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

# OPINION

CONCERNING

# UVASORB® K2A

Adopted by the SCCNFP on 1 July 2004
By means of the written procedure

# 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 is requested to answer the following questions :

* *Does the SCCNFP consider that the provided information confirm that UVASORB® K2A is safe for use in cosmetic products, when used as UV-filter up to 10% in the finished product?*

* *Does the SCCNFP propose any restrictions or conditions for its use in cosmetic products based on the provided information?*

## 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

## 2.1. General

### 2.1.1. Primary name

INCI name : pending

### 2.1.2. Chemical names

2,4-Bis-[4-[5-(1,1-dimethyl-propyl)benzoxazol-2-yl]phenylimino]-6-[(2-ethylexyl)imino]-1,3,5-triazine

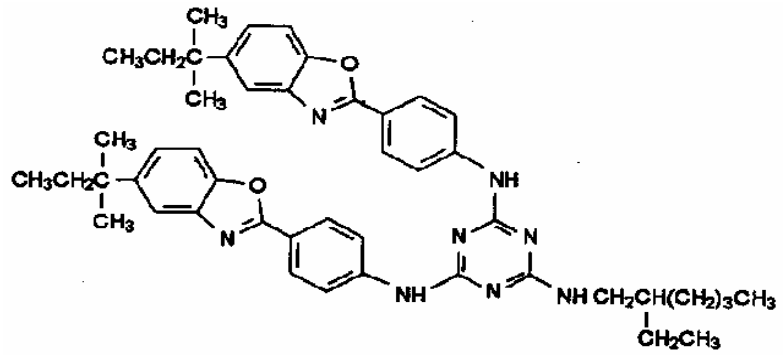
### 2.1.3. Trade names and abbreviations

Trade name : UVASORB® K2A
R&D name : ZN3044

### 2.1.4. CAS/EINECS no.

CAS No. : 288254-16-0
EINECS : /

### 2.1.5. Structural formula



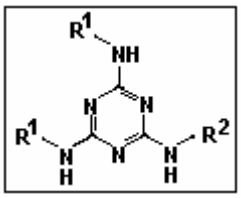
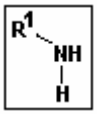
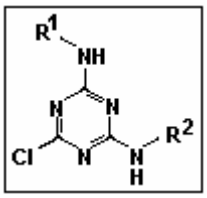
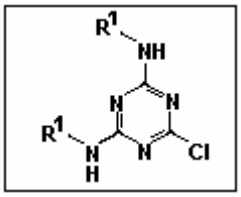
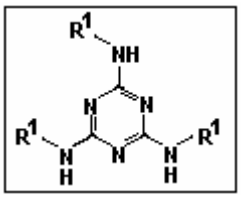
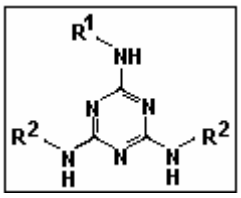

### 2.1.6. Empirical formula

Emp. Formula : $C_{47}H_{56}N_8O_3$
Mol weight : 765

### 2.1.7. Purity, composition and substance codes

Purity : 97 %

Impurities :

| | | | |
|---|---|---|---|
|  | UVASORB® K2A | 2,4-Bis-[4-[5-(1,1-dimethyl-propyl)benzoxazol-2-yl] phenylimino]-6-[(2-ethylexyl)imino]-1,3,5-triazine | 97 % |
|  | Impurity 1 PM 280 | 4-[5-(1,1-Dimethyl-propyl)benzoxazol-2-yl] phenylamine | < 1.0 % |
|  | Impurity 2 PM 520,5 | 2-[4-[5-(1,1-Dimethyl-propyl)benzoxazol-2-yl] phenylimino]4-chloro-6-[(2-ethylexyl)imino]-1,3,5-triazine | < 0.1 % |
|  | Impurity 3 PM 671,5 | 2,4-Bis-[4-[5-(1,1-Dimethyl-propyl)benzoxazol-2-yl] phenylimino]-6-chloro-1,3,5-triazine | < 0.6 % |
|  | Impurity 4 PM 613 | 2,4,6-Tris-[4-[5-(1,1-dimethyl-propyl)benzoxazol-2-yl] phenylimino]-1,3,5-triazine | < 1.0 % |
|  | Impurity 5 PM 915 | 2-[4-[5-(1,1-Dimethyl-propyl)benzoxazol-2-yl] phenylimino]-4,6-bis-[(2-ethylexyl)imino]-1,3,5-triazine | < 1.0 % |
| | Unknown | | < 0.6 % |

