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

OPINION

CONCERNING

ZINC OXIDE

COLIPA n° S 76

Adopted by the SCCNFP during the 24th plenary meeting
of 24-25 June 2003

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.

Request for inclusion of Zinc oxide in Annex VII, part 1 – List of permitted UV Filters which Cosmetic Products may contain – to Council Directive 76/768/EEC.

1.2 Request to the SCCNFP

The SCCNFP is requested to answer the following questions :

* Is Zinc oxide safe for use in cosmetic products as a UV filter up to 25 %?

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

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

Throughout this document, it is assumed that the biological and toxicological effects are determined by the zinc cation. As such, data from any inorganic zinc compound has been used to assess the toxicological potential of ZnO.

Zn^{2+} is generally considered a non-toxic metal. This view, as reviewed by Walsh et al., is based on the knowledge that Zn^{2+} (5) :

* is an essential nutrient present in virtually every cell and critical for numerous physiological functions;
* must be consumed in the diet and absorbed to maintain human health;
* does not appear to accumulate with age;
* is regulated such that deficiencies lead to an increase in absorption whereas excessive intake is associated with decreased absorption and increased excretion ;
* administration for therapeutic reasons in man at doses above the recommended daily allowance (RDA) has not produced significant pathology; and
* long history of safe use in sunscreen products in US and other countries.

In contrast to states of zinc toxicity, there is much known about the consequences of Zn^{2+} deficiency and procedures for correcting such conditions. Acute and chronic Zn^{2+} deficiency due to nutritional factors and several diseases produce clinical symptoms, which are varied ranging from mild to severe and affecting several organ systems including skin (6). Correcting such deficiencies is accomplished through dietary supplementation.

Zn^{2+} and many of its salts are ingredients in multivitamins. ZnO is available as a dietary supplement and generally recognised as safe in most countries. Importantly, as a dietary supplement there were no studies located that suggested any adverse effects in humans as a result of excess Zn^{2+} consumed through the diet.

2.1.1. Primary name

Zinc oxide (INCI name)

2.1.2. Synonyms

Zinc oxide (IUPAC)
Zinkoxid neutral H&H
Zinkoxid NDM

2.1.3. Trade names and abbreviations

Zcote HPI
MZO 350

2.1.4. CAS no.

CAS n° : 1314-13-2
EINECS : 215-222-5

2.1.5. Structural formula

/

2.1.6. Empirical formula

Emp. Formula : ZnO
Mol weight : 81.38

2.1.7. Purity, composition and substance codes

Assay : ≥ 95% (based on uncoated materials)
≥ 92% (based on coated materials)

Coatings : Commonly used coatings for ZnO include dimethicone, vegetable oils, metal salts and fatty acids. These materials when combined with ZnO can make the particle hydrophobic, rendering it more compatible with waterproof or anhydrous systems and more convenient for the formulator. ZnO has been used as a coating to photostabilize other pigments.

Comment : no information is given on metals and other impurities.

2.1.8. Physical properties

Substance code : S76
Appearance : ZnO is odourless, non-flammable, white or faintly yellowish, amorphous powder. Microfine powders, used in sunscreen products, have an average particle size of approximately 0.20 microns or less with a narrow distribution.
Melting point : 1975 °C
Boiling point : /
Density : 5.47 g/cm²
Rel. vap. dens. : /
Vapour Press. : /
Log P_{ow} : /

2.1.9. Solubility

Practically insoluble in water (0.00016 g/100 ml water), soluble in diluted mineral acids.

2.2. Function and uses

Requested use : up to 25% in sunscreen formulations.

Main industrial uses of ZnO are rubber, ceramics, optical glass, paint and plastics. Cosmetics & pharmaceuticals: ointments, baby and skin creams, toothpaste, deodorants, sunscreens. ZnO is added to animal food and fertilisers, as essential trace element.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

Species	:	Wistar-II rats,
Number of animals	:	10 males and 10 females
Test substance	:	ZnO
Dose	:	Single oral dose of 5000 mg/kg bw by gavage
Results	:	LD_{50} > 5000 mg/kg. No clinical signs of toxicity were observed during this study. No deaths occurred.
Conclusion	:	ZnO is non-toxic after a single oral ingestion.

Ref. : 7

2.3.2. Acute dermal toxicity

Method	:	According to the JO CEE recommendations, 19/09/1984 (L251/103.105)
Species	:	Sprague Dawley rats
Number of animals	:	5 males and 5 females
Test substance	:	ZnO
Concentration and vehicle	:	The test substance was prepared as a 50% w/v paste in distilled water
Dose	:	Single dose of 2000 mg/kg applied uniformly on approximately 10% of the total surface of the animals.
Observation period	:	14 days
Results	:	LD_{50} > 2000 mg/kg. No clinical signs of toxicity were observed during this study. No deaths occurred.
Conclusion	:	ZnO is non-toxic after a single dermal application

Ref. : 8

2.3.3. Acute inhalation toxicity

In Great Britain, the occupational exposure standards of ZnO fume are 5 mg/m³ (long term) and 10 mg/m³ (short term).

In the USA, the permissible exposure limit is 5 mg/m³ (long term) and the recommended exposure limits are 5 mg/m³ (long term) and 15 mg/m³ (maximum; short term).

Ref. : 65

2.3.4. Repeated dose oral toxicity

Species	:	Sprague-Dawley female rats
Number of animals	:	64 in 4 groups of 16 rats each
Test substance	:	ZnO
Dose	:	400 ppm ZnO in the diet (approx. 40 mg/kg bw/day) with or without co-administration of 184.000 µg Vitamin A equivalents /kg feed (basal diet as control group)
Exposure period	:	120 days

The study was designed to investigate the effects of chronic feeding of high levels of vitamin A in combination with zinc on total lipids, cholesterol, vitamin A and zinc concentrations in the liver and serum cholesterol.

Results
The administration of ZnO oxide at a dose level of 40 mg/kg bw/d for a period of 120 days in the diet did not lead to any noticeable clinical toxicity. In the high zinc groups a significant elevation of serum cholesterol was observed as the only biologically relevant effect which was independent from the vitamin A co-administration.

Ref. : 13

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

Animal study

Method	:	standard toxicological study of 13-week duration
Species	:	ICR mice and Wistar rats
Number of animals	:	12 animals of each sex
Test Substance	:	$ZnSO_4 .7 H_2O$
Dose	:	0, 300, 3000 and 30,000 ppm in the diet (mice: 42.7/46.4; 458/479 and 4927/4878 mg/kg/d for males/females; rats : 23.2/24.5, 234/243 and 2514/2486 mg/kg/d for males/females)
Exposure period	:	13 weeks

The clinical signs of the animals were observed every day, they were weighed weekly and their diet and water intake measured twice a week. Dead and moribund animals were necropsied and samples

for histopathological observations were taken. Blood for haematological and biochemical examinations was taken.

