EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

OPINION ON

PHENYLBENZIMIDAZOLE SUFONIC ACID AND ITS SALTS

COLIPA S45

Opinion adopted by the SCCP during the 10th plenary of 19 December 2006

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1. BACKGROUND

Submission I on the UV-filter 2-Phenylbenzimidazole-5-sulfonic acid and its salts was submitted December 2005.

The substance is currently regulated in the cosmetics directive in annex VII, part 1 list of permitted UV filters under entry 6, which cosmetic products may contain. 2-Phenylbenzimidazole-5-sulfonic acid is used as an UV-filter in cosmetic products at a maximum concentration at 8% weight/weight (expressed as acid).

According to the preamble to annex VII the authorised UV-filters "*may be added to other cosmetic products within the limits and under the conditions laid down in this annex*."

2. TERMS OF REFERENCE

1. *Does the SCCP consider the use of 2-Phenylbenzimidazole-5-sulfonic acid and its salts in a concentration up to 8% w/w in sunscreen products safe for the consumer?*

2. *Does the SCCP consider the use of 2-Phenylbenzimidazole-5-sulfonic acid and its salts in a concentration up to 8% w/w in other products than sunscreen products safe for the consumer?*

3. *Does the SCCP foresee any other restrictions to the safe use of 2-Phenylbenzimidazole-5-sulfonic acid and its salts?*

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Phenylbenzimidazole Sulfonic Acid (INCI)

3.1.1.2. Chemical names

Ensulizole (INN)
2-Phenylbenzimidazole-5-sulfonic acid
Benzimidazole, 2-phenyl, 5-sulfonic acid

3.1.1.3. Trade names and abbreviations

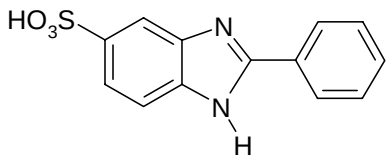
Trade name:	Novantisol®	Neo Heliopan Typ Hydro
	Novantisolsaeure	Eusolex® 232
	Neo Heliopan® Hydro	Parsol® HS
COLIPA n°:	S45	

3.1.1.4. CAS / EINECS number

CAS: 27503-81-7

EINECS: 248-502-0

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_{13}H_{10}N_2O_3S$

3.1.2. Physical form

White to pale beige coloured powder

3.1.3. Molecular weight

Molecular weight: 274.3

3.1.4. Purity, composition and substance codes

Purity: > 98%

3.1.5. Impurities / accompanying contaminants

Impurities:	water:	max. 2%
	heavy metals:	< 10 ppm
	sulphated ash:	max. 0.1%

3.1.6. Solubility

As sodium or triethanolammonium salt at 20°C:

Water: > 30%
Ethanol: miscible

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: -1.1 (pH 5), calculated (ACD logD-Suite)
-2.1 (pH 8), calculated (ACD logD-Suite)

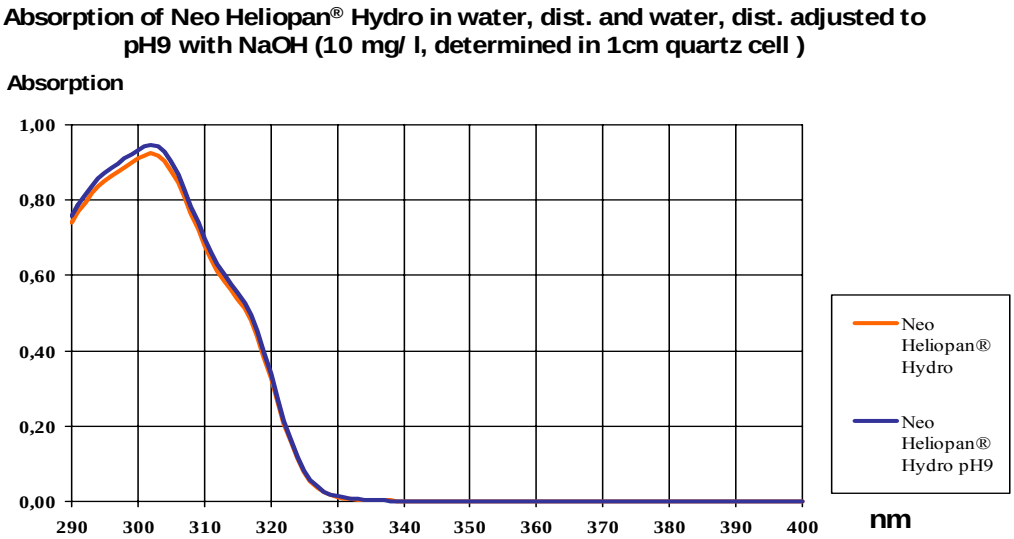
3.1.8. Additional physical and chemical specifications

- melting point: /
- flash point: > 300°C
- vapour pressure: /
- boiling point: /
- density at 20 °C: /
- viscosity: /
- pKa: /
- pH: 4,2 (sat. aqueous solution)
- UV/visible absorption spectrum: /

- Refractive index at 20 °C: /

Spectra:

UV-vis: PBSA λ_{max} = 302 nm E_{max} = 920-990
PBSA-Na λ_{max} = 302 nm E_{max} = 920-990



3.1.9. Stability

No data

General Comments on Physico-chemical characterisation

* Several batches have been used without a proper physico-chemical characterisation.
* Log $P_{ow,}$: calculated values cannot be accepted as estimates of the true physical constants without justification, indicating that the reported values are realistic.
* No data on stability or photo-stability were provided.

3.2. Function and uses

2-Phenylbenzimidazole-5-sulfonic acid (PBSA) shows high absorption (E_{max}= 920-990) in the UVB-region of the spectrum. It is used as a UV-filter in cosmetic sun protection preparations. Maximum use concentration is 8% w/w.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

The acute toxicity of 2-Phenylbenzimidazole-5-sulfonic acid was assessed by the oral route in rats and mice and by the dermal and i.p. routes in rats. All studies are old.

3.3.1.1. Acute oral toxicity

Rat

Guideline: /
Species: male rats, strain Wistar
Group: not started but 'housed in groups of 5'
Substance: Novantisol-Na (a 16.5% solution)
Batch: Pt. 8/70
Purity: /
Dose: 40 ml/kg of the 16.5% aqueous solution via gavage
Observation: 14 days
GLP: not in compliance

6600 mg/kg of Novantisol-Na was the maximum administered. No signs of toxicity were observed and at necropsy, 14 days after administration, no macroscopic changes were observed.

