substance is teratogenic in rats at oral dose levels of 25 mg/kg and above. Dermal absorption is low in rats but may be considerable in rabbits. Dermal application of 25 mg/kg did not show deleterious effects in this species. A short-term oral study with dose-levels sufficiently high to induce a systemic effect, information on dermal absorption through human skin, and a chromosomal aberration test is needed to evaluate this substance. Information is also requested on the purity of the substance and on the presence of the trans-isomer.

However, the Committee sees no objections to maintaining the use of this substance as preservative in cosmetic products.

Information : - Colipa, Submission I, September 1983
Submission II, September 26, 1984
- Dossier The Dow Chemical Company
- Data Sheet National Institute Public Health, the Netherlands, December 1983

N° 49 1-(4-Chlorophenoxy)-1-(imidazol-1-yl)-3,3-dimethylbutan-2-one



$C_{15}H_{17}ClN_2O_2$
MW : 292.8

Synonym : Climbazole

Poorly soluble in water, soluble in ethanol.

Used at levels up to 0.5%.

Oral LD_{50} values (in mg/kg) are 400 in rats, 664 in mice, 250 in rabbits and 250-500 in dogs.

Eye irritation tests in rabbits with 0.5% in isopropanol/water showed marked to severe reactions, but virtually no reactions occurred with 0.5% in polyethyleneglycol. Eye irritation also occurred when tested at a level of 0.5% in formulations of hair lotions.

No sensitization was observed in guinea pigs by induction treatment with 10% and challenge treatment with 0.3, 1.0 or 3.0%.

In a sub-chronic (13-week) oral study, rats received daily doses of 0, 5, 15 or 45 mg/kg b.w. suspended in 0.5% aqueous tylose. The top-dose induced slight growth depression, decreased alkaline phosphatase and creatinine values, and increased activity of n-demethylase. Increased liver weights, not accompanied by microscopical changes, were seen in the 15, and 45 mg/kg group.

A sub-chronic (13-week) oral study in dogs was conducted with dose-levels of 0, 5, 10 and 20 mg/kg, administered daily by gelatine capsule. The only change was an increased n-demethylase activity in the top-dose group.

In a sub-acute dermal study, rabbits received 2 ml 0.5% in polyethylene glycol 400 or in isopropanol/water, daily for 21 days. In addition to skin changes, the test rabbits showed a slightly increased incidence of liver changes (dilatation, proliferation and fibrosis).

In a teratogenicity study, pregnant rats were treated daily with 0, 10, 30 or 100 mg/kg in 0.5% aqueous tylose by gavage. With 30 and 100 mg/kg growth depression occurred. Clinical signs of toxicity and an increased number of resorptions were observed only with 100 mg/kg. There were no indications of teratogenic properties.

An Ames test with up to 2 mg/plate was negative.
A micronucleus test in mice with the very high dose levels of 150 and 300 mg/kg was also negative.

Upon single oral administration of 50 mg/kg b.w. of rats, maximum blood levels of climbazole and its main metabolite were 5 and 6 µg/ml respectively. The half life in rats was 3 - 4 hours.
In the 90-day oral dog study the maximal blood levels of the compound and the main metabolite in the top-dose group (20 mg/kg) were respectively 0.4 and 1.3 µg/ml.
Upon repeated dermal application of 1 ml 0.5% solution to rabbit skin peak blood levels were 0.013 and 0.010 µg/ml.
Human volunteers, after single or repeated exposure to hair lotion with 0.5% or shampoo with 2% showed maximum plasma levels of 10 ng/ml.

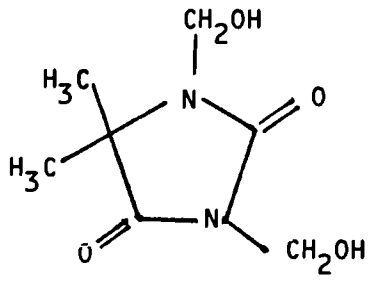
The substance is moderately toxic upon acute and sub-acute oral exposure. The no-effect level in short-term oral studies in rats and dogs is around 10 mg/kg b.w. Dermal absorption has been observed in animals and man. Upon rigourous dermal exposure of humans to formulations with 0.5 or 2% climbazole the maximum blood levels of the compound and its main metabolite (< 35 ng/ml) were considerably lower than the blood levels found in those rats and dogs (1000 - 10.000 ng/ml) showing slight signs of toxicity upon repeated oral exposure.

Because the substance shows structural relationship with Clotrimazole (which is used medicinally against infections with Candida and fungi) the Committee discussed the possibility of resistance or cross-resistance to imidazole compounds. Available evidence suggests that secondary resistance and cross resistance of fungi to imidazole derivatives are rare and inconsistent. The Committee noted that the compound is used in certain cosmetics for purposes other than preservation at concentrations higher than 0.5%.

The use of this substance as preservative in cosmetic products at a maximum level of 0.5% can be permitted.

Information : - Colipa dossier
- Data sheet National Institute of Public Health, the Netherlands

N° 50 1,3-Bis(hydroxymethyl)-5,5-dimethylimidazolidine-2,4-dione



$C_7H_{12}N_2O_4$ CAS N° 6440-58-0
MW : 188

Synonyms : - Dimethylol dimethylhydantoin
- Glydant

Easily soluble in water.
This substance is used in cosmetics up to a concentration of 0.2% expressed as formaldehyde. In the remaining of this report dose levels refer to the 55% aqueous solution of the compound in question and not to formaldehyde.

The oral LD_{50} in rats was between 3 and 5 g/kg b.w. for females and between 2 and 3.65 g/kg b.w. for males. In a one hour inhalation toxicity test in male rats the LC_{50} was $>$ 204 mg/liter. A 4-hour inhalation study in rats of both sexes resulted in an LC_{50} value of $>$ 377 mg/liter. The acute dermal LD_{50} in rabbits was $>$ 20 g/kg.

In a skin irritation test in rabbits application of 0.5 ml of the test substance to the intact or abraded skin was not irritating.

