OPINION OF THE SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD PRODUCTS INTENDED FOR CONSUMERS

CONCERNING

2,4-DICHLOROBENZYL ALCOHOL
(DCBA)

COLIPA n° P74

adopted by the SCCNFP on 10 January 2003
by means of the written procedure

1. Terms of Reference

1.1 Context of the question

The Cosmetic Directive 76/768/EEC, adopted on 27 July 1976 (published OJEC on 27 September 1976) has been amended six times and at present covers more than 25 adaptations to technical progress. Technical Annexes (II – VII) are a set of lists, qualifying the use of certain ingredients for the safety of the final preparation.

In Annex VI, a positive list of preservatives which may be used is laid down. Besides the intended use as a preservative some of these substances are also destined "for other uses", mostly in other concentrations as for preservative purposes. These substances are marked by (+). At present 26 different substances are listed in Annex VI with(+) "other uses" in addition.

In order to guarantee consumer's health protection and safety of the respective products and following the 6th amendment to the Cosmetic Directive 76/768/EEC - document 93/35/EEC (14 June 1993) - those already evaluated substances have to be judged concerning the "other uses" (+) separately, especially related to the toxicological characteristics of the probably different concentration applied in the other uses than as listed as preservative (Annex VI).

In order to fulfil these demands a special submission (IV) for 2,4-Dichlorobenzyl Alcohol describing appropriate investigations and their results has been presented for an evaluation.

1.2 Request to the SCCNFP

Scientific evaluation and opinion on the "other uses" (+) of 2,4-Dichlorobenzyl Alcohol; see also Annex VI,1 - No. 22.

* Is 2,4-Dichlorobenzyl Alcohol, especially up to 0.5% safe for use in cosmetic products, as in creams, lotions and shampoos?

* Does the SCCNFP propose any restrictions or condition of the use of 2,4-Dichlorobenzyl Alcohol in cosmetic products?

1.3 Definitions of terms where appropriate

2,4-Dichlorobenzyl Alcohol has been already subject for evaluations on the basis of earlier submissions (submission I, October 1980; submission II, June 1982; submission III, April 1984).

The results of the respective evaluation and the opinion of the SCC is laid down in the opinion of the SCC of 1 July 1986. COLIPA presented Submission IV (March 2001) in order to defend the use of 2,4-Dichlorobenzyl Alcohol as "other uses" in creams, lotions and shampoos at a maximum concentration of 0.5%.

1.4. Statement on the toxicological evaluation

The SCCNFP is the scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food products intended for consumers.
The Commission's general policy regarding research on animals supports the development of alternative methods to replace or to reduce animal testing when possible. In this context, the SCCNFP has a specific working group on alternatives to animal testing which, in co-operation with other Commission services such as ECVAM (European Centre for Validation of Alternative Methods), evaluates these methods.

SCCNFP opinions include evaluations of experiments using laboratory animals; such tests are conducted in accordance with all legal provisions and preferably under chemical law regulations. Only in cases where no alternative method is available will such tests be evaluated and the resulting data accepted, in order to meet the fundamental requirements of the protection of consumer health.

2. Toxicological Evaluation and Characterisation

2.1. General

2.1.1. Primary name (INCI)

Dichlorobenzyl Alcohol

2.1.2. Chemical names

2,4-Dichlorobenzyl alcohol, 2,4-Dichlorobenzene-methanol

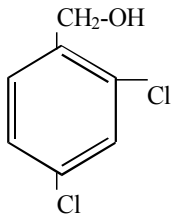
2.1.3. Trade names and abbreviations

Myacide SP, DCBA, Dybenal

2.1.4. CAS number

CAS n° : 1777-82-8
EINECS/ELINCS : 217-210-5

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : $C_7H_6Cl_2O$
Mol weight : 177,04

2.1.7. Composition, purity and identification

Purity : > 98 %

Spectroscopic data :

Wavelength	Absorbance
265 nm	0.88
272 nm	1.35
280 nm	1.26

Sulphated Ash : < 0.5 %

Impurities : Maximum individual impurity (HPLC) < 0.1 %
Total impurities (HPLC) < 0.3 %
Tetrabutyl ammonium hydrogen sulphate < 0.3 %
Heavy metals < 20 ppm

2.1.8. Physical properties

Appearance : White or slightly yellow crystals
Melting point : 57 – 60 °C
Boiling point : 150°C at 25 mmHg
Density : Specific gravity 1.45
Vapour Press. : 1.545×10^{-1} Pascals
Log P_{ow} : 1.87 at pH 6.8