## 2.1.8. Physical properties

Appearance : Off-white powder
Melting point : /
Boiling point : /
Flash point : /
Density : /
Rel. vap. density : /
Vapour pressure : /
Log $P_{ow}$ : 4.7 ± 0.1

### 2.1.9. Solubility

| | | |
|---|---|---|
| Solubility in water | : | Insoluble |
| in Ethyl alcohol | : | 9 g/100 ml at 25°C |
| in Mineral oil | : | Insoluble |
| in Caprylic/caprictriglyceride | : | > 50 g/100 ml at 25°C |
| in C12-15 Alkyl benzoate | : | 20 g/100 ml at 25°C |
| in Isopropyl Palmitate | : | 20 g/100 ml at 25°C |
| in Octyl Palmitate | : | 20 g/100 ml at 25°C |
| in Octyldodecanol | : | > 50 g/100 ml at 25°C |

### 2.1.10. Stability

Exposure of the test substance to simulated solar radiation for either 9'20" (10 MED) or 10'44" through a Melinex filter, absorbing UVB light, did neither change $\lambda_{max}$ nor reduce the absorbance compared to the sham-irradiated control. Although under the conditions tested, the substance was determined photo-stable, the experimental conditions were insufficiently rigorous.

General Comment

- 2,4-Bis-[4-[5-(1,1-dimethyl-propyl)benzoxazol-2-yl]phenylimino]-6-[(2-ethylexyl)imino]-1,3,5 triazine (UVASORB® K2A) belongs to the class of secondary amines and thus it is prone to nitrosation. No data are provided on the nitrosamine content of UVASORB® K2A.

- The photo-stability testing is inadequate.

## 2.2. Function and uses

Maximum requested concentration: 10 % in finished cosmetic product (as a UV filter).

## TOXICOLOGICAL CHARACTERISATION

## 2.3. Toxicity

### 2.3.1. Acute oral toxicity

| | | |
|---|---|---|
| Guideline | : | OECD 432 (1996) |
| Species/strain | : | Rat, Wistar outbred (Crl: (WI) WU BR) |
| Group size | : | 3 males + 3 females |
| Test substance | : | ZN3044 |
| Batch no | : | 0100L1 |
| Purity | : | 97.2% |
| Dose | : | 2000 mg/kg bw |
| Vehicle | : | maize oil |
| GLP | : | In compliance |

Results
No mortality or distinct clinical signs. No treatment- related macroscopic changes. The oral $LD_{50}$ exceeds 2000 mg/kg bw.

Ref.: 1

### 2.3.2. Acute dermal toxicity

No data

### 2.3.3. Acute inhalation toxicity

No data

### 2.3.4. Repeated dose oral toxicity

No data

### 2.3.5. Repeated dose dermal toxicity

No data

### 2.3.6. Repeated dose inhalation toxicity

No data

### 2.3.7. Sub-chronic oral toxicity

| | | |
|---|---|---|
| Guideline | : | OECD 407 (1998) |
| Species/strain | : | Rat, Wistar outbred (Crl: (WI) WU BR) |
| Group size | : | 4 groups; 10 rats/sex/group |
| Test substance | : | ZN3044 |
| Batch no | : | 0100L1 |
| Purity | : | 97.2% |
| Dietary levels | : | 0, 0.2, 0.6 and 2.0%<br>(equal to overall intakes of 0.13, 0.4 and 1.4 g/kg bw/day) |
| Vehicle | : | diet |
| Exposure | : | 13 weeks |
| GLP | : | In compliance |

Results
Clinical signs: no mortality, no treatment-related findings
No treatment-related findings regarding arena testing, FOB and motor activity assessment, ophthalmoscopy, body weight, food/water intake, haematology, clinical chemistry, urinalysis, organ weights, macroscopy and histopathology.

Conclusion
The NOAEL was ≥ 2% ZN3044 in the diet (≥ 1.4 g ZN3044/kg bw/day).