Results
ICR mice : at the high dose level 4 males and I female were found dead or killed in extremis. High dose animals showed a moderate decrease in hematocrit, haemoglobin and leucocyte counts (only males). Total protein, glucose and cholesterol were reduced and alkaline phosphatase and urea nitrogen were increased in high dose animals. High dose females showed reduced ALAT and increased calcium levels, ASAT was increased in high dose males. Absolute and relative thyroid weights of males and kidney weights of females were increased at the highest dose. Gross pathology and histopathology showed changes in kidneys, thyroids, pancreas (degeneration/necrosis of acinar cells, clarification of nucleoli), gastrointestinal tract, and spleen.

The NOEL for mice in this study was found at the mid dose at 3000 ppm (about 470 mg $ZnSO_4.7$ H_2O/kg bw (104 mg Zn^{2+}/kg bw).

Wistar rats : at the high dose level a moderate reduction in leucocyte counts, slightly decreased hematocrit and haemoglobin values (males only) and decreased total protein and cholesterol values were recorded. Absolute and relative liver weights and relative kidney weights were decreased in high dose males. Histopathology showed pancreatic damage (degeneration, necrosis of acinar cells, clarification of centroacinar cells and interstitial fibrosis) in high dose animals.

The NOEL for rats was found at the mid dose at 3000 ppm (about 240 mg $ZnSO_4.7$ H_2O/kg bw for males and females, respectively (53.5 mg Zn^{2+}/kg bw).

Ref. : 14

Human studies

Since zinc is an essential trace element for all mammalian species, its presence in the diet of humans and animals as well as in drinking water is required. In this regard, the mammalian organism is used to handle varying quantities of externally administered zinc, such that Zn^{2+} homeostasis is regulated via control mechanisms of absorption and excretion. In humans, the average daily intake is estimated at about 15 mg of Zn^{2+}. The available evidence from human studies points to a NOEL of about 0.5 mg/kg/day and thus confirms the safe exposure level, which could be predicted from the animal data.

A series of human experimental studies have been published which report adverse effects from oral administration of zinc salts :

* 47 healthy volunteers ingested $ZnSO_4$ tablets three times a day for 6 weeks, resulting in a total daily dose of 2 mg Zn^{2+}/kg bw. Headaches, nausea, vomiting, loss of appetite and abdominal cramps were seen in 26 of the volunteers.

Ref. : 15

* Oral doses of 160 mg $ZnSO_4$/day (2.3 mg Zn^{2+}/kg bw/day) given to 12 healthy adult men for 35 days resulted in decreased HDL cholesterol, total serum cholesterol showed no change.

Ref. : 16

* $ZnSO_4$ tablets were administered twice daily to I I adult men for a total intake of 300 g/day (4.3 mg Zn^{2+}/kg bw/day). There was a significant increase in serum zinc levels and reduction in lymphocyte stimulating response to PHA after 4 and 6 weeks of treatment. In addition, a slight increase in LDL and a significantly reduced level of HDL cholesterol were observed.

Ref. : 17

* After 6 weeks oral administration of 150 mg $ZnSO_4$/day (2.1/2.5 mg Zn^{2+}/kg bw/day for males/females, respectively), the total plasma and HDL cholesterol remained unchanged, whereas the LDL cholesterol was significantly decreased in female volunteers.

Apart from these studies using dosages close to the threshold for adverse systemic effects, the following reports confirm safe exposure levels in humans: In a double-blind trial, 13 humans received 200 mg zinc sulphate (corresponding to about 1.35 mg Zn^{2+}) three times a day for 18 weeks, while 14 humans received a placebo. No signs of toxicity associated with the zinc treatment were reported (18). In a study of Greaves and Skillen also no toxicity was seen in 18 humans after administration of 220 mg zinc sulphate (about 150 mg Zn^{2+}) 3 times a day for 16-26 weeks.

Ref. : 19

Finally, the administration of 50 mg Zn^{2+}/day (as zinc gluconate) in a 10-week study with female volunteers is considered as a LOAEL for humans after repeated dosing, being 0.83 mg Zn^{2+}/kg bw/day (taking into account the dietary background level of 9.72 mg Zn^{2+}/day for females, the LOAEL would be 0.99 mg Zn^{2+}/kg bw/day) (Ref. : 55). Based on this human study, the Health Council of the Netherlands (1998) established in agreement with the recommendation of the Commission of the European Communities (1993), a Maximal Tolerable Daily Intake (MTDI) of 30 mg zinc/day (0.5 mg/kg/day).

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)

Method : OECD 404
Species : New Zealand rabbits

Number of animals	:	3 males
Test Substance	:	ZnO (purity unknown)
Site of application	:	Right flank, shaved 24 hours before the test application for 4 hours.
Concentration	:	The test substance was suspended 1:1 (w/w) in distilled water. 0.5 ml of this test article was applied with the help of hydrophilic gauze.
Observation times	:	1, 24, 48 and 72 hours after removal of the patch.

Results

Animal Number	Erythema			
	1h	24h	48h	72h
0280	0	0	0	0
0302	0	0	0	0
0309	0	0	0	0

Animal Number	Oedema			
	1h	24h	48h	72h
0280	0	0	0	0
0302	0	0	0	0
0309	0	0	0	0

Conclusion : ZnO is not a skin irritant.

Ref. : 20

Methods	:	Repeated patch test		
Species	:	New Zealand White rabbits, TO (outbred) and AG2 (inbred) mice, Dunkin-Hartley white guinea pigs		
Number of animals	:	8 rabbits, 6 mice, 8 guinea pigs		
Test substance	:	ZnO (98% purity)		
Application	:	Once daily on 5 consecutive days		
Site of application	:	Shaved dorsal skin sites of 5 cm 2		
Concentration	:	Application of 0.5 ml/day as a 20% aqueous suspension in 0.1% Tween 80.		
Application conditions:		Rabbits, mice, Guinea pigs	:	open patch test.
		Second rabbit study	:	occlusive patch test
Observations	:	24 hours after last application, samples for histological evaluation were taken		

Results

Macroscopic observations in rabbit, mouse and Guinea-pig skin exposed to ZnO for 5 consecutive days

Treatment	Rabbit (open patch test)		Rabbit (occlusive patch test)		Mouse		Guinea pig	
	N	Irritancy	N	Irritancy	N	Irritancy	N	Irritancy
ZnO	4	-	4	-	6	-	8	-
0.1 %Tween 80	4	-	4	-	6	-	8	-
Deionized H_2O	4	-	4	-	6	-	8	-

ZnO exhibited no overt skin irritation or other histological effects in rabbit, mouse or Guinea pig.

