



EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Public Health and Risk Assessment
C7 - Risk assessment

SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS

SCCP

Opinion on

4-Aminobenzoic acid (PABA)

COLIPA n° S1

Adopted by the SCCP
during the 8th plenary meeting of 20 June 2006

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1. BACKGROUND

PABA or 4-Aminobenzoic acid (PABA) is listed in Annex VII part 1 no. 1 on the List of permitted UV filters that may be used in cosmetic products. The maximum authorised concentration in finished cosmetic products is 5 %. No other limitations and requirements or conditions of use and warnings are required by the SCCP.

In 2001, the Commission received inquiries from Denmark expressing its concern about the use of certain UV filters in cosmetic products. In this connection an opinion on PABA was missing, as the substance has been introduced into the Annexes of the cosmetics legislation based on safety evaluations from the Member States prior to the existence of the SCCNFP.

Submission I on the UV-filter 4-Aminobenzoic acid (PABA) was submitted December 2005.

2. TERMS OF REFERENCE

- 1. Is 4-Aminobenzoic acid (PABA) safe for continued use as a UV filter in cosmetic products under the current restriction of 5.0 % as a maximum concentration?*
- 2. Does the SCCP consider that the use of 4-Aminobenzoic acid (PABA) is safe for the consumer in a concentration up to 5 % when used in other cosmetic products than sunscreen products?*
- 3. Does the SCCP propose any further restrictions or conditions for its continued use in cosmetic products?*

Opinion on 4-Aminobenzoic acid (PABA)

3. OPINION**3.1. Chemical and Physical Specifications**

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Primary name: 4-Aminobenzoic acid
INCI name: PABA

3.1.1.2. Chemical names

p-Aminobenzoic acid, Aniline-4-carboxylic acid, *p*-Carboxyaniline

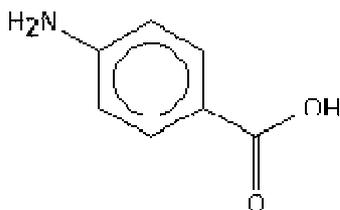
3.1.1.3. Trade names and abbreviations

Trade name: PABA
COLIPA n°: S1

3.1.1.4. CAS / EINECS/ELINCS number

CAS: 150-13-0
EINECS: 205-753-0

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: C₇H₇NO₂

3.1.2. Physical form

Crystalline powder

3.1.3. Molecular weight

Molecular weight: 137.13

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3.1.4. Purity, composition and substance codes

The PABA used is a pharmaceutical grade with a specified purity of $\geq 98.5\%$; actually the purity is 99.5-99.9%,

3.1.5. Impurities / accompanying contaminants

According to the product specification the impurities are:

Heavy metals: $\leq 0.002\%$

Elements identified:

by sweep electron microscopy: Ca, Na, Al, Si, S

Volatile diazotizable substances: $\leq 0.002\%$

Loss on drying: $\leq 0.2\%$

Residue on ignition: $\leq 0.1\%$

Total amount of impurities (TLC): $\leq 0.5\%$, actually $\leq 0.1\%$ ¹⁾, primarily azo-4,4'-dibenzoic acid

¹⁾ An unpublished trend analysis for the last five years (2000-2005), which shows that the total amount of impurities in the quality of PABA used has steadily decreased over this period from $<0.5\%$ to $<0.1\%$.

3.1.6. Solubility

The solubility of PABA is 6.1 g/l at 30°C in water, 125 g/l alcohol and 17 g/l ether. PABA is soluble in ethyl acetate and glacial acetic acid, slightly soluble in benzene, and practically insoluble in petroleum ether.

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: 0.68

3.1.8. Additional physical and chemical specifications

Appearance: Odourless crystalline powder; may turn slightly yellow on prolonged exposure to light and air

Melting point: 188.5°C

Boiling point: /

Density: /

Rel. vap. density: /

Vapour Pressure: 2.7×10^{-4} mm Hg

3.1.9. Stability

PABA dissolved in alcohol was irradiated with UV-light in cuvettes. The UV-spectrum of PABA was practically unchanged after 3 hrs of irradiation. The calculated half-life time of PABA at an irradiation of 15 J/cm² was 177 min. The experiments were carried out in a very

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weak solution of PABA (10^{-5} M). The UV-dose in the experiment was high compared to natural sunlight, and further the spectrum of the sun lamp differed from natural sunlight emitting wavelengths beyond 290 nm. Therefore, the authors discussed that only a minor degree of photo-degradation of PABA will take place after doses comparable to outdoors conditions.

Ref.: 1

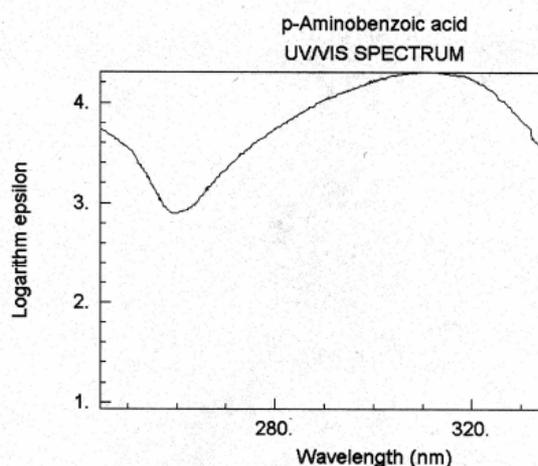
PABA dissolved in 70% alcohol with/without 10% glycerol and phosphate buffer or in pure water was irradiated with UV-light in cuvettes or applied on five volunteers before irradiation. The PABA solutions were stored in darkness at room temperature for 3 months. No colour change was observed after 3 months stored in darkness or after irradiation in different media. PABA in 70 % alcohol was stable for 3 months in darkness. PABA dissolved in alcohol, buffer, and water was not decomposed under any of these conditions. It was concluded that PABA is stable in alcohol/glycerol solutions for 3 months and after UV-irradiation *in vitro*.

Ref.: 2

PABA dissolved in water was irradiated with UV-light in cuvettes. The experiments were carried out with a 7×10^{-5} M solution of PABA. The experiments indicated that UV-irradiation of PABA gives rise to formation of azodibenzoic acid. The UV-dose required to form 10% of azodibenzoic acid is 2.3 J/cm^2 corresponding to 77 times the minimal erythemal dose for human skin (MED).

Ref.: 3

3.1.10. UV-light absorption spectrum



3.2. Function and uses

PABA is known as a sunscreen agent in cosmetics. It absorbs UVB radiation strongly. In the 1940's, dermatologists began to prescribe it in 2-5 % strengths in aqueous creams or in 70 % alcoholic solutions as a sunscreen. PABA was very popular as a sunscreen agent through the 1950's and 1960's and used as such throughout the world. The use of PABA has declined during the last decade.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Acute toxicity of PABA was determined. Mice rats and dogs were employed.

Guideline:	/
Species/strain:	Mice, rats, and dogs (strain not specified)
Group size:	Mice: 5 – 10 animals; rats: 8 animals; dogs: 1 – 3 animals
Test substance:	PABA, free acid
Batch:	/
Purity:	not stated
Dose levels:	Mice (g/kg bw): 2.5; 3.0; 3.5; 4.0 Rats (g/kg bw): 2.0; 2.5; 3.0; 3.6; 4.2; 5.0; 6.0 Dogs (g/kg bw): 0.5; 1.0; 1.5; 2.0; 3.0
Route:	Oral, gavage
Exposures:	1
GLP:	not in compliance

The animals were administered PABA only once and thereafter observed until death occurred, for dogs this could take 1-2 days. Toxic sign in mice were weakness and loss of normal posture, death occurred in several hours. In dogs toxic signs were tremors, weakness, and 2 dogs vomited. Convulsions were observed with the largest dose. Acute gastro-enteritis with haemorrhages was seen with lethal doses. Acute necrosis of the liver was seen with the two largest doses.

Oral LD ₅₀ for rats:	> 6 g/kg bw
Oral LD ₅₀ for mice:	2.85 g/kg bw
Oral LD ₅₀ for dogs:	1–3 g/kg bw

Ref.: 13

3.3.1.2. Acute dermal toxicity

No data submitted

3.3.1.3. Acute inhalation toxicity

No data submitted

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3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

Human

Guideline:	/
Species/strain:	/
Group size:	50 human volunteers with healthy skin; 45 females, 5 males
Test substance:	PABA, 5% in a sunscreen formulation
Batch:	/
Purity:	not stated
Dose levels:	5%
Application:	20 µl was applied on the back of each volunteer under occlusive cutaneous test plaster. Blank plaster, demineralised water, and two well tolerated products were tested to standardize the test. Positive control was sodium lauryl sulfate 0.3%
Route:	Dermal application
Exposure period:	The test plasters were removed after 48 h and test area assessed. Further assessment followed after 72 hr and 96 hr.
GLP:	not in compliance

All volunteers were included in the study as none discontinued. The test substance scored 0 reaction point, whereas the positive control scored 70 points.

It was concluded that the substance including 5% PABA is well-tolerated and showed very good skin compatibility.

Ref.: 15

In a patient with no prior history of cutaneous reactions to other sunscreens or topical anaesthetics, PABA did not show contact allergic reactions when patch tested were protected from sunlight. When PABA was tested in alcohol and when the site was exposed to UVA irradiation the patient showed photo-contact allergic reactions. PABA has not shown irritation in patch test sites without irradiation.

Ref.: 16

3.3.2.2. Mucous membrane irritation

It is stated in the submission that reports of several hundred persons who had used 5% PABA in alcohol solutions on their face and body did not show any eye or skin irritation. Further details on the possible study reports are not found.

Ref.: 17

3.3.3. Skin sensitisation

Test on animals**Local Lymph Node Assay**

Guideline:	OECD 429
Animals:	Female CBA/Ca or female CBA/JHsd mice, 6-12 weeks old
Group size:	4 – 5 animals
Test substance:	PABA
Batch:	/
Purity:	99%
Concentrations:	PABA was tested in 4:1 acetone/olive oil in concentrations: 0 (control) 0.5, 1.0, 2.5, 5.0, and 10.0%
Application:	Quantity of test formulation applied: 25 µl
Treatment scheme:	Frequency and total duration of treatment: daily for 3 (lab. A, B and E) or 4 (lab. C and D) consecutive days followed by rest for 2 days (lab. A, B and E) or no rest (lab. C and D) prior to analyses. Anatomical site: dorsum of both ears
Preparation procedure:	Termination: On day 6 (lab. A, B and E) or 5 (lab. C and D), the mice were injected intravenously with ³ H-TdR (or ¹²⁵ IUdR for lab. E). Five hours later the mice were sacrificed, and the auricular lymph nodes were excised
Isolation of lymph nodes:	Mechanical disaggregation through 200-mesh stainless steel gauze, wash with excess of PBS, precipitation with 5% TCA (4°C). Transferred to 10 ml scintillation fluid (Optiphase MP). Lab A and B pooled the lymph nodes for each group (pooled treatment approach), and lab. C, D and E analysed the lymph nodes from individual mice (individual animal approach)

An international trial in which the performance of the assay has been evaluated using seven test materials, including PABA, in five independent laboratories (A, B, C, D, and E). The Local Lymph Node Assay was performed according to the standard protocol stated by Kimber and Basketter (1992) with a few modifications, e.g. some of the participating laboratories pooled the lymph nodes and other tested individual mice. Generally, the protocols comply with the newly adopted OECD test guideline No. 429 “Skin Sensitisation: Local Lymph Node Assay”.

The applicant concluded that PABA induced no increase in isotope incorporation, relative to vehicle control, whereas other chemicals, known to be skin sensitising, elicited clear positive results.

Ref.: 52

Guideline:	OECD 429
Animals:	Female CBA/Ca mice, 6 – 12 weeks old
Group size:	4 animals
Test substance:	PABA

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Batch:	/
Purity:	99%
Concentrations:	PABA was tested in 4:1 acetone/olive oil in concentrations: 0 (control), 5.0, and 10.0 %
Application:	Quantity of test formulation applied: 25 µl
Treatment scheme:	PABA was applied to the dorsum of both ears daily for 3 consecutive days followed by 2 days without treatment
Preparation procedure:	Termination: On day 6, the mice were injected intravenously with ³ H-TdR, and five hours later the mice were sacrificed, and the auricular lymph nodes were excised
Isolation of lymph nodes:	Isolation of lymph nodes: excised and pooled for each experimental group. Mechanical disaggregation through 200-mesh stainless steel gauze, wash with excess of PBS, precipitation with 5% TCA (4°C). Centrifuged and re-suspended. Transferred to 10 ml scintillation fluid (Optiphase "Hisafe3", Wallac, Turku, Finland)

The aim of the experiments was to explore the utility of the production of the cytokines interferon-gamma and interleukin 12 (IL-12) by draining lymph node cells as alternative readouts for the LLNA.

