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

CONCERNING

LAWSONE

Colipa n° C146

adopted by the SCCNFP during the 19th plenary meeting
of 27 February 2002

1. Terms of Reference

1.1 Context of the question

The adaptation to technical progress of the Annexes to Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products.

1.2 Request to the SCCNFP

The SCCNFP was requested to evaluate the additional genotoxicity data submitted and to inform the Commission whether these new results justify a modification of the opinion on Lawsone adopted by the SCCNFP during the 16th plenary meeting of 13 March 2001.

1.3. 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.
The extent to which these validated methods are applicable to cosmetic products and its ingredients is a matter of the SCCNFP.

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

Introduction

The additional data submitted on concern one *in vitro* and two *in vivo* genotoxicity studies on Lawsone.

The studies are summarised and evaluated in the present report.

The submission includes as well several published scientific papers on general issues of genetic toxicology, not related with the evaluation of genotoxic potential of Lawsone.
No attention has been given by this report to these scientific publications.

2.8. Mutagenicity/Genotoxicity

2.8.1. Mutagenicity/Genotoxicity *in vitro*

Mammalian cell gene mutation assay

OECD guidelines:	OECD 476 (1984)
Species/strain	V79 Chinese Hamster cell/ HPRT locus
Replicates:	3 independent tests
Test Substance:	2-HYDROXY-1,4-NAPHTHOQUINONE in DMSO
Batch No.:	008160: FE (99.4% purity)
Concentr. scored:	15-5000 □g/ml; 1000-5000 □g/ml; 500-5000 □g/ml (with and without met. act.)
GLP:	OECD G.L.P. (1981)

Colipa C146 has been investigated for induction of cell mutations at the HPRT locus in Chinese hamster V79 cells. Liver S9 fraction from Aroclor 1254 induced rats were used as the exogenous metabolic activation system. The maximum concentration was determined on the basis of preliminary tests which showed some toxicity at 5000 □g/ml concentration.
Negative and positive controls were in accordance with the literature (MNNG; BaP). The updated version of the OECD Guideline indicates for this cell system (EMS; EMU; NMDA).

Results

The mutation frequency in cells treated with Colipa C146 was higher than 3 times the vehicle control value for some concentrations (with and without metabolic activation).
Although this effect was reproduced in the different experiments, there wasn't any dose-effect relationship. According to OECD this study provides equivocal results which should be clarified by further testing preferably using a modification of experimental conditions. The 476 OECD Guideline has been updated inn 1997 (the study presented has been developed during 1996).

Ref. : 19

2.8.2. Mutagenicity/Genotoxicity *in vivo*

28-day *in vivo* cytogenetic assay

OECD guidelines:	does not exist
Literature guidelines:	Unknown
Species/strain	Sprague-Dawley CD, male and female rats
Replicates:	3 animals/sex/dose
Doses:	vehicle control; 12.5 –25.0-50-100 mg/kg/day x 28 days
Genotoxic endpoint:	chromosome aberrations in rat peripheral lymphocytes
Test Substance:	2-hydroxy-1,4-naphthoquinone Lot 600522-32 of unknown purity
GLP:	Internal Quality Ass. Unit.

Colipa C146 was given daily orally to 4 groups of animals, one group of animals was treated with vehicle (arachis oil B.P.), one groups of animals was treated with Cyclophosphamide.

At the end of the treatment period (28 days) a sample of fresh blood was taken from the orbital sinus of each rat; the blood cultures were incubated for 48 hours and then treated with demicolcine. Slides prepared from the cultures were analysed for the presence of cytogenetic effects.
Data from only one group of Colipa C146 treated animals are reported in the table, for the dose of 100 mg/kg/day.
No cytogenetic effect could be observed in the cultures derived from treated animals (vehicle=4.3%; C146=2.8%; CP=14.0%).
It is not possible to compare data obtained in this experiment with the data of literature, as the methodology employed has not been evaluated in general literature.

Ref. : 18

Bone marrow micronucleus test

OECD guidelines:	not followed. This study has been performed according to Schimd (1975) and to Salamone *et al.* (1980). The study has been completed on July 6th 2001 at the CIT Laboratory (France). The OECD 474 Guideline has been adopted on May 26th 1986 and updated on July 21st 1997.
Species/strain	Mice/Swiss/co:0F1 (IOPS Caw)
Group size:	10 male mice treated; 5 male mice with vehicle (control)
Dose level:	250 mg/kg (one oral treatment)
Test Substance:	2-hydroxy-1,4-naphthoquinone CAS No. 83-72-7 (>98.5% HPLC) ACROS Organics, Belgium
Batch No.	A0147608
Negative control:	0.5/ methylcellulose. Animals were sacrificed 72 hours after treatment
Positive control:	absent
GLP:	OECD GLP, 1997

The animals were treated orally and killed after 72 hours.

For each animal hematology parameters were evaluated and the number of the micronucleated polychromatic erythrocytes (MPE) was counted in 2000 polychromatic erythrocytes. The PE/NE ratio was also evaluated.

Results

Micronucleus : in the group of animals treated with Colipa C146 the frequency of MPE as well as the PE/NE ratio was similar to that of the vehicle control group.
The study indicates that the chemical is non-toxic for the bone marrow cells of mice.

Hematology investigations
In the animals treated with Colipa C146 there were no treatment-related abnormalities, such as erythrocytes (RBC), haemoglobin (HB), mean cell volume (MCV), packed cell volume (PCV), mean cell haemoglobin concentration (MCHC). mean cell haemoglobin (MCH).

Conclusion

The aim of this study was to evaluate the potential of the test substance to induce damage to the chromosome or to the mitotic apparatus in bone marrow cells of mice.
Under the experimental conditions, no conclusions can be drawn on the potential genotoxic effect of Colipa C146 *in vivo* on mice, due to the absence of a positive control.

2.13. Opinion

The SCCNFP is of the opinion that the information submitted is insufficient to allow an adequate risk assessment to be carried out. Consequently, there is no scientific basis to modify the opinion on Lawsone adopted on 13 March 2001.

2.14 References

18. Wright N.P. 2-Hydroxynaphthoquinone : metaphase analysis of lymphocyte chromosomes of rats from a 28-day toxicity range finding study (Project no.436/7) *in vivo* (Project no. 436/8); Safepharm Lab. Ltd. P.O. Box n°45 Derby, UK, 1992
19. De Jouffrey S. In vitro mammalian cell gene mutation tests (HPRT/V79 system). Centre International de Toxicologie, Evreux, France. Report n° 13589 MVA, 26.11.1996
31. Haddouk H., Bone marrow micronucleus test by oral route in mice. CIT BP 563-27005 Evreux France, Laboratory Study no. 21786 MAS, 6 July 2001