Ref.: 12

## 2.3.8. Sub-chronic dermal toxicity

No data

## 2.3.9. Sub-chronic inhalation toxicity

No data

## 2.3.10. Chronic toxicity

No data

## 2.4. Irritation & corrosivity

## 2.4.1. Irritation (skin)

*Irritation*

| | | |
|---|---|---|
| Guideline | : | OECD 404 (1992) |
| Species/strain | : | New Zealand albino white rabbit |
| Group size | : | 3 males |
| Test substance | : | ZN3044 |
| Batch no | : | 0100L1 |
| Purity | : | 97.2% |
| Dose level | : | 0.5 g moistened with 0.5 ml water |
| Application | : | Cup fixed with adhesive gauze, 4 hour exposure |
| Skin readings | : | 1, 24, 48, and 72 hours after removing the patches according to the Draize scoring system. |
| GLP | : | In compliance |

Results
In one rabbit very slight erythema was observed 24 hour after treatment. No other signs of irritation were noted. The substance is not irritating to the skin

Ref.: 3

*Phototoxicity*

| | | |
|---|---|---|
| Guideline | : | OECD 432 (draft 2002) |
| Test system | : | Balb/c 3T3 cells |
| Test substance | : | ZN3044 |
| Batch no | : | 0100L1 |
| Purity | : | 97.2% |
| Quantities/vehicle: | | The test substance was dissolved in DMSO (5mg/ml) and further diluted in PBS to 50 µg/ml. Serial dilutions of 100% - 0.005% of this solution were used for treatment of the 3T3 cells. |
| Positive control | : | Serial dilutions of 8-MOP in DMSO/PBS |
| Treatment | : | After 1 h preincubation, cells were exposed to 5 Joules UVA/$cm^2$ |

After 24h recovery, they were incubated for 3 hours with neutral red.

GLP : In compliance

Conclusion
Under the conditions of the test ZN3044 was not phototoxic. The photo-toxicity factor (PTF) was 1. The positive control showed phototoxicity. PTF was > 5 (viz. 63.9).

Ref.: 8

## 2.4.2. Irritation (mucous membranes)

| | | |
|---|---|---|
| Guideline | : | OECD 405 (1987) |
| Species/strain | : | New Zealand albino white rabbit |
| Group size | : | 3 males |
| Test substance | : | ZN3044 |
| Batch no | : | 0100L1 |
| Purity | : | 97.2% |
| Dose level | : | 0.061 g (equivalent to a volume of 0.1 ml) |
| Eye readings | : | 1, 24, 48, and 72 hours after treatment |
| GLP | : | In compliance |

Results
Slight redness and swelling of the conjunctivae and slight ocular discharge were noted in all rabbits one our after treatment. One rabbit still showed slight redness and swelling of the conjunctivae 24 hour after treatment. No other signs were noted. The substance is not irritating to the eye.

Ref.: 2

## 2.5. Sensitisation

## 2.5.1 Test for capacity to induce sensitisation (maximization test)

| | | |
|---|---|---|
| Guideline | : | OECD 406 (1992) |
| Species/strain | : | Guinea pigs (Dunkin Hartley Crl:(HA)BR) |
| Group size (main test) | : | 10 males (test group), 5 males (control group) |
| Test substance | : | ZN3044 |
| Batch no | : | 0100L1 |
| Purity | : | 97.2% |
| Dose levels | : | On the basis of preliminary tests, intradermal induction was conducted with 10% (using FCA) , while topical induction was done with 30% concentration in maize oil. Since a 30% concentration was not irritating, the induction site was pretreated with 10% sodium lauryl sulphate in vaseline. Challenge was conducted with a 30% concentration in vaseline. |
| GLP | : | In compliance |

Results
The challenge did not cause any skin reactions. ZN3044 is not a sensitizer.

Ref.: 4

## 2.5.2 Test for capacity to induce photo-sensitisation

| | | |
|---|---|---|
| Guideline | : | / |
| Species/strain | : | Guinea pigs (Dunkin Hartley Crl:(HA)BR) |
| Group size (main test) | : | 10 males (test group), 5 males (control group) |
| group size (pos. contrl) | : | 5 males (test group), 5 males (control group) |
| Test substance | : | ZN3044 |
| Batch no | : | 0100L1 |
| Purity | : | 97.2% |
| Positive control | : | 10% Musk ambrette |
| Irradiation conditions | : | UVB : 0.1 Joules/$cm^2$, UVA: 10 Joules/$cm^2$ |
| Induction | : | Five induction treatments (over 10 days) by topical application of 10% solution in acetone, followed by UVB- and UVA-irradiation. The first induction was preceded by intradermal injections of Freund's complete adjuvant. |
| Challenge | : | Topical application of 10% solution in acetone, 12 days after the last induction, both with and without subsequent irradiation. |
| GLP | : | In compliance |

Results
The experiment was performed essentially according to the method described by Ichikawa *et al.,* (1981). OECD Guideline 406 and EEC Guideline B.6 regarding 'normal sensitisation' were followed wherever possible.