Conclusion
ZnO is not a skin irritant after repeated application in different animal species.

Ref. : 21

2.4.2. Irritation (mucous membranes)

Method	:	Directive 92/69/EEC B.5 and OECD 405
Species	:	New Zealand White rabbits
Test substance	:	ZnO (particle size unknown)
Site of application	:	Conjunctiva sac of one eye
Concentration	:	Approximately 64 mg of the test substance (0.1 ml) were instilled into one eye of the rabbit, the other eye remained untreated and served as control. After 24 hours both eyes of two animals were rinsed with water.
Observation times	:	1, 24, 48 and 72 hours after instillation

Results
No symptoms of systemic toxicity were observed and no mortality occurred. Slight iridial irritation (grade 1) was observed in one animal, at 1 hour only. Slight irritation of the conjuctivae (grade 1-2) was seen as redness (mean scores over 24-72 hours 0.7, 1 and 1), which had completely resolved at 72 hours in all animals. Chemosis (grade 2) and discharge (grade 1) were also observed in all animals, but at 1 hour only. No corneal opacity or epithelial damage was observed in any of the animals.

Conclusion
According to EU criteria, ZnO is considered non irritating to the eyes.

Ref. : 22

Method	:	Draize test
Species	:	Japanese white rabbits.
Number of animals	:	6 males
Site of application	:	Into the eye lids
Test substance	:	Ultra-fine ZnO, white powder

Concentration : The test substance was prepared as a 6% w/w aqueous suspension and instilled at a volume of 0.1 ml. The eyelids were closed for approximately one second. The eyes of 3 animals were rinsed with tap water after 30 seconds of the instillation.

Observation times : 1, 2, 3, 4 and 7 days after instillation of the test substance referring to Draize criteria.

Results
Total Score of Ocular Lesions with ultra-fine ZnO (6% w/w aqueous suspension)

Animal No.		Total Score Rate				
Not rinsed		After 24 h.	After 48 h.	After 72 h.	After 4 d.	After 7 d.
	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	average	0	0	0	0	0
Rinsed	7	0	0	0	0	0
	8	0	0	0	0	0
	9	0	0	0	0	0
	average	0	0	0	0	0

Conclusion
Ultra-fine ZnO was evaluated as negative irritant for the eye according to the test result of a single dose instillation eye irritation test in the rabbit.

Ref. : 23

2.5. Sensitisation

Method : Directive 96/54/EC B.6 and OECD 406
Species : Female Dunkin Hartley Guinea pigs
Test substance : ZnO (purity 99.69%)
Number of animals : 10 per test group, 5 in control group
Induction : Intradermal injection of 20% test substance in water and epidermal exposure to a 50% concentration (highest practically feasible concentration). Similar treatment of control group with water alone. Approx. 24 hours before epidermal induction exposure all animals were treated with 10% SDS (sodium dodecyl sulfate).
Challenge : Two weeks after epidermal application all animals were challenged with a 50% test substance concentration and the vehicle.

Results
In the first study, in response to the 50% test substance concentration skin reactions of grade 1 were observed in 4/10 experimental animals 24 hours after the challenge, while no skin reactions were evident in the controls. In the second study no skin reactions were evident in the experimental animals, while a skin reaction grade I was seen in one control animal. Combining both studies 4/20 animals (20%) showed skin reactions

Conclusion
According to EU criteria, ZnO is a non-sensitiser to the skin and, as such, does not have to be classified for skin sensitisation.

Ref. : 24a, 24b

Method	:	Directive 96/54/EC B.6 and OECD 406 (Guinea pig max. test)
Species	:	Female Dunkin Hartley Guinea pigs
Number of animals	:	10 per test group, 5 per control group
Test substance	:	ZnO (Zinkweiss Pharma A, purity 99.9%)
Induction	:	Intradermal injection of 2 % test substance in water (highest reproducibly injectable concentration) and epidermal exposure to a 50% concentration (highest practically feasible concentration). Similar treatment of control group with water alone. Approx. 24 hours before epidermal induction exposure all animals were treated with 10 % SDS.
Challenge	:	Two weeks after epidermal application all animals were challenged with a 50% test substance concentration and the vehicle.
Results	:	None of the treated animals showed a skin reaction.

Conclusion
According to EU criteria ZnO does not have to be classified for skin sensitisation and is a non-sensitiser to the skin.

Ref. : 25

Method	:	Human Repeat Insult Patch Test.
Number of volunteers	:	60 (56 completed the test)
Test substance	:	Microfine ZnO coated with 2.5% dimethicone [25% suspension of the test substance in corn oil (dimethicone content of the test article: 0.625%)]
Concentration	:	0.2 ml of a 25% suspension of the test substance in corn oil
Induction	:	9 occlusive 24-hour exposures every other day for 3 consecutive weeks
Challenge	:	After a 10-14 day rest period, a single treatment was applied to a previously unexposed test site.
Observations	:	Reactions were scored 24 and 48 hours after application.
Results	:	No adverse reactions were noted during the course of the study.

Conclusion
ZnO as tested was neither a skin irritant nor a skin sensitiser.

Ref. : 26

2.6. Reproductive toxicity

2.6.1. Teratogenicity

Species	:	Female Wistar rats

Number of animals	:	25 animals per group
Test substance	:	$ZnSO_4$ (unspecified) Aspirin (positive control)
Doses	:	Zinc sulfate : 0, 0.4, 2.0, 9.1 and 42.5 mg/kg by gavage in water; Aspirin : 250 mg/kg
Exposure period	:	Day 6-15 of gestation
Procedure	:	The effects of $ZnSO_4$ were assessed using daily clinical observations in the pregnant dams, body weight determination on day 0, 6, 11, 15 and 20 p.c., and food consumption throughout the experimental period. Necropsy was performed on day 20 p.c. The pregnant uteri and ovaries were examined for numbers of corpora lutea, implantations, viable foetuses and resorptions. All foetuses were weighed, sexed and examined for gross external defects. In addition, skeletal examinations (in two-thirds of the foetuses) and soft tissue examinations (in the remaining one-third of foetuses) were performed.