PABA induced no increase in isotope incorporation, relative to vehicle control, whereas other chemicals, known to be skin sensitising, elicited clear positive results. In addition, exposure of mice to PABA did not stimulate secretion by local lymph node cells at levels of p40 IL-12 higher than those observed with concurrent vehicle or of detectable levels of interferon gamma. The applicant concluded that PABA showed no sensitizing potential according to the specified method.

Ref.: 53

Test in humans

In a dermatological clinic in Norway 23 patients with reactions to sunscreen preparations were seen during the years 1980-1982. The symptoms were eczema, redness, stinging or burning of the face following sun exposure. Patch tests and photopatch tests were carried out on the patients. Allergy to PABA was demonstrated in 11 patients (48%), of which 6 had plain contact allergy (26%) (PABA on non irradiated test sites) and 5 (21.7%) reacted to PABA only after irradiation.

Ref.: 18

Dermatology departments in Scandinavia performed photopatch testing on 1993 patients with suspected photodermatosis from 1980-1985, using the standard photopatch test procedure of the Scandinavian Photodermatology Research Group (SPDRG). In the test both irradiated and non-irradiated test sites were examined, and the results from the non-irradiated test were interpreted as plain contact allergic reactions. 369/1993 (18.5%) showed positive reactions in patch and photopatch tests. 44/1993 (2.2%) reacted against PABA (plain contact allergy against PABA). 749 of the patients had PMLE (Polymorphic light eruption) and 120 (16.0%) showed positive reactions in patch and photopatch tests. 9/749 (1.2%) reacted against PABA (plain contact allergy against PABA).

63 of the patients had other skin diseases (PLR/AR (Persistent light reaction/Actinic reticuloid) and 27 (42.9%) of them showed positive reactions in patch and photopatch tests. 2/63 (3.2%) reacted against PABA (plain contact allergy against PABA).

Concomitant and cross-sensitivity reactions were particularly observed between the PLR/AR and PMLE group of patients. This indicates susceptibility to contact and photo contact sensitivity among such patients.

In the 1993 patients with suspected photodermatosis about 40 % of the patients (749 + 63) were suffering from skin diseases, which may influence susceptibility.

Ref.: 19, 20

3.3.4. Dermal / percutaneous absorption

One of the purposes of this study was to examine the behaviour of PABA on the skin.

Guideline:	/
Test substance:	5% PABA sunscreen product (Presun, Westwood Pharmaceuticals, Inc)
Batch:	/
Purity:	not stated
Dose applied:	2 µg/cm ²
Skin:	Human skin, back of the hand of one of the investigators and freshly excised hairless mice skin
Experimental:	<i>Human skin:</i> 5% PABA was applied to the back of the hand of one of the investigators at an application density of approximately 2 µl/cm ² . The area was examined with a reflectance microscope <i>Hairless mice skin:</i> 5% PABA was applied at 2 µl/cm ² to an area of freshly excised hairless mice skin. The product was rubbed in well to stimulate use conditions. The area was examined with a light microscope and for further examination a reflectance electron microscope
Skin preparation:	/
Exposure period:	/
Donor chamber:	/
Receptor fluid:	/
Control:	/
Skin integrity:	/
Reproducibility:	/
Recovery:	/
GLP:	not in compliance

The behaviour of PABA on the skin was examined using microscope. The absorption spectrum of PABA micro-crystalline aggregates on quartz plates was determined and compared with the spectrum of PABA dissolved in alcohol and PABA in water Crystals could be seen on the human skin, but it was difficult to investigate the crystals further on live human skin due to shallow depth of focus and subject movement.

As the PABA product dried on the hairless mice skin many small crystals could be observed with a light microscope. Examination of the skin with a reflectance microscope revealed needle-like crystals scattered over the surface, more concentrated in some areas than in others.

The absorption spectrum of PABA changed significant in shape and intensity for the 5% PABA sunscreen product, which formed crystalline deposits on the skin compared to the spectrum

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determined after applying a solution of PABA (PABA dissolved in alcohol) not forming crystalline deposits on the skin. The maximum absorbance of the PABA crystals occurred between 300 and 305 nm, whereas the absorption maximum of PABA in alcohol dissolution was 289 nm. The peak absorbance of PABA crystals is broad and relatively flat throughout the entire UVB region.

Because skin contains a large quantity of water and PABA is moderately soluble in water, the absorption spectrum of PABA in water solution was also determined. The maximum peak was found at 278 nm. The peak for PABA dissolved in alcohol was 289 nm.

Discussion

Crystals of PABA was observed on the skin surface after application of PABA on skin at concentrations of 5% PABA product. The authors pointed out that the reservoir effect of the skin contrary to other studies might be due to PABA crystals on the surface acting as the reservoir of PABA instead of a reservoir in the horny layer. The authors concluded that the protective action of PABA against sunburn in humans derives from a combination of its absorption as crystalline deposits on the skin surface together with the contribution of the absorption of PABA in solution within the skin layers.

Ref.: 22

Studies of the mechanism of the protective action of PABA

Guideline:	/
Test substance:	An ethanol solution of ¹⁴ C-labeled PABA (California Bionuclear Corp, Sun Valley, CA)
Batch:	/
Purity:	not stated
Dose applied:	20 – 50 µg/cm ²
Skin preparation:	Full-thickness human skin
Experimental:	The skin was placed on one half of a diffusion chamber with the dermis towards the cell (inner side). An ethanol solution of ¹⁴ C-labelled PABA was applied onto the horny layer. After evaporation of the ethanol the other half of the chamber was placed on the skin and the chamber was clamped. At the end of the study the epidermal surface was washed and the skin removed from the chamber. The epidermis (including the horny layer) was separated from the dermis and both parts analysed for the amount of PABA
Skin temperature:	Room temperature
Exposure period:	2 hr
Donor chamber:	/
Receptor fluid:	/
Control:	/
Skin integrity:	/
Reproducibility:	/
Recovery:	/
GLP:	not in compliance

An *in vitro* method using diffusion chambers to measure the entry of PABA into excised human skin was carried out. In this study the amount of PABA penetrating into the skin was measured in the epidermis and not in the horny layer as would have been preferred. It was observed that

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PABA enters the horny layer rapidly and diffuses through it quite readily. Approximately 40% of the applied amount of PABA was recovered on the skin surface, 40% in the epidermis and a small amount in the dermis (less than 5%) 2 hours after application. However, large biological variation was observed from study to study. The remaining 15% of the applied dose is not recovered and the authors do not state where the amount of PABA may be found. The applicant stated that 15% of the applied dose of PABA was not recovered and might have penetrated the skin. However, the amounts of PABA recovered in the different compartments (surface, epidermis and dermis) are not stated. The authors pointed out that in the setup they normally only recover 85 – 90% of the applied dose.

Ref.: 21

Percutaneous absorption and metabolism of PABA was studied *in vivo* in humans

Guideline:	/
Test substance:	5% PABA in three different preparations: <ol style="list-style-type: none"> 1. Hydroalcoholic gel containing methylcellulose 2. Anionic O/W emulsion (octanol/water) at pH 4.2 in which PABA is mainly suspended 3. Anionic O/W emulsion at pH 6.5 in which PABA is dissolved
Batch:	/
Purity:	/
Dose applied:	20 g preparation applied to the face, neck, trunk and upper extremities of the volunteers corresponding to 1 g of PABA
Experimental:	6 Healthy male volunteers received topically 20 g of preparations on the face, neck, trunk and upper extremities. Each subject received each preparation topically in random order. Urine was collected immediately before the application of the sunscreen preparation and 2, 4, 8, 12, 16, 24, 28, 32, 36, 40, and 48 hours thereafter One week before starting the topical experiments each subject received a 500 mg oral dose of PABA dissolved in 8 ml of ethanol and 150 ml water. Urine was collected before dosage and at 1, 2, 4, 6, 8, 10 and 24 hours thereafter
Skin preparation:	/
Skin temperature:	/
Exposure period:	48 h
Donor chamber:	/
Receptor fluid:	/
Control:	/
Skin integrity:	/
Reproducibility:	/
Recovery:	/
GLP:	not in compliance

An *in vivo* study using healthy male volunteers was carried out. Both oral administration and skin application with subsequent urine collection and analyses were performed. Large intersubject variations were observed regarding the amount of PABA (measured as PABA or acetylated PABA) excreted in the urine measured as the cumulative urinary excretion. The amounts measured in the urine ranged from 15.8 mg for one subject to 96.3 mg for another subject corresponding to 1.6 to 9.6% of the applied amount of PABA.

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After oral administration of PABA a quick excretion of PABA was observed. Six hours after administration 66.9% of the administered dose had been excreted. 50.5% to 83.1% was excreted as acetylated derivative when PABA was given orally. However, when the compound was applied topically the level of acetylated PABA excreted fluctuated between 69.1% and 90.2%.

Discussion

Skin absorption of PABA corresponding to 1.6 to 9.6% of the applied amount of PABA was measured in the urine of six male volunteers after application of PABA in three different preparations. No significant difference was observed between the three preparations.

Ref.: 23

Comments

The results show that up to 9.6% PABA was recovered in the urine. After oral administration 66.9% was recovered. This indicates that at least $(9.6/0.669)$ 14.3% is absorbed and bioavailable.

Percutaneous absorption and metabolism of PABA was studied in an *in vitro* test using hairless guinea pig

Guideline:	/
Test substance:	¹⁴ C-PABA (p-amino [carboxy- ¹⁴ C]benzoic acid (55.6 mCi/mmol) (Amersham Corporation, Arlington, Heights, IL)).
Batch:	/
Purity:	97%
Dose applied:	PABA was applied in ethanol to skin at approximately 2 µg/cm ²
Skin preparation:	Skin from 3 to 6-month old hairless guinea pig (Charles River Laboratories, Boston, MA) was prepared with a Padgett dermatome at a thickness of 200 µm
Experimental:	Absorption and metabolism experiments were conducted <i>in vitro</i> using flow-through diffusion cells Lactate formation from the metabolism of glucose was used as an index of viability and was measured in the collected receptor fluid 24 hours after application of compound to the skin, the skin surface was washed with soap and water to remove unabsorbed material. Experiments were continued for another 24 hours to allow additional absorbed material to enter the receptor fluid Fluid samples (0.2 ml) were collected in 6-hour intervals. The fluid samples and skin homogenates were analyzed for radioactivity.
Skin temperature:	/
Exposure period:	48 hr
Donor chamber:	/
Receptor fluid:	HEPES-buffered Hanks' balanced salt solution
Control:	Control experiment were conducted with skin made nonviable by using distilled water receptor fluid
Skin integrity:	/
Reproducibility:	/
Recovery:	The total recovery of radioactivity (amount absorbed + amount removed in the 24-wash) ranged from 85 to 95% of the applied amount
GLP:	not in compliance

Skin penetration of PABA was studied in hairless guinea pigs *in vitro* using radiolabelled substances. The study showed that $5.0 \pm 0.7\%$ of PABA was recovered in the receptor fluid, $20.7 \pm 4.9\%$ was recovered in the skin so a total of $25.7 \pm 5.6\%$ was recovered in total in the viable skin. In nonviable skin, the results were: $18.7 \pm 4.8\%$ in the receptor fluid, $14.7 \pm 3.2\%$ in the skin and $33.4 \pm 8.0\%$ in total recovery. The difference between viable and nonviable skin regarding the amount penetrating the skin can be the pH changes that occur in degeneration. A lower pH would increase the amount of PABA that are non-ionized and would, therefore, enhance skin penetration. PABA was extensively N-acetylated during percutaneous absorption. The authors reported that an amount of 5% and 18% of PABA could penetrate the guinea skin in viable or in nonviable skin respectively after topical application.

Ref.: 25

Comment

A non-guideline method was used. Moreover the substance was dissolved in ethanol. The relevance of the results is questionable.