Ref.: 1

Guideline: /
Species: Wistar rats, AF/HAN-EMD-SPF
Group: 5 male 5 females per dosage (30 animals in total)
Substance: Eusolex 232 sodium salt
Batch: /
Purity: /
Dose: 1000, 1280 and 1600 mg/kg body weight by gavage of 20% aqueous solution.
GLP: not in compliance

Immediately after dosing, all animals showed hypokinesia, staggering gait, deep slow breathing. In the highest dose group, 25 minutes after administration all were prone, had bristled fur, dyspnoea and fits. 3 of the 10 animals in the 1600 mg/ky bw died within 2.25 hours. No abnormalities were observed in animals in the two lower does groups at 24 hours. The surviving animals in the highest does group showed no abnormalities by the second day.
At 14 days after administration the surviving animals were killed and no macroscopic changes were observed

Ref.: 2

Guideline: /
Species: Wistar rats, AF/HAN-EMD-SPF
Group: 5 male 5 females (10 animals in total)
Substance: Eusolex 232 triethanolamine salt
Batch: /
Purity: /
Dose: 1600 mg/kg body weight by gavage of 20% aqueous solution.
GLP: not in compliance

Immediately after dosing, all animals showed hypokinesia, staggering, deep slow breathing. By 15 minutes after administration they had bristled fur, and by 30 minutes they were fitting. No abnormalities were observed at 24 hours. At 14 days after administration, the animals were killed and no macroscopic changes were observed.
The LD_{50} oral rat is >1600 mg/kg bw.

Ref.: 3

Mice
Two studies have also been performed.

Guideline:	/
Species:	male mice, strain CF1
Group:	not started but 'housed in groups of 5'
Substance:	Novantisol-Na (a 16.5% solution)
Batch:	Pt. 8/70
Purity:	/
Dose:	40ml/kg of 16.5% aqueous solution via gavage
Observation:	14 days
GLP:	not in compliance

6600 mg/kg of Novantisol-Na was the maximum administered. No signs of toxicity were observed and at necropsy, 14 days after administration, no macroscopic changes were observed.

Ref.: 1

Guideline:	/
Species:	mouse, NMRI strain
Group:	10 males per dose
Substance:	Eusolex 232
Batch:	/
Purity:	/
Doses:	8 doses between 640 – 5000mg/kg bw
Route:	Oral as 5 or 10g Eusolex 232 suspended in 100ml 0.5% carboxymethylcellulose in water.
GLP:	not in compliance

There was 1 death at 1600 mg/kg caused by a dosing-lesion and not by the test substance. No effects were observed up to 5000 mg/kg. The LD_{50} oral mouse is > 5000 mg/kg bw.

Ref.: 4

3.3.1.2. Acute dermal toxicity

Guideline:	/
Species:	Wistar rats, AF/HAN-EMD-SPF
Group:	5 male 5 females)
Substance:	Eusolex 232 sodium salt
Batch:	/
Purity:	/
Dose:	30% aqueous solution applied to 6x6cm shaved skin (equivalent of 3000mg/kg bw of test substance), occluded with foil and sealed with rubber sleeve for 24 hours.
GLP:	not in compliance

No abnormality was observed after removal of the application. There was no abnormality at 14 days.

Ref.: 2

Guideline: /
Species: Wistar rats, AF/HAN-EMD-SPF
Group: 5 male 5 females (10 animals in total)
Substance: Eusolex 232 triethanolamine salt
Batch: /
Purity: /
Dose: 30% aqueous solution applied to 6x6cm shaved skin (equivalent of 3000mg/kg bw of test substance), occluded with foil and sealed with rubber sleeve for 24 hours.
GLP: not in compliance

No abnormality was observed after removal of the application. 1 animal died at 24 hours and since all the other animals in both experiments did not show any signs of toxicity, it was considered probable that this mortality was not substance-related. At 14 days there were no pathoanatomical findings. The LD_{50} dermal rat is >3000 mg/kg bw.

Ref.: 3

3.3.1.3. Acute intraperitoneal toxicity

Rat
Two studies by the intraperitoneal (i.p.) route are available.

Guideline: /
Species: Wistar rats, AF/HAN-EMD-SPF
Group: 5 male 5 females per dosage (70 animals in total)
Substance: Eusolex 232 sodium salt
Batch: /
Purity: /
Dose: 640 – 1600 mg/kg body (10g test substance in 100ml water)
GLP: not in compliance

Immediately after dosing, all animals showed hypokinesia, staggering, deep slow breathing which lasted for several hours. By 24 hours surviving animals still showed hypokinesia and deep slow breathing but by 2-3 days the surviving animals exhibited no abnormalities. There were no pathoanatomical findings.

Dose (mg/kg)	**Dead rats/total number of rats after days**			**Of these**	
	1	**7**	**14**	**male**	**female**
640	0/10	0/10	0/10	0/5	0/5
800	0/10	0/10	1/10	1/5	0/5
860	1/10	2/10	2/10	2/5	0/5
1000	4/10	4/10	4/10	0/5	4/5
1250	7/10	7/10	7/10	4/5	3/5
1400	10/10	-	-	5/5	5/5
1600	10/10	-	-	5/5	5/5

The LD_{50} was 1046 mg/kg bw.

Ref.: 2

Guideline: /
Species: Wistar rats, AF/HAN-EMD-SPF
Group: 5 male 5 females per dose (60 animals in total)
Substance: Eusolex 232 triethanolamine salt
Batch: /
Purity: /
Dose: 1000 – 2000 mg/kg bw (10g test substance in 100ml water)
GLP: not in compliance

Immediately after dosing, all animals showed staggering and by 15 minutes hypokinesia, staggering, deep slow breathing which lasted for several hours. By 24 hours surviving animals still showed hypokinesia and staggering but by second day the surviving animals exhibited no abnormalities. There were no pathoanatomical findings attributed to the test substance.

Dose (mg/kg)	Dead rats/total number of rats after days			Of these	
	1	7	14	male	female
1000	0/10	0/10	0/10	0/5	0/5
1250	0/10	1/10	1/10	1/5	0/5
1400	2/10	3/10	3/10	3/5	0/5
1600	6/10	7/10	7/10	4/5	3/5
1800	8/10	8/10	8/10	5/5	3/5
2000	10/10	-	-	5/5	5/5

The LD_{50} was 1513 mg/kg bw.

Ref.: 3

The LD_{50} i.p. rat is in the range 1000 – 1500 mg/kg bw.

Summary of acute toxicity results:

Species	Route	LD_{50} [mg/kg bw] PBSA	PBSA-Na	PBSA-TEA
rat	dermal	-	> 3000	> 3000
	oral	-	> 1600	> 1600
	i.p.	-	1046	1513
mouse	oral	> 5000	> 6600	-

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline: /
Species: rabbit. New Zealand
Group: 3 males intact skin; 3 males abraded skin
Substance: Eusolex 232 – sodium salt
Batch: /
Purity: /
Dose: 0.5ml of 30% aqueous solution applied to Left side of back; water control to Right side, occluded for 24 hours.
GLP: not in compliance

No abnormalities were recorded over a 7 day observation period.

Ref.: 2

Guideline:	/
Species:	rabbit. New Zealand
Group:	3 males intact skin; 3 males abraded skin
Substance:	Eusolex 232 – triethanolamine salt
Batch:	/
Purity:	/
Dose:	0.5ml of 30% aqueous solution applied to 4cm^2 Left side of back; water control to Right side, occluded for 24 hours.
GLP:	not in compliance

No abnormalities were recorded over a 7 day observation period

Ref.: 3

Guideline:	OECD 404
Species:	rabbit, SPF albino
Group:	3 female
Substance:	Neo Heliopan typ hydro (NB. The test substance was not fully described but elsewhere was said to be the sodium salt at 10%)
Batch:	H&R 1991016
Purity:	/
Dose:	0.5 ml preparation on 2.5 cm^2 gauze, 4, 10 or 25 hour exposures
GLP:	in compliance

No reactions were observed up to 72 hours of observations.