Results
No compound-related effects were observed at any dose level. In particular, there was no indication of any specific teratogenic activity of $ZnSO_4$ under the conditions of this experiment.

Conclusion
The No Observed Effect Level (NOEL) with regard to maternal and developmental toxicity of $ZnSO_4$ was 42.5 mg/kg (corresponding to about 17 mg/kg if expressed as zinc).

Ref. : 27

Species	:	Female Dutch rabbits
Number of animals	:	14-19 animals per group
Test substance	:	$ZnSO_4$ (unspecified) 6-amino-nicotinamide (positive control)
Doses	:	$ZnSO_4$: 0, 0.6, 2.8, 13.0 and 60.0 mg/kg by gavage in water 6-amino-nicotinamide : 2.5 mg/kg by gavage
Exposure period	:	$ZnSO_4$: day 6-18 of gestation (p.c.) 6-amino-nicotinamide: single application at day 19 of gestation
Procedure	:	The effects of $ZnSO_4$ were assessed using daily clinical observations in the pregnant dams, body weight determination on day 0, 6, 12, 18 and 29 p.c., and food consumption throughout the experimental period. Necropsy was performed on day 29 p.c. The pregnant uteri and ovaries were examined for numbers of corpora lutea, implantations, viable foetuses and resorptions. Foetuses were weighed, sexed and examined for gross external defects, in addition skeletal examinations and soft tissue examinations were performed in all foetuses.

Results
No compound-related effects were observed at any dose level. In particular, there was no indication of any specific teratogenic activity of $ZnSO_4$ under the conditions of this experiment. The positive control substance 6-aminonicotinamide induced the expected embryotoxic effects (increased number of resorptions and birth defects).

Conclusion
The No Observed Effect Level (NOEL) with regard to maternal and developmental toxicity of Zinc Sulfate was at least 60.0 mg/kg (corresponding to about 24 mg/kg if expressed as zinc).

Ref. : 28

Species	:	Female Sprague Dawley: CFE rats
Number of animals	:	10 animals per group
Test substance	:	ZnO

Doses and exposure period
7 groups received the test substance at levels of 2000 (about 100 mg/kg/d) or 4000 ppm (about 200 mg/kg/day) in the diet. 7 equally sized control groups received the basal diet without test substance. 4 groups were fed the test diets at 4000 ppm from day 0 through day 15, 16, 18 or 20 of gestation. In a separate experiment, 3 dose groups (2 at dietary levels of 4000 and 1 at 2000 ppm) received test diets starting 21 days prior to the mating until day 15 or 16 of gestation.

Procedure
The effects of ZnO were assessed using reproduction and litter data as well as zinc, iron and copper content in maternal and foetal tissues.

Results
The dietary level of 2000 ppm ZnO administered from day 21 prior to mating until day 15 of gestation induced no adverse effects on foetal development. In contrast, marked embryotoxic (embryo-lethal) effects were observed after the different treatment regimens at the high dietary level of ZnO. In dose groups which received the test diet from day 21 prior to mating until day 15 or 16 of gestation, complete resorption (100% prenatal death) were observed in all treated dams. Determination of tissue levels revealed a dose related increase in maternal whole body and liver zinc concentration and decreased copper concentrations particularly in maternal livers. Similar changes in electrolyte concentrations were observed in foetal whole body samples. These analytical results support the interpretation that the observed developmental effects of exaggerated high zinc doses are mediated by depletion of certain essential minerals such as copper.

Conclusion
ZnO, 2000 ppm, administered from day 21 prior to mating until day 15 of gestation induced no adverse effects on foetal development. This dietary level corresponds to a daily dose of about 100 mg/kg expressed as ZnO, or about 80 mg/kg expressed as zinc which represents the NOEL in this study.

Ref. : 29

2.7. Toxicokinetics (incl. Percutaneous Absorption)

Method	:	*In vivo*, human
Volunteers:	:	6 healthy males
Test substance	:	ZnO
Site of application	:	Chest, upper and lower legs
Exposure	:	Daily application of 40% ZnO in ointment for a of period 10 days

Procedure	:	Following the application, blood samples were drawn from each subject at 1-hr intervals for a total of 3 hours. Serum zinc concentrations were determined by flame atomic absorption spectrometry.

Result
Topical application of the ZnO preparation had no statistically significant effect on serum zinc concentrations.

Conclusion
Topical application of ZnO in ointment does not affect systemic levels of zinc.

Ref. : 30

Method	:	*in vivo*, human
Volunteers	:	31 subjects with chronic psoriasis
Test substance	:	ZnO
Concentration	:	ZnO -containing ointments were applied during 14 day at a daily dose of 2.68 g Zn^{2+}
Procedure	:	Blood samples were obtained prior to treatment (i.e., baseline) and 7 and 14 days of treatment. Plasma Zn^{2+} concentrations were determined using a flame-ionisation atomic absorption spectrophotometer.

Results
There was no significant increase in plasma Zn^{2+} concentrations in any individual over the 2-week study period.

Conclusion
Even in diseased skin, dermal Zinc oxide application did not increase plasma zinc levels.

Ref. : 31

Method	:	*in vitro*, human skin
Skin samples	:	Obtained from the abdomen of surgery patients.
Test substance	:	Mixtures of ZnO, $ZnSO_4$, ZnPC (zinc 2-pyrrolidone 5-carboxylate) and CuPC (copper 2-pyrrolidone 5carboxylate), $CuSO_4$
Concentration	:	5 formulations were used (3 emulsions and 2 ointments) :

Emulsion A (water/oil, 0.25% (w/w) of ZnPC + 1.5% (w/w) ZnO + 0.5% (w/w) CuPC;
Emulsion B (water/oil, 0.25% (w/w) of $ZnSO_4$ + 1.5% (w/w) ZnO + 0.5% (w/w) $CuSO_4$;
Emulsion C (water/oil, 0.1% (w/w) of $ZnSO_4$ + 7% (w/w) ZnO + 0.2% (w/w) $CuSO_4$;
Ointment D 0.1% (w/w) $ZnSO_4$ + 10% (w/w) ZnO + 0.35% (w/w) $CuSO_4$;
Ointment E 10% (w/w) ZnO + 0.2% $CuSO_4$.

16 mg/cm^2 (1.88 mg Zn^{2+}/cm^2) of each formulation was applied to the outer skin surface.