An eye irritation test in rabbits with 0.1 ml of the 55% aqueous solution was positive, but a 1% dilution of this material was not irritating.

In a sensitization test in 50 humans, following repeated dermal applications of 0.5 ml of a 0.1% aqueous dilution of the test material for 24 hours (nine times in an induction period of 3 weeks), the challenge treatment, after a 12 day rest period, did not induce irritation or sensitization. In a

similar test in 202 subjects treated 10 times with 0.2 ml of a 1.0% aqueous dilution, the challenge treatment induced signs of sensitization in 3 subjects.

A photosensitization test was conducted in 25 persons by dermal application of 0.3 ml of a 0.4% aqueous dilution in occluded patches on 10 days in 2 successive weeks each time followed by UV irradiation at twice the minimal erythema dose. The challenge treatment, conducted after a 14-day rest period, did not provide indications of phototoxicity or photosensitization.

In a sub-acute (28-day) dermal toxicity study (conducted by IBT - Chicago) rabbits received 20 daily applications of 0, 0.4 or 40% aqueous solutions; or 0, 8 or 800 mg/kg b.w. No changes attributed to treatment were reported with respect to body weight, haematology, clinical chemistry of blood or urine, or in the weight and pathology of the internal organs. Animals of the high dose group showed signs of mild to moderate irritation of the treated skin. Practically no irritation occurred in the low-dose group.

In a short-term (21-week) oral rat study, dose levels of 0, 100, 200 and 400 mg/kg were applied daily by gavage for 8 weeks, and at levels of 0, 100, 300 and 600 mg/kg for the remaining 13 weeks of the study. No significant, dose-related changes were found. An increase in adrenal weight of the high-dose males was not attributed to treatment. The target organ was not established.

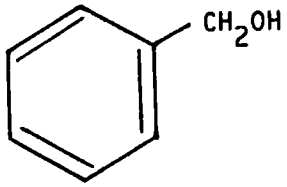
Mutagenicity tests with S.typhimurium and Saccharomyces cerevisiae, with and without metabolic activation were negative.

The Committee knows that the substance resembles phenytoin and methoin hydantoin, which are known drugs with a narrow-therapeutic range. Due to the low toxicity of the compound, no further information is requested.

The use of this substance as preservative in cosmetic products can be permitted at a maximum level of 0.2% expressed as formaldehyde.

Information : - Colipa dossier
- Data sheet National Institute of Public Health, the Netherlands

N° 51 Benzyl alcohol



C_7H_8O CAS N° 100-51-6
MW : 108.13

Synonym : Phenyl carbinol.

Soluble in water, easily soluble in ethanol and ether.

Used in cosmetics at levels up to 1.0%.

Oral LD_{50} values for rats were 3.1, 2.08 and 1.23 g/kg. For mice and rabbits the values were 1.58 and 1.94 g/kg respectively. The i.p. LD_{50} in rats and guinea pigs was between 0.4 and 0.8 g/kg; the i.v. LD_{50} in rats was 0.3 g/kg. In guinea pigs the dermal LD_{50} was $<$ 5 ml/kg.
Symptoms of intoxication included paralysis, convulsions and narcosis.
The substance possesses local anaesthetic properties.

Skin irritation was moderately strong in guinea pigs treated with the undiluted substance.

A sensitization test in 25 volunteers with 10% in petrolatum was negative.
Cross-sensitization with Peru balsam has been reported.

Exposure to the vapour phase results in dermal absorption and 100 ppm in air may induce systemic effects. No fatalities or symptoms were produced in rats exposed to 61 ppm for 6 hours.

The body readily oxidizes benzyl alcohol to benzoic acid which, after conjugating with glycine, is eliminated as hippuric acid in the urine.

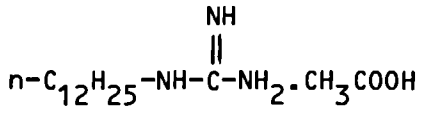
A negative result of an Ames test has been reported.

No information is available on concentrations which are non irritant to the skin. Information on systemic toxicity may become available from the oral studies in rats and mice on sub-chronic and chronic toxicity and on teratogenicity which are being conducted in the framework of the US National Toxicology Program.

The Committee sees no objection to maintaining the use of this substance as preservative in cosmetic products.

Information : - Colipa dossier
- NTP FY 82
- Opdyke Monographs, Fd. Cosmet. Toxicol. 11 (1973) 1011-1013

N° 52 1-Dodecylguanidinium acetate (Dodine - ISO)



$C_{15}H_{33}N_3O_2$ CAS N° 2439-10-3
MW : 287

Moderately soluble in alcohols (7-23%), poorly soluble in water (0.063%), insoluble in most other solvents.

Used in cosmetics at levels of 0.5% in rinsed off products and 0.1% in other products.

The oral LD_{50} values for rats and mice vary from 0.27 to 1.72 g/kg; for rabbits and guinea pigs values of 0.535 and 0.176 g/kg were reported; for quail and pheasants 0.848 and 0.950 g/kg respectively. Acute effects in rats are hypoglycaemia, increased ATP-ase in blood and decreased ATP-ase in the liver. Dermal LD_{50} values for rabbits are 2.1 and 1.5 g/kg (1, 2), for rats $>$ 6.0 g/kg (5)

Considerable erythema and oedema developed in rabbit skin treated with an aqueous paste of dodine (2).

Very severe eye irritation occurred in rabbits treated with 10 mg undiluted substance, but 0.1 ml of a 0.12% aqueous dispersion produced only weak changes (2).

In a sub-chronic (14-week) rat study the feeding of 3200 ppm in the diet induced marked reduction in growth rate and food intake, and increased irritability and spontaneous activity. However, no changes in haematology or in gross and microscopic pathology were found. Feeding levels lower than 3200 ppm were not examined in this study (2);

In a two-year rat feeding study with dietary levels of 50, 200 and 800 ppm, growth rate and food intake were reduced in the top-dose group. Haematology, organ weights, or gross and microscopic pathology did not reveal compound-related changes. Clinical chemistry of blood was not reported (2).