2.1.9. Solubility

Water : 0.1 g/100ml at 20°C
Propylene Glycol : Soluble 45 g/100ml at 20°C
Acetone : Soluble 95 g/100ml at 20°C
N-methyl Pyrrolidone : 103 g/100ml at 20°C

2.2. Function and uses

Cosmetics :
- permitted as a preservative up to 0.15 %
- Current 'other uses' are as an anti-microbial in skin gels, creams, and lotions, deodorants and shampoos up to 0.5 %

Others :
- throat lozenges containing DCBA at 1.2 mg per 2.6 g lozenge are widely available 'over the counter'. One lozenge can be taken every 2 hours.
- antiseptic creams at 0.5%

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

LD_{50} oral in rats 3 g/kg bw, in male mice 2.3 g/kg bw.
Subcutaneous LD_{50} in mice 1.7 g/kg bw.

Ref. : 1 to 4

2.3.2. Repeated dose oral toxicity

A short term (3 wk) oral study in rats with dose levels of up to 500 mg/kg bw and an incomplete, sub-chronic (13 wk) study with 0.36 and 0.72 mg/kg bw were negative.

A short-term (4 wk) oral study in guinea pigs treated with 1.2 mg/kg bw/day was likewise negative.

Ref. : 1 to 4

A new study (1989) was presented which tested the sub-chronic toxicity of 2,4-dichlorobenzyl alcohol in rats when dosed by oral gavage for 13 weeks.

Groups of 16 male and 16 female rats were dosed by oral gavage with solutions of 2,4-dichlorobenzyl alcohol in propylene glycol at dose levels of 0, 200 and 400 mg/kg/day.

Overt signs of toxicity were recorded throughout the study, body weight and food consumption were recorded weekly. Haematology, blood biochemistry and urine analysis were conducted after 6 weeks and at the end of the treatment period. An ophthalmic examination was conducted at the beginning of the treatment and during week 13.

The animals were killed and dissected at the end of the treatment period, followed by a macroscopic examination and recording of organ weights. Samples of bone marrow were taken. A microscopic examination was conducted on a comprehensive range of tissues from the control and 400 mg/kg /day group as well as on livers and stomachs of the 200 mg/kg/day group.

Treated rats showed increased incidence of post-dose salivation and rales or uneven respiration. Treated females had slightly lower body weight gain, treated males had higher food consumption than the respective control groups.
No treatment related effect was seen on haematology or ophthalmoscopy.
Treated animals showed changes in blood biochemistry: increased serum ALP activity, decrease in gamma globulin, increased serum cholesterol and, only at week 7, increased serum urea and creatinine levels. Urinary pH was slightly decreased in treated males and mean urinary flow was slightly increased in rats given 400 mg/kg/day. Treated males had increased epithelial cell counts and cellular detritus in the urine sediment.

Liver weights were increased in rats given 400 mg/kg/day, kidney weight was increased in male rats given 400 mg/kg/day.

Macroscopic examination revealed treatment-related changes in the stomach, most marked in all males and 6/17 females given 400 mg/kg in form of wrinkled, rough or discoloured appearance of the forestomach mucosa.

Microscopic examination revealed the following :

Distinctive swelling of the keratin layer of the forestomach epithelium in most rats treated with 2,4-dichlorobenzyl alcohol.

Treatment-related changes in the forestomach and liver of some of the rats in the 400 mg/kg/day dose group : ulceration, erosion and necrosis (6/16), submucosal oedema (12/16), hyperplasia and hyperkeratosis.

Rats from the 200 mg/kg/day dose group showed a generalised but minimal thickening of the forestomach epithelium. This was also seen in the high dose group (400 mg/kg) and surprisingly in one animal of the control group.

In the liver, centrilobular hepatocyte enlargement and centrilobular glycogen loss were recorded in rats given 400 mg/kg/day.

The epithelial damage, hyperplasia and hyperkeratosis in the forestomach at 400 mg/kg/day were considered to be due to the irritant action of the test article, as were the increased incidence of minimal epithelial thickening in rats given 200 mg/kg/day, and increased post-dose salivation and serum ALP activities in treated rats.

Ref. : 7

Further studies

The pathogenesis of the forestomach lesions and their relevance for human safety have been further investigated in two more studies. In the first, 56 rats were given 400 mg/kg of 2,4-dichlorobenzyl alcohol in propylene glycol daily, and killed at intervals over a 13 week period. In the second study, rats were given 2,4-dichlorobenzyl alcohol in propylene glycol daily at 25, 100 or 400 mg/kg/day or in 0.4 % aqueous cellosize solution for seven weeks. Some rats from the 400 mg/kg/day dose groups were subsequently maintained off-dose for another four to eight weeks.