Procedure
After 72hrs, any remaining formulation persisting on the treated skin surface was gently removed using cotton swabs. The receptor solution (isotonic saline) was replaced after 2, 4, 12, 24, 48 and 72hrs. The stratum corneum was stripped twice using adhesive tapes. Samples of dermis and whole epidermis were taken from treated skin. Zinc and copper concentrations were determined in receptor solution and in the skin preparations by atomic absorption spectrometry. Values were normalized by dividing Zn^{2+} concentration in control skin (percent of Zn^{2+} increase) and then divided by applied dose of Zn^{2+}, mg/cm^2.

Results
The maximum concentration of Zn^{2+} was 0.34 ± 0.22% of the applied dose of ZnO recovered in the receptor fluid after 72 hr. The relative increase of Zn^{2+} was 0.5% in the epidermis and 1.1'% in the dermis.

Conclusion
The systemic availability of dermally applied ZnO as tested (determined as fraction analysed in the receptor fluid) was found to be 0.34%.

Ref. : 33

Method	:	*in vitro,* pig skin
Test substance	:	$ZnSO_4 \times H_2O$ and ZnO
Concentration	:	Aqueous solutions of Zinc sulfate monohydrate and ZnO were applied to the skin preparations at a concentration of 40 mg/ml

Procedure
Skin preparations measuring 1 mm in thickness with stratum corneum, stratum germinativum and blood vessel containing parts of the dermis were obtained from pigs using a modified dermatome. In two independent experiments for each compound 7 skin preparations were mounted in Teflon flow-through diffusion chambers, which were continuously rinsed with physiological receptor fluid (0.9% NaCl). After checking the integrity using caffeine as a marker substance, each of the test formulations was applied to 6 skins at a dose of 1 mg/cm² for 8 hours without occlusion and subsequently washed off with a neutral shampoo. After 0, 2, 4, 6, 8, 16, 24, 40, 48, 64 and 72 hours, the cutaneous permeation was determined by quantifying zinc with atomic absorption spectroscopy (detection limit 10 ng/ml) in the receptor fluid. The experiment was stopped at 72 hours. Furthermore, zinc was analysed in the skin preparations and the rinsing fluids. The amounts of zinc detected in receptor fluid and different layers of the skin were corrected for background levels of endogenous zinc.
Results
The following dermal absorption rates (% of applied dose) through pig skin in vitro within 72 hours were determined (corrected for background levels of zinc)

Skin compartment	Zinc sulfate	ZnO
Receptor fluid	0.3 %	0.03%
Horny layer	1.3 %	12.3 %
Residual skin	0 %	2.6 %

Total recovery of applied zinc in both experiments ranged from 82.0-109%.

Conclusion
Based on the amount recovered at 72 hours from the receptor fluid, the systemically available amount of zinc can be considered to be < 1 % of the applied dose.

Ref. : 34

2.8. Genotoxicity/Mutagenicity

Existing literature data on the *in vitro* and *in vivo* studies on mutagenicity and/or genotoxicity of Zinc Oxide and Zinc salts have not been included in the report.
In its general summary, the COLIPA submission I of 26 November 2001 includes a table that has been prepared by TNO. This table reports the summary data on mutagenicity/genotoxicity studies. From the table, it possible to conclude that Zinc Oxide and Zinc salts have mutagenic and /or genotoxic *in vitro* activity (gene mutation, chromosome aberrations and UDS in mammalian cell).
In vivo studies presented are not conclusive (some positive results have been reported : bone marrow cytogenetic assay in rodents and host-mediated assay in mice).
We presume that all these studies refer to the non-micronized material. It is possible that zinc oxide under the micronized form may display a genotoxic profile of some concern.

Ref. : 3

2.9. Carcinogenicity

No data

2.10. Special investigations

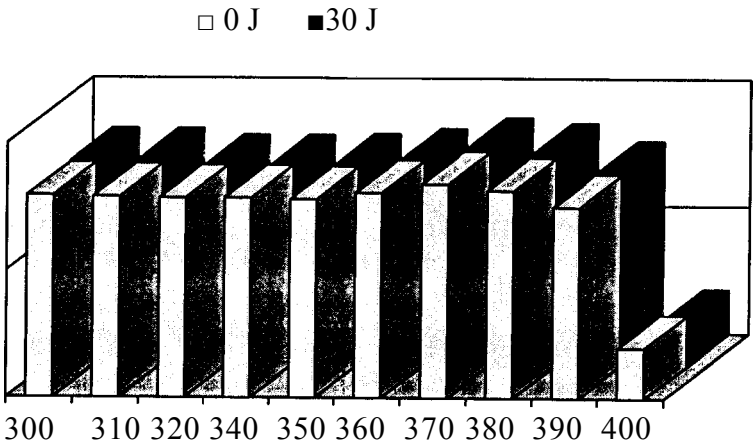
No data

2.10.1. Photo-chemical properties

Method	:	Photostability as measured by UV absorption
Test substance	:	15% microfine (< 0.2μm) ZnO (non-coated)
Light source	:	Oriel Xenon Arc Solar Simulator

A thin film, 2 mg/cm^2, of the sunscreen product was applied to a synthetic substrate and, using a using a solar simulator, irradiated. The absorption curves were measured after exposure to 0 and 30 joules /cm^2 solar-simulated UV.

Results



ZnO based sunscreen before and after UV irradiation

Conclusion
As can be seen, the absorption curve for ZnO was unchanged following exposure to 30 J/cm^2 solar simulated UV by pre-irradiation demonstrating complete photo-stability.

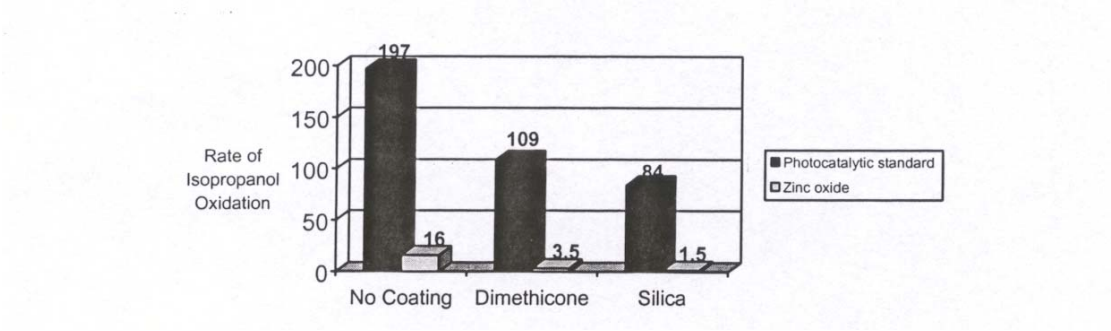
Ref. : 39

2.10.2. Photo-reactivity

Photoreactivity can be defined as the propensity of a given molecule/crystal to react with neighboring molecules when irradiated. The photoreactivity of pigments (i.e. ZnO) can be easily measured using a well-recognised assay. Briefly, the tests are performed in a small batch photoreactor (200 cm³ capacity), held at a constant temperature (24 °C), and under constant focused photon flux from a 500W medium pressure mercury arc lamp (Priel Products Ltd). The photoactivity index is measured as the zero order rate of the photocatalytic oxidation (reactive oxygen species) of liquid propan-2-ol to propanone, under oxygenated conditions for a fixed mass of particulate. Photoactivities are expressed in terms of moles converted per gram of particulate per hour of irradiation. Propanone analysis was conducted at regular intervals using gas chromatography (Perkin Elmer GC 1830).