A one-year feeding study in dogs with dietary levels of 0, 50, 200 or 800 ppm did not reveal changes in growth, behaviour, haematology or gross pathology. The weight of the thyroid was slightly increased at all dose levels. Microscopically, an activated appearance of this organ was seen in 1/4 dogs at 200 ppm and in 4/4 dogs at 800 ppm, but in none of the dogs at 50 ppm (2).

No significant increase in incidence of tumours was seen in mice treated orally with 21 mg/kg b.w./day from day 7 to day 28 after birth and then with 82 ppm in the diet for 18 months (3).

No detrimental effects on reproduction were found in a three-generation study in mice with dietary levels of 400 and 800 ppm. The lactation index was reduced in the top-dose group. Gross and microscopic examination was negative (1).
In a two-generation study in rats, 800 ppm did not affect reproduction but litter size was slightly reduced (2).

Mutagenicity tests in Salmonella typhimurium, Escherichia coli, Streptomyces coelicolor, and Saccharomyces cerevisiae were all negative.
The N-nitroso derivative of dodine showed mutagenic activity with E. coli K 12, but not with S. typhimurium his G 46.

Metabolism of dodine differs amongst different species. While elimination of the compound in rats is rapid, it accumulates in guinea pigs and mice (4).

Occupational exposure of workers involved in the production of dodine produced minor effects (acute dermatitis, eye irritation) which were readily reversible (1).

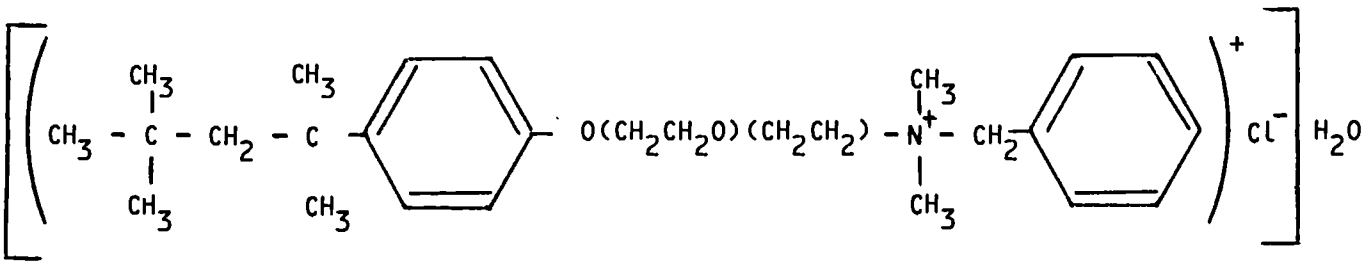
Because dodine is a severe irritant to the mucous membrane of the eye, it should not be used in eye cosmetics, and its use in intimate sprays should be called in question.

The no effect level in dogs was relatively low (1.25 mg/kg b.w.) and the Joint WHO/FAO Expert Committee on Pesticides allocated a temporary ADI of 0.01 mg/kg.
More information on dermal absorption, preferably in man, is required.

However, the Committee sees no objection to maintaining the use of this substance as preservative in cosmetic products.

Information : (1) WHO Pesticide Residues Series, N° 4 (1975) 265-284
(2) Levinskas et al. Toxicol. appl. Pharmacol. 3 (1961) 127-142
(3) Innes et al. J. Natn. Cancer Inst. 42 (1969) 1101-1114
(4) Vettorazzi G. Intern. Regulatory Aspects for Pesticide Chemicals. Vol. I. Toxicity profiles, CRS Press Inc. 1979
(5) Data sheet University of Pisa - Genetics Laboratory

N° 53 Benzethonium chloride (INN)



$C_{27}H_{42}NO_2.Cl$ CAS N° 121-54-0
MW : 448

Synonyms : - 4'-(1,1,3,3-tetramethylbutyl)phenoxy-ethoxyethylene-dimethylbenzyl-ammonium chloride
- Phemerol chloride
- Hyamine 1622

Soluble in water, alcohols and other organic solvents.

Used in cosmetics at levels of 0.1%.

LD_{50} values for the rat are : oral 420 mg/kg, i.p. 33 mg/kg, and i.v. 19 mg/kg. Intranasal administration of 0.06 ml of a solution of 0.25% or more was lethal to rats.

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.

A sensitization test in humans with 0.12% in formulations applied to the skin under closed patches was negative.

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

In a one year feeding study in groups of 3 dogs 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.

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. It is not clear whether this result is an indication of carcinogenic properties of the test substance.

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.

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.
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.
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.

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.
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.
Peri- and postnatal effects were examined in rats doses 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.

Dermal absorption was examined by applying 1.0 ml of a 10% aqueous solution of the 14C-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.
Maternal and foetal absorption of the 14C-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.

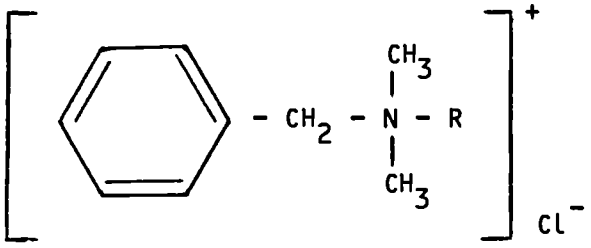
An Ames test with up to 100 nmoles/plate was negative. Another Ames test with up to 7500 µg Hyamine 1622/plate was likewise negative.

The available data indicated that the substance has significant toxic properties including pharmacological activity, embryotoxicity, teratogenicity, interference with reproductive performance and inhibition of ileum motility at lower than use levels. A clear no-effect level had not been demonstrated. The Committee notes that insufficient information had been provided on dermal absorption, and on the mutagenic properties of the

substance. Because, moreover, further studies are in progress and other studies are planned, the Committee decides to await the results before recommending any classification.