Percutaneous absorption of PABA. Comparison of an *in vitro* test (Isolated Perfused Porcine Skin Flap/IPPSF) and *in vivo* percutaneous absorption in man

Guideline:	OECD 427 (<i>in vitro</i> part); OECD 428 (<i>in vivo</i> part)
Test substance:	^{14}C -PABA (^{14}C]p-aminobenzoic acid) (0.05 mCi/mg) (Sigma. St. Louis, MO). PABA was dissolved in ethanol
Batch:	/
Purity:	99.9%
Group size:	The <i>in vivo</i> study included five or six normal volunteer outpatients per group (males, age 18-85 years and postmenopausal women ages 50-65 years)
Dose applied:	PABA dose was $21.5 \mu\text{g}/\text{cm}^2$ <i>in vitro</i> and <i>in vivo</i>
Skin:	<i>In vitro</i> : Porcine skin flaps in a 10-cm^2 area (5 per each chemical) perfused in a non-recirculating chamber <i>In vivo</i> : Ventral forearm skin <i>in vivo</i> in a 10-cm^2 skin surface area Full-thickness pig skin (epidermis and dermis)
Experimental:	<i>In vitro</i> : The flaps were dosed with radiolabelled compound in a manner similar to the <i>in vivo</i> human experiment. Monitoring of glucose utilization assessed skin flap viability. Venous effluent was collected at 30-min increments for 8 hour after which the perfusions were terminated <i>In vivo</i> : The subjects were topically dosed with ^{14}C -chemical on the ventral forearm in a 10-cm^2 skin surface area. The ethanol vehicle was allowed to air dry and the site was not occluded. Subjects were instructed to not touch or wash the study area for 24 hours. They were allowed to wear clothing over the dosing area. All urine was collected for 7 days. After 24 hours the skin-dosing site was washed and after 7 days the site was stripped with cellophane tape 10 times to collect residual ^{14}C -chemicals in the skin. Percutaneous absorption was determined from the ^{14}C -urinary excretion
Skin temperature:	/
Exposure period:	24 h
Donor chamber:	/

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Receptor fluid:	/
Control:	Five different chemical including PABA
Skin integrity:	/
Reproducibility:	/
Recovery:	/
GLP:	not in compliance

In vitro

The experimental skin penetrated dose of PABA was $5.9\% \pm 3.7\%$ (mean \pm SD) (Highest value 11.9) after topical application to pig skin *in vitro*. $56.2\% \pm 10.1\%$ (mean \pm SD) was recovered in skin surface wash and $12.3\% \pm 6.3\%$ by tape stripping. Recovery: $76.9 \pm 5.2\%$.

In vivo

After topical application of PABA to man, $11.5\% \pm 6.3\%$ (mean \pm SD) (Highest value 18.3%) was found in the urine, $29.5\% \pm 12.8\%$ (mean \pm SD) was measured in skin surface wash and $0.56\% \pm 0.47\%$ was recovered by tape stripping. The study showed that porcine skin *in vitro* did not give the same results as human skin *in vivo* indicating that the pig skin *in vitro* model is not suitable for studying the skin penetration of PABA. The porcine *in vitro* model did show comparable results to *in vivo* results for other substance than PABA.

The skin penetration of PABA in 5 voluntary subjects was measured to $11.5 \pm 6.3\%$ with a large intersubject variation as indicated by the standard deviation. The largest amount of PABA was found after washing the skin after 24 hours indicating that PABA is not penetrating the skin but remains on the skin surface and later can be washed off.

Ref.: 24

Comment

On the basis of the absorption studies submitted, it can be concluded that $11.5 \pm 6.3\%$ (highest value measured 18.3%) represents a minimum amount of PABA that is systemically available as it is based on excretions in the urine.

3.3.5. Repeated dose toxicity

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

Oral, rat

Guideline:	/
Species/strain:	Rats, strain, and sex not stated, 6 weeks old
Group size:	6 – 7 rats
Test substance:	PABA as free acid
Batch:	/
Purity:	/
Dose levels:	0, 0.6 g/kg bw/d, and 1.4 g/kg bw/d
Route:	Oral, gavage
Exposure period:	28 days
GLP:	not in compliance/

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Rats are resistant to oral administered PABA and tolerate 1.4 g/kg bw for about a month. All rats survived and the gain in body weight for all 3 groups was the same. The autopsies on all of the animals were negative. NOAEL is larger than 1.4 g/kg body weight.

Ref.: 13

Determination of accumulation of PABA in blood, liver, and kidney and the overt toxicity of PABA in rats

Guideline:	/
Species/strain:	Sprague-Dawley rats
Group size:	12 animals (sex not given) (5 weeks old at start of study, weight 150 g)
Test substance:	PABA as potassium salt (High grade, Sigma Chemical Co., St. Louis, MO, USA)
Batch:	/
Purity:	/
Dose levels:	0, 0.1, 0.5, and 1% PABA in deionised water
Route:	Oral, in drinking water
Exposure period:	4 week
Experimental:	Three rats from each group at week 1 and 2 and the remaining six rats from each group at week four were killed by decapitation and blood was collected and tissues were removed immediately
GLP:	not in compliance

PABA in the drinking water did not affect the growth of rats as indicated by the similar body weight gain during the 4-week feeding period. PABA had no significant effects on organ (liver, kidney, and spleen) weights. The study showed that PABA might accumulate in tissues and blood, but only to a relatively limited extent. Plasma aspartate aminotransferase activities in rats given 0.5% and 1% PABA were significantly lower ($p < 0.05$) than that of control rats at week 2, but not at week 1 or 4. 1% PABA in the drinking water, significantly decreased t-butylhydroperoxide-induced lipid peroxidation in the liver ($p < 0.05$) but not in the kidney.

Ref.: 26

Comment

Based on the effect on the effect on plasma aspartate aminotransferase activities, a NOEL could be equal to 0.1% PABA in drinking water (According to Sprague Dawley – Ace Animals, Inc <http://WWW.aceanimals.com>) daily intake 10 – 12 ml/100 g bw/d. From the paper, the body weight at the end of the experiment was 320 g. This would correspond to a water intake of $10 \times 3.2 = 32.0$ ml, weight 320 g; $1 \text{ mg} \times 32.0/0.320 = 100 \text{ mg/kg/d}$).

Humans

A case report regarding a 51-year-old man admitted to the hospital with a seven-day history of progressive myalgias, generalized weakness and fever. The man had been treated with PABA 3 g four times a day for four weeks for Peyronie's disease. After discontinuation of PABA his fever abruptly returned to normal, the myalgias and weakness resolved and a decrease in the transaminases occurred within 24 hours. Normalization of his transaminases occurred in 10 days. Hepatic injury has not been widely considered to be potential adverse effect of PABA's administration. However, this case report shows that PABA may cause hepatic injury.

Ref.: 27

A case report described that the potassium salt of PABA (administered orally 4 g three times a day for about 2 months) to a 64-year-old woman led to hepatotoxicity in a patient with scleroderma, as evidenced by elevated serum alanine aminotransferase and aspartate aminotransferase.

Ref.: 28

Comment by the applicant

Only few studies have investigated the repeated dose toxicity of PABA. It is concluded from the studies available that PABA does not show any adverse effects after repeated oral administration. However, two case reports indicate hepatic injury after oral intake of PABA in doses of 12 g/day, corresponding to 200 mg/kg body weight for a human of weight 60 kg. In the two cases there were signs of hepatic injury when administered daily in about four weeks.

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

Oral, rat

Guideline:	/
Species/strain:	Wistar female rats
Group size:	4 rats (200 – 220 g)
Test substance:	PABA as potassium salt
Batch:	/
Purity:	/
Dose levels:	Control, PABA alone (1.2 g/kg bw/d), Hexachlorobenzene (HCB) alone (0.05% in feed), HCB-PABA prophylactic administration (1.2 g PABA/kg bw/d + 0.05% HCB in feed) HCB-PABA therapeutic administration (from 52 day to day 108 in study, 1.2 g PABA/kg bw/d + 0.05% HCB in feed during 108 days)
Route:	Oral, by gavage
Exposure period:	108 days
GLP:	not in compliance

In a 108-days study the influence of PABA on porphyria in rats was investigated. The influence of PABA on porphyria in rats induced by hexachlorobenzene (HCB) is investigated in different ways: a prophylactic administration (simultaneously), and a therapeutic administration (application after manifestation of the HCB-porphyria). The urine of the animals was collected and porphyrin and precursors of porphyrin were determined by ion-exchange chromatography. Porphyrins were separated by thinlayer chromatography.

Neither a simultaneous HCB-PABA (Prophylactic administration) nor PABA application after manifestation of the HCB-porphyria (therapeutic administration) influenced significantly the excretion of urinary porphyrins or precursors. In conclusion, PABA has not shown any effects on the excretion of urinary porphyrins or precursors. No toxic sign were described after 108 days of oral administration of 1.2 g/kg bw of PABA, administered as potassium salt, and NOEL or NOAEL could be stated as equal or larger than 1.2 g/kg bw of PABA.

Ref.: 29

Humans

A case report described a 19-year-old woman, who suffered from renal failure and low grade fever when she was submitted to the hospital. Eight months before admission, she received PABA orally (0.75 g/day; 12.5 mg/kg/d) in a 6-months period prior to the illness. She received PABA for the treatment of localized vitiligo. On admission blood test showed: white blood cell count 5,500/ml, haemoglobin 10.6 g/dl, creatinine 282 µmol/l (7.2 mg/dl). Her renal function worsened steadily and her serum creatinine rose to 620 µmol/l (erratum in article, where 620 mol/l was stated) two years after the initial diagnosis. Although the association between PABA administration and the CTI (chronic tubulointerstitial nephritis) could simply be fortuitous, a possible pathogenic link cannot be excluded.

Ref.: 30

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity *in vitro***Bacterial Reverse Mutation Test**

Guideline: /
 Species/strains: *Escherichia coli* (streptomycin resistance, histidine, methionine, and tryptophane requirement)
 Test substance: PABA dissolved in growth medium
 Batch: /
 Purity: /
 Replicates: /
 Concentrations: 1 mg/ml, no positive or negative control
 Preincubation test: /
 Test conditions: Only without metabolic activation
 Solubility: /
 GLP: not in compliance

PABA did not induce reverse mutation in *E. coli* without metabolic activation.

Ref.: 31

PABA was tested in a bacteria reverse mutation assay with *S. typhimurium* (TA100) as test organism. The test was set up as a photomutagenic test and is therefore referred later in part 3.3.10.2. PABA did not induce mutations in the test.

Ref.: 32

Bacterial Reverse Mutation Test

Guideline: OECD 471, except for the choice of positive controls and the maximum PABA concentration tested

Opinion on 4-Aminobenzoic acid (PABA)

Species/strains:	<i>Salmonella typhimurium</i> TA97, TA98, TA100, TA1535, TA1537
Test substance:	PABA
Batch:	/
Purity:	99%
Replicates:	/
Concentrations:	0 (control), 100, 333, 1000, 3333, 10000 µg/plate (in DMSO) (the maximum test concentration is recommended to be below 5000 µg/plate. In this test 10000 µg/plate was used as the maximum test concentration)
Controls:	As positive controls sodium azide (strain TA1535 and TA100), 4-nitro-o-phenylenediamine (strain TA98), 9-aminoacridine (strain TA97 and TA 1537) and 2 aminoanthracene were used. Rat and hamster liver S-9 fraction were added to all strains. Three mutagens were delivered coded to each test laboratory; 9-aminoacridine hydrochloride H20 (13 dose levels from 0 – 333 µg/plate), 4-nitro-o-phenylenediamine (13 dose levels from 0 – 3333 µg/plate) and tris(1,3-dichloro-2-propyl)phosphate (10 dose levels from 0 – 10000 µg/plate). As negative control potassium chloride was used (9 dose levels from 0 – 10000 µg/plate).
Preincubation test:	4 µg – 2.500 µg/plate (in DMSO)
Test conditions:	Standard plate test and preincubation test both with and without metabolic activation (Aroclor-induced rat liver S9-mix)
Solubility:	/
GLP:	In compliance

In a study, the mutagenic effect of 270 chemicals, including PABA, was determined in bacteria reverse mutation assays using different *Salmonella* strains in the presence or absence of the S9 metabolic activated system.

Incubation with PABA up to 10000 µg/plate did not cause an increase in the number of revertants neither in the presence nor the absence of the S9 fraction from hamster or rat liver. The formation of revertants was inhibited at the highest concentrations of PABA on 10000 µg/plate. It was concluded that PABA was not mutagenic with or without the S-9 fraction.

Ref.: 33

***In vitro* Chromosome Aberration Assay in CHO Cells**

PABA was tested for induction of chromosome aberration in CHO cells. The test was performed both with UV-exposure and without UV-exposure and is therefore also discussed later in part 3.3.10.2.

Guideline:	/
Species/strains:	Chinese Hamster Ovary (CHO) cells
Test substance:	PABA
Batch:	/
Purity:	/
Replicates:	Triplicate
Cell system:	CHO cells without metabolic activation
Concentrations:	1500, 1700, and 1900 µg/ml in DMSO
Controls:	Positive control: MMS 100 µg/ml
GLP:	not in compliance

Results

PABA significantly increased the incidence of chromosomal aberrations at 1900 µg/ml.

Ref.: 34

Comment

PABA did not induce mutations in bacteria, but induced an increased incidence of chromosome aberrations in CHO cells at 1900 µg/ml (the highest dose tested; no scoreable metaphases could be found at higher doses).

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

No data submitted

3.3.7. Carcinogenicity

Topical application, mice

Guideline:	/
Species/strain:	Swiss, female mice
Group size:	50 animals
Test substance:	PABA
Batch:	/
Purity:	/
Dose level:	1, 5, and 10% PABA dissolved in acetone
Control:	Acetone only and 0.5% of 7,12-dimethylbenzanthracene (DMBA)
Route:	Topical, 2 applications weekly
Exposure period:	Up to 110 weeks
GLP:	not in compliance

The chemicals (0.02 ml) were applied on the dorsal skin between the flanks twice a week on a 1-inch square area, which was shaved regularly. The animals were checked weekly, and all lesions as well as tumours were recorded. Animals were allowed to die spontaneously or were killed when moribund. Complete autopsies were performed on all animals and the skin from all animals, all grossly observed tumours and other lesions in the lungs, livers, kidneys, etc., from treated as well as control groups were studied histologically.