The challenge did not cause positive signs of photosensitization. Challenge with Musk Ambrette caused clear signs of photosensitization in all animals. ZN3044 is not a photosensitizer.

Ref.: 9

## 2.6. Reproductive Toxicity / Teratogenicity

No data

## 2.7. Toxicokinetics (incl. Percutaneous Absorption)

| | | |
|---|---|---|
| Guideline | : | OECD 248 (draft 2000) |
| Test system | : | Human skin membranes. Sterile glass rings(0.64$cm^2$) were glued to the epidermal side |
| Contact time | : | 24 hours |
| Test substance | : | 'Cold ZN3044' and [ring-$^{14}$C] ZN3044 |
| Purity | : | > 97% |
| Radiochem. purity: | | 99.5% |
| Batch | : | 0100L1 and 0138GM02 (labelled material) |
| Positive control | : | [4-$^{14}$C] testosterone (13.8 µg/$cm^2$) |
| Formulation | : | formulation containing cetearyl alcohol and cetearyl glucoside, glyceryl stearate, caprylic/capric triglyceride |
| Application | : | emulsions (8.8 mg/$cm^2$) containing 10%, 5% and 2.5% ZN3044 (821, 528 and 245 µg/$cm^2$) |
| Receptor fluid | : | (1.2 ml) DMEM and Ham's F12 Culture medium (3:1) supplemented with EGF (10 µg/ml), hydrocortisone (400 g/ml), gentamicin (50 µg/ml), L-glutamine (2mM), polyoxyethylene 20 oleyl ether (6% w/v) and foetal |

calf serum (10% v/v). This receptor fluid is known to support viability of the skin membranes (OECD guidance document, December 2000).

GLP : in compliance

Results

The absorption characteristics of the positive control (testosterone) were comparable to the historical data of the laboratory. The relative skin absorption of ZN3044, as measured in the receptor fluid after 24 h was 0.013% (10% emulsion), 0.012% (5% emulsion) and 0.016% (2.5% emulsion). Only a low percentage of the dose was detected in the skin membrane (after tape stripping); 0.32% (10% emulsion), 0.51% (5% emulsion) and 0.89% (2.5% emulsion).
The flux constants in human skin membranes were 0.0043 $\mu g/cm^{-2}.h^{-1}$ (10% emulsion), 0.0031 $\mu g/cm^{-2}.h^{-1}$ (5% emulsion) and 0.0020 $\mu g/cm^{-2}.h^{-1}$ (2.5% emulsion).

Conclusion

The in vitro absorption of 2.5-10% ZN3044 was low: after 24 hours continuous exposure, only 0.01-0.02% of the dose was detected in the receptor fluid, while 0.32 to 0.89% was associated with the skin membrane. Based on the amounts in receptor fluid and remaining in the skin 0.333% x 821 $\mu g /cm^2 = 2.7 \mu g/cm^2$ is available.

Ref.: 5

## 2.8. Mutagenicity/Genotoxicity

### 2.8.1. Mutagenicity/Genotoxicity *in vitro*

**Reverse Mutation Testing Using Bacteria**

| | | |
|---|---|---|
| Guideline | : | OECD/471 (1997) |
| Test substance | : | ZN3044 |
| Batch no | : | 0100L1 |
| Purity | : | 97.2% |
| Test system | : | *Salmonella typhimurium* TA1535, TA1537, TA98, and TA100<br>*Escherichia coli* WP2 *uvr*A |
| Vehicle | : | DMSO |
| Metabolic act. | : | S9 from rat liver homogenate (rats were treated with Aroclor 1254) |
| Doses | : | 62[1], 185[1], 313[2], 556[1], 625[2], 1250[2], 1667[1], 2500[2] and 5000[2] µg/plate<br>[1] first experiment<br>[2] second experiment |
| Replicate | : | 2 experiments, both in presence and absence of S9 mix |
| Positive controls | : | According to OECD/471 Guideline |
| GLP | : | In compliance |

Results

No toxicity was observed in any strain. In both assays, with and without S9 mix, there was neither a two fold increase nor a dose-related response in the mean number of revertant colonies. The positive controls gave the expected increase in the mean number of revertant colonies.