The photoreactivity of microfine ZnO and a photocatalytic standard was measured using the method described above. Non-coated, dimethicone coated and silica coated versions of both pigments were measured.

Results



Measurement of the photoreactivity of microfine ZnO and a photocatalytic standard. Non-coated, dimethicone coated and silica coated versions of both pigments were measured

Conclusion
As shown in figure 2, microfine ZnO is virtually non-photoreactive. Note that the error on the test is ~3.0.

Ref. : 39

Sunscreen formulations (2 mg/cm^2) were placed upon a hydrated collagen substrate (Vitro-Skin). Using a solar simulator (Oriel Xenon Arc Solar Simulator), the thin film was irradiated and samples taken at 0, 10, 20 and 30 joules/cm^2. The samples were digested and the percent octyl methoxycinnamate (OMC) remaining was determined by HPLC. A 50:50 mix (coated:noncoated) was used to test the hypothesis that ZnO does not need to be coated to be non-reactive.

Test substance
Two formulations were studied. One contained only 7.5% OMC as the sunscreen and the other contained 7.5% OMC in combination with 10% microfine ZnO. The microfine ZnO used was a 50:50 mix of commercially available coated (dimethicone) and non-coated particles. The dimethicone coating contributed 1 % of the weight of the powder.
Results
Lack of interaction of microfine ZnO and octyl methoxycinnamate (OMC) under UV irradiation

Formula	Percent OMC Remaining		
	10 J/cm^2	20 J/cm^2	30J/cm^2
7.5 % OMC	85.1	80.7	78.1
7.5% OMC + 10% Microfine ZnO	87.5	85.8	83.8

Conclusion
ZnO was shown to be non-photoreactive.

Ref. : 39

2.10.3. Phototoxicity / Photoirritation

Method	:	Neutral Red Uptake Phototoxicity Test in *vitro* (EU Directive 67/548/EEC, Annex V, B41)
Cell type	:	Balb/c 3T3 mouse fibroblasts, Clon 31
Test substance	:	Micronised ZnO (uncoated) Chlorpromazine (positive control)
Test vehicle	:	ZnO in EBSS/ 1% (v/v) ethanol solubilised/dispersed in a concentration range of 1.0 to 100.0 mg/l. Chlorpromazine in EBSS
Light source	:	Metal-halide lamp with cut-off filters according to Spielmann et al. (40, 41). UVA 1667 mW/cm^2 for 50 min (5.0 J/cm^2), UVB: non-detectable.

Results and conclusion
Both ZnO irradiated and unirradiated had similar cytotoxic concentration response curves. Both curves were similarly shaped with nearly identical NR_{50} values at approximately 9 mg/L. The ratio of [NR_{50} (-UV)/ NR_{50} + UV)] PIF equals 1, demonstrating that ZnO does not exhibit a phototoxic potential in this test.

Ref. : 42

Method	:	Occlusive patch test on humans
Volunteers	:	30
Test substance	:	Micronised ZnO
Concentration	:	25% test substance in cosmetic w/o emulsion
Application	:	Test articles were applied for 24 hours to the skin of the back of the volunteer by using HAYES-test chambers (provide occlusive patch effect), consisting of a plastic capsule 10 x 10 mm, providing a test area of 100 mm² and holding a volume of approx. 50 µ1. These chambers were arranged in strips of 2 x 5 patches forming a patch set.
Irrad. Cond.	:	One duplicate patch site on the right back or arm within 10 min after patch removal with 18 J/cm² UV-A.
Observ. Period	:	Skin reactions were read 0, 24 and 48 hours after patch removal in the pretest for phototoxicity.

Results
No phototirritant skin reaction in any volunteer was observed.

Conclusion
ZnO as tested was not photo-irritant.

Ref. : 43

Method	:	Occlusive patch test in humans
Volunteers	:	10
Test substance	:	25% ZnO (dimethicone coating)

Application and irradiation conditions
Test sites were tape stripped with hypo-allergenic tape 3 times to remove several layers of cornified epithelium. 0.2 g or 0.2 ml of the test material or hydrophilic ointment USP (negative control) was placed onto a 2 cm x 2 cm occlusive patch, then applied to the non-irradiated

control site (left arm) and allowed to remain in place for 24 hours. Concurrently the test material or negative control was applied to the right arm directly onto the skin. The site was then irradiated with non-erythemogenic UV-A irradiation at a distance of 10 cm of the source and receiving a UV-A light dosage of greater than 4.4 mW/cm^2 UV-A. The test site was then covered with occlusive patch containing additional test material (0.2 g or 0.2 ml).

Observation times
Skin reactions were read 0, 24, 48 hours and I week after patch removal.

Results
No adverse effects were observed.

Conclusion
ZnO as tested was not photo-irritant.

Ref. : 44

2.10.4. Photosensitisation / Photoallergy

Method : Photo maximisation/photoallergy test
Volunteers : 30
Test substance : micronised ZnO
Concentration : 25% test substance in a cosmetic emulsion (w/o)

Application : Test articles were applied to the skin of the back of the volunteer by using HAYES-test chambers (provide occlusive patch effect), consisting of a plastic capsule 10 x 10 mm, providing a test area of 100 mm² and holding a volume of approx. 50 µl. These chambers were arranged in strips of 2 x 5 patches forming a patch set.

Pretest : Testing of irritant and photo-irritant properties by applying the test articles once for 24 hours in duplicate and irradiating with UV-A immediately after patch removal.

Induction : 6 times under occlusion for 24 hours within three weeks and irradiation with UV-B immediately after patch removal. The patch site on the right back or arm was exposed to approximately twice the MED (minimal erythema dose) of UV-B within 10 min. after patch removal. If the skin became tanned after several exposures, irradiation time was increased in order to induce sufficiently intense erythema. A non-irradiated patch site was deemed unnecessary during induction and was not applied.