Information :
- Data sheet National Institute of Public Healt, the Netherlands
- Colipa dossier, Submission I, April 1984
- Colipa dossier, Submission II, September 1985
- Cosmetic Ingredient Review (C.I.R.). Final report on the safety assessment of benzethonium chloride and methyl-benzethonium chloride. J. Amer. College Toxicol. 4 (nr 5) 65-106 (1985)

N° 54 Benzalkonium chloride (INN)



$C_{21}H_{38}N.Cl$ CAS N° 139-07-1
MW : 339,99

Synonym : Alkyl(C_8-C_{18})-dimethylbenzylammoniumchloride, -bromide and -saccharinate

Soluble in water and alcohols, poorly soluble in hydrocarbons, oils and fats.

Used in cosmetics at levels of 0.5%.

Oral LD_{50} values for rats and mice obtained for commercial products with different alkyl groups usually vary between 0.5 and 1.0 g/kg b.w. Intravenous LD_{50} values in mice of 12.8 - 26 mg/kg have been reported. Intranasal administration of 0.06 ml of a 0.125% solution was lethal for rats.

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 of 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% benzalkoniumchloride + 0.01% EDTA induced severe irritation when brought into contact with 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.

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

In a 2 year rat study, 0.5% in the diet (250 mg/kg b.w.) caused mortality and pathological changes in the gastrointestinal tract. Pathological changes 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 b.w.

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. 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.

In an oral teratogenicity study, groups of 15 pregnant rabbits were treated by gavage with 0, 10, 30 or 100 mg/kg/day 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.

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.

Skin penetration tests in vitro with pieces of human skin were conducted in aqueous solutions of 0.005M to 0.1M benzalkoniumchloride. 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.
No penetration was found in vitro with skin from hairless rats exposed to 2.5% ^{14}C-dimethylbenzylammoniumchloride for 4.5 hours.
In a similar in vitro test with human epidermis the mean penetration was 1.47% of the dose applied.

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.

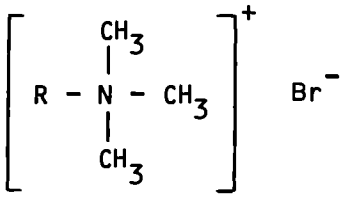
A mutagenicity test with S. typhimurium His G 46-uvr B exposed to 10-100 µg/plate was negative.
A micronucleus test in mice treated i.p. with 20 mg/kg b.w., twice, with an interval of 24 hours did not reveal increased numbers of micronuclei.
The substance was found to induce repairable DNA damage in the E. coli DNA polymerase A assay, but no mutagenic properties were observed. No forward muations were induced in Schizosaccharomyces pombe P_1 with or without metabolic activation. A chromosome aberration test with CHO-cells in vitro was negative.

Benzalkoniumchloride possesses considerable irritative properties for the eye and the gastrointestinal tract, and was highly toxic under certain conditions of acute exposure. The oral no-toxic effect level in rats was less than 12.5 mg/kg/day, while mortality occurred in dogs and rats with 25 mg/kg. The use of this preservative in all kinds of cosmetics could lead to a human exposure of c. 1 mg/kg b.w./day. Even if the use level is reduced from 0.5 to 0.25% the Committee is unwilling to make any evaluaton until a

no-effect level for systemic toxicity has been established, and more information is available on dermal absorption. In addition information should be made available on other uses than as a preservative.

Information : - Data sheet National Institute of Public Health, the Netherlands
- Colipa dossier. - Partial submission (I), September 1983
- Submission II, September 1985
- Submission III, October 1985

N° 55 Alkyl(C12-C22) trimethylammonium bromide and chloride (including Cetrimonium bromide) (INN)



R = alkyl

$C_{19}H_{42}NBr$ CAS N° 57-09-0
MW : 364.48

Soluble in water and alcohols, insoluble in ether.

Used in cosmetics up to 0.1%.

LD_{50} values reported (in mg/kg b.w.) are : oral for rats 1000; intraperitoneal for rats 56, for mice 39.8, for rabbits 125; subcutaneous for mice 75-80.
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.

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 to moderate erythema and oedema, while with 50% these 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.

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 b.w. in the 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.

A teratogenicity study in mice, treated once intraperitoneally with 10.5 or 35.0 mg/kg b.w. 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.

The percutaneous absorption of the ^{14}C-labeled 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.).

Upon oral administration of the ^{14}C-labeled 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 poor 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.

It was observed that this substance may be teratogenic under ordinary conditions of use.

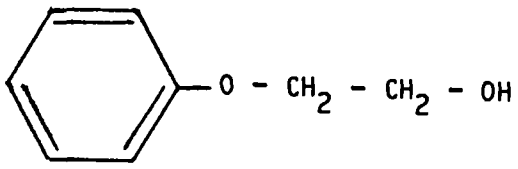
The Committee recommended that information be requested as follows :

1. A no effect level for teratogenicity on oral and dermal administration.
2. Genotoxicity information by a gene mutation test and a chromosomal aberration test on mammalian cells in vitro.

No opinion can be expressed.

Information : - Data sheet National Institute of Public Health, the Netherlands, September 1978
- Colipa dossier, Submission I, April 1984

N° 56 1-Phenoxypropan-2-ol



$C_9H_{12}O_2$ CAS N° 770-35-4
MW : 153.11

Synonyms : - Phenoxypropanol
- Propylenephenoxyethol

Soluble in water (1%), miscible with alcohol, ether and chloroform.

Used up to 1.0% in cosmetics.

Oral LD_{50} values in rats are 2720 and 2544 mg/kg.

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.

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.

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.

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 effects were noted with 100 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 changes occurred with 50 mg/kg.

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).

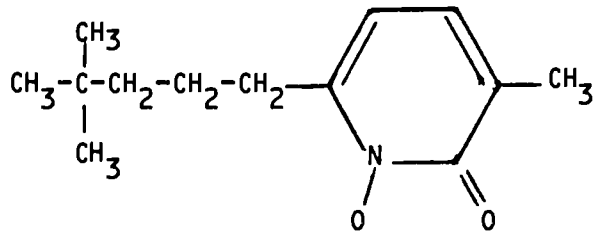
In teratogenicity studies, rats and rabbits were treated orally during pregnancy with dose levels of 0, 80, 160 or 320 mg/kg/day for rats and 0, 25, 50 or 100 mg/kg/day for rabbits. In neither of these two studies were there adverse effects on foetal development.