Conclusion

ZN3044 was not mutagenic in this test.

Ref.: 6

**Photomutagenicity**

**Reverse Photo-Mutation Testing Using Bacteria (1)**

| | | |
|---|---|---|
| Guideline | : | / |
| Method | : | Modified method to include solar simulated light (Dean *et al*., 1991, 1992) |
| Test substance | : | ZN3044 |
| Batch no | : | 0100L1 |
| Purity | : | 97.2% |
| Test system | : | *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA102 *Escherichia coli* WP2 (pKM101) |
| Vehicle | : | DMSO |
| Doses | : | 5, 10, 25, 50, 100 and 500 µg/plate both in the absence and in the presence of two doses of solar simulated light. (The dose levels were based on a dose-range finding study with 10 doses ranging from 6.67 to 5000 µg/plate). All doses were plated in triplicate. |
| Irradiation | : | Filter with UV-cut-off at 290 nm was applied. Two doses of solar simulated light (one dose producing a 2-4 fold increase in the number of revertants per plate, the other one half of this dose) |
| Replicate | : | two independent experiments using identical exposure conditions |
| Positive control | : | 8-MOP (10, 33, 100 and 333 µg/plate) in strains TA102 and WP2 (pKM101) |
| GLP | : | In compliance |

Results
In both experiments, with and without solar simulated light, there were no positive increases in the mean number of revertant colonies per plate. The positive control gave the expected increase in the mean number of revertant colonies in strains TA102 and WP2 (pKM101).

Conclusion
ZN3044 was not photomutagenic in this test.

Ref.: 10

**Reverse Photo-Mutation Testing Using Bacteria (2)**

| | | |
|---|---|---|
| Guidelines | : | OECD 471 (1997) |
| Test substance | : | ZN3044 |
| Batch no | : | 0100L1 |
| Purity | : | 97.22% |
| Test system | : | *Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 *Escherichia coli* WP2 *uvrA* |
| Vehicle | : | DMSO |
| Doses | : | 417 (max due to solubility) and 139 µg/ml both in the absence and in the presence of four doses of solar simulated light. |
| Irradiation | : | Four doses of UV irradiation ranging from 38.6 to 6168 mJ/cm$^2$. The highest UV irradiation dose resulted in a weak mutagenic response. |
| Replicate | : | one experiment was performed |

| | | |
|---|---|---|
| Positive controls | : | 8-MOP (3 µg/ml) in *E. coli* WP2 *uvrA*<br>chloropromazine (10µg/ml) in TA98, TA100, TA1535 and TA1537 |
| GLP | : | In compliance |

Results
The positive controls showed the expected positive results. The test substance was not phototoxic to any strain (no decrease in the number of revertant colonies). The test substance did not cause an increase in the mean number of revertant colonies with UV radiation compared to the background reversion rate without UV irradiation.

Conclusion
ZN3044 was not photomutagenic in this test.

Ref.: b

### *In Vitro* Mammalian Chromosome Aberration Test

| | | |
|---|---|---|
| Guideline | : | OECD 473 |
| Test substance | : | ZN3044 |
| Batch no | : | 0100L1 |
| Purity | : | 97.2% |
| Test system | : | CHO cells |
| Vehicle | : | DMSO |
| Metabolic Act. | : | S9 from rat liver homogenate (rats were treated with Aroclor 1254) |
| Doses/treatment | : | The selection of the highest concentration scored was based on toxicity of the test substance on cells. The following concentrations were tested 40.0, 50.0, 75.0, 100 and 125 µg/ml<br>Treatment/harvesting times were:<br>without S9 mix: first experiment 18h/18h<br>second experiment 18h/18h and32h/32h<br>with S9 mix: first experiment 4h/18h (pulse treatment)<br>second experiment 4h/18h and 4h/32h (pulse treatment) |
| Replicate | : | 2 independent experiments, both in presence and absence of S9 mix |
| Positive controls | : | mitomycin C (absence S9 mix); cyclophosphamide (presence S9 mix) |
| GLP | : | In compliance |

Results
In both the first and the second (independent) tests, no statistically significant increase in the number of cells with structural chromosomal aberrations was observed at any concentrations and time points. The positive and negative controls gave the expected results.