The percentage of tumour-bearing mice exposed to PABA was 36, 44, and 40 for the concentrations 1, 5 and 10%, respectively. The percentage of tumour-bearing mice exposed to acetone only (vehicle) was 44%, and in the group exposed to the positive control (DMBA) 78% of the mice were bearing a tumour.

As compared to the control group PABA did not produce a statistically significant increase in skin tumour incidence or tumours in other examined organs.

Ref.: 35

In a study on mice the effect of pre-irradiated PABA and non-irradiated PABA on UV-induced skin cancer was tested. Hairless mice were exposed to UVB with or without protection with PABA or with photodegraded PABA daily for 30 weeks and were observed for a further 10

weeks. The test is discussed in section 3.3.10.3. None of the animals in the non-irradiated groups developed cancer.

Ref.: 36

Comment

PABA did not induce tumours in a skin painting study with Swiss mice.

3.3.8. Reproductive toxicity

3.3.8.1. One generation reproduction toxicity

The effect of PABA a on the reproduction of the rats

Guideline:	/
Species/strain:	Sexually mature, virgin Long-Evans rats
Group size:	6 mated rats about 60 days old
Test substance:	PABA
Batch:	/
Purity:	/
Dose level:	0, 1, and 2% PABA and 1% PABA + 1% inositol
Route:	Oral in feed
Exposure period:	From day 1 p.c.
GLP:	not in compliance

Rats were mated and afterwards exposed to PABA alone and plus inositol in the feed. The litter size and the capability of lactation were determined. All litters were reduced to 6 pups before the lactation period. The size of the litters was identical in all groups and so was the birth-weight of the pups. All pups looked normal in all respects. One litter in the group receiving 2% PABA was omitted as all was born dead. The lactation was inadequate both in control and test groups, and it was suggested that this was caused in parts by insufficiency of dietary fat. It was concluded that PABA in a concentration of 1% or 2% did not affect the reproduction in rats.

Ref.: 37

The resorption of foetuses in the rat during exposure to compounds with an antioxidant effect

Guideline:	/
Species/strain:	Sexually mature, female rats (strain not specified)
Group size:	9 mated rats
Test substance:	PABA
Batch:	/
Purity:	/
Dose level:	Not stated, but the total dose during the experiment was 500 mg
Route:	Oral in feed
Control:	129 mated rats
Exposure period:	Day 1 – 22 p.c.
GLP:	not in compliance

Opinion on 4-Aminobenzoic acid (PABA)

Rats were mated and the percentage of resorptions after exposure to various antioxidants in the feed was determined. 29 compounds, among these PABA were tested. The number of resorptions was counted and the degree of resorption was recorded. The two horns of the uterus were examined separately. PABA caused 77.7% litters with resorptions and 23.8% of the implantations had terminated in resorption. Among the untreated pregnant rats, 40.8% had one or more resorptions and 10.6% of all recognized implantations had terminated in resorptions. Thus PABA caused both an increased number of resorptions in the single litter and an increased number of litters with resorptions. From the results the applicant concluded that although PABA has increased the number of resorptions, the effects is questionable as other extrinsic or intrinsic factors may also affect the prenatal mortality.

Ref.: 38

3.3.8.2. Two generation reproduction toxicity

No data submitted

3.3.8.3. Teratogenicity

The effect of PABA on the development of *Drosophila melanogaster*

Guideline:	/
Species/strain:	Larvae of <i>Drosophila melanogaster</i> line D-32
Group size:	/
Test substance:	PABA
Batch:	/
Purity:	/
Dose level:	50 mg to 500 mg, up to 1 gram at most of PABA was dissolved in 100 g of hot nutrition medium
Control:	No PABA added
Route:	In nutrition medium
Exposure period:	The development of the larvae was followed to adult flies
Observations:	Changes in the development of eyes, wings, melanin inclusions, and legs were recorded
GLP:	not in compliance

PABA showed the larval transformation beginning at PABA 1:2000 (50 mg/100 g). A dose of 1:200 caused sharp inhibition of development. In a study of adult males PABA caused no fewer than 30 different types of morphological modifications. The changes primarily concerned the imaginal disks of the eye and wing. The authors point out that the results leave no doubt that a biogenetic agent with appreciable potentialities for interferences in biological processes has been found.

Ref.: 40

Guideline:	/
Species/strain:	<i>Drosophila melanogaster</i> strain Oregon R, Canton S109, and Canton S
Group size:	/
Test substance:	PABA is dissolved in modified Schneider's medium supplemented with foetal calf serum and added to the cell suspension

Opinion on 4-Aminobenzoic acid (PABA)

Batch:	/
Purity:	/
Dose level:	PABA: 10^{-3} M
Control:	No PABA added
Route:	In nutrition medium
Exposure period:	Drosophila eggs are exposed to 100 different chemicals in the nutrition medium, and the stages of development of the separated cells are record
Observations:	The number of myotubes and gangliae were recorded
GLP:	not in compliance

Prepared myotubes and gangliae were incubated for 24 hours. Then the cells were fixed, stained with hematoxylin and counterstained with Evan's blue. The number of myotubes and gangliae in the PABA exposed eggs were reduced as compared to the controls. According to the applicant the effect of PABA on the egg cells was a false positive.

Ref.: 41

Comment

It is difficult to draw any conclusion from this study.

Intraperitoneal injection and oral administration

Guideline:	/
Species/strain:	White female (strain not specified)
Group size:	11 – 13 female rats
Test substance:	PABA
Batch:	/
Purity:	/
Dose level:	5, and 15 mg/kg bw/d and 50 mg/kg bw/d by gavage. PABA dissolved in physiological salt solution
Route:	Injection or gavage
Exposure period:	Day 1 – 16 p.c.
Observation period:	Day 1 – 20 p.c.
GLP:	not in compliance

The effect of PABA on the reproduction of rats was studied. Female rats, 11-13 in each group were housed overnight with one male. The first day of pregnancy was recorded as the day when spermatozoa were identified in the vaginal smear. The pregnant rats were exposed to PABA from day 1 to day 16 p.c. On day 20 the dams were sacrificed. The number of yellow bodies, implantation sites, resorbed/dead fetuses and the state of external development of the live fetuses were examined. Then the fetuses were fixed in Bouin's solution or in alizarin red in order to study internal organs and bones/cartilages respectively. Two effects were observed in the treated groups as compared to the controls: PABA had a positive effect on the growth processes in fetuses by narrowing the dispersion of extreme values in the size of the litters, and a slight, insignificant decrease in the body weight was observed at the 15 mg/kg and 50 mg/kg levels. It was concluded that PABA did not exhibit adverse effects on the reproduction of rats in the doses used in this experiment.

Ref.: 39

3.3.9. Toxicokinetics

Absorption of PABA from the small intestine. It has been found that PABA is absorbed rapidly from the rat small intestine. The results indicate that PABA is transported through the intestine wall by a carrier-mediated transport system, and that the molecular structure of PABA is important for the absorption and its biotransformation characteristics.

Ref.: 43

Transport across the placenta. In one study performed with 40 healthy obstetric patients, procaine was injected into the mother at the time of delivering the baby. The doses ranged from 1 to 10 mg/kg bw. PABA is a metabolite of procaine and the biotransformation of procaine to PABA is rapid (in sec). The concentration of PABA found in the foetal circulation (the umbilical cord veins) was 40 – 60% as compared to the concentration in the maternal circulation. Thus, PABA can cross the placenta rapidly, but the transport mechanism still remains unknown. However, it is suspected that the mechanism for transport across the placental barrier is the same as for the intestine, i.e. carrier-mediated.

Ref.: 46

Biotransformation of PABA. PABA is extensively acetylated on the primary amino group during percutaneous absorption in the hairless guinea pig and human.

Ref.: 117

A study with rabbits showed that PABA administered intravenously was excreted mainly as the acetyl conjugate, *p*-acetamidobenzoic acid. Smaller amounts of *p*-aminohippuric acid and *p*-acetamidohippuric acid were also formed. The *p*-aminohippuric acid, formed by conjugation with glycine can be further acetylated to *p*-acetamidohippuric acid. In rabbits, 30-40% of the acetylation to *p*-acetamidobenzoic acid occurred in the kidney. Human studies have shown that PABA is being biotransformed primarily by acetylation and glycine conjugation and in smaller amounts by glucuronidation.

Ref.: 11

Elimination of PABA. PABA has long been an accepted objective marker to verify completeness of 24 hour urine sampling as PABA is rapidly and almost completely eliminated with the urine. For this reason PABA has been used clinically for long as the indicator substance in pancreas and liver function tests.

In a study with 4 volunteers, 93% PABA was recovered in the urine during the first 5 hours after a single oral dose of 80 mg PABA. In another study, 33 volunteers ingested 80 mg PABA in connection with meals. Mean urine recovery over a 24 hour period was 93±4% of the administered dose. With age of the volunteers a gradual delay in PABA recovery was observed. These studies show that PABA is absorbed rapidly from the gut, and that it is fast and almost completely eliminated with the urine within 24-hours.

Ref.: 7, 8

Opinion on 4-Aminobenzoic acid (PABA)

Conclusion on toxicokinetics

The toxicokinetics of PABA is characterized by fast oral absorption, biotransformation by the major routes acetylation and glycine conjugation, the minor route by glucuronidation in the liver and kidney, and a fast and almost complete elimination via the urine within 24-hours. PABA is extensively acetylated during percutaneous absorption in humans. Studies have shown that PABA can cross the placenta rapidly. Furthermore, the results of one study indicate that the human placenta has a significant capacity for acetylation of PABA. The dermal absorption is discussed in part 3.3.4, Dermal/percutaneous absorption.

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

Cytotoxicity Assay *in vitro*: Neutral Red (NR) Assay at simultaneous Irradiation with Artificial Sunlight

Guideline:	/
Species/strain:	Balb/c 3T3 cells clone 31
Test substance:	PABA
Batch:	/
Purity:	/
Concentrations:	Eight concentrations (not stated). The test substance was dissolved in Earl's balanced salt solution
Artificial sunlight:	Dr. Honle Sol 500 solar simulator. Wavelength of the solar simulator with the filter H1. Dose: 1.7 mW/cm ² . Total exposure: 5 J/cm ² UVA (50 min)
Control:	Positive control; cells exposed to chlorpromazin
GLP:	not in compliance

The effect was given as EC₅₀ (µg/ml), which is the concentration of chemical that caused a reduction of NRU (neutral red uptake) by 50 % compared NRU by the untreated controls. The EC₅₀ of the positive control was between 0.4 µg/ml and 0.84 µg/ml with UVA and between 10 µg/ml and 24 µg/ml without UVA. The EC₅₀ of PABA was > 1000 µg/ml both with and without UVA. The applicant concluded that PABA did not cause phototoxic effects.

Ref.: 50

Guideline:	/
Species/strain:	Human foreskin keratinocyte
Test substance:	PABA
Batch:	/
Purity:	/
Concentrations:	Eight concentrations (not stated). The test substance was dissolved in Earl's balanced salt solution
Artificial sunlight:	Dr. Honle Sol 500 solar simulator. UVA. Dose: 1.7 mW/cm ² . Total exposure 5 J/cm ² (50 min)
Control:	No positive control
GLP:	not in compliance

Opinion on 4-Aminobenzoic acid (PABA)

The phototoxic effect of PABA was studied in human foreskin keratinocytes. 72 hrs after irradiation, cell viability was determined as a reduction in neutral red uptake at 50% compared to control. The EC50 of PABA was 3599 ($\mu\text{g}/\text{ml}$) when irradiated and 3680 ($\mu\text{g}/\text{ml}$) non-irradiated. The applicant concluded that the small difference in the EC50 values indicates that PABA is non-phototoxic.