Challenge : One application under occlusion for 24 hours after 10 to 14 days in duplicate. UV-A irradiation of one duplicate immediately after patch removal. Patch areas on an untreated site were irradiated with 18 J/cm^2 of UVA within 10 min. after patch removal.

Skin Evaluations
Pretest : 0, 24 and 48 hours after patch removal.
Induction : 0, 24, 48 and 72 hours after patch removal, “Thursday”-patches immediately and 72 hours after patch removal.
Challenge : 0, 24, 48 and 72 hours after patch removal.
Results
ZnO did not produce any photoallergic skin reaction.

Conclusion
ZnO can be considered as non-photosensitising under the conditions of this study.

Ref. : 43

Method	:	Photo maximisation/photoallergy test
Volunteers	:	25
Test substance	:	25% ZnO (dimethicone coating)
Irrad. System	:	150 W xenon arc solar simulator (Solar Light Co., Philidelphia PA)

Determination of Minimal erythema dose (MED) : Prior to the testing of sunscreen products, the sensitivity of the unprotected skin of each subject was determined in seconds, based on the length of exposure, which first elicits a slight reddening of the skin, as observed 24 hour following exposure.

Induction : Duplicate test areas (2.5 x 2.5 cm), to which 62.5 µl of the test material was evenly applied (density 10 mg/cm^2), were delineated on the subject's back. Sites were then covered with 2 cm x 2 cm occlusive patches. After 2 hours the test sites were wiped dry and one set was exposed to 3 MED's (minimum erythema dose) of solar simulating radiation while the alternate set was again left non-irradiated. This sequence was repeated for a total of 6 exposures twice weekly.

Challenge : 10-14 days after the final induction exposure. Similar occlusive applications are made for 2 hours to a previously unexposed untreated, area of normal skin; the sites were then irradiated with 4.0 J/cm^2 of UVA radiation.

Skin Evaluation : 48 and 72 hours post challenge irradiation.

Results
No adverse effects or unexpected reactions of any kind were observed on any of the subjects.

Conclusion
ZnO can be considered as non-photosensitising under the conditions of this study,

Ref. : 45

2.10.5. Photo-mutagenicity

Bacterial Reverse Mutation Test

Guideline	:	Notes of Guidance 2000
Species/strain	:	*Salmonella typhimurium,* TA98, TA100, TA1537, *E. coli* WP2
Replicates	:	Triplicate plates, only 1 experiment performed
Test substance	:	HR96/104702 dissolved in 10 % emulsion in 3 % Tego Care 450
Batch no	:	(Batch 50108745, purity 97.2 %)
Concentrations	:	direct plate incorporation assay without S9 mix. TA 98 : 2, 6.32, 20, 63.25 and 200 µg/plate TA 1537 : 20, 63.25, 200, 632.5 and 2000 µg/plate TA 100 and WP2 : 50, 158.1, 500, 1581 and 5000 µg/plate

Positive Control : According to the strains + 8-MOP
UV irradiation : Heraeus Suntest CPS light
: Dose of UV :
UVA 35/41 mJ/cm^2/min
UVB 2.3/2.6 mJ/cm^2/min
GLP : Quality Assurance statement included

Results

Without UV
TA 98 : From 0 to 200 µg/plate no increase in the number of revertants was observed.
TA 100 : From 0 to 5000 µg/plate no increase in the number of revertants was observed.
TA 1537 : From 0 to 200 µg/plate no increase in the number of revertants was observed.
E. coli WP2 : From 0 to 5000 µg/plate no increase in the number of revertants was observed.

With UV
UVA 8 mJ/cm^2/min
TA 98 : From 0 to 200 µg/plate no increase in the number of revertants was observed.

UVA 4 mJ/cm^2/min
TA 100 : From 0 to 5000 µg/plate no increase in the number of revertants was observed.
TA 1537 : From 0 to 200 µg/plate no increase in the number of revertants was observed.

UVA 90 mJ/cm^2/min
E. coli WP2 : From 0 to 5000 µg/plate no increase in the number of revertants was observed.
8-MOP at dose of 500 µg/plate and irradiated with UVA 90 mJ/cm^2/min gives an increase of more 10 times in the number of revertants

Conclusions
Based on the reversion rate, the test agent HR96/104702 dissolved in 10 % emulsion in 3 % Tego Care 450 at different concentrations, with or without UV irradiation, it is concluded that no mutation were induced in *Salmonella typhimurium* and *E. coli* tester strains.
Micronized material has no mutagenic activity on bacteria cells; it is not photomutagenic in the same cell system.

Ref. : 50

***In vitro* Mammalian Chromosomal Aberration Test**

Guideline : Notes of Guidance 2000
Species/strain : Chinese Hamster Ovary Cells
Replicates : Duplicate cultures, independent experiment
Test substance : HR96/104702 dissolved in 10 % emulsion in 3 % Tego Care 450
Batch no : Batch 50108745, purity 97 %
Positive Control : 4-NQO and 8-MOP.
Concentrations : 7 doses from 95.74 - 813.8 µg/ml
UV irradiation : Heraeus Suntest CPS light
: Dose of UV :

UVA 35/34 mJ/cm^2/min
UVB 2.1/2.2 mJ/cm^2/min
Doses of UV : 375 and 750 mJ/cm^2/min
GLP : Quality Assurance statement included

Exposure and concentrations
* Samples were exposed during 3 hours with or without UV.
* Cultures were prolonged during 17 hours before harvest.

Results
Structural chromosome aberrations
Without UV
A dose dependence trend of the number of aberrations was found (chromatid and chromosome deletions, chromosome and chromatid exchanges).

With UV (375 and 750 mJ/cm^2/min)
A higher (3 - 4 x) dose dependence trend of the number of aberrations was found than without UV(chromatid and chromosome deletions, chromosome and chromatid exchanges).
Results were repeated in the independent experiment
Statistical analysis of the data indicates that the frequency of aberrant cells excluding gaps is statistically significant at the highest 2 doses in the absence of UV light as compared to controls.
In the presence of UV light, in the first experiment, statistical significance was observed for the highest 3 doses.
In the second experiment with or without UV light, only the highest doses induced a statistically significant increase in the percentage of cells displaying structural aberrations.

Polyploidy
Into account that no specific positive control agent has been used in this assay, and that only metaphases with 19 - 23 chromosomes were considered for scoring, polyploidy means a near tetraploid karyotype.
While not statistically significance has been determined, a biologically relevant increase in the number of poliyploid and endoreduplicated metaphases was observed.