Conclusion
ZN3044 was not clastogenic in this test.

Ref.: 7

**Photoclastogenicity**

***In Vitro* Mammalian Chromosome Aberration Photomutagenicity Test (1)**

| | | |
|---|---|---|
| Guideline | : | / |
| Method | : | Recommendations for photochemical genotoxicity testing (Gocke et al., 2000). In line with OECD 473 (1997), EEC Directive 67/548/EEC and EMEA notes for guidance on photo-safety testing |
| Test substance | : | ZN3044 |
| Batch no | : | 0100L1 |
| Purity | : | 97.2% |
| Test system | : | CHO cells |
| Vehicle | : | DMSO |
| Doses | : | First experiment:<br>Based on results of mitotic index scoring, at least three concentrations (125,100, 50, 25 µg/ml) were tested each with 0, 8, 16 and 32 minutes) UV radiation periods.<br>Second experiment:<br>Five concentrations (75, 50, 30, 20, 10 µg/ml) were tested each with 0, 12, 32, 42 or 52 minutes solar simulated UV radiation. |
| Irradiation | : | Philips TL super professional sunlamps, emitting UV radiation similar to sunlight (mean dose rating during all exposures 162 mJ/cm$^2$/min in first experiment and 128 mJ/cm$^2$/min in second experiment). |
| Replicate | : | 2 independent experiments |
| Positive controls | : | 8-MOP |
| GLP | : | In compliance |

Results
The positive controls gave the expected results.

First experiment: The number of cells with structural chromosomal aberrations was increased at 125 µg/ml following 32 min. of UV radiation, compared to the concurrent control. A slight (not statistically significant) UV-dose-related increase was observed with this concentration. Treatment with lower concentrations did not result in structural chromosomal aberrations.

Second experiment: At 22 minutes of UV radiation, the highest concentration (75 µg/ml) showed a significant increase in cells with chromosomal aberrations. A concentration-related increase (not statistically significant) was observed at this UV exposure period.
At 32 minutes of UV radiation, all tested concentrations (75, 50, 30, 20, 10 µg/ml) showed an increase in cells with chromosomal aberrations. There was no concentration-response relationship. A UV dose-related increase in chromosomal aberrations was only observed at the concentration of 75 µg/ml.

Conclusion
ZN3044 was photo-cytotoxic and photo-clastogenic under the conditions of this test.

Ref.: a

***In Vitro* Mammalian Chromosome Aberration Photomutagenicity Test (2)**

| | | |
|---|---|---|
| Guideline | : | / |
| Test substance | : | ZN3044 |
| Batch no | : | 0100L1 |
| Purity | : | 97.2% |
| Test system | : | CHO cells |
| Vehicle | : | DMSO |
| Doses | : | Based on a dose range finding study, the following doses were used<br>With UV: 1.25, 2.5, 5*, 10*, 25*, 50*, 75 and 100 µg/ml,<br>Without UV: 2.5, 5, 10*, 25*, 50*, 75*, 100, 150 and 200 µg/ml<br>The levels marked with an asterisk* were analyzed for chromosomal aberrations.<br>Treatment/harvesting times were 3h/20h in both experiments. |
| Irradiation | : | 170-191 mJ UVA/$cm^2$ and 13.5-13.7 mJ UVB/$cm^2$ (radiation during 2 minutes in the dose range finding study and during 75 seconds in the main study). A UV Filter with UV-cut off at 290 nm was applied |
| Replicate | : | 2 experiments, (the second experiment was only performed in the presence of UV irradiation) |
| Positive controls | : | 8-MOP (with UV); mitomycin C (without UV) |
| GLP | : | In compliance |

Results
In both tests, no significant increase in the number of cells with structural chromosomal aberrations was observed. The positive control 8-MOP gave positive results. ZN3044 was not photo-clastogenic under the conditions of this test.

Remark
Only one UV dose was applied for 75 seconds. This dose did not induce any toxicity. Although the pos. controls gave adequate results, it may be argued that the system was not stressed to the limit.