Ref.: 51

3.3.10.1.2 Photosensitisation

Test in animals

Contact Photoallergy in Guinea Pig

Guideline:	/ The test was modified according to a method by Horio (1976)
Animals:	Albino Hartley guinea pigs, 250 – 300 g.
Group size:	10 females, no control.
Substance/ test formulation:	PABA, 10% in ethanol
Batch:	/
Purity:	/
<i>Induction phase</i>	
Application:	A 20% solution of sodium lauryl sulfate (SLS) was applied as induction of a local irritation. After 1 hour, 10% PABA in ethanol (unknown quantity) was topically applied to the site and immediately afterwards irradiated. The application of chemicals and exposure to light was repeated for a total of 5 exposures at 48 hours intervals.
UV radiation:	Black lights emitting 300-420 nm (mainly UVA at 360 nm), 2 hours at a distance of 15 cm. Energy output: $4.5 \text{ mW}/\text{cm}^2$
Irradiation dose:	$4.5 \text{ mW}/\text{cm}^2 \times 7200 \text{ s} = 32.4 \text{ J}/\text{cm}^2$.
Resting phase:	After an interval of 14 days, the animals were challenged.
<i>Challenge phase</i>	
Application:	Single 0.05 ml application of a 5% ethanol solution of PABA to the depilated back area. This area was then irradiated with the black lights at a distance of 15 cm for 1 hour.
Irradiation dose:	$4.5 \text{ mW}/\text{cm}^2 \times 3600 \text{ s} = 16.2 \text{ J}/\text{cm}^2$
Observation of symptoms:	The tests sites were examined for erythema 24 and 48 hours following irradiation

None of the animals revealed photo contact sensitivity. The applicant concluded that it was not possible to induce photo contact sensitization to PABA in guinea pigs with the described procedure.

Ref.: 57

Guideline:	/
Animals:	Hartley outbred guinea pigs, weighing 350 – 450 g
Group size:	8 groups of 5, 10 or 19 animals, including both females and males
Substance/ test formulation:	PABA

Opinion on 4-Aminobenzoic acid (PABA)

Batch:	/
Purity:	> 95%
<i>Induction phase</i>	
Application:	A 20% solution of sodium lauryl sulfate (SLS) was applied as induction of a local irritation. After 1 hour, 10% PABA in ethanol (unknown quantity) was topically applied to the site and immediately afterwards irradiated. The application of chemicals and exposure to light was repeated for a total of 5 exposures at 48 hours intervals.
UV radiation:	Black lights emitting 300-420 nm (mainly UVA at 360 nm), 2 hours at a distance of 15 cm. Energy output: 4.5 mW/cm ²
Irradiation dose:	4.5 mW/ cm ² x 7200 s = 32.4 J/ cm ² .
Resting phase:	After an interval of 14 days, the animals were challenged.
<i>Challenge phase</i>	
Test formulation:	A primary irritation study was performed before the main study. PABA was tested at 4 concentrations, 2 conc./animal. After 2h±15min of dermal exposure under occlusive patches at the lumbar region, the treated sites were exposed to 10 J/cm ² UVA radiation. A solution of 5% w/v PABA in methanol was slightly irritating and was chosen for the induction phase
Application:	The induction exposures were conducted 3 times per week for 2 weeks (6 inductions in total). On the first day, 4 intradermal injections were made with FCA (1:1 in water), defining the corners of an area of approximately 2x2 cm, on the clipped and depilated nuchal area, which was then stripped with cellophane. A 25 ml Top Hill Chamber containing the test material (or vehicle or nothing) was applied to the nuchal area. The patched area was occluded with rubber dental dam for approximately 2 hours. After removal of patches the lumbar region was shielded with aluminium foil and test sites were irradiated with 10 J/cm ² UVA.
Irradiation dose:	Light source: UVA radiation (primarily 320-400 nm) was supplied by a bank of 6 fluorescent blacklight lamps (Sylvania F20T12/BL). The distance from the light source to the animal test sites and radiometer detector was approximately 6 inches (≈15 cm)
Resting phase:	After the induction phase the animals were rested for 10-14 days
<i>Challenge phase</i>	
Test formulation:	A solution of 0.5% w/v PABA in acetone was not irritating in the primary irritation study and was chosen for the challenge phase
Application:	The lumbar region was clipped and depilated. Two patches containing either the test material or vehicle was applied to the dorsal area (one on each side). The patched area was occluded with rubber dental dam for 2 hours. After removal of patches a hole was cut in the right site of the dental dam, exposing the treated site. The site was wiped with a disposable paper wetted with the vehicle. The left site was shielded with aluminium foil and the animals were then irradiated
Irradiation dose:	10 J/cm ² UVA
Observation of symptoms:	At 24 and 48 hours post challenge the severity of reaction (erythema) of the sites were read

A guinea pig photoallergy model, described by Harber and associates was used. The model has been used to detect photoallergenicity of known human photoallergens. The use of Freund's complete adjuvant (FCA) and cellophane tape stripping has detected weak human photoallergens (musk ambrette and 6-methylcoumarin). Validation of the test assay is limited. The test was modified according to a method by Horio (1976). Significant numbers of positive skin reactions in animals induced and challenged with PABA plus UVA (9/19 in test group no. 1). No significant responses were found in the control groups (empty patches or vehicle only, groups 2 – 8). The applicant concludes that the data indicated that PABA has a photoallergic potential in guinea pigs. The potency of this photoallergic response seems to be of the same level as for musk ambrette.

Ref.: 55

Comment

While the first experiments with guinea pigs were negative, PABA demonstrated a photoallergic potential in the second study. One difference between the two studies was that Freund's complete adjuvant (FCA) was used in the second study.

Test in humans

Published data on human sensitisation are based on patch tests on patients consulting a dermatological clinic with suspected contact allergy (CA) or photoallergy (PA). As sunscreens are known to cause CA or PA, they are often included in patch test series. Consequently, the literature on sensibility and photosensitivity to PABA is substantial, including several publications describing case reports (16, 57, 59, 60) and retrospective studies on a large number of patients from dermatological clinics, mainly in Europe.

Table 1 summarises the results from papers in which sunscreens, including PABA, are used in the test battery. When comparing the number of PA-reactions to sunscreens in general and to PABA specifically in the various countries, the published data are not assessed in the same way. Thus data are limited by referral bias. Furthermore, experimental conditions are not always comparable. Choice of vehicle, irradiation dose and test concentration may influence the result of the photopatch test. In a paper from 1978, it is stated that false negative results may occur when photopatch tests with PABA is performed with other vehicles than alcohol (16). However, in a paper from 2003 a German, Austrian, and Swiss photopatch test group (DAPT) recommends using petrolatum as a vehicle. Furthermore, they recommend using irradiation doses of 5 J/cm² UVA and a test concentration of PABA of 10% (61). In the summarized papers, different irradiation doses and concentrations of PABA are used. According to several papers this does not influence the results (62) (63).

Table 1. Overview of photopatch tests performed in various parts of the world during the period 1980-2003 in which sunscreens including PABA were used in the test battery.

Test period	Country	No. of patients	PABA conc (%) and vehicle	PA to PABA (%) ¹	PA to sunscreens (%) ²	Leading sunscreen agent causing PA (%) ¹	Ref
1980-1982	Norway	23	1 alc ⁸	21.7	-	PABA (21.7)	18
1980-1985	Scandinavia	1993	5 alc	2.2	-	-	20

Opinion on 4-Aminobenzoic acid (PABA)

1985-1990	USA	187	1/5 pet ⁹	2.73	31 ^{3,5,7}	Pentyl dimethyl PABA (18.2 ³)	67
1987-1989	Thailand	274	5 pet	1.8	4.1 ^{5,7}	PABA (1.8)	68
1985-1990	Europe (DAPT)	1129	5 pet	0.09	1.7	4-isopropyl-dibenzoyl-methane (0.89)	69,70
1982-1992	France	283	2 pet	1.1	16.3 (21.6 ⁵)	Benzophenone-3 (12.4)	71
1981-1996	Netherlands	402	5/10 pet	0.5 ⁶	20.6 ^{5,7}	Isopropyl-dibenzoyl methane (7.8)	72
1990-1992	France	370	5/10 pet	0.3	12.7 ^{3,5}	Benzophenone-3 (6.8 ³)	65
1990-1993	France	108	2/5 pet	0.9	6.5	Benzophenone-3 (3.7 ³)	73
1987-1992	France	270	2 pet	0	17.4	Benzophenone-3 (11.9)	74
1986-1993	USA	138	5/10 pet/alc	2.2	31.8 ⁵	Amyl dimethyl PABA (3.6)	75
1991-1993	Singapore	116	5 pet	0.9	1.7	Benzophenone-3 (0.9) / PABA (0.9)	76
1990-1996	Sweden	355	5 alc	0-6	10.1 ⁵	Benzophenone-3 (4.2)	63
1991-1997	Europe (DAPT)	1261	10 pet	0.24	2.15 ⁵	Benzophenone-3 (0.63)	77, 70
1983-1998	UK	2715	2/10 pet	0.18	1.5 (1.9 ⁵)	Benzophenone-3 (0.5)	78
1996-1998	UK	167	-	0	11.4 ⁵	Butylmethoxydibenzoyl-methane (4.8)	79
1989-1999	Netherlands	99	5 pet	0	31.5 ³	Isopropyl-dibenzoyl methane (113)	64
1991-1999	Australia	81	10 pet	0	34.6	-	80
1992-1999	Australia	19	10 pet	0	100	Benzophenone-3 (47.4)	66
1998-1999	Belgium	11	-	9	54.5 ⁵	Benzophenone-3 (36)	81
1993-2000	Australia	172	5 pet	1.8	6.4 ⁷ (14.25 ^{3,5,7})	4-isopropyl-p-dibenzoyl-methane (3.52 ³)	82
2003	Germany	24	5 alc	0	-	-	83

¹ The percent is calculated as the total number of reactions of the total number of photopatch-tested patients regardless of skin disease unless otherwise stated.

² The percent is calculated as the total number of reactions to sunscreen agents of the total number of photopatch-tested patients regardless of skin disease unless otherwise stated.

³ The calculation is based on both PA and combined PA/CA reactions.

⁴ Percent not stated but reactions to PABA occurred.

⁵ % PA is based on total positive reactions.

⁶ The test period is from 1986 to 1996.

⁷ The individual sunscreens were not tested in equal number of patients.

⁸ Alc: alcohol.

⁹ Pet: petrolatum.

The level of positive photo allergy reactions to PABA is in the same order of magnitude in the various countries, except for a Norwegian study that showed a level 10 times higher than results from the other countries (see table 1). The number of photo allergy reactions to PABA tends to decline in European studies during the period 1990-2000 compared to the period 1980-1989. Overall, the decline is insignificant since the retrospective studies spanning both decades have chosen to publish one value for the entire time span. Despite the insignificant decline, many papers explain the tendency to decline by the reduced use of PABA during the last decade.

Ref.: 64, 65, 66, 63, 62

Comment

Data from studies in animals are inconsistent. One study with guinea pigs was negative while another using Freund's complete adjuvant (FCA) was positive.

Human tests performed on patients contacting a dermatological clinic with a suspected skin disease shows that PABA has a potential to cause photo allergy reactions. Considering the fact that the results are based on patients with suspected dermatological problems rather than the general population, the prevalence is likely to be lower in the general population than in the studied groups. The highest photo allergy reaction to PABA was found in a Norwegian study from 1980 – 82 on 23 patients were 21.7% reacted. In most study the reaction to PABA was in the range 0 – 3%.

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

3.3.10.2.1 Phototoxicity / photomutagenicity / photoclastogenicity *in vitro*

In separate experiments it has been demonstrated that PABA in addition to absorbing UVB also act as a radical scavenger.

Ref.: 101, 102

Isolated nucleotides and DNA

Guideline:	/
Species/strains:	Thymine or thymidine
Test substance:	PABA
Batch:	/
Purity:	/
Replicates:	/
Concentrations:	0 – 12 mM PABA
UV irradiation:	Osram HBO 500 W high pressure Hg-lamp combined with a 324 nm filter (Acton Res. Corp.) Irradiation conditions: 6 hr
GLP:	not in compliance

The dimer yields were determined by HPLC. The UV-light induced formation of thymine and thymidyl-3',5'-thymidine (TpT) dimers in aqueous solutions of thymine or thymidine were studied. Aqueous solutions of thymine or thymine-dimer and PABA were irradiated with UV-light (324 nm). Aqueous solution of PABA, thymine, and thymidine were adjusted to pH 7 and pH 3 respectively.

Thymine-dimerization

At pH 7.0, the dimer yield increased with the PABA concentration up to 2 mM PABA. Afterwards the yield was constant. The yield of the thymine-dimer as a function of time was linear up to 15 h followed by a slight decline. The dimer yield as a function of thymine at constant PABA concentration (10 mM) was linear up to 10 mM thymine. At pH 3.0, the dimer yield decreased exponentially with the PABA-concentration up to 20 mM PABA. At constant concentrations of PABA (1 mM) and thymine (10 mM) the yield of the thymine-dimer as a function of time was linear up to 20 h. At constant PABA-concentration (1 mM) and an irradiation time of 6 h the yield of the dimer as a function of thymine is linear up to 10 mM thymine.