During both studies, overt signs of toxicity were recorded throughout the study, body weight and food consumption were recorded weekly. After killing, the stomachs of the animals were removed and investigated macroscopically and microscopically.
The main four points from these studies can be summarised as follows :

2,4-dichlorobenzyl alcohol produced ulceration, hyperplasia and hyperkeratosis in the forestomach of rats given 400 mg/kg bw/day in form of an 8% solution in propylene glycol. Such changes are typical of the reactive response to an irritant. Formulations containing 2,4-dichlorobenzyl alcohol at similar concentrations are known to be irritant to the skin and mucous membranes of laboratory animals. When administered in an aqueous system, the reactive response in the forestomach was less marked than with propylene glycol, so it appears that propylene glycol exacerbated these effects of 2,4-dichlorobenzyl alcohol.

At 200 mg/kg bw/day in form of a 4% solution in propylene glycol, 2,4-dichlorobenzyl alcohol did not cause hyperplasia but, instead, elicited minimal thickening of the forestomach epithelium; this change was also present (albeit at a lower incidence) in rats given propylene glycol alone. Thus, when 2,4-dichlorobenzyl alcohol was administered in propylene glycol, 200 mg/kg was the NOEL for hyperplasia, and 100 mg/kg was the NOEL for forestomach epithelial thickening.

All of the lesions identified after 13 weeks' of treatment were present by week 7 and no increase in the severity of the hyperplastic response appeared despite a further 6 weeks' dosing. Once

treatment with 2,4-dichlorobenzyl alcohol was discontinued, the epithelium rapidly returned to normal, showing that all of the lesions were fully reversible and with no evidence of autonomy.
Ref. : 8, 9

A review and discussion of the study results and their relevance for human safety has been carried out. The NOAEL has been identified at a dose of 100 mg/kg/day.
Ref. : 10

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

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.
Ref. : 1 to 4

Skin irritation studies carried out in a limited number of guinea pigs showed irritation with 5% and above in polyethyleneglycol, but not with 1%.

In preliminary rabbit studies repeated dermal application of 0.5% caused very slight erythema.
Ref. : 1 to 4

2.4.2. Irritation (mucous membranes)

Preliminary studies in rabbits showed no eye irritation at 0.08% in aequeous solution, or 0.1% in propyleneglycol.
Ref. : 1 to 4

Results of rabbit-eye tests showed no signs of irritation at concentrations of 0.05 % and 0.1 %. Concentrations of 0.5 % and 1 % in propylene glycol induced slight irritation and discharge two hours after testing.
A concentration of 5 % in polyethylene glycol 400 caused immediate lacrymation followed by discharge of a cloudy viscid fluid. There was no further lacrymation after two hours and the eyes were normal in appearance during further observation. The same degree of lacrymation was observed by instillation of polyethylene glycol 400 alone.

In addition to these experiments, a Draize test, following OECD Guideline 405, has been carried out with neat 2,4-dichlorobenzyl alcohol.

Initially, the test article (63 mg, the weight equivalent to 0.1 ml) was instilled into one conjunctival sac of a sentinel New Zealand Rabbit. Ocular reactions were assessed for ten days after treatment. The material elicited a strong response, including stinging, injection of conjunctival vasculature, marked ocular discharge and slight corneal opacity. These effects, had generally resolved by Day 3. A persistent area of disrupted epithelium showed slow resolution, which was not completed by Day 6. Other effects included symblepharon as well as an area of

granulation by Day 10. This was confirmed on day 11 and the rabbit was terminated since no resolution was expected.

A second rabbit was dosed after local administration of an analgesic and within one minute after instillation, the eye was rinsed. No initial stinging response was observed. In the first hours a considerable discharge and conjunctival chemosis were observed. On Day 2 the corneal surface was translucent over most of its area and the iris, while still responding to light, was hyperaemic. Injection of the conjunctival vasculature, slight chemosis and slight discharge were also apparent. Partial symblepharon was observed on Day 3. Reactions showed some improvement on Day 3 and 4 and the eye had reverted to normal on Day 6.

Two more rabbits were dosed likewise. On Day 1 the treated eyes were slightly opaque, eyelids swollen and there was marked ocular discharge. The reactions had largely resolved by Day 2, other than slight chemosis and slight opacity in one treated eye. Symblepharon was apparent in both treated eyes but had resolved completely by Day 4 and Day 7, respectively.