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 µg/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.

In view of the relatively low no-effect level, the relatively high use level and the considerable dermal absorption, the Committee cannot recommend the use of this substance at the proposed concentrations, even if its use is restricted to rinsed off products. The Committee is willing to reconsider its opinion, if further data become available.

Information : - Colipa submission I, February 14, 1980
- Colipa submission II, September 1985

N° 57 1-Hydroxy-4-methyl-6(2,4,4-trimethylpentyl) 2-pyridon and its monoethanolamine salt



$C_{16}H_{30}N_2O_3$
MW : 298.4

CAS N°

Synonyms : - Octopyrox
- Pyroctone olamine

Poorly soluble in water, well soluble in ethanol.

Used up to 0.5% in cosmetics, but up to 1.0% in rinsed off products.

Oral LD_{50} values are 8100 mg/kg in rats, $>$ 1000 mg/kg in mice, and $>$ 4000 mg/kg in dogs.
Dermal LD_{50} values in rats are $>$ 2000 mg/kg, and $>$ 750 mg/kg.

In skin irritation tests with two strains of rabbits 1% in aqueous PEG (75%) was found to be slightly irritating. A 1% solution in 1,2-propylene glycol was likewise slightly irritating in two strains of rabbits. A formulation with the test substance was not more irritating than the same formulation without the test substance.

Eye irritation tests with a formulation with and without octopyrox showed a similar degree of irritation. With 50% aqueous isopropanol the degree of eye irritation was the same as with 0.2% octopyrox in 50% aqueous isopropanol. A shampoo with 0.5% octopyrox was not more irritating than the same shampoo without octopyrox.

In a sensitization test in guinea pigs by the Buehler method, 9 epicutaneous applications of a 40% aqueous concentration in 3 weeks, followed after 14 days by a challenge of the same concentration with a closed patch, did not induce signs of sensitization.
A maximization test in guinea pigs [using as induction treatment 0.05% in propyleneglycol (PG) for injection, and 5% in PG on a patch, and as challenge treatment (after 2 weeks rest) 0.4 ml, 0.05 and 0.1% in PG on a patch during 48 hours], did not result in signs of sensitization.

In a photosensitization test, guinea pigs were treated topically 10 times in 2 weeks with 0.05 ml 5.0% in PG followed by irradiation for 2 hours. After 2 weeks rest, 0.2 ml of 0.03% up to 1.0% were applied topically. No dermal changes occurred either at the irradiated sites or at the non-irradiated sites.

In a repeated insult patch test with 50 humans, 0.5% in a vehicle containing 20% disodium laurylalcohol polyglycol ether sulfosuccinate did not induce skin irritation, skin fatigue or sensitization.
In a use test in more than 300 volunteers, 0.2 to 1.0% in shampoos applied seven times in 4 weeks provided no evidence of adverse reactions.

A phototoxicity test in humans treated topically with a 0.1% aqueous solution in isopropanol was negative both with UV-A and UV-B.

Short-term (30-day) oral treatment of rats with 0, 4, 15, 55, 210 or 800 mg/kg b.w./day in the diet induced growth retardation with 210 and 800 mg, mortality (2/20 rats), and signs of anaemia with 800 mg. No changes were detected at lower levels.
A 90-day oral rat study with 0, 40, 100 and 250 mg/kg b.w. by stomach intubation on 5 days/week showed growth depression and decreased haemoglobin levels in the top-dose group. Clinical chemistry, urine composition and microscopy of c. 20 organs examined did not reveal treatment-related changes.

Short-term (30-day) diet administration to dogs of 0, 16, 40 or 100 mg/kg b.w./day did not induce any adverse effects.
The feeding of the same dietary levels to dogs for 90 days likewise failed to induce any abnormalities.

Dermal treatment of rabbits with 0.5 mg/day in shampoo, or with 0.1 mg/day in hair lotion 30 times in 36 days or 30 times in 64 days respectively did not induce any dermal or systemic effect.
A 5-week subcutaneous injection study was conducted in rats with 0, 100, 500 or 2000 mg/kg b.w./day in 0.5% aqueous carboxymethylcellulose. The two higher dose levels caused haematological and clinical chemical changes and inflammation at the injection site. The no-toxic effect level was 100 mg/kg (Summary only).
In a 6 months dermal study, rats were treated topically with 0, 1 or 2 mg/rat/day administered in 0.2 ml, 0.05 and 1.0% in PG 6 times weekly [for 4 weeks ?]. The only change was thickening of the epidermis which was attributed to the vehicle.
A similar study was conducted [with 4 weeks treatment ?] for one year. The treated skin sites showed thickening and necrosis.
(The English version of the reports on the latter two Japanese dermal studies do not contain a description of the study design).

An Ames test with up to 500 µg/plate was negative. Another Ames test with up to 200 µg/plate (which also included a test with E.coli) was likewise negative. A micronucleus test in mice treated orally with 125, 250 or 500 mg/kg, and a second micronucleus test in mice with up to 125 mg/kg (given intraperitoneally once, or once daily for 4 days) were negative.
No increase in chromosomal aberrations was seen in bone narrow-cells of Chinese hamsters upon single oral treatment with 3.5 g/kg b.w.
Subcutaneous administration of the 6-^{14}C-labelled compound to rats at a level of 35 mg/kg b.w. did not reveal any measurable DNA binding.

No indications of teratogenic properties were obtained in a study in rabbits treated with 0, 16, 32 or 63 mg/kg b.w./day by stomach intubation from day 7 - 19 of gestation.
In a rat teratogenicity study, with subcutaneous injection of 100, 500 or 2000 mg/kg b.w./day on days 7 through 17 of gestation, growth depression occurred in the top-dose group. No treatment-related increase in foetal abnormalities (external, visceral or skeletal) was observed.
In another rat teratogenicity study 2000 mg/kg was administered subcutaneously on days 6 through 20, 7 through 17, or 9 through 17. The type and incidence of foetal abnormalities were not affected by treatment.
No evidence of teratogenic potential was observed in a hen's egg test by single injection of 0.93 mg/egg.