Ref.: 5

Guideline:	OECD 404
Species:	rabbit
Group:	3 female, 3 male
Substance:	Eusolex 232 TS liquid
Batch:	G306086
Purity:	/
Doses:	0.5 ml preparation on 4 cm^2 gauze to left side, 4 occlusion 0.5 ml of 16% substance in water on 4 cm^2 gauze to right side, 4 occlusion
GLP:	in compliance

[Although not stated in the study report, Eusolex 232 TS liquid is a 72% solution of the tromethamine [Tris(hydroxymethyl)aminomethane] salt. The diluted test product would be 11.5%.]

Eusolex 232 TS liquid caused slight irritation with 1 rabbit showing erythema on day 4 and slight scaling on days 6 and 7. A second rabbit showed slight scaling on days 5 and 6.
No reactions were seen to the diluted product.

Ref.: 6

Guideline:	/
Species:	rabbits
Group:	6

Substance: Novantisol-Na (a 16.5% solution)
Batch: Pt. 8/70
Purity: /
Dose: 1ml of 10% aqueous solution applied on 3x4 cm gauze to each side of back; one site scarified; 24 hour occlusion. Water to control sites.
Observation: 24 and 72 hours
GLP: not in compliance

No reaction was observed at the site of application of 10% aqueous Novantisol-Na on either intact or scarified skin.

Ref.: 1

Guideline: /
Species: rabbits
Group: 6
Substance: Novantisol-Na (a 16.5% solution)
Batch: Pt. 8/70
Purity: /
Dose: 1ml of 10% aqueous solution applied on 3x4 cm gauze to each side of back; one site scarified; 24 hour occlusion. Application repeated daily for 5 days.
Observation: daily
GLP: not in compliance

No reaction was observed at the site of application of 10% aqueous Novantisol-Na on either intact or scarified skin.

Ref.: 1

Phenylbenzimidazole Sulfonic Acid and its salts can be considered as non-irritant to the skin.

[A report on file, describing an irritation study of PBSA at 3% in the Guinea pig is of limited value for assessment purposes, because:

- the test concentration was too low,
- the species used is not ideal for this type of experiment,
- the test article was only poorly described.

Ref.: 7

3.3.2.2. Mucous membrane irritation

Guideline: /
Species: rabbit. New Zealand
Group: 3 males
Substance: Eusolex 232 – sodium salt
Batch: /
Purity: /
Dose: 0.1ml of 30% aqueous solution instilled into conjunctival sac of left eye; right eye untreated control.
GLP: not in compliance

No abnormalities were recorded over a 7 day observation period.

Ref.: 2

Guideline: /
Species: rabbits

Group: albino, 12
Substance: Novantisol-Na (a 16.5% solution)
Batch: Pt. 8/70
Purity: /
Dose: 0.ml of 10% aqueous solution instilled into conjunctival sac of right eye; left eye untreated control. After 1 minute, the right eyes were rinsed in 6 animals but not in the other 6.
GLP: not in compliance

No reaction was observed at the single 24 hour observation period.

Ref.: 1

Guideline: /
Species: rabbit. New Zealand
Group: 3 males
Substance: Eusolex 232 – triethanolamine salt
Batch: /
Purity: /
Dose: 0.1ml of 30% aqueous solution instilled into conjunctival sac of left eye; right eye untreated control.
GLP: not in compliance

No abnormalities were recorded over a 7 day observation period.

Ref.: 3

Guideline: OECD 405
Species: New Zealand white rabbit
Group: 2 male, 1 female
Substance: Eusolex 232 TS liquid (containing 40% Eusolex 232)
Batch: G353175
Purity: /
Doses: 0.1ml of 30% aqueous solution instilled into conjunctival sac of left eye; right eye untreated control.
GLP: in compliance

Slight reddening of the conjunctiva was observed in 1animal on day 1 of the study only. No other effects were observed during the 8-day observation period.

Ref.: 8

Conclusion
Phenylbenzimidazole Sulfonic Acid and its salts can be considered as non-irritant to the conjunctiva.

3.3.3. Skin sensitisation

Guideline: /
Species: Guinea pig. Pirbright white
Group: 5 male and 5 females in each group (apart from untreated – 9 males and 11 females)
Substance: Eusolex 232 – sodium salt
Batch: /
Purity: /
Dose: Induction

	Group 1:	30% Eusolex 232 – sodium salt at 30% solution applied to left flank 5 times weekly for 2 weeks
	Group 2:	5% Eusolex 232 – sodium salt at 5% solution applied to left flank 5 times weekly for 2 weeks
	Group 3:	2% DNCB solution applied to left flank 5 times weekly for 2 weeks
	Group 4:	water control
	Group 5:	untreated
	Elicitation	
	10% of the induction concentration applied 7 days after above.	
GLP:	not in compliance	

No reaction observed at the site of application of the trial substance. All animals tested with DNCB produced an erythematous reaction 3 hours after application of the elicitation dose.

Ref.: 2

Guideline:	/	
Species:	Guinea pig. Pirbright white	
Group:	5 male and 5 females in each group (apart from untreated – 9 males and 11 females)	
Substance:	Eusolex 232 – triethanolamine salt	
Batch:	/	
Purity:	/	
Dose:	Induction	
	Group 1:	30% Eusolex 232 – triethanolamine salt at 30% solution applied to left flank 5 times weekly for 2 weeks
	Group 2:	5% Eusolex 232 – triethanolamine salt at 5% solution applied to left flank 5 times weekly for 2 weeks
	Group 3:	2% DNCB solution applied to left flank 5 times weekly for 2 weeks
	Group 4:	water control
	Group 5:	untreated
	Elicitation	
	10% of the induction concentration applied 7 days after above.	
GLP:	not in compliance	

No reaction observed at the site of application of the trial substance. All animals tested with DNCB produced an erythematous reaction 3 hours after application of the elicitation dose.

Ref.: 3

Magnusson and Kligman

Guideline:	OECD 406	
Species:	Guinea pig	
Group:	10 male and 10 female per group	
Substance:	Eusolex 232 –TS liquid (containing 40% Eusolex 232)	
Batch:	G353175	
Purity:	/	
Dose:	Group 1:	negative controls
	Group 2:	intradermal injections of Freunds adjuvant, Eusolex 232 – TS liquid (12.5%) and Freunds + Eusolex 232-TS liquid (12.5%) into three separate sites. After 1 week, a single topical application of undiluted Eusolex 232-TS liquid. Challenge after 1 week with Eusolex 232 TS liquid 25%.
GLP:	in compliance	

No reactions were observed. There was no contemporaneous positive control but historical controls with DNCB available.