Ref. : 11

2.5. Sensitisation

Sensitisation tests in guinea pigs showed no evidence of sensitisation at concentrations of 2% in acetone, 0.1% in water or 2.5 % in a cream formulation.

Ref. : 1 to 4

In addition to the experiments submitted earlier, a guinea pig maximisation test (Magnussen & Kligman) has become available. Induction was carried out with 0.1 % in ethanol/PEG 400/ DOBS (intradermal) and 10 % in ethanol (dermal patch). The challenge concentration was 2.5 % in ethanol (dermal patch). 0 out of 10 guinea pigs showed evidence of sensitisation after 3 challenge treatments. A slight reaction in one guinea pig at the second challenge was not confirmed at the third challenge.

The authors concluded that 2,4-Dichlorobenzyl alcohol is not regarded as sensitising.

Ref. : 12

2.6. Teratogenicity

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

Ref. : 1 to 4

2.7. Toxicokinetics (incl. Percutaneous Absorption)

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.

Ref. : 1 to 4

2.8. Mutagenicity/Genotoxicity

2.8.1. Mutagenicity/Genotoxicity *in vitro*

Bacterial Reserve Mutation Test
(OECD 471: 21.07.97 (1983); EC B.14: 19.05.00)

The first study presented in 1980 reports the use of 5 strains of *Salmonella* (G46, TA1535, TA1536, TA1537 and TA1538) which are the least sensitive, in the absence and in the presence of a metabolic activation system (rat-liver homogenate) induced by phenorbital; the test was performed only at 1 concentration (1,000 µg/plate).
This study is completely inadequate for an assessment of the mutagenic hazard because of an unsound methodology (only one dose, only weak sensitive strains).

The second study presented in 1984 reports the use of 5 strains of *Salmonella* (TA1535, TA1537, TA9118 and TA100) tested in the presence of S-9 fraction (the inducer not identified) at doses up to 500 µg/plate (the chemical must be antibacterial).
This study is also inadequate for an assessment of the mutagenic hazard of P74, because only a summary was included.
The two studies were performed by the Company (internal reports) .

Ref. : 1 to 4

***in vitro* Mammalian Chromosome Aberration Test**
(OECD 473; 21.07.97 (1983); EC: B10: 19.05.00)

Strains/species/cell	:	Human lymphocytes
Metabolic activation	:	Rat liver homogenate induced by Aroclor (indicated in the summary but not in the section Material and Methods)
N° of experiments	:	one
N° of metaphases analysed	:	in the presence of MA 100 metaphases per dose were analysed; in the absence of MA for the lowest dose 100 metaphases were analysed, whereas for the second dose only 38, due to low mitotic index
Positive controls	:	Mytomycin C (-MA) and Cyclophosphamide (+MA)
Test substance	:	2,4-DCBA
Batch No/Purity	:	845127.ZZ; 99% (analysis carried out between 8.1.85 and 23.1.85)
Concentration	:	100, 200 µg/plate (-MA); 100, 200, 400 µg/plate (+ MA)
Treatment	:	24h (+MA); 2h (+MA)
GLP/Guideline	:	None
Study issue	:	1985

Results :
In the absence of metabolic activation the only dose analysed (100 µg/plate) presented a ca 50% reduction of Mitotic Index and a presence of 6% of cells with aberration gaps (2% aberrations only) against a control with 4% of a total of aberrations and gaps.

In the presence of metabolic activation there was no reduction in the mitotic index at the three doses evaluated (100, 200, 400 μg/ml); there was not observed a difference in the total no. of abnormalities, between the treated and the control. The positive controls produced the expected results.

This study is not adequate for the assessment of the mutagenic hazard of P74, due to the lack of a recognized guideline and the condition of the treatment (only one dose analysed without MA).