Conclusions
Based on the structural and/or numerical chromosome aberrations observed in the absence and in the presence of UV light, the test agent HR96/104702 dissolved in 10 % emulsion in 3 % Tego Care 450 at different concentrations showed positive effects in cultured Chinese Hamster Ovary (CHO) cells under the conditions of the study.

Micronized material has clastogenic activity on mammalian cells cultured *in vitro*; it has been also shown, that the test agent displays aneugenic activity; it is also photoclastogenic and possibly photoaneugenic in the same cell system.

Ref. : 51

***In vitro* Mammalian Chromosomal Aberration Test**

Guideline : Notes of Guidance 2000

Species/strain	:	Chinese Hamster V79 Cells
Replicates	:	Duplicate cultures, independent experiment
Test substance	:	HR99/104702 dissolved in culture medium
Batch no	:	Batch 50376240, purity > 95 %
Positive Control	:	4EMS and 8-MOP.
Concentrations	:	7 doses Experiment #1 0.4, 1.1, 3.3, 10, 30, 90, 150 µg/ml with or whithout irradiation Experiment # 2 – Without irradiation 2, 4, 7, 10, 13, 16 µg/ml Experiment # 2 – With irradiation 0.5, 1, 2, 3, 6, 12 µg/ml
UV irradiation	:	Xenon lamp Suntest CPS light
Doses of UV	:	Intensity of irradiation UVA/UVB 0.3/0.015 mW/cm^2 Irradiation period : 20 min 50 sec Dose UVA/UVB : 375/18.75 mJ/cm^2

Total treatment time with the chemical : 3 hours

Recovery	:	15 hours.
GLP	:	Quality Assurance statement included

Exposure and concentrations

* Samples were exposed during 3 hours with or without UV.
* Cultures were prolonged during 18 hours before harvest.

Results

Cytotoxicity

Experiment # 1 : the maximum dose gave a reduction of survival of 76 % without irradiation and 61 % with irradiation.

Experiment # 2 : survival was in the range of the control with or without irradiation

Structural chromosome aberrations

Experiment # 1

Without UV : A dose dependence trend of the number of aberrant cells was found (chromatid breaks and chromatid exchanges; a few cells displayed chromosome type aberrations).

With UV : A higher dose dependence trend of the number of aberrant cells was found than without UV (chromatid breaks and chromatid exchanges; a few cells displayed chromosome type aberrations). Statistical analysis showed that the difference was significant as compared to the controls.

Experiment # 2

The results confirmed clearly the results found in the first experiment.

Polyploidy

No difference in the percentage of polyploid cells have been observed as compared to the controls in both experiment.

Conclusions
Based on the structural chromosome aberrations observed in the absence and in the presence of UV light in both experiment, the test agent HR99/104702 dissolved in culture medium at different concentrations, displayed positive effects in cultured Chinese Hamster V79 cells under the conditions of the study. It should be noted that the observed effects were induced with low doses.
This micronized material has clastogenic activity on mammalian cells cultured *in vitro*; it is also photoclastogenic in the same V79 cell system.

Ref. : 49

Photocomet assay *in vitro*

Guideline	:	/
Species/strain	:	HaCaT cells (human keratinocytes)
	:	Chinese Hamster V79 Cells
Replicates	:	Duplicate cultures, only 1 experiment
Test substance	:	HR00/106407 dissolved in deionized water
Batch no	:	Batch 50508802, purity : 93.3 %
Positive Control	:	EMS and Chlorpromazine
Treatment time	:	Not indicated Three sets of data were presented for HaCaT cells: #1 : Without irradiation #2 : Directly after irradiation (with and without pre-incubation in the dark). #3 : 20 hours after irradiation (with pre-incubation in the dark). One set of data was presented for V79 cells: #1: Directly after irradiation (with pre-incubation in the dark).
Concentrations	:	5 doses HaCat - without irradiation 3.9 – 62.5 µg/ml HaCat – directly and 20 hours after irradiation 1.95 – 31.3 µg/ml V79 cells - directly after irradiation 1.95 – 31.3 µg/ml
UV irradiation	:	Heraeus lamp Suntest CPS light HaCaT cells with and without pre-incubation 450/20 mJ/cm^2 UVA/UVB V79 cells with and without pre-incubation 980/40 mJ/cm^2 UVA/UVB HaCaT cells “variation 20 hours” 230/10 mJ/cm^2 UVA/UVB
GLP	:	Quality Assurance statement included

Results
In the presence of UV, V79 cells indicate the induction of DNA damage in both experiments as evidenced by the mean tail length.
HaCaT cells did not showed any induction of DNA damage in the absence of UV irradiation and in all conditions after UV irradiation.

Conclusions
Based on the mean tail length, the test agent HR00/106407 dissolved in deionized water is considered photogenotoxic in cultured Chinese Hamster V79 cells under the conditions of the photocomet assay *in vitro*.

Ref. : 54

Overall conclusions
The micronized material has been found clastogenic, possibly aneugenic and inducing DNA damages in cultured mammalian cells *in vitro*, under the influence of UV light.

2.11. Safety evaluation

NOT APPLICABLE

2.12. Conclusions

A considerable part of the investigations and their results submitted have been performed 15 or more years ago and consequently cannot fulfil modern requirements. However, there is a broad basic knowledge on Zn^{2+} and its compounds, e.g. ZnO.

The physico-chemical specifications of ZnO used in many of the studies are incomplete, the purity/impurities not specified. On the other hand, ZnO is practically insoluble in water. Thus, in general, ZnO may be considered as a non-toxic substance, including when used in cosmetic products.

The main concern of the present evaluation is related to the risk assessment of micronised (approximately 0.2 μm) ZnO, which may be coated by other compounds, and which is used as an ingredient in sunscreen formulations.

Micronised ZnO has been demonstrated to be photoclastogenic, possibly photo-aneugenic, and a photo-DNA damaging agent in mammalian cells cultured *in vitro*. The relevance of these findings needs to be clarified by appropriate investigations *in vivo*.

There is a lack of reliable data on the percutaneous absorption of micronised ZnO and the potential for absorption by inhalation has not been considered.

As to a safety assessment of an use of UV-filters by children over the age of 1 year, the SCCNFP issued a position statement (SCCNFP/0557/02).

2.13. Opinion

Based on the conclusions (point 2.12), the SCCNFP is of the opinion that there more information is needed to enable a proper safety evaluation of micronised Zinc oxide for use as a UV filter in cosmetic products. Consequently, an appropriate safety dossier on micronised ZnO itself, including possible pathways of cutaneous penetration and systemic exposure, is required.

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