Ref.: 9

Magnusson and Kligman

Guideline:	OECD 406
Species:	Guinea pig, Dunkin Hartley
Group:	20 test, 10 negative control
Substance:	HR 92/103089 (Phenylbenzimidazole Sulfonic Acid)
Batch:	/
Purity:	/
Dose:	Group 1: negative controls Group 2: intradermal injections of 0.1ml Freund's complete adjuvant in water, HR 92/103089 1% w/v in arachis oil, and Freund's + HR 92/103089 1% w/v in arachis oil (1:1) into three separate sites. After 1 week, a single topical application of 0.2 – 0.3 ml HR 92/103089 50% w/w in arachis oil under occlusion for 48 hours. Challenge on day 21 with 0.l-0.2 ml HR 92/103089 25 and 10% w/w in arachis oil.
GLP:	in compliance

No reactions were observed. There was no contemporaneous positive control but historical controls with DNCB available.

Ref.: 10

Magnusson and Kligman

Guideline:	OECD 406
Species:	Guinea pig, Dunkin Hartley
Group:	20 test; 10 negative control
Substance:	HR 92/103089 Na (Phenylbenzimidazole Sulfonic Acid sodium salt)
Batch:	/
Purity:	/
Dose:	Group 1: negative controls Group 2: intradermal injections of 0.1ml Freund's complete adjuvant in water, HR 92/103089 Na 25% w/v in water, and Freund's + HR 92/103089 Na 25% w/v in water (1:1) into three separate sites. After 1 week, a single topical application of 0.2 – 0.3 ml HR 92/103089 Na as supplied under occlusion for 48 hours. Challenge on day 21 with 0.l-0.2 ml HR 92/103089 Na as supplied and at 75% v/v in water.
GLP:	in compliance

No reactions were observed. There was no contemporaneous positive control but historical controls with DNCB available.

Ref.: 11

The overall conclusion of the available studies is that they showed no evidence of the test substance being a skin sensitiser.

3.3.4. Dermal / percutaneous absorption

Clinical study in humans using labelled PBSA-Na[2-^{14}C]

Guideline	/
Group:	6 male

Test substance: ^{14}C – Eusolex 232, sodium salt
Test article: EMD 36463 ^{14}C-Eusolex sun protection cosmetic formulation
8% ^{14}C-Eusolex 232/ Eusolex 232 (29 mMol) in cosmetic gel
Radioactivity: 1.76 MBq/g Gel
Vehicle: cosmetic gel
Amount applied: 1g approximately (but accurately weighed) per volunteer
Application site: upper forearm
Exposure: 6 hours (unoccluded)
Sampling: blood: 5 min before dosing, 1, 3, 6, 12, 24, 48, 72, 96, 120 hrs. post dosing
Urine: 5 min before dosing , 0-6, 6-12, 12-24, 24-48, 48-72, 72-96, 96-120 hours post dosing
Faeces: after each defaecation
Recovery: skin washing: 6 hrs. p.d. (cotton swabs/ether)
Skin stripping: 6 hrs. p.d. (immediately after skin washing), 120 hrs. p.d.

Results
Plasma levels were practically 0.

Excretory and recovery data are summarized in the following table:

Subject	Excretion (% of applied dose)			Recovery from (% of applied dose)			Total Recovery
	Urine	Faeces	Total	skin surface[1)]	horny layer[2)]		
					6 hrs.	5 days	
1	0.217	0.042	0.259	66.386	10.472	0.218	77.335
2	0.142	0.028	0.170	52.980	27.020	0.370	80.540
3	0.115	0.030	0.145	43.409	35.637	0.023	79.214
4	0.104	0.046	0.150	54.586	22.916	0.343	77.955
5	0.068	0.039	0.107	70.587	13.933	0.220	84.847
6	0.085	0.038	0.123	50.862	22.055	0.287	73.327
Mean	**0.122**	**0.037**	**0.159**	**56.468**	**22.006**	**0.244**	**78.876**

[1)] by washing with cotton swabs soaked with ether
[2)] by skin stripping

Conclusion
Penetration rates could not be established. However, cumulative penetration can be derived from total excretion. It amounts to 0.159% (0.107 – 0.259) of the applied dose.

Total recovery was low - 78.8%. It is known from animal experiments (ref. 22 and 23) that radioactivity (i.e. test article) is not lost by metabolism to CO_2 or by accumulation elsewhere in the body. The loss must have occurred during exposure. The test article was formulated as a gel which tends to dry off during exposure and become brittle.

From this experiment, the SED (systemic exposure dose) for safety margin calculation would be 0.259 % of the applied dose (maximum observed).

Ref.: 12

***In Vitro* Percutaneous Absorption**

Guideline: OECD 428.
Tissue: female pig skin (whole skin discs, diameter 5cm, thickness 3 – 4 mm, temp. 32°C)

Test substance:	Phenylbenzimidazole Sulfonic Acid (Neo Heliopan Hydro)
Test articles:	4% in w/o and o/w cosmetic emulsions
Amount applied:	150 – 200 µg/cm^2 / disc
Exposure:	1.5 and 24 hrs.
Number of replicates:	3 per test article and exposure time
Method of analysis:	HPLC (detection limit: 0.03 µg Phenylbenzimidazole Sulfonic Acid /ml)
GLP:	not in compliance

Results
The recovery for the w/o preparation was 96.6% (range 89.9 – 100.4). The maximum absorbed (epidermis, dermis, receptor fluid) was 6.6 µg/cm^2 at 24 hours. Product present in the receptor fluid was below the limit of detection).

The recovery for the o/w preparation was 100.9% (range 90.8 – 101.6). The maximum absorbed (epidermis, dermis, receptor fluid) was 10.9 µg/cm^2 at 24 hours. Product present in the receptor fluid was below the limit of detection).

Ref.: 33

Comments
- The skin samples used were too thick.
- Only 3 chambers per test article were used.
- The concentration of the test substance in the test articles was too low (4% instead of the requested 8%)

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity

/

3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity

Guideline:	OECD 408
Species/strain:	Rat, Wistar W.74 (SPF)
Group size:	Test: 30 animals [15♂, 15♀]/ dose group) Control: 30 animals [15♂, 15♀]
Observation:	13 weeks (7 days/week, application volume: 10 ml/kg bw)
Test substance:	Novantisol saure (2-Phenylbenzimidazole-5-sulfonic acid)
Batch:	414 512
Purity:	/
Vehicle:	5% aqueous tylose
Dose levels:	0, 100, 330 and 1000 mg/kg bw
GLP:	not in compliance

Animals were treated as given above and examined with respect to in-life-observations, clinical pathology and anatomic pathology.
In-life-observations were carried out daily on all animals for mortalities and clinical signs of toxicity. Body weight and food-/water consumption were recorded weekly.
Clinical pathology, viz. haematology, clinical chemistry and urinalysis, was performed in week 6 and at termination of the study using 5 males and 5 females of each group.
Anatomic pathology examinations comprised organ weights, necropsy, histopathology. Organ weights (thymus, thyroid, heart, lung, liver, spleen, kidneys, adrenals, testes, ovaries) were determined for all animals. All animals were also subjected to macroscopic inspection after necropsy. Histopathological examination was carried out in 5 male and 5 female animals of the control- and the 1000 mg/kg-group. The organs looked at were: heart, lung, liver, pancreas, spleen, kidneys, pituitary, thyroid, thymus, adrenals,

testes/epididymes, prostate gland, seminal vesicles, ovaries, uterus, salivary gland (head), oesophagus, stomach, intestines (duodenum, jejunum, ileum, olon), lymph nodes, bladder, brain, eyes, aorta, trachea, skeletal muscles, bones, bone marrow).