Ref. : 13

***in vitro* Mammalian Cell Gene Mutation Test**
(OECD 476; 21.07.97 – 1984; EC: B10: 19.05.00)

Strains/species/cell	:	Chinese hamster, V79
Mutagenic system	:	Forward Mutations to 6-Thioguanine Resistance
Metabolic activation	:	S-9 from livers of M.Charles River Wistar Rats treated with Aroclor 1254
N° of experiments	:	2 (5-10 plates)/condition
Method of analysis	:	Scoring of 6TG resistant mutants
Test substance	:	2,4-DCBA
Batch No/Purity	:	845127.ZZ; 98.5%
Concentrations	:	25, 50, 100, 200, 400 μg/ml (-MA); 25, 50, 100, 200, 400 μg/ml
Positive controls	:	MNNG (-MA); BMBA (+MA)
Treatment time	:	3h
GLP/Guideline	:	None

Results :
In the absence of MA, for the control it is indicated the spontaneous mutation frequency (equal to =; no resistant colonies); 25 μg/ml = resistant; 50 μg/plates = 4 resistant = $3.3x10^{-6}$ MF; the other doses were toxic; the PC (MNNG) induced a MF of 180.7 per 10^{-6} cells. In a second test on the absence of MA the respective MF were: 50 μg=$1.4x10^{-6}$; 100 μg = $1.5x10^{-6}$; 200 μg = 4.4 x 10^{-6}; 400 μg = $6.2x10^{-6}$; the control = 0% MF).

In the presence of MA for the control a MF of $2.4x10^{-6}$; was observed, whereas the treated group presented a MF of $5.2x10^{-6}$ (50μg), $1.2x10^{-6}$ (100μg), $5.6x10^{-6}$ (200μg), $1.4x10^{-6}$ (400μg); only one test could be evaluated, because of the absence of resistant colonies.
The number of the plates for scoring the 6TG resistant clones have been seeded with 10^5 cells which made a total population of $10x10^5 = 10^6$ clones; this condition does not seem suitable for scoring an induction of MF of the order of 10^{-6} in the control. In this type of testing the cell culture use must have a stable mutation frequency, but not equal to 0, that does not permit a correct evaluation. In 1984 a guideline was adopted by OECD that has not been taken into consideration by the AA. In their methodology they refer to a protocol not supported by OECD and modified according to their internal report.

The study is not adequate for the assessment of the mutagenic hazard of P74, because the methodology, not according to OECD guidelines, was not sufficiently sensitive for an adequate evaluation.

Ref. : 15

Unscheduled DNA Synthesis in Mammalian Cells *in vitro*
(OECD 482; 23.10.1986; EC B17: 18.11.1987)

Strains/species/cell	:	HeLa cells (source not indicated)
Metabolic activation	:	S-9 from livers of male Charles River Wistar Rats treated with Aroclor 1254
N° of experiments	:	3
Replicates	:	Not indicated
Method of analysis	:	incorporation of ^{3}H-thymidine and scintillation counting
Test substance	:	2,4-DCBA
Batch No/Purity	:	845127.ZZ; 99.3%
Concentrations	:	10^{-7} - 10^{-3} (±MA) (the tests ± MA were not conducted concurrently)
Positive controls	:	NMNG; B(a)pyrene
GLP/Guideline	:	None

Results :
No induction of DNA repair was observed in the three experiments. Among the positive controls only NMNG resulted clearly DNA-repair inducer, whereas B(a)P induced a DNA repair less than twice the control value in the presence of metabolic activation system. It could be concluded that the S-9 mix was not fully efficient, as no control was made of its specific activity by known methods.

This study is not adequate for the assessment of the mutagenic hazard of P74, due to a protocol not according to international guidelines, and the weak conditions used.

Ref. : 14

2.9. Carcinogenicity

No data available

2.10. Special investigations

No data available

2.11. Safety evaluation

If the normal exposure assessment for a preservative is used as the starting point and its preservative concentration (0.15%) is replaced by its “other uses” concentration (0.5%) for all concerned product types (shampoo, deodorant, cream, face cream), the topical exposure to 2,4-dichlorobenzyl alcohol would be estimated at 43 mg/day. The worst case systemic exposure would be estimated at 0.64 mg/kg bw/day, assuming a 90% dermal penetration at a body weight of 60 kg.

CALCULATION OF THE MARGIN OF SAFETY

Not applicable

2.12. Opinion

Presently, 2,4-Dichlorobenzyl alcohol (DCBA) is regulated in the Cosmetic Directive 76/768/EEC, Annex VI, part 1, reference n° 22 and can therefore be used as a preservative up to a maximum authorised concentration of 0.15% in the finished cosmetic product.

However, in response to the current mandate, the SCCNFP is of the opinion that the information submitted is insufficient to allow an adequate risk assessment of this substance to be carried out.

The genotoxicity/mutagenicity studies are inadequate (inappropriate protocols).

Consequently, and before any further consideration, the following are required :

- adequate genotoxicity/mutagenicity data;
- the very high absorption rate (90%) should be further investigated using modern testing methods;
- cumulative exposure via other routes of application should be included in the assessment.

2.13. References

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