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Thymidine-dimerization

At pH 7.0 and a constant PABA-concentration (1 mM) the yield of thymidine-dimer increased with the thymidine concentration up to 10 mM but at a lower yield. At pH 7 and a constant PABA-concentration (1 mM) the yield of thymidine-dimer increased with the thymidine concentration up to 10 mM. At constant concentrations of thymidine (6.7 mM) and PABA (1 mM) and 6 hr irradiation the yield of the thymidine-dimer as a function of time was linear. At pH 3 and a constant PABA concentration (1 mM) the thymidine-dimer yield as a function of the thymidine concentration up to 10 mM increased. The thymidine-dimer yield as a function of constant concentration of PABA (1 mM) and thymidine (6.7 mM) and 3 hr irradiation was linear.

The paper describes the reaction mechanisms leading to different dimers, but it does not mention the fate or effects in biological systems.

Ref.: 104

Guideline:	/
Species/strains:	[³ H]-Thymine-labelled <i>E. coli</i> DNA (193 cpm/μmol)
Test substance:	PABA
Batch:	/
Purity:	/
Replicates:	/
Concentrations:	0.05% PABA in phosphate-buffered saline (PBS)
UV irradiation:	Johns monochromator at 310 nm with a Mylar filter to exclude UVR < 310 nm. Maximum irradiation time: < 12 min. Output: 1.0 J/cm ²
GLP:	not in compliance

Samples of [³H]-labelled DNA was irradiated in PBS in the presence or absence of 0.05% PABA in a quartz cuvette. The formation of pyrimidine dimers (CPD) was determined chemically followed by thin layer chromatography. The distribution of radioactivity on the chromatogram was determined. In another experiment the dimer-DNA was further irradiated with UV-light.

Irradiation of DNA-molecules in solution (PBS) increased the amount of pyrimidine dimers in the DNA-molecule. In addition, irradiation of the dimer-containing DNA with UV-light (254 nm) split the dimers formed in the DNA molecules.

Conclusion

The applicant concluded that PABA induced the formation of thymidine dimer in DNA and it was mentioned that the formation of pyrimidine dimers might indicate that other damages to DNA may occur.

Ref.: 103

Guideline:	/
Species/strains:	Human neonatal foreskin fibroblast (SUNY Stone Brook)
Test substance:	PABA
Batch:	/
Purity:	/
Replicates:	Triple or quadruple

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Concentrations:	0.05% PABA in phosphate-buffered saline (PBS)
UV irradiation:	FS-40 sunlamp behind a plastic-filter with cut-off at 325 nm, exposure rate: 3.5 J/cm ² /s. Dose: 0.35 J/cm ² . The cells were irradiated for 1000 sec.
GLP:	not in compliance

The PABA-induced formation of pyrimidine dimers (CPD) *in vitro* in DNA from foreskin fibroblasts radiated with UV-light was studied.

Neonatal foreskin fibroblast cells were plated and incubated in phosphate-buffered saline (PBS) with or without PABA and irradiated. DNA was extracted and tested for pyrimidine dimer formation after treatment with UV-endonuclease. The transformation to anchorage independent cells (AIC) was determined after irradiation in the presence or absence of PABA. After irradiation, the cells were plated and grown for 9 days. On day 9 the cells were trypsinised and counted. After 14 days growth in soft agar the number of AIC were counted. AIC cells were irradiated on days 2, 3 and 4. Over the cells were a the plastic-filter (0.35 mm) and PBS with PABA (0.05%) or without PABA

It was shown that the effect of PABA depends on the experimental design. Irradiation through PABA in the plastic decreased the production of dimers whereas irradiation with PABA in the cell suspension increased the production. Furthermore, when the cells were irradiated with PABA in the PBS but without the plastic membrane, the amount of AIC increases.

Conclusion

The applicant concluded that PABA absorbed UV light but also gave rise to production of pyrimidine dimers. Furthermore, the amount of AIC increased when small amounts of PABA were present.

Ref.: 105

Bacteria

Guideline:	/
Species/strains:	<i>Escherichia coli</i> K12 AB2480 (uvrA recA)
Test substance:	PABA
Batch:	/
Purity:	/
Replicates:	/
Concentrations:	0.001 – 0.5% in buffer
UV irradiation:	200W Hg-lamp (Wotan, Germany) + Bausch & Lomb monochromator + Mylar filter (>300 nm, band peaking at 313 nm) Dose: 0.3 – 0.6 mW/cm ² Reactivation light: 500 w tungsten-halogen lamp, WL: 350-425 nm
GLP:	not in compliance

The survival of bacteria exposed to UV-irradiation and PABA was assayed. The bacteria were exposed to UV-irradiation in the presence or absence of non-irradiated or irradiated PABA. Furthermore, the bacteria were exposed to visible light for reactivation of repair mechanisms.

The photosensitising effect on the survival of *E. coli* was shown to be dependent on the concentration of PABA. At PABA concentrations above 0.35% the quenching effect of PABA on UV-radiation overwhelms the photosensitising effect of PABA. At 0.1% PABA, the survival of *E. coli* is unchanged whether or not it is irradiated, both before and after the addition to the

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cell suspension. Furthermore, PABA increases the reactivation of visible light in *E. coli*. The reactivation effect was measured as the survival ratio between reactivated/non-reactivated bacteria. The applicant concluded that PABA concentrations above 0.5% protects directly and stimulates an inborn protection mechanism in the bacteria.

Ref.: 85

Guideline: /
 Species/strains: *Escherichia coli* WP2 and WP2pKM101
 Test substance: PABA
 Batch: /
 Purity: /
 Replicates: /
 Concentrations: 5000 µg/plate (in DMSO)
 Positive controls: Chlorpromazine (CPZ) and 8-methoxypsoralen (8-MOP)
 UV irradiation: Osram Ultra-Vitalux sun lamp. Irradiation was performed both with unfiltered light (UVA/UVB) and filtered with a 3 mm glass sheet which removes UVB light. UVA/UVB: 5.5/1.7 mJ/cm²; and 11.4/3.6 mJ/cm². UVA: 230 mJ/cm² and 460 mJ/cm²
 GLP: not in compliance

The bacteria-experiments with PABA were carried out only as preliminary studies. The applicant claims that PABA has not the capability to induce photomutagenicity.

Ref.: 34

Guideline: /
 Species/strains: *Salmonella typhimurium* TA100
 Test substance: PABA
 Batch: /
 Purity: /
 Replicates: At least twice
 Concentrations: 0 (control) 2, 4, and 8 mM (in buffer, pH 6.5)
 Positive controls: N-methyl-N-nitrosoguanidine (MNNG), sodium azide, and 3-azidoglycerol
 UV irradiation: A 254-nm 30-W germicidal lamp (Tungsram, Hungary) with energy flux density of 1 mW/cm²
 GLP: not in compliance

PABA and UV-photoproducts of PABA were all negative in the bacteria gene photomutation assay both irradiated and non-irradiated.

Ref.: 32

Guideline: /
 Species/strains: *Salmonella typhimurium* TA98, TA100, TA102, TA1535, TA1537 and *Escherichia coli* WP2 and WP2 (pKM101)
 Test substance: PABA
 Batch: /
 Purity: /
 Replicates: /

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Concentrations:	On plate: 0, 0,5, 5, 50, 150, 1500, 5000 µg/plate (dissolved in DMSO) In suspension: 0, 625, 1250, 2500 µg/ml (dissolved in DMSO)
Positive controls:	Chlorpromazine (CPZ) and 8-methoxypsoralen (8-MOP)
UV irradiation:	Xenon arc lamp (Oriel) with 2 mm Schott WG320 UV filter, 8 cm water filter and a UV reflecting dichromic mirror Irradiances (waveband 300-400 nm): 25 mW/cm ² (250 J/s/m ²) (suspension) and 5 mW/cm ² (50 J/s/m ²) (plate) Mercury-metal halide arc (Honle) lamp with an H1 (UVA) filter (in some experiments). Irradiances (waveband 300-400 nm): 2 mW/cm ² (20 J/s/m ²)
GLP:	not in compliance

S. typhimurium and *E. coli* were suspended in phosphate buffered saline with PABA for 30 min. prior to exposure to UV-light. After irradiation, 0,1 ml samples were plated out on agar plates without biotin, tryptophan or histidine. The procedure was repeated with direct irradiation of the plates. The number of revertants was determined. A phototoxicity assay was performed in order to choose a range of UV doses and doses of test compound.

Both CPZ and 8-MOP in combination with light increased the number of revertants and decreased the survival rate of *S. typhimurium* and *E. coli*, but differences between strains were observed.

When PABA was added to the suspension or to the plates the number of revertants decreased, in proportion to the concentration of PABA, but differences between strains were still observed. In none of the tests PABA increased the number of revertants. The applicant concludes that PABA is not mutagenic under the tested conditions.

Ref.: 86

Guideline:	/
Species/strains:	<i>Salmonella typhimurium</i> TA102
Test substance:	PABA
Batch:	/
Purity:	/
Replicates:	Quadruple
Concentrations:	0 – 3160 µg/plate
Positive controls:	8-MOP
UV irradiation:	Suntest CPS, Heraeus, Hanau, Germany, Xenon lamp 200-800 nm simulating solar radiation
GLP:	not in compliance

8-MOP increased the number of revertants in proportion to the concentration of 8-MOP and the amount of UV-light. PABA decreased the number of revertants in proportion to the concentration of PABA. The applicant concludes that PABA is not photo-mutagenic. Contrary to that, PABA shielded the cells against the harmful UVB effects reducing the mutation frequencies.

Ref.: 87

Guideline:	/
Species/strains:	<i>Escherichia coli</i> AB1157, GC3216 tif ⁺ sfiA ⁺ , KS1192 trpA58 strs (λ)

Opinion on 4-Aminobenzoic acid (PABA)

Test substance:	PABA
Batch:	/
Purity:	/
Replicates:	Triplicate
Concentrations:	0 – 20 mM
Positive controls:	N-methyl- and N-ethyl-N-nitrosourea (MNU and ENU), methyl- and ethylmethanesulfonates (MMS and ENU), chloramphenicol
UV irradiation:	0.2 mJ/cm ²
GLP:	not in compliance

Bacteria were grown to 5×10^8 cells per ml in appropriately supplemented minimal-glucose medium. In one part of the study chloramphenicol was added to the medium. Bacteria strains were irradiated with UV-light or incubated with the mutagenic substances before and after the addition of PABA.

The mutagenic efficiency of MNU was suppressed up to 100-fold when PABA was administered to *E. coli* cells concurrently with the mutagen or prior to the mutagenic treatment. The antimutagenic effect is not caused by a chemical reaction between PABA and MNU. PABA significantly decreased the frequency of reversion of Arg⁺ revertants induced by UV-irradiation or incubation with mutagenic substances indicating an antimutagenic effect of PABA. The effect was suggested to be an induction of the SOS system. According to the applicant the results indicate that pretreatment with PABA may induce the SOS DNA repair pathway found in *E. coli*. Furthermore, administration of PABA simultaneously to the UV-light decreases the mutagenic response in *E. coli*. PABA appears to be an effective antimutagen, reducing mutagenesis by induction of the SOS response.

Ref.: 88

Mammalian cells

Guideline:	/
Species/strains:	mouse lymphoma cell line L5178Y
Test substance:	PABA
Batch:	/
Purity:	/
Replicates:	/
Concentrations:	0.25% w/v in phosphate buffer and 0.5% w/v in 5% w/w DMSO
Positive controls:	/
UV irradiation:	200W Hg-lamp (Wotan, Germany) + Bausch & Lomb monochromator + Mylar filter (>300nm, band peaking at 313 nm). Output: 0.6 – 1 mW/cm ²
GLP:	not in compliance

Irradiation of mouse lymphoma cells in Fischer's medium. The cell suspension was irradiated with UV light in a quartz cuvette, and then plated and incubated for 10 days without exposure to visible light. The colonies were counted for each plate.

The presence of PABA during irradiation caused a PABA concentration-dependent reduction in survival of the mouse lymphoma cells. Up to a PABA-concentration of 0.2%, the reduction in survival increased proportionally, at concentrations of more than 0.2%, the effect remained approximately constant. It was suggested that the only effect of DMSO was an increase of the

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cell membrane permeability of PABA. The effect of PABA on survival of the cells was suggested to be due to a DNA-damaging effect, e.g. by thymidine triplet formation *in vitro*.

The applicant concludes: PABA reduced the survival of lymphoma cells at simultaneous UVR, but it is emphasized that these results may not apply to human skin *in vivo*. Furthermore, as the end-point in the study is not DNA-damage but cell survival, it cannot be concluded that the killing effect is caused by DNA-damage.

Ref.: 89

Guideline:	/
Species/strains:	V79 Chinese hamster cells and <i>Saccharomyces cerevisiae</i> XV185-14C
Test substance:	PABA
Batch:	/
Purity:	/
Replicates:	6 dishes
Concentrations:	0 – 10 ⁻³ M
Positive controls:	/
UV irradiation:	Westinghouse FS20 “Sunlamp” low pressure tubular lamp, dose 2.2 J/cm ² /h 275-313 nm
GLP:	not in compliance

Yeast-cells and hamster cells were cultured in YPG-agar medium and in Eagles medium, respectively. Both types of cells were incubated for 10 min. in the dark and then irradiated. The cells were irradiated to the same level of survival as the cells exhibited different sensitivity to UV-light. After irradiation, the yeast-cells were spread on complete medium for survival and medium for indication of revertants. Hamster cells were seeded in culture medium for survival and indication of HPRT mutants.