Results
In-life-observations: Three mortalities (1♂, week 5, control group; 1♀, week 13, 330 mg/kg-group; 1♀, week 6, 1000 mg/kg-group) occurred intercurrently but were not substance related. No clinical signs of toxicity were seen in any group. Food- and water consumption were the same in test- and control groups.
Clinical pathology: Haematology results (total and differential blood count) of test animals did not differ from controls throughout the experiment. The same holds for clinico-chemical parameters, except for protein content in serum in females of the 1000 mg/kg-group, which was increased as compared to controls. However, the level found was within the biological variability range on record for female rats of this age and was therefore not considered substance related.
Anatomical pathology: Necropsy-findings in the animals which had died intercurrently, revealed no pathological changes attributable to the test substance. Neither did the animals which were sacrificed pursuant to protocol at the end of the study exhibit any such changes. Differences in organ weights, if observable at all (e.g. spleen in ♀'s), were small and not dose dependent. Histopathology did not reveal any organ change or –damage in any one of the dose groups.

NO(A)EL: 1000 mg/kg bw/day

Ref.: 13

3.3.5.3. Chronic (> 12 months) toxicity

/

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

Bacterial gene mutation assays

Guideline:	EEC guideline in Annex V of Directive 67/548/EEC
Species/strain:	*Salmonella typhimurium* (TA100, TA1535, TA98, TA1537, TA1538) *Escherichia coli* (WP2, WP2 uvrA)
Replicates:	
Test substance:	Eusolex 232 (2-Phenylbenzimidazole-5-sulfonic acid)
Solvent:	DMSO
Batch:	G-196572, article n° 1/05372-6
Purity:	99.7%
Concentrations:	0, 50, 250, 1250, 2500, 5000, 10000 µg/plate
Treatment:	
Metabolic activation:	S9-Mix, with and w/o
GLP:	in compliance
Controls:	positive: 2-AA; 9-AA; DAUN; ENNG; MNNG; MMS; 4-NP; 2-NF negative: vehicle

A preliminary screen for bacterotoxicity was run with a series of concentrations of which the ones depicted above were selected for the main study.
The main study was performed in duplicate. Each run was carried out with and w/o metabolic activation and with quadruplicate plating.

Results

The test article did not show mutagenic activity, neither in the absence nor in the presence of the metabolic activation system. All positive controls exhibited the expected mutagenic effects.

Ref.: 14

Guideline: 84/449/EEC
Species/strain: *Salmonella typhimurium* (TA100, TA1535, TA98, TA1537, TA1538)
Replicates:
Test substance: Neo Heliopan Type Hydro (2-Phenylbenzimidazole-5-sulfonic acid)
Solvent: DMSO
Batch: WE 161022
Purity: 100%
Concentrations: 0, 300, 1000, 3000, 10000, 30000, 75000 µg/plate
Treatment:
Metabolic activation: S9-Mix, with and without
GLP: in compliance

In the first experiment, all doses could be used for assessment. Bacteriotoxic effects were not seen and there was no indication of mutagenic activity of the test article.
The second, confirmatory experiment, which was run under the same conditions (except for a lower concentration of S9-mix) gave the same results.

All positive controls exhibited the expected mutagenic effects.

Ref.: 15

***In vitro* chromosome aberration test**

Guideline: OECD 473 and method B.10 of Annex V to Directive 76/548/EEC.
Cells: Human peripheral blood lymphocytes
Test substance: Neo Heliopan Hydro (2-Phenylbenzimidazole-5-sulfonic acid)
Solvent: distilled water
Batch: 56
Purity: 100%
Concentrations: 0, 0.33, 1.0, 3.3, 10 mM
Metabolic activation: S9-Mix, with and without
Control: positive: with S9 – cyclophosphamide (25 µg/ml)
without S9 – mitomycin C (0.5 µg/ml)
negative: distilled water
GLP: in compliance

The design is depicted in the following table:

Experiment	Test conc. [mM]	Test conc. [µg/ml]	Metabolic activation	Treatment [hrs] +t/a	Treatment [hrs] ./.t/a	Treatment [hrs] total	Sampling [hrs]	Mitotic index*) [%]	Toxicity [%]
1	0	0	w/o	24	0	24	24	5.1	0
	0.33	90.4						5.1	0
	3.3	904						2.4	53
	10.0	2740						1.6	69
	0	0	S9	3.5	20.5	24	24	5.5	0
	1.0	27.4						5.3	4
	3.3	904						2.6	53
	10.0	2740						2.3	58

Experiment	Test conc. [mM]	Test conc. [µg/ml]	Metabolic activation	Treatment [hrs] +t/a	Treatment [hrs] ./.t/a	Treatment [hrs] total	Sampling [hrs]	Mitotic index*) [%]	Toxicity [%]
2	0	0	w/o	4	20	24	24	6.7	0
	1.0	27.4						6.3	6
	3.3	904						5.8	13
	10.0	2740						4.9	27
	0	0	S9	3.5	20.5	24	24	6.4	0
	1.0	27.4						5.7	11
	3.3	904						4.2	34
	10.0	2740						3.1	52

*) 2000 cells examined

Two independent experiments were run. All cultures were established in duplicate. Test concentrations were selected based on the results of a preliminary cytotoxicity test. Positive controls (mitomycin C in experiments with metabolic activation; cyclophosphamide in those without) were included and showed the expected frequencies of aberrations, thus confirming the efficacy of the test system.

The test article did not induce an increase in the number of structural chromosome aberrations as compared to controls. Numerical aberration was not observed.

Ref.:16

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

/

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

3.3.8.1. Prenatal developmental toxicity

Guideline: /
Species/strain: rat, Wistar BOR:Wisw (SPFcpb)
Group size: virgin females, 25 test + 25 control
Test substance: Eusolex 232 sodium salt
Batch: K 90083872
Purity: /
Dose: 1000 mg/kg bw by daily gavage
Vehicle: distilled water
Treatment: exposure: day 6 to 15 post coitum (application: 5ml daily)
GLP: in compliance

Two dose groups (25 animals each) were used, one test- (1000 mg/kg bw) and one control group (0 mg/kg bw). They were dosed from day 6-15 post coitum and kept off-dose from day 16-19. There were no signs of toxicity in dams. Animals were sacrificed on day 20 and necropsied. Reproductive parameters are shown in the table below:

Parameters		Control total	Control mean litter	Control mean %	Test total	Test mean litter	Test mean %
Animals	total	25	-	-	25	-	-
	pregnant	23	-	-	24	-	-
Corpora lutea		279	12.1	-	269	11.2	-
Implantations		260	11.0	-	233	9.7	-
Resorptions	complete	0	0	0*	1	0.04	0.3*
	early	6	0.26	2.5*	5	0.21	1.9*
	late	3	0.13	1.2*	3	0.13	1.2*
Foetuses	dead	0	0	0*	0	0	0*
	live	251	10.9	96.3*	224	9.3	96.6*
	male	128	5.6	50.3**	132	5.5	58.4**
	female	123	5.3	49.7**	92	3.8	41.6**

*) relative to number of implantations (avg. of individual dams)
**) relative to number of live foetuses (avg. of individual dams)

Foetuses were weighed, inspected macroscopically for external malformations and prepared for inspection for visceral and skeletal malformations (by transverse section and double staining respectively). They did not show any such malformations. Skeletal variations were the same (nature and frequency) in test- and control group.