The kinetics were studies in dogs and rats. Dogs received a single oral dose of 50 or 100 mg/kg b.w. Maximum blood levels were respectively 22.9 and 33.0 µg/ml. The elimination occurred with a half-life of 2.7 hours. Upon single oral treatment of rats with the compound as the monoethanolamine salt (100 mg/kg), the sodium salt (87.2 mg/kg) or the free acid (79.9 mg/kg) no noticeable differences in serum levels were observed. Maximum levels were 12.3 µg/ml, which were reached after 1.2 hours. The elimination occurred in 2 phases with half lives of 0.5 hour and 4.5 hours.

Dermal absorption was examined in rats treated topically with 2 mg of the ^{14}C-labelled compound on 4 cm^2, and rubbed in for one minute. Then the treated site was cleaned with wet cotton wool pads, which removed 43.1% of the dose applied. During the next 7 days 1.4% of the dose was excreted in the urine and 3.1% in the faeces.
After oral administration of 0.24 mg/kg, maximum blood levels were between 0.006 and 0.014 µg/ml between 3 and 8 hours after treatment.

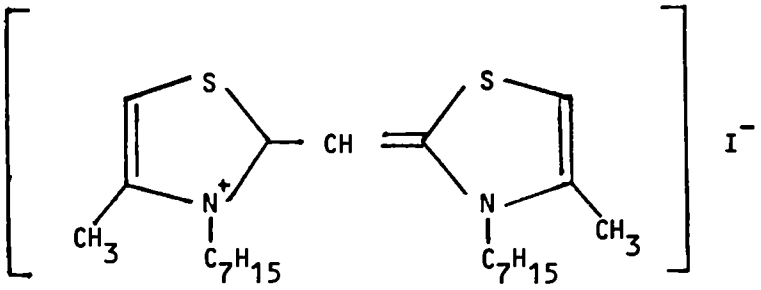
Skin penetration in rats of 1% in a shampoo, applied without rinsing, was 65.1 µg/cm^2 under occlusion, and 38.2 µg/cm^2 without occlusion. When rinsed after 109 minutes of contact, penetration was reduced to 3.4 µg/cm^2 under occlusion and 2.0 µg/cm^2 without occlusion.
Skin penetration increased with increasing concentration and also with increasing duration of contact up to 10 minutes.
Topical application under occlusion without rinsing resulted in tissue levels similar to those after oral intubation.

This compound has been well examined, and showed only moderate toxicity. From adequate short-term (90-day) studies in rats and dogs, 100 mg/kg b.w. was found to be a no-toxic effect level. No indications of mutagenic or teratogenic properties were obtained in appropriate tests. Dermal absorption is not considerable ($<$ 10%). A dermal dose is rapidly eliminated, partly with the urine and partly with the faeces.

The use of this substance as preservative in cosmetic products can be permitted.

Information : - Colipa dossier, Submission I, December 1979
Submission II, January 1985
- Data Sheet National Institute of Public Health, the Netherlands, October 1980

N° 58 3-Heptyl-2-(3-heptyl-4-methyl-4-thiozolin-2-ylidenemethyl)-4-methylthiazolinium iodide



$C_{23}H_{39}N_2S_2I$ CAS N°
MW : 534.61

Synonym : Kankohso 201

Poorly soluble in water and ethanol. Replacing the iodine ion of Kankohso 201 by an orotate ion (Kankohso 201-OA) improves the solubility in water with a factor 10, and in ethanol with a factor 28.

Used at levels up to 0.002%.

In mice, the oral LD_{50} of the iodide was 1.1 g/kg in males and 1.7 g/kg in females; the intradermal LD_{50} of the iodide was > 40 mg/kg. An intraperitoneal LD_{50} of 110 mg/kg for mice was obtained with the orotate.

A skin irritation test in rabbits with 0.3 ml of a 0.3% solution of the iodide in polyethylene glycol applied to intact and abraded skin resulted in slight oedema in 1 out of 4 animals treated on the abraded skin, and in none out of 4 treated on the intact skin.
Closed patch tests in 10 humans with 20 - 200 µg of the iodide in 0.1 g of a hydrophylic ointment, or with 0.05 ml of 0.03% in polyethylene glycol, were negative.

An eye irritation test in rabbits with 0.00075% of the iodide in saline was negative. With 0.1 ml of a 0.02% or a 0.05% dilution of the orotate (diluent not mentioned) in the rabbit eye, no irritation was produced with

0.02% while with 0.05% minimal changes were observed. The undiluted iodide derivative produced considerable changes, but with 0.3% in polyethylene glycol only slight signs of irritation were observed.

In a maximization test in guinea pigs treated topically and by intradermal injection with a 1% solution in the induction period, the challenge treatment with 0.3% produced a slight response in 1 out of 10 animals, whereas none of the animals showed a response with lower concentrations. (The document concerned, does not mention the compound or the salt with which this test was conducted).

Several phototoxicity tests were conducted with the iodide. In a rabbit test 0.02 ml of 0.3% in polyethylene glycol or 0.3% in ethanol was negative. Negative results were obtained also in a phototoxicity test in 10 humans treated with 0.1 g ointment containing 20 or 200 µg. A photopatch test in 18 humans treated with 20 or 100 µg in water-soluble ointment was also negative.

In a 90-day dermal study in rabbits daily application of 0.5 ml, of a 0.05% solution of the orotate in a glycerine/alcohol/water mixture to the ear skin did not induce any changes other than a yellow discolouration and keratolyses of the application site (summary report only). In another dermal rabbit study (only 2 rabbits per group) 0.5 ml of a 0.01% or a 0.1% solution of the iodide in glycerin-ethanol-water mix applied daily to the ear, 6 days a week, for 13 or 26 weeks, no changes in growth rate, haematology, biochemistry, or histopathology were observed. Only very slight changes occurred at the application site (e.g. thickening and desquamation of horny layer). Only a summary report is available.