In both yeast and V79 cells, the addition of PABA increased the survival in a concentration-dependent way. The amount of revertants/mutants decreased in a concentration-dependent way to the control level at the highest concentration of PABA. The applicant concluded that with a sufficient amount PABA protects single cell systems against lethal as well as mutagenic effects of UV-light.

Ref.: 90

Mammalian photo-chromosome aberration assays

Guideline:	/
Species/strains:	Chinese hamster ovary cells (CHO)
Test substance:	PABA
Batch:	/
Purity:	/
Replicates:	Triplicate
Concentrations:	1500, 1700, and 1900 µg/ml cell suspension
Positive controls:	8-MOP and MMS
UV irradiation:	Osram Ultra-Vitalux sun lamp. Irradiation was performed both as unfiltered light (UVA/UVB) and filtered with a 3 mm glass sheet which removes UVB light. UVA/UVB: 200/37.5 mJ/cm ² . UVA: 700 mJ/cm ²
GLP:	not in compliance

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Cultures in triplicate were incubated with PABA in solvent (DMSO), 8-MOP or MMS in culture flasks and incubated before irradiation. After treatment the cells were rinsed with buffer and grown for further 18 h. Afterwards chromosome preparations were made for the evaluation of chromosome aberrations and the mitotic index.

In preliminary experiments the highest concentration of PABA on 1900 µg/ml caused chromosome aberrations. This effect was enhanced by concomitant exposure to UVA/UVB.

Conclusion

The applicant concluded that PABA may cause chromosome aberrations in high concentrations.

Ref.: 34

3.3.10.2.2 Phototoxicity / photomutagenicity / photoclastogenicity *in vivo*

Skin application tests in mice

Guideline:	/
Species/strains:	Albino hairless mice, Ucsd strain, aged 2 – 3 months
Test substance:	PABA
Batch:	/
Purity:	/
Group size:	8 mice
Dose:	5% PABA in 70% 2-propanol + 30% propylene glycol and 5% PABA in PreSun Lotion
Applications:	20 µl of 5% PABA applied on 10 cm ² dorsal skin 1 hr before irradiation
Positive controls:	/
UV irradiation:	2 FS20 Westinghouse sunlamp housed in a non-reflecting holder. Used at a distance of 13-cm. UV output 240 – 325 nm, with max. at 313 nm. Dose: 3 times the minimal erythema dose (MED). Test results at a dose of 0.07 J/cm ² were reported
Observation period:	48 hrs
GLP:	not in compliance

The test substances were applied on the dorsal area on mice, and 25µCi of tritiated thymidine were injected intraperitoneally 1 h before UV-irradiation. The mice were sacrificed 48 h after irradiation with UV-light. Samples of the exposed skin were homogenized, and epidermal DNA synthesis was measured as incorporated tritiated thymidine.. Non-irradiated mice were included in each experiment. The protective effect was determined as the ratio between DNA synthesis in irradiated/non-irradiated mice. DNA-synthesis was determined 6 and 48 hours after irradiation. Erythema/oedema-ratio in the skin was determined 24 and 48 hours after irradiation.

PABA-containing sunscreens protected against UVB, which is determined as an unchanged DNA-synthesis measured 6 hours after irradiation. At 48 hours after irradiation the protective effect remained. The erythema/oedema ratio was higher in animals without protection. However, the accurate reading is difficult in hairless mice, because of the dermal oedema that these animals may elicit. In addition, the results show that the vehicle itself is important for the protecting effect. The erythema/oedema ratio was determined after 24 and 48 hours. The

Opinion on 4-Aminobenzoic acid (PABA)

applicant concludes that PABA sunscreens protect against UV-induced DNA-damage in the skin.

Ref.: 92

Guideline:	/
Species/strains:	C3Hf/HeN female mice, 6 weeks old
Test substance:	PABA (PreSun, Westwood Pharmaceuticals Inc.)
Batch:	/
Purity:	/
Group size:	5 – 8 animals
Concentrations:	/
Applications:	/
Positive controls:	Croton oil
UV irradiation:	6 FS40 Westinghouse fluorescent sunlamps emitting principally (> 60 %) wavelengths between 280 and 320 nm with a total energy output of 0.179 mW/cm ² . Duration of exposure: 30 min, 5 times per week. The dose is 0.32 J/cm ² /exposure
Observation period:	30 days
GLP:	not in compliance

Half an hour prior to UV-irradiation, the PABA-containing sunscreen (0.3 – 0.5 ml) was applied and rubbed onto the shaved dorsal surface, ears and tail of the susceptible mice, which are mice with the UV-induced tumour RD87. After irradiation, animals were randomly chosen and small skin sections were surgically excised, and a histological examination was performed. The mice, which were photoprotected by prior application of PABA showed no evidence of any parakeratosis or increased melanogenesis, and only slight nuclear change or increase of the number of epidermal cell layers. The unprotected mice, however, demonstrated marked changes in all these parameters.

The applicant concluded that the treatment with PABA prior to irradiation with UVB light was effective in preventing the pathological skin changes associated with UVB irradiation.

Ref.: 93

3.3.10.3. Photocarcinogenicity

Skin application tests in mice

Guideline:	/
Species/strains:	Hairless mice, Jackson Labs, Bar Harbor, Maine 13 weeks old
Test substance:	PABA (PreSun, Westwood Pharmaceuticals Inc.)
Batch:	/
Purity:	/
Group size:	5 or 10 animals
Concentrations:	5%
Applications:	A single application of a thin coat of 5 % PABA or the other dose groups was applied before each irradiation. DMBA: 5 mice DMBA + sunscreen base (55 % ethanol in water with added emollients): 10 mice

Opinion on 4-Aminobenzoic acid (PABA)

	DMBA + sunscreen base + UVR: 10 mice
	Sunscreen base + UVR: 10 mice
	Sunscreen base: 10 mice
	DMBA, UVR + PABA: 5 mice
Positive controls:	0.5 % 9,10-dimethyl benz-[a]-anthracene (DMBA)
UV irradiation:	Westing house FS-40 lights. Output: 0.305 mW/cm ² (3.05 x 10 ³ erg/cm ² sec). The total energy during the test was 21.51 J/cm ² (2.39 x 10 ⁸ erg/cm ² sec). Duration of exposure: 15 min
Observation period:	33 weeks + 22 weeks
GLP:	not in compliance

The protecting effect of PABA on UV- light induced skin tumours and actinic damage in hairless mice irradiated with UV was studied. Treatment started with a single application of a thin coat of 0.5% DMBA in acetone, or acetone alone. Treatment with DMBA before exposure to ultraviolet light is known to accelerate the development of squamous cell carcinomas. Four weeks later, the remainder of the treatment schedule was instituted. Before each exposure to UVR, the sunscreen base or the complete formula with 5% PABA was painted on the area with DMBA or acetone. One hour later, the appropriate animals were irradiated 15 min, 3 times a week for 29 weeks. Following the 33-week treatment period, all mice treated with DMBA, all mice exposed to DMBA, UVR + PABA, and half of the other survivors were included in a follow-up study. The mice were left 22 weeks with no further treatment or irradiation. The changes in the incorporation of [³H]TdR in DNA were used as biomarker.

The hairless mice treated with 5% PABA were almost totally protected from skin cancer induced by chronic exposure to UVR in conjunction with a chemical carcinogen – only one mouse developed a small tumour in PABA-treated skin. DNA synthesis and histological observations of all surviving mice showed that unprotected animals had elevated levels of [3H]Thymidine which has been associated with precancerous conditions of the skin, as well as a hyperplastic epidermis and hypergranulosis. However, mice treated with PABA also synthesised DNA on the upper side of the reported normal range, and showed some hyperplasia and hypergranulosis.

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Conclusion

The applicant concludes that due to the small number of animals in this study the results should be considered preliminary. The result, however, showed that PABA could protect against skin tumours induced by ultraviolet light, but not against some hyperplasia and hypergranulosis. It was suggested that the UV-light itself induces mechanisms protecting against cancer.

Ref.: 91

Guideline:	/
Species/strains:	Female hairless (Hr/Hr) mice, 8 – 12 weeks old (Bomholdtgaard, Denmark)
Test substance:	PABA
Batch:	/
Purity:	/
Group size:	30 mice
Test formulation:	1. PABA, 5% in a vehicle consisting of 70% ethanol and 5% glycerol in water 2. Photodegraded PABA: PABA, 5% in a vehicle consisting of 70% ethanol and 5% glycerol in water. This solution was irradiated with UVB to a dose of 27 J/cm ²
Test conditions:	A and B: Without treatment, +/- UV irradiation C and D: PABA, +/- UV irradiation E and F: Vehicle, +/- UV irradiation G and H: Pre-irradiated PABA +/- UV irradiation 3 min. irradiation with 155 mJ/cm ² . Every second week the dose was increased 25-30% to a constant dose of 360 mJ/cm ² corresponding to 7 min. irradiation. The mice were irradiated five days a week for 30 weeks and then observed for 10 weeks. The total UV dose of the mice was 49 J/cm ² UVB.
Controls:	Irradiation without PABA
UV irradiation:	Philips T1 40 W/12 light source UVB (290-320 nm) Output: 0.86 mW/cm ² . Distance: 70 cm UVA (320-400 nm) Output: 0.01 (<i>it is probably 0.1</i>) mW/cm ² . Distance: 70 cm MED (mice): 175 mJ/cm ²
Observation period:	40 weeks
GLP:	not in compliance

Hairless mice were exposed to UVB with or without protection with PABA or with photodegraded PABA daily for 30 weeks and were observed for a further 10 weeks. The effect was assessed as the development of tumour and the weight of dorsal skin. Tumour was defined as a papule >1x1x1 mm.

The death rate of the animals was not significant in any group as compared to the control group. No animals in the non-irradiated groups developed cancer. All animals in the irradiated unprotected groups developed cancer. Protection with both pre-irradiated PABA (about 40% degradation) and non-irradiated PABA delayed the tumour induction time significantly compared to the unprotected groups. The mean weight of the dorsal skin of protected animals was lighter compared to the unprotected animals. All tumours registered were squamous cell carcinomas (SCC). Neither basocellular nor malignant melanomas were found. No metastases were found.

Opinion on 4-Aminobenzoic acid (PABA)

Conclusion

The applicant concludes that both PABA and the photodegradation product DABA (diazobenzoic acid) protected against the development of squamous cell carcinoma.

Ref.: 36

Guideline:	/
Species/strains:	Female hairless (Hr/Hr) mice, 8 – 12 weeks old (Bomholdtgaard, Denmark)
Test substance:	PABA
Batch:	/
Purity:	/
Group size:	30 mice
Test formulation:	PABA, 5% in a vehicle consisting of 70% ethanol and 5% glycerol in water
Test conditions:	A and B: Without treatment, +/- UV irradiation C and D: PABA, +/- UV irradiation E and F: Vehicle, +/- UV irradiation 3 min. irradiation with 155 mJ/cm ² . Every second week the dose was increased 25-30% to a constant dose of 360 mJ/cm ² corresponding to 7 min. irradiation. The mice were irradiated five days a week for 30 weeks and then observed for 10 weeks. The total UV dose of the mice was 49 J/cm ² UVB.
Controls:	Irradiation without PABA
UV irradiation:	Philips T1 40 W/12 light source UVB (290-320 nm) Output: 0,86 mW/cm ² . Distance: 70 cm UVA (320-400 nm) Output: 0,1 mW/cm ² . Distance: 70 cm MED (mice): 175 mJ/ cm ²
Observation period:	40 weeks
GLP:	not in compliance

The purpose of the experiment was to study the short-term effect of PABA on UV-induced photo carcinogenesis using the same test method as described above. Female mice were divided into 6 groups with 30 mice each, one of the animal test groups were treated with PABA on the skin during one-third of the induction period (weeks 16-26) to investigate the effect of short-term application. As is the case with the remaining animal test groups, these animals were irradiated without PABA up to week 30 and then observed up to 10 weeks.

The institution of PABA treatment in week 16 resulted in an increase in tumour induction time, which was significant ($p < 0.05$) following 10 weeks of irradiation. The tumour induction time was increased after 10 weeks of radiation. At the end of the study, there were more animal tumour-free animals in the PABA treated group than in the unprotected group.

The number of tumours in every class was significantly higher in unprotected animals than in all other animals. The skin weight was significantly higher in unprotected animals. All tumours found were squamous cell carcinoma, no metastases were found.

Opinion on 4-Aminobenzoic acid (PABA)

Conclusion

The applicant concludes that PABA protects against skin tumours provoked by UVB-light. Apparently, PABA inhibits the development of benign tumours to invasive carcinomas even if PABA is used intermittently.