Therefore, 1000 mg/kg bw is a NO(A)EL for dams and foetuses.

The study was conducted as a limit test and conformed to OECD 414 and method B.31 of Annex V to Directive 67/548/EEC although not stated in the report.

Ref.: 17

3.3.8.2. Teratogenicity

/

3.3.9. Toxicokinetics

Rat screening experiment, in vivo stability and excretory pathways

Guideline: /
Species/strain: male Chbb:THOM (Wistar) rat
Group size: 4 (oral and intra-venous, 2 animals each)
Test substance: Eusolex 232 sodium salt [2-^{14}C]
Batch: /
Purity: /
Dose: single dose of 0.378 MBq/animal (approx. 0.2 mg) PBSA-Na[2-^{14}C]
Vehicle: distilled water
GLP: not in compliance

In the screening experiment, rats were given orally and i.v. (2 animals each) a single dose of 0.378 MBq/animal (approx. 0.2 mg) Eusolex 232 sodium salt [2-^{14}C]. They were put in metabolism cages and exhaled air, urine and faeces collected over a period of 72 hrs. All animals were sacrificed after 72 hours and residual radioactivity in the carcasses determined. The following chart shows the radioactivity 'balance':

Route	Animal	% applied dose found in	Total

		exhaled air	urine	faeces	carcass	
oral	1	<0.002	15.6	81.5	0.03	97.1
	2	<0.002	14.9	80.7	0.02	95.7
	mean	***<0.002***	***15.2***	***81.1***	***<0.1***	***96.3***
intravenous	3	<0.002	77.9	22.5	0.2	100.6
	4	<0.002	79.9	20.4	0.2	100.5
	mean	***<0.002***	***78.8***	***21.5***	***0.2***	***100.5***

*) based on total radioactivity

The conclusions to be drawn from this experiment are:

- elimination of test article is virtually complete by 72 hours;
- elimination occurs via urine and faeces only;
- the test article is not metabolized to CO_2.

Ref.: 22

Absorption, distribution and excretion in pregnant rats

Guideline: /
Species/strain: pregnant Wistar rat; BOR:WISW
Group size: 30 (oral and intra-venous, 15 animals in each group)
Test substance: ^{14}C labelled Eusolex 232 sodium salt. (6.44 MBq/mg or 174 µCi/mg)
Batch: K 900 83872
Purity: > 98% (chemical and radio-chemical)
Dose: single dose
intra-venous group : 1 mg/kg bw on day 18 of gestation
oral group: 1000 mg/kg bw on day 18 of gestation
Vehicle: distilled water
GLP: in compliance

The following table outlines the study design and shows all experiments and examinations carried out within the scope of this study:

Experiment	Route	Sampling [hrs. p.d.]	Animals	Dose ranges
Plasma concentrations	i.v.[1)]	0.1, 0.5, 1, 2, 4, 8, 24	3	i.v.: 0.98 – 1.03 mg/kg 1.90 – 2.15 MBq/kg p.o.: 962 – 1030 mg/kg 1.81 – 2.15 MBq/kg The doses actually given were recorded individually for each animal
	p.o.[2)]	0.25, 0.5, 1, 2, 4, 8, 24	3	
Distribution in plasma and selected organs[3)]	i.v.	0.1 1 8	3 3 3	
	p.o.	0.25 1 8	3 3 3	
Excretion in urine and faeces	i.v.	0 - 48	3	
	p.o.	0 – 48	3	

[1)] injection into caudal vein
[2)] gavage
[3)] liver, kidneys, brain, amniotic fluid, foetuses

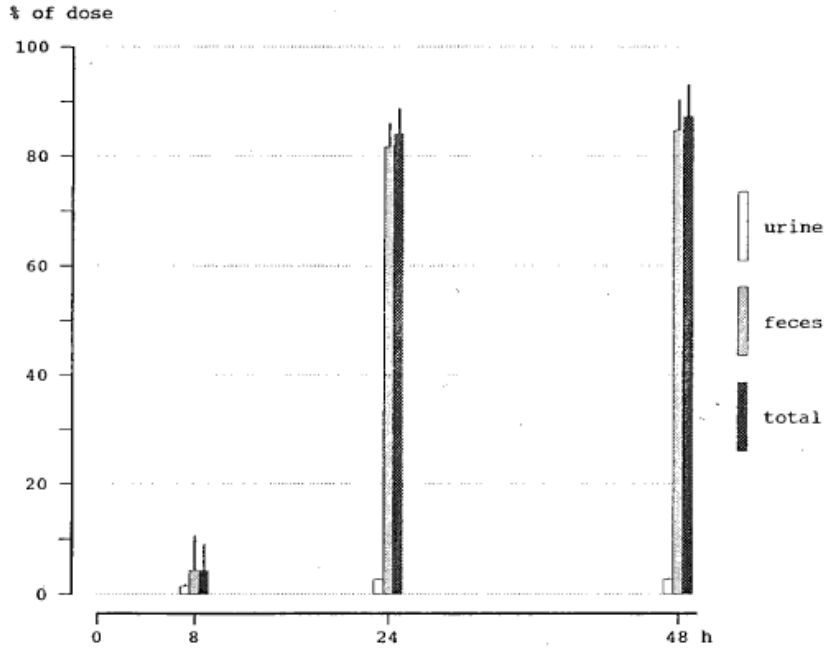
Plasma concentrations found were as follows:

	Route	Time [hrs.] 0.1	0.25	0.5	1	2	4	8	24
Plasma concentration*) [µg/ml]	i.v.	4.12		0.63	0.14	0.02	0.005	0.006	<0.001
	p.o.		19.20	12.90	6.64	4.44	3.54	3.07	0.16

*) means of 3 animals based on total radioactivity

After i.v. administration (dose: 1 mg/kg approx.) the highest blood level was measured 5 min post dosing and amounted to 4.12 µg/ml which corresponds to a V_d = 0.25 lit.. Plasma elimination half-life ($t_{½}$ between 5 min and 4 hrs.) was 0.4 hours.

Following oral administration (dose: 1000 mg/kg approx.) the maximum blood level was reached after 15 min already. The amount of absorption from the gastrointestinal tract was estimated to be 3 – 4%. 24 hours post dosing radioactivity was virtually eliminated from plasma.