In a 15 wk oral study (with only 5 rats/group), administration of the iodide by gavage of 4 or 40 mg/kg b.w./day, 6 days a week, induced growth depression and decreased weight and pathological changes of the liver and spleen. The liver was the organ most markedly affected. (The report shows many deficiencies in the conduct of the study).

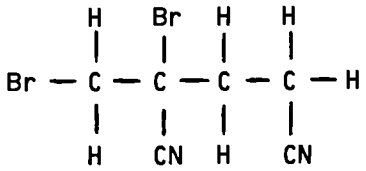
An Ames test with the iodide at a maximum level of only 10 µg/plate was negative.

It was noted that Kankohso 201 is the iodide.
A short-term oral study in rats is requested with the iodide to establish the highest dose that does not induce growth depression or liver damage. Depending on the results, information on dermal absorption, teratogenicity and further mutagenicity tests may be desirable.

No opinion can be expressed.

Information : Colipa dossier, Submission I, March 1984

N° 59 1,2-Dibromo-2,4-dicyanobutane



MW : 265.9

Synonym : Tektamer 38

Soluble in water 0.27% at 0 °C; easily soluble in organic solvents.

Used in cosmetics up to 0.1%.

Oral LD_{50} values in rats were 720 mg/kg, and 541 mg/kg. The dermal LD_{50} in rabbits was > 5000 mg/kg. The LC_{50} in rats (exposed to the powder for one hour) was > 200 mg/liter air.

A skin irritation test in rabbits with 0.5 g of undiluted powder produced erythema and oedema. The substance was classified as a moderate irritant. A 0.3% aqueous solution was not irritaing to the rabbit skin. A 0.3% dilution in oil was neither irritating nor sensitizing when applied to the skin of humans.

The undiluted substance was found to be a severe irritant to the eye of rabbits, but when applied as a 0.1% aqueous solution no irritation was noticed.

In a 90-day study in rats with 0, 83.5, 500 or 3000 ppm in the diet (given to the offspring of rats which had already been fed these diets through the periods of mating, pregnancy and lactation) an increased weight of the thyroid was observed in the top-dose group and growth rate was decreased in males fed 500 or 3000 ppm. Increased haematopoiesis was observed

microscopically in the spleen of the top-dose females. No diseased or malformed pups were seen in the litters of the parent rats, which suggests absence of teratogenic properties.

In a 90-day study in dogs with feeding levels of 0, 167, 1000 or 4000 ppm, the top-dose group showed growth retardation, diarrhoea, changes in haematology and blood biochemistry, enlarged thyroids (hyperplasia) and increased haematopoiesis in the liver and spleen.
The lowest feeding level (4.2 mg/kg b.w./day) was a no-effect level in the rat and possibly also in the dog.
A second 90-day dog study with feeding levels of 0 and 167 ppm, and designed to examine thyroid function, showed increased thyroid weights in treated females. These changes were not accompanied, however, by changes in T_3 or T_4 values or in the microscopy of the thyroid.

An oral dose (50 mg/kg) given to rats, was mainly excreted in the urine (84 - 91%). A small part (6 - 10%) was excreted in the faeces, and a very small part (0.5%) in the respiration air. Seven days after a single intragastric dose 0.4% remained in the organs, 0.3% of the dose was in the liver.

The Ames test (with and without activation) and a dominant lethal assay in male mice were negative.

Although there is adequate information for accepting the use of the substance in cosmetics in general, information on dermal absorption and pharmacokinetics is needed to justify its use in sunscreening agents.

The use of this substance as preservative in cosmetic products can be permitted, except for sunscreening agents.

Information : Colipa dossier, March 4, 1981
Submission II, Sept. 1984

N° 60 4,4-Dimethyl-1,3-oxazolidine

See report EUR 8634.

PART 2

LIST OF PRESERVATIVES PROVISIONALLY ALLOWED

Reference number	Substance	Maximum authorized concentration	Limitations and requirements	Conditions of use and warnings which must be printed on the label
a	b	c	d	e
1	2,6-Dimethyl-1,3-dioxan-4-yl acetate (Dimethoxane)	0·2 %		
2	Boric acid (*)	(a) 0·5 % (b) 3 %	(a) Products for oral hygiene (b) Other products	
3	Chlorphenesin (INN) (*)	0·5 %		
4	3-Acetyl-6-methylpyran-2,4(3*H*)-dione (Dehydroacetic acid) and its salts	0·6 % (acid)		
5	Formic acid (*)	0·5 % (acid)		
6	4-Hydroxybenzoic acid benzyl ester	0·1 % (acid)		
7	Hexamidine (INN) and its salts (including isethionate (INN) and 4-hydroxybenzoate) (*)	0·1 %		
8	3,3′ Dibromo-4,4′-hexamethylene-dioxydibenzamidine (Dibromohexamidine) and its salts (including isethionate)	0·1 %		
9	Dibromopropamidine (INN) and its salts (including isethionate (INN)	0·1 %		

Reference number	Substance	Maximum authorized concentration	Limitations and requirements	Conditions of use and warnings which must be printed on the label
a	b	c	d	e
10	Thiomersal (INN)	0·007 % (of Hg) If mixed with other mercurial compounds authorized by this Directive, the maximum concentration of Hg remains fixed at 0·007 %	For eye make-up and eye make-up remover only	Contains thiomersal
11	Phenylmercuric salts (including borate)	Ditto	Ditto	Contains phenylmercuric compounds
12	Esters of sorbic acid (Hexa-2,4-dienoic acid) (*)	0·6 % (acid) If mixed with sorbic acid and/or its salts, the maximum total concentration of the acids, salts and esters remains fixed at 0·6 % (acid)		
13	Undec-10-enoic acids: salts, esters, the amide, the mono- and bis(2-hydroxethyl) amides and their sulphosuccinates (*)	0·2 % (acid)		
14	2,6-Diacetyl-1,2,3,9b-tetrahydro-7,9-dihydroxy-8,9b-dimethyldibenzolfuran-1,3-dione (usnic acid) and its salts (including the copper salt (*)	0·2 %		
15	Hexatidine (INN) (*)	0·2 %		
16	Benzylformal (a 1:1 mixture of benzyloxymethanol and (benzyloxymethoxy) methanol)	0·2 %		
17	Clorofene (INN)	0·2 %		
18	5-Bromo-5-nitro-1,3 dioxane (*)	0·1 %	Only for products rinsed off after use	