Ref.: 11

**Comparison between the two *in vitro* Mammalian Chromosome Aberration Photomutagenicity tests**

1. Strain:

| | | |
|---|---|---|
| Lab1 | CHO-K1 | (Leiden) |
| Lab2 | CHO-VBL | (San Francisco) |

2. Guideline:

| | | |
|---|---|---|
| Lab1 | Dean et al., 1992; Gocke et al., 2000 | (OECD) |
| Lab2 | SCCNFP/321/00 | (OECD) |

3. UV Lamp:

| | |
|---|---|
| Lab1 | Philips TL Sunlamp |
| Lab2 | Xenon Lamp Heraeus 56001794 |

4. Calibration:

| | | |
|---|---|---|
| Lab1 | Digital radiometer | |
| Lab2 | Radiometer/Photometer | |

5. UVA:

| | | |
|---|---|---|
| Lab1 | 2.70/2.70 mW/cm² | (1st exp.) |
| | 2.16/2.12 mW/cm² | (2nd exp) |
| Lab2 | 2.55 mW/cm² | (1st exp) |
| | 2.26 mW/cm² | (2nd exp) |

6.UVB:

| | | |
|---|---|---|
| Lab1 | not indicated | |
| Lab2 | 180 µW/cm² | (1st exp) |
| | 183 µW/cm² | (2nd exp) |

7. Doses:

| | | |
|---|---|---|
| Lab1 | 162/128.4 mJ/cm² | |
| Lab2 | 191/13.5 mJ/cm² | (1st exp) |
| | 170/13.7 mJ/cm² | (2nd exp) |

8. Time Irradiation

| | | |
|---|---|---|
| Lab1 | 0, 8, 16, 32 min. | (1st exp) |
| | 0, 12, 32, 42 min. | (2nd exp) |
| Lab2 | 75 sec. | |

9. Mitotic Index (toxicity):

| | | | |
|---|---|---|---|
| Lab1 | NO UV: | between 71 and 83 % | (1st exp) |
| | 4 min UV: | between 83 and 91% | |
| | 8 min UV: | between 92 and 110% | |
| | 16 min UV: | between 55 and 82% | |
| | 32 min UV: | between 56 and 92% | |
| | NO UV: | between 94 and 117% | (2nd exp) |
| | 12 min UV: | between 78 and 102% | |
| | 22 min UV: | between 76 and 105% | |
| | 32 min UV: | between 49 and 56% | |

42 min UV: between 32 and 38%

MOP (positive control) : 1 min UV 4.2/4.8%

Lab2 75 sec UV: between 100 and 96%

10. Doses (UVASORB):

Lab1 10-125 µg/ml (5 doses)
10-75 µg/ml (5 doses)
Lab2 1, 25, 100 µg/ml

11. Chromosome aberrations

Lab1: 1st exp.: 32 min UV (125,100,50,25 µg/ml)
The dose of 125 µg/ml was found photoclastogenic. 5.5% of cells with aberrations
2nd exp : 22 min UV (75, 50, 30, 20, 10 µg/ml)
All analyzed doses were found photoclastogenic. 6.0% cells with aberrations
In both experiments MOP was found photo-clastogenic.

Lab2: All doses analyzed (5, 10, 25, 50 µg/ml, 1st exp; and 5, 10, 25, 50 µg/ml, 2nd exp) were found not photo-clastogenic compared with the negative control treated cultures, or treated in the absence of UV. MOP induced 58-48% of cells with chromosome aberrations.

**Discussion**

The studies conducted by Lab1 for photomutagenicity on bacteria (ref. b), for phototoxicity on mammalian cells (ref.8) and for photoclastogenicity on mammalian cells (ref. a) have employed the same solar simulator lamp (Philips TL Sunlamp), calibrated with a digital radiometer. Although only the UVA dose is indicated in these three reports, the dose for evaluating the photoclastogenicity, the induction of photomutagenicity and phototoxicity is adequate.
The results indicate that the test item, UVASORB/K2A is not photomutagenic on bacteria, nor phototoxic on mammalian cells, but is photoclastogenic on mammalian cells (positive results in two independent experiments).
The study for evaluating the photoclastogenicity of the test item was made under conditions suitable for the evaluation of this type of hazard: UV light doses, duration of the exposure, conditions expressing toxicity on the cells (reduction of the Mitotic Index). The result, indicating a photoclastogenicity activity of the chemical, is based on adequately developed experimental conditions.
UVASORB K2A is photo-clastogenic on mammalian cells treated *in vitro*.

The study conducted by Lab2 for photoclastogenicity on the same cell line, but of different origin, was insufficiently stringent. The study is inadequate for an evaluation. There was

inadequate exposure inducing almost no toxicity on the cells with the mitotic index being similar in the treated and untreated cultures.

**Evaluation of the overall results**

UVASORB K2A has been tested for mutagenicity on bacteria and for clastogenicity on mammalian cells and found to be non-mutagenic /clastogenic.
UVASORB K2A has been tested for photomutagenicity on bacterial cells and for photoclastogenicity on mammalian cells and found photoclastogenic.

CHO cell lines have been used in many *in vitro* studies for evaluating the photoclastogenicity of chemicals of different types, including sunscreen products. For a total of 23 tested chemicals on this cell line, 15 chemicals were reported photoclastogenic and 8 chemicals negative: these latter included 5 sunscreens and three other types of chemicals (reported in:S.BRENDLER-SCHWAAB et al, 2004):

According to EMEA (2002) "compounds found to be photogenotoxic can be considered as potential photocarcinogens and a specific testing in rodent photocarcinogenicity studies is normally not required"

After a positive photogenotoxicity test, photocarcinogenic potential is assumed, otherwise photocarcinogenicity testing is required.

According to BRENDLER-SCHWAAB et al. (2004) "*In vivo* testing for photochemical genotoxicity is problematic since the skin cannot be easily utilized in standard approaches. Transgenic mutagenicity models may be useful in this area in that they allow determination of mutations in skin cells."
The psoralens and uroquinolones, that have been tested for photochemical carcinogenesis and have produced positive results, are photochemical genotoxins. Hence, the correlation of experimental data between photochemical carcinogens and photochemical genotoxins is convincing.

There are, however, other experimental conditions that could be applied to the further evaluation of the potential potential of UVASORB® K2A, such as, for example, the use of other *in vitro* tests employing different photoclastogenic end points, methods, and cell lines (Photo – MLA assay for the evaluation of both gene mutations (large colonies) and chromosome aberrations (small colonies), or the use of some newly developed *in vivo* tests: *In vivo* photomicronucleus test), etc.
In the presence of several contrasting results obtained by different methods, these data could be evaluated on the weight of evidence.

Ref.: d, e, f

## 2.8.2 Mutagenicity/Genotoxicity *in vivo*

No data

## 2.9. Carcinogenicity

No data

## 2.10. Special investigations

The capacity to prevent induction of DNA damage and expression of p53 protein after exposure to solar simulated radiation (SSR) was examined in a two-compartment organ culture model of human skin. Four substances labelled A, B, C and D were tested. The best protection to SSR-induced DNA damage was observed with test substance C, followed by B, A and D.
Because the identity of substance A, B, C and D was not revealed, this study is unsuitable for evaluation.

Ref.: c

## 2.11. Safety evaluation

Not applicable

## 2.12. Conclusions

2,4-Bis-[4-[5-(1,1-dimethyl-propyl)benzoxazol-2-yl]phenylimino]-6-[(2-ethylexyl)imino]-1,3,5 triazine (UVASORB® K2A) belongs to the class of secondary amines and thus it is prone to nitrosation. No data are provided on the nitrosamine content of UVASORB® K2A.
The photo-stability testing is inadequate.

There is no data on reproduction toxicity. The NOAEL in a subchronic toxicity study was more than 1400 mg/kg bw/day.

The test substance was not irritating to the eyes and the skin. It is not a sensitiser.

The skin absorption was 2.7 µg/cm².

The test substance was not mutagenic on bacteria nor in a chromosome aberration test. It was, however, photoclastogenic on mammalian cells treated *in vitro*.

## 2.13. References

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c. Vinck A;A; Effect of 3V Sigma sunscreens on induction of CPD and expression of p53 after exposure to ultraviolet radiation. Report V99.226, January 2000. TNO, NL-Zeist
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e. BRENDLER-SCHWAAB, S. et al.: Photochemical genotoxicity: principles and test methods. Report of a GUM task force. Mutation research 566, 65-91, 2004.
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## 3. Opinion of the SCCNFP

The SCCNFP is of the opinion that the information submitted is insufficient to assess the safe use of the substance.

Before any further consideration, the following information is required:

* data on photo-stability and nitrosamine formation in a prototype formulation under simulated in-use conditions;
* data on reproduction toxicity;
* further photo-mutagenicity/photo-carcinogenicity testing.

## 4. Other considerations

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## 5. Minority opinions

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