Excretion of ^{14}C-radioactivity (% of dose) with urine and feces after single po administration of 1000 mg/kg Eusolex 232-^{14}C to pregnant Wistar rats

Distribution and excretion results are summarized in the table below:

in	% of applied dose found *) hours. after i.v. application 0.1	1	8	24	48	hours after p.o. application 0.25	1	8	24	48
Organs/tissues:										
Plasma	12.8	0.66	0.01			0.02	0.01	0.007		
Liver	8.7	0.53	0.02			0.03	0.01	0.016		

in	% of applied dose found *) hours. after i.v. application					hours after p.o. application				
	0.1	1	8	24	48	0.25	1	8	24	48
Kidneys	13.5	0.44	0.01			0.02	0.01	0.007		
Brain	0.06	0.006	n.d.			n.d.	n.d.	n.d.		
Amniotic fluid	0.37	0.15	0.11			0.001	0.003	0.002		
Placenta	1.04	0.08	0.02			0.001	0.001	0.002		
Foetuses	0.03	0.01	0.003			n.d.	n.d.	n.d.		
Carcass	54.8	35.9	18.6			88.7	92.3	69.2		
Excreta:										
Urine			50.9	61.4	61.8			1.4	2.5	2.6
Faeces			1.8	25.9	26.3			4.4	81.6	84.6
Carcasses					0.1					n.d.

*) means of 3 animals

Conclusions from this experiment:

- There is some absorption from the gastro-intestinal tract (estimate: 3 – 4%) after oral application;
- there is no indication for accumulation in any of the organs investigated (both routes);
- trace amounts of radioactivity are found in brain and foetuses after i.v. application; nothing is found in these organs in the oral experiment. This indicates that both blood/brain- and placental barriers are not passed;
- elimination of radioactivity from the body is virtually complete by 48 hours.

Ref.: 23

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

Photo-irritation

Guideline: /
Species: mouse; NMRI
Group: 5 female, 5 male per group
Substance: Eusolex 232
Batch: /
Purity: /
Dose: 100µl of 5% aqueous solution.
GLP: not in compliance

The test design was as follows:

Group		Animals	Treatment	
			material	irradiation
Test	1a	10 (5 ♂ and 5 ♀)	test article	yes
	1b	10 (5 ♂ and 5 ♀)	test article	no

Control	2a	10 (5 ♂ and 5 ♀)	vehicle	yes
	2b	10 (5 ♂ and 5 ♀)	vehicle	no

Animals were irradiated with light from a quartz lamp (Q600); the dose of UV is not stated.

No skin effects were recorded in non-irradiated animals.

In both irradiated groups, viz. test and controls, slight to moderate erythema was seen 48 and 72 hours post irradiation. However, these effects were the same both in intensity and frequency and are therefore not indicative of a photoirritation potential of the test article.

Ref.: 24

Guideline: /
Species: Guinrea pig; BOR:DHPW (SPF)
Group: 10 test , 5 in non-irradiated control
Substance: Neo Heliopan Typ Hydro
Batch: 157711
Purity: /
Dose: 100% and 50% test substance in water. Positive control 5% 8-MOP
GLP: in compliance

Group	Number of animals	Test site	Treatment	
			material	irradiation
Test	10 ♀	1	blank	UVA and UVB
		2	test article 100%	
		3	test article 50%	
		4	positive control	
Control	5 ♂	1	blank	No (dark)
		2	test article 100%	
		3	test article 50%	
		4	positive control	

Animals of the test and the non-irradiated control group were treated once with the test article and positive control (8-MOP). Irradiated was in two stages 30 min post dosage (stage 1: 0.1 J/cm^2 UVB; stage 2: 10.26 J/cm^2 UVA).

Skin reactions were evaluated 24 and 48 hrs. post irradiation.

In the non-irradiated group no skin reactions were observed.

In the irradiated group, the test sites carrying the positive control exhibited slight erythema (24 hrs. – 3/10; 48 hrs. – 7/10); all the other test sites remained unaffected.

Ref.: 27

3T3-NRU-PT

Guideline: OECD 432, method B.41 af Annex V of Directive 67/548/EEC
Substance: Phenylbenzimazol sulfonic acid
Batch: 31244

Purity: /
Dose: 8 concentrations from 391 to 50,000 mg/l (5%) diluted in Earle's buffered salt solution
GLP: in compliance

Test concentrations for this experiment were chosen high and cytotoxicity could be seen both in the irradiated and non-irradiated sample. The calculated photo-irritation-factor (PIF) was 1.4, which according to current interpretation standards means non-phototoxic. The negative control (sodium lauryl sulphate had a PIF of 1.1; the positive control, chlorpromazine a PIF of 52.9)

Ref.: 32

Photosensitization

Guideline: /
Species: Guinea pig, Pirbright white, BOR:DMPW
Group: 10 test, 5 positive control
Substance: Neo Heliopan, Typ Hydro
Batch: 157711
Purity: /
Dose: 30µl test substance applied; after 30 minutes irradiation with combined Philips TL20 W/09 and TL20 W/12 lamps. Repeated daily for 10 applications. 10.44 J UV. Two weeks after induction, 20 µl of test substance at 100, 75, 50 and 25% dilutions were applied followed by 10.06J UV from TL20 W/09 lamp with 6mm glass filter to absorb UVB. Control was hexachlorophene 2% in acetone for induction and 1% for elicitation.
GLP: in compliance

The positive control animals showed erythema but no reaction was seen in any of the test animals at 24 and 48 hour observations.

Ref.: 25

A study with Eusolex 232 triethanolamine salt describes the experiment rather poorly and fragmentary so that the results are difficult to interpret. Therefore it has not been used for risk assessment purposes.

Ref.: 26

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

Guideline: /
Substance: Parsol HS
Batch: 196429
Purity: 98.1%
GLP: in compliance

Parsol HS was tested in three different assays:

- A bacterial reverse mutation assay (photo Ames test),
- a yeast gene conversion assay,
- an *in vitro* chromosome aberration assay in CHO cells.

In the photo Ames test, tester strains were *Salmonella typhimurium* (TA 1537 and TA 102) *E. coli* WP2 and WP2pkM101. Test concentrations ranged from 25 – 2500 µg/plate and irradiation doses from 10/0.34 – 1800/61.2 mJ/cm^2 UVA/UVB. Solvent- and dark controls,

as well as appropriate positive controls for each strain were included. The result was negative.

In the yeast assay, the *Saccharomyces cerevisiae* D7 system was used. The concentration range tested was 50 – 1000 µg/ml, the administered irradiation doses ranged from 168/2.5 – 3360/50.0 mJ/cm^2 UVA/UVB. Appropriate positive and negative controls were run concurrently and showed the expected effects. The test article did not produce any evidence for photomutagenic activity.

In the chromosome aberration assay (CHO cells), test concentrations ranged from 250 – 1000 µg/ml and irradiation doses from 200/6.8 – 800/27.2 mJ/cm^2 UVA/UVB. A positive control was included. The rate of structural aberrations was not increased by Parsol HS at any UV exposure.

These results provide evidence for the absence of a photomutagenic potential of Parsol HS.

Ref.: 28

3.3.11. Human data

A 3% solution of Navantisol was tested in 60 volunteers who received a 24 hr. closed patch; skin effects were read 24, 48 and 72 hours after patch removal. After this first reading, 10 panellists were patched 5 more times in the same way every other day. After a 10 day break, one more patch was applied. No skin reactions were seen in the course of this experiment.

Ref.: 7

Neo Heliopan (at concentrations 5 and 10%) was applied under Finn chambers to the backs of 50 volunteers and kept in place for 48 hours. Skin reactions were assessed 48 and 72 hours after termination of exposure. No skin effects were observed at any point of the study.