Reference number	Substance	Maximum authorized concentration	Limitations and requirements	Conditions of use and warnings which must be printed on the label
a	b	c	d	e
19	Bronopol (INN) (*)	0·1 %		
20	6,6-Dibromo-4,4-dichloro-2,2′-methylene-diphenol (Bromochlorophen) (*)	0·1 %		
21	Tetrabromo-ó-cresol (*)	0·3 %		
22	2-Chloroacetamide	0·3 %		Contains chloroacetamide
23	3,4-Dichlorobenzyl alcohol (*)	0·15 %		
24	2,4-Dichlorobenzyl alcohol (*)	0·15 %		
25	Triclocarban (INN) (*)	0·2 %		
26	4-Chloro-*a*-cresol (*)	0·2 %		
27	Halocarban (INN) (*)	0·3 %	Maximum concentration in aerosols: 0·2 %	
28	Triclosan (INN) (*)	0·3 %		
29	Dichlorophen (INN)	0·2 %		Contains dichlorophen
30	N-(Trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide (Captan — (ISO)) (*)	0·5 %		

Reference number	Substance	Maximum authorized concentration	Limitations and requirements	Conditions of use and warnings which must be printed on the label
a	b	c	d	e
31	Chlorhexidine (INN) and its digluconate, diacetate and dihydrochloride (*)	0·3 %		
32	4-Chloro-3,5-xylenol (*)	0·5 %		
33	2,4-Dichloro-3,5-xylenol (*)	0·1 %		
34	Quinolin-8-ol and its salts (*)	0·3 %	Not to be used in products applied after sunbathing, or in talcum powders for children under three years of age	Not to be used for children under three years of age
35	1,3,5-Tris (2-hydroxyethyl)hexa-hydro-1,3,5-triasine	0·3 %		Contains 1,3,5-Tris (2-hydroxyethyl) hexahydro-1,3,5-triazine
36	3,3'-Bis (1-hydroxymethyl-2.5-di-oxoimidazolidin-4-yl)-1,1'-methyl-enediurea ("Imidazolidinyl urea") (*)	0·6 %		
37	4-Isopropyl-*m*-cresol	0·1 %		
38	2-Chloro-*N*-(hydroxymethyl) acetamide	0·3 % for the chloracetamide	For products rinsed off after use	
39	1-Hydroxymethyl-5,5-dimethyl-hydantoin (*)	0·2 % expressed as free formaldehyde or theoretically available formaldehyde	For products rinsed off after use	Contains formaldehyde (1)
40	Pyrithione sodium (INM) (*)	0·5 %		

(1) Only if the concentration is greater than 0·05 %.

Reference number	Substance	Maximum authorized concentration	Limitations and requirements	Conditions of use and warnings which must be printed on the label
a	b	c	d	e
41	2,2'-Dithiobis(pyridine 1-oxide), addition product with magnesium sulphate trihydrate (*)	0·5 %		
42	Poly(1-hexamethylenebiguanide hydrochloride) (*)	0·3 %		
43	2-Phenoxyethanol (*)	1 %		
44	Hexamethylenetetramine (*) (methenamine) (INN)	0·2 % expressed as free formaldehyde or theoretically available formaldehyde		Contains formaldehyde (1)
45	Mixture of 5-Chloro-2-methyl-isothiasol-3(2*H*)one and 2-methylisothiazol-3(2*H*)-one with magnesium chloride and magnesium nitrate	0·005 % (of a mixture in the ratio 3:1 of 5-chloro-2-methylisothiazol 3(2*H*)one and 2-methylisothiazol-3 (2*H*)-one		
46	Pyridin-2-ol 1-oxide (*)	0·5 %	Only for products rinsed off after use	
47	Pyrithione aluminium camsilate (INNM)	0·2 %		
48	Methanamine 3-chloroallylochloride (INNM)	0·2 %		

(1) Only if the concentration is greater than 0·05 %.

Reference number	Substance	Maximum authorized concentration	Limitations and requirements	Conditions of use and warnings which must be printed on the label
a	b	c	d	e
49	1-(4-Chlorophenoxy)-1-(imidazol-1-yl)-3,3-dimethylbutan-2-one (*)	0·5 %		
50	1,3-Bis(hydroxymethyl)-5,5-dimethylimidazolidine-2,4-dione (*)	0·2 % expressed as free formaldehyde or theoretically available formaldehyde		Contains formaldehyde (1)
51	Benzyl alcohol (*)	1 %		
52	1-Dodecylguanidinium acetate (Dodine — ISO) (*)	0·5 % 0·1 %	For products rinsed off after use For other uses	
53	Benzethonium chloride (INN) (*)	0·1 %		
54	Benzalkonium chloride (INN), 1-2-Benzisothiazol-3-(2*H*)-one 1,1 dioxide) (*), its bromide and saccharinate	0·5 %		
55	Alkyl (C12-C22) trimethylammonium bromide and chloride (including Cetrimonium bromide (INN) (*)	0·1 %		
56	1-Phenoxypropan-2-ol	1 %		

(1) Only if the concentration is greater than 0·05 %.

Reference number	Substance	Maximum authorized concentration	Limitations and requirements	Conditions of use and warnings which must be printed on the label
a	b	c	d	e
57	1-hydroxy-4-methyl-6(2,4,4-trimethylpentyl) 2-pyridon and its monoethanolamine salt	1·0 % 0·5 %	For products rinsed off after use For other uses	
58	3-Heptyl-2-(3-heptyl-4-methyl-4 thiozolin-2-ylidenemethyl)-4-methyl-thiazolinium iodide	0·002 %	Creams, toilet lotions, shampoos'	

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