Ref.: 94

Guideline:	/
Species/strains:	Female hairless (Hr/Hr) mice, 8 – 12 weeks old (Bomholdtgaard, Denmark)
Test substance:	PABA
Batch:	/
Purity:	/
Group size:	30 mice
Test formulation:	PABA, 5% in a vehicle consisting of 70% ethanol and 5% glycerol in water
Test conditions:	3 min. irradiation with 155 mJ/cm ² . Every second week the dose was increased 25-30% to a constant dose of 360 mJ/cm ² corresponding to 7 min. irradiation. The mice were irradiated five days a week for 30 weeks and then observed for 10 weeks. At the age of 48-52 weeks the animals were killed and a blood sample was taken. Liver and spleen were removed and weighed. Skin, tumour, liver, spleen and bone marrow were taken for histological examination.
Controls:	Irradiation without PABA
UV irradiation:	Corona-2 lamp with 3 Philips T1 40 W/12 light tubes. UVB (280-320 nm) Output: 0,86 mW/cm ² . Distance: 70 cm UVA (320-400 nm) Output: 0,1 mW/cm ² . Distance: 70 cm UVC (250-280 nm) Output: 0.06 mW/cm ² . Distance: 70 cm MED (mouse): 175 mJ/ cm ²
Observation period:	40 weeks
GLP:	not in compliance

Hairless mice were exposed to UVB-irradiation with and without PABA protection. PABA was applied on the back immediately before the irradiation. The blood was analysed and liver and spleen were weighed and examined histologically.

In the unprotected group, 25 out of 28 developed squamous cell carcinoma SCC (one to eight per animal). In the partly protected groups 11 out of 30 mice developed SCC. In the fully protected groups only one developed SCC. No SCC was seen in the control groups. In the unprotected groups, a significantly higher number of peripheral blood granulocytes and a significantly higher mean weight of both spleen and liver were found after irradiation. Treatment with topical PABA during the whole period of UV-exposure prevented the peripheral blood granulocytosis. The protected mice also had a significantly lower mean weight for the liver and spleen than UV-exposed and non-protected mice.

Conclusion

The applicant concludes that topically applied PABA protects against UVB-induced changes in the blood. Furthermore, PABA protects against SCC even when the protection is intermittent.

Ref.: 95

Opinion on 4-Aminobenzoic acid (PABA)

Guideline:	/
Species/strains:	Female hairless (Hr/Hr) mice (Bomholdtgaard, Denmark)
Test substance:	PABA
Batch:	/
Purity:	/
Group size:	/
Test formulation:	PABA, 5% in a vehicle consisting of 70% ethanol and 5% glycerol in water
Test conditions:	The mice were divided in the following groups: Non-irradiated +/- PABA (A, and D), irradiated +/- PABA (B+C), and vehicle +/- irradiation (E+F). The mice were irradiated five days a week for 30 weeks and then observed for 10 weeks. For the first two weeks, the dose was 155 mJ/cm ² (3 min. exposure time). Every second week the dose was increased 25-30% to a constant dose at 360 mJ/cm ² corresponding to 7 min. exposure time
Controls:	Irradiation without PABA
UV irradiation:	Philips TI 40 W/12 light source UVB (290-320 nm)
Observation period:	40 weeks
GLP:	not in compliance

Hairless mice were exposed to UVB-irradiation in the presence or absence of PABA. PABA was applied on the back immediately before irradiation. MED was estimated. At the end of the study, the animals were killed, weighed and dorsal skin, left femoral lymph node and internal organs were sectioned and examined for tumours and metastases.

In the unprotected groups (+/- vehicle) 100% of the animals were tumour-bearing, 90% were malign tumours. In the protected groups, 12% of the animals were tumour-bearing, 4% were malign tumours. No animals in the non-irradiated groups developed tumours. The weight of the dorsal skin in the irradiated and protected group was significantly lower than in the irradiated and unprotected group. All cancers were squamous cell carcinoma (SCC) without metastases. No basal cell carcinoma (BCC) or melanomas were found.

Conclusion

The applicant concludes that topically applied PABA protected against UVB-induced changes. The induction of erythema was significantly inhibited and so is the tumour development. It was suggested that an incomplete blocking of carcinogenic radiation caused tumours to develop in the protected group.

(This reference was submitted) Ref.: 98

3.3.11. Human data

Skin application tests in humans

Erythema was used as biomarker in this study. The protecting effect was assessed as erythema protecting score (EPS)

Guideline:	/
Test group:	175 adult human beings with fair skin and of both sexes
Test substance:	PABA

Opinion on 4-Aminobenzoic acid (PABA)

Batch:	/
Purity:	/
Group size:	/
Test formulation:	PABA, 5% in 70 – 95% ethanol
Test conditions:	After application of PABA, the volunteers were exposed to sunlight under different conditions after application of PABA: The subjects laid inactive in the sun for 60, 120, 150, and 180 min. Application was followed by 30 min. heavy exercise (profuse sweating) The subjects swam 10, 15, and 20 min. in freshwater after application but before sun exposure The skin was washed manually or under a shower before sun exposure The subjects were engaged in “normal” activities on a summer day on the beach
Dose level:	5% PABA in ethanol was applied to six areas, 1.5 by 1.5 inches on the back and the legs
Controls:	Untreated (unprotected) skin areas were used as positive control Irradiation without PABA
UV irradiation:	Sunlight. The volunteers were exposed to sunlight at two geographical areas, Arizona and Switzerland (3000 m altitude)
Observations:	The effect was determined as EPS (% skin reflectance) and protection assessed by visual inspection
GLP:	not in compliance

From these human experiments, the results indicate that 5% PABA in ethanol caused prolonged protection of the skin under different conditions as mentioned above in “Test conditions”, even under sweat-producing exercise and swimming. Friction from clothing did not reduce the protecting effect. The prolonged effect of PABA is caused by a chemical attachment of PABA to the horny layer. Only the free acid in ethanol reacts with the horny layer.

Conclusion

The applicant concludes that PABA is able to protect fair skin against damage (erythema) from UV-light even under marginal conditions such as mid-day sun at high altitude.

Ref.: 97

A manufacturer of a sunscreen product based on PABA in alcohol recorded all received complaints on skin problems received from the customers. Each complaint was evaluated by a dermatologist based on the symptoms described in a follow-up questionnaire. In an unpublished personal communication, the dermatologist has evaluated the record of complaints during the period 1995-2004.

Method:	Evaluation of the complaints on skin problems based on the description of the symptoms described in a follow-up questionnaire.
Persons:	Normal users of a commercial sunscreen product, i.e. the general population.
Substance:	PABA.
Test formulation:	Commercial product. 5% PABA in a sunscreen formulation, based on alcohol.

Opinion on 4-Aminobenzoic acid (PABA)

Application: The users applied the sunscreen product routinely to their skin to protect against sun burn.

Observation of symptoms: A dermatologist assessed the received complaints in follow-up questionnaires and rated the complaints as certain or dubious allergy.

The result of the evaluation of the received complaints may be seen from Table 2.

Table 2. Sunscreen (PABA)-related records of complaints. Registered allergy cases (including photoallergy).

Year	Units sold of product	Product related symptoms					
		Certain allergy		Dubious allergy		Total number of allergy records	
		Number	%	Number	%	Number	%
1995	485,000	3	0.0006	15	0.0031	18	0.0037
1996	370,000	8	0.0022	18	0.0049	26	0.0070
1997	235,000	3	0.0013	5	0.0021	8	0.0034
1998	240,000	6	0.0025	11	0.0046	17	0.0071
1999	305,000	4	0.0013	11	0.0036	15	0.0049
2000	400,000	7	0.0018	13	0.0033	20	0.0050
2001	605,000	15	0.0025	13	0.0021	28	0.0046
2002	565,000	11	0.0019	15	0.0027	26	0.0046
2003	710,000	14	0.0020	22	0.0031	36	0.0051
2004	780,000	15	0.0019	14	0.0018	29	0.0037

The percentage is the relation between units sold of the sunscreen product and the sum of certain allergy and dubious allergy records. Each unit of the sunscreen product contains 100 or 200 ml of 5% PABA.

Through the years 1995-2004 complaints related to the use of the sunscreen product amounted 22 certain or dubious allergies on average per year (range 8-36), corresponding to 0.0049% (range 0.0034-0.0071%) complaints per unit of sold sunscreen product. The frequencies of certain or dubious allergies were on average 0.0018% and 0.0031%, respectively.

Conclusion

The applicant concludes that the frequencies of certain and dubious allergies are related to the number of sold units of sunscreen product and not to the number of persons using the product. Even though, these unpublished data indicate that PABA in the form of this commercial sunscreen product only presents a limited risk when applied to the skin of the general population.

Ref.: 99

3.3.12. Special investigations

The applicant summarised the special investigations:

In aqueous, cell-free *in vitro* systems with thymine, thymidine or naked DNA, UV-activated PABA was able to induce formation of cyclobutane pyrimidine dimers (CPD). However, as these systems lack cellular repair mechanisms, the results cannot be transferred directly to living organisms. In living cells *in vitro*, CPD induce several DNA repair mechanisms and the effect of the repair systems is prominent. Thus PABA has the capability to form CPD, but also to induce DNA repair systems in the cells that scavenge these adducts. In addition, PABA exhibited an antimutagenic effect in prokaryotic cells. This effect was enhanced by UV-light.

CPD excised by the excision repair systems activates the melanogenesis thus stimulating the tanning process in the skin and in turn increasing the protection of the skin. Furthermore, when PABA is applied to the skin it will primarily stay in stratum corneum. Less than 10% is penetrating into the deeper, living skin tissue. Thus stratum corneum plus PABA will act as a filter that absorbs UV-radiation reducing or preventing the radiation from reaching the vulnerable, living skin tissue.

3.3.13. Safety evaluation (including calculation of the MoS)**CALCULATION OF THE MARGIN OF SAFETY**

Not applicable

3.3.14. Discussion

The safety has only been considered for dermal exposure

PABA has low acute oral toxicity, more than 2 g/kg bw in rodents.

Applications of 5% PABA in sunscreen formulation on human skin have shown that irritation is negligible.

Only few studies have investigated the repeated dose toxicity of PABA, a NOEL based on the transient effect on plasma aspartate aminotransferase activities after 2 weeks was 100 mg/kg bw/d. In two patients who were administered PABA orally for about four weeks (12 g/day) signs of hepatic injury were observed (LOAEL = 200 mg/kg bw/d).

PABA did not induce mutations in bacteria, but induced an increased incidence of chromosome damage to CHO cells.

PABA did not induce tumours in a skin painting study with Swiss mice.

PABA has been tested for reproductive toxicity in rats and *Drosophila melanogaster*. PABA did not affect the reproduction negatively.

PABA is acetylated in human skin. The toxicokinetic of PABA is characterized by fast oral absorption, biotransformation by the major routes acetylation and glycine conjugation, the minor

Opinion on 4-Aminobenzoic acid (PABA)

route by glucuronidation in the liver and kidney, and a fast and almost complete elimination via the urine within 24-hours. Studies have shown that PABA can cross the placenta rapidly.

Data from photosensitisation studies in animals are inconsistent. One study with guinea pigs was negative while another using Freund's complete adjuvant (FCA) was positive.

Human tests performed on patients contacting a dermatological clinic with a suspected skin disease shows that PABA has a potential to cause photoallergy (PA-reactions). Considering the fact that the results are based on patients with suspected dermatological problems rather than the general population, the prevalence is likely to be lower in the general population than in the studied groups. The highest PA-reaction to PABA was found in a Norwegian study on 23 patients from 1980 – 82 where 21.7% reacted. In most studies the reaction to PABA was in the range 0 – 3%.

PABA showed no photomutagenic potential *in vitro* in bacteria reverse photomutation assays but showed chromosomal damage in CHO cells at high concentrations. PABA showed no photomutagenic nor photogenotoxic effects in skin application studies *in vivo*. PABA has the capability to form cyclobutane pyrimidine dimers (CPD) in cellular DNA.

PABA protects mice against skin tumours provoked by UVB-light even if PABA is used intermittently.

PABA is able to protect humans with fair skin against damage (erythema) from UV-light even under marginal conditions such as mid-day sun at high altitude.

4. CONCLUSION

Although 4-aminobenzoic acid is presently permitted and used as a sunscreen, it became apparent in the process of evaluation of the dossier that much of the information did not conform to current standards and guidelines.

Before any further evaluation of the use of 4-aminobenzoic acid, both as a UV-filter and for purposes other than a UV filter, the SCCP requires a new dossier in which data to all relevant toxicological endpoints and conform to modern standards and SCCNFP/SCCP guidelines to be submitted before 1 July 2007.

The applicant should also specify for what other purposes the substance should be used.

5. MINORITY OPINION

Not applicable

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