Ref.: 29

A repeat open application test (ROAT) was conducted with Neo Heliopan at 5 and 10%. 0.1ml of two test articles were rubbed into the antecubital fossae (left and right respectively) of 20 volunteers twice a day for 14 consecutive days. No skin effects were observed at any point of the study.

Ref.: 30

8% Eusolex 232 H in gel was tested in 20 volunteers received 2 occlusive 24 hr. patches (Hayes chambers) on contralateral sites of the back. This treatment was carried out twice a week for 3 consecutive weeks (on the same sites). After the last application one side was irradiated with 2 MED's. This irradiation was repeated after 3 days. Following a 10 day rest period, two more occlusive 24 hr. patches were applied to naïve sites. After patch removal, these sites were irradiated with 10 J/cm^2 UVA and skin reactions assessed 24 and 48 hours afterwards. No skin effects were seen.

Ref.: 31

3.3.12. Special investigations

Two *in vitro* screening assays (both GLP compliant), one for oestrogen- and one for androgen receptor binding properties are described:

The oestrogen (ER)-binding screen used human recombinant ER, α-subtype as receptor and radiolabelled oestradiol [2,4,6,7,16,17-^{3}H(N)] as ligand. Neo Heliopan Hydro sodium salt

(Batch HRO2Na 103089; purity 100%) was tested at concentrations ranging from 0.296 – 296 µg/ml. β-Oestradiol (as reference compound with high ER-affinity) and genistein (reference with low ER-affinity) were used as positive controls.
The test article did not replace labelled ligand from the ER. IC_{50}-values could not be calculated.
β-Oestradiol and genistein displaced the labelled ligand as expected (IC_{50}'s: $5.2x10^{-4}$ µg/ml and $4.7x10^{-2}$ µg/ml respectively) thus proving the efficacy of the test system.

Ref.: 19

In the androgen receptor (AR)-binding screen, rat recombinant fusion protein to thioredoxin was the receptor and labelled methyltrienolone[17α-methyl-^{3}H] the ligand. Neo Heliopan Hydro sodium salt (Batch HRO2Na 103089; purity 100%) was tested at concentrations ranging from 0.296 – 296 µg/ml. Positive controls were dehydrotestosterone (DHT) (as reference compound with high AR-affinity) and androstenedione (ANDRO) (reference with low AR-affinity).

Neo Heloppan Hydro sodium salt showed absolutely no affinity for the AR-receptor, so that IC_{50}-values could not be calculated. The positive controls ascertained the efficacy of the test system by producing IC_{50}'s of $1.4x10^{-3}$ µg/ml (DHT) and 0.6 µg/ml (ANDRO)

Ref.: 20

One *in vivo* test for oestrogenic effects was undertaken by an *in vivo* uterotrophic assay in immature rats.

Guideline: OECD draft protocol of April 20th, 2000.
Species: rat, HsdCpd:WU
Group: 6 juvenile females per group
Substance: Neo Heliopan Hydro sodium salt
Batch: 50698414
Purity: 100%
Dosing: as shown in the following chart:

Group		Animal	Treatment				
			Doses	Application (day 1 to 3)			Sacrifice
				route	frequency	volume	
1	Negative control	6♀	0 (untreated)	-	-	-	day 4
2		6♀	0 (vehicle)	s.c.	daily	4 ml/kg	day 4
3	Positive control	6♀	0.3 µg/kg 17α-ethinylestradiol	s.c.	daily	4 ml/kg	day 4
4		6♀	1.0 µg/kg 17α-ethinylestradiol	s.c.	daily	4 ml/kg	day 4
5	Test	6♀	50 mg/kg test article	s.c.	daily	4 ml/kg	day 4
6		6♀	200 mg/kg test article	s.c.	daily	4 ml/kg	day 4

Corn oil was the vehicle.
GLP: in compliance

There were no clinical signs of toxicity throughout the dosing period. Food consumption and body weight development was the same in test and control groups. At necropsy no gross pathological findings except enlargement of uteri in positive controls became manifest.
Ref.: 21

These studies produce evidence for the absence of an oestrogenic potential Neo Heliopan Hydro sodium salt. 200 mg/kg bw (highest concentration tested) is a NO(A)EL for oestrogenic effects.

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Maximum absorption through the skin	A (µg/cm^2)	=	0.416 µg/cm^2
Skin area surface	SAS (cm^2)	=	18000 cm^2
Dermal absorption per treatment	SAS x A x 0.001	=	7.488 mg
Typical body weight of human		=	60 kg
Systemic exposure dose (SED)	SAS x A x 0.001/60	=	0.125 mg/kg
No observed adverse effect level (rat, oral, 90-day: 1000 mg/kg bw, 4% absorption)	NOAEL	=	40 mg/kg bw

Margin of Safety	**NOAEL/SED**	**=**	**320**

3.3.14. Discussion

Physico-chemical specifications
Several batches were used without a proper physico-chemical characterisation.

General toxicity
The LD_{50} i.p. rat is in the range 1000 – 1500 mg/kg bw. The LD_{50} dermal rat is >3000 mg/kg bw. The NO(A)EL was set at 1000 mg/kg bw/day (13 week oral study in rats). In a teratogenicity study, the NO(A)EL was set at 1000 mg/kg bw for the dams and foetuses.
The submitted studies produce evidence for the absence of an oestrogenic potential. 200 mg/kg bw (highest concentration tested) is a NO(A)EL for oestrogenic effects.

Absorption, distribution and excretion
An absorption, distribution and excretion study in pregnant rats showed that there was no indication for accumulation in any of the organs investigated (both routes); trace amounts of radioactivity are found in brain and foetuses after i.v. application; nothing is found in these organs in the oral experiment. This indicates that both blood/brain- and placental barriers are not passed; elimination of radioactivity from the body is virtually complete by 48 hours.

Irritation/sensitisation
Phenylbenzimidazole sulfonic acid and its salts can be considered as non-irritant to the skin and the conjunctiva. It has no photo-irritating potential.
The available studies showed no evidence of the test substance being a skin sensitiser/photo-sensitiser.

Dermal absorption

From a clinical study in humans using labelled PBSA-Na[2-^{14}C], the SED (systemic exposure dose) for safety margin calculation would be 0.416 µg/cm² (0.259% of the applied dose) (maximum observed).

Mutagenicity
The test article did not show mutagenic activity in 2 Bacterial gene mutation assays, neither in the absence nor in the presence of the metabolic activation system. It did not induce an increase in the number of structural chromosome aberrations as compared to controls. Numerical aberration was not observed.
The submitted studies provide evidence for the absence of a photomutagenic potential.

Carcinogenicity
No data were submitted.

4. CONCLUSION

Based on the information provided, the SCCP is of the opinion that the use of phenylbenzimidazole sulfonic acid and its salts as a UV-filter at a maximum concentration of 8.0% in the cosmetic sun protection preparations does not pose a risk to the health of the consumer.

Uses of phenylbenzimidazole sulfonic acid and its salts in other types of cosmetic products at concentrations up to 8.0% also does not pose a risk to the health of the consumer.

5. MINORITY OPINION

Not applicable

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