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

Benzoic Acid and Sodium Benzoate

Adopted by the SCCP
during the 4th plenary of 21 June 2005

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1. BACKGROUND

1.1. Background

The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) was requested to review the data submitted to support the safety of benzoic acid, its salts and esters (COLIPA[1] No. P 2), when used at concentrations other than those laid down in Annex VI to Directive 76/768/EEC as preservatives, for other specific non-preservative purposes apparent from the presentation of the products.

The SCCNFP adopted an opinion on 4 June 2002 on Benzoic acid and Sodium benzoate (SCCNFP/0532/01, final). In its opinion the SCCNFP stated, that "*the SCCNFP can only assess the safety of substances for which appropriate data has been submitted for evaluation. Safety assessment is specific and not generic. Only toxicological data for benzoic acid and its salt sodium benzoate have been made available for review. Therefore, there is no review of other salts of benzoic acid or any of its esters. These will require separate evaluation when the necessary data have been made available.*"
It was stated furthermore, that *"the SCCNFP does not find the submission appropriate for a safety evaluation of benzoic acid and sodium benzoate for the applied "other uses" in cosmetic products".*

Recently, the European Commission received Submission II on Benzoic acid, its salt and esters.

2. TERMS OF REFERENCE

1. *On the basis of provided data the SCCP is asked to assess the risk to consumers when Benzoic acid, its salts and esters are used for non-preservative purposes in cosmetic rinse-off products at a maximum concentration of 2.5 % and in cosmetic oral-care products at a maximum concentration of 1.7 %, and in leave-on products up to 0.5%.*

2. *Does the SCCP recommend any further restrictions with regard to the use of Benzoic acid, its salts and esters safe when used for non-preservative purposes in cosmetic products?*

[1] COLIPA – European Cosmetics Toiletry and Perfumery Association

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Benzoic acid (INCI name)
Sodium benzoate (INCI name)

3.1.1.2. Chemical names

Benzoic acid Benzene carboxylic acid; benzene formic acid; carboxybenzene; benzene carboxylic acid; phenylcarboxyl acid; phenylformic acid, E210
Sodium benzoate E211

3.1.1.3. Trade names and abbreviations

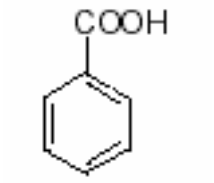
/

3.1.1.4. CAS / EINECS / ELINECS number

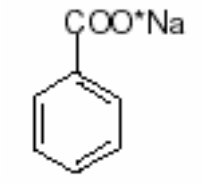
	CAS	EINECS
Benzoic acid :	65-85-0	200-618-2
Sodium benzoate :	532-32-1	208-534-8

3.1.1.5. Structural formula

Benzoic acid



Sodium benzoate



3.1.1.6. Empirical formula

Benzoic acid : $C_7H_6O_2$
Sodium benzoate : $C_7H_5O_2Na$

3.1.2. Physical form

Benzoic acid : solid
Sodium benzoate : solid

3.1.3. Molecular weight

Benzoic acid : 122.13
Sodium benzoate : 144.11

3.1.4. Purity, composition and substance codes

No data

3.1.5. Impurities / accompanying contaminants

No data

3.1.6. Solubility

Benzoic acid : 2.91 g/l in water at 20°C
Sodium benzoate : 556 g/l in water at 20°C, hygroscopic

3.1.7. Partition coefficient (Log P_{ow})

Benzoic acid : 1.88
Sodium benzoate : -2.269

3.1.8. Additional physical and chemical specifications

Benzoic acid

Organoleptic properties	:	/
Melting point	:	122 °C
Boiling point	:	249.2°C
Flash point	:	/
Vapour pressure	:	0.0011hPa
Density	:	1.321 g/cm^3 at 20°C
Viscosity	:	/
pKa	:	-2.269
Refractive index	:	/
Stability	:	/

Sodium benzoate

Organoleptic properties	:	
Melting point	:	>300.0 °C
Boiling point	:	464.9°C
Flash point	:	/
Vapour pressure	:	0.0011hPa
Density	:	
Viscosity	:	/
pKa	:	-2.269
Refractive index	:	/
Stability	:	/

3.2. Function and uses

Benzoic acid is a natural ingredient occurring in many foodstuffs and in plant extracts.
Benzoic acid, its salts and esters are used as preservatives in cosmetic products, with a maximum concentration of 0.5 %, (calculated as acid), as regulated by the EU Cosmetics directives 76/768/EEC.

This request is for use for non-preservative purposes in cosmetic rinse-off products, at a maximum concentration of 2.5 %, and in cosmetic oral-care products, at a maximum concentration of 1.7 %, and in leave-on products, up to 0.5%. However these purposes are not specified.

Other uses for benzoic acid and its salts include regulated use as food preservatives, most suitable for foods, fruit juices, and soft drinks in an acidic pH range. In the EU, there are regulations controlling the maximum levels of benzoic acid and its salts for use in foodstuffs ready for consumption and the specific purity criteria of food additives. The levels are expressed as the free acid.

Non alcoholic drinks	150 mg/l
Alcoholic drinks	200 mg/l
Jams & jellies	500 mg/kg
Aspic	500 mg/kg

Ref.: AR 1, AR 2

In the United States, benzoic acid and sodium benzoate are on the FDA list of substances that are generally recognized as safe (GRAS). Both may be used as antimicrobial agents, flavouring agents and as adjuvants with a current maximum level of 0.1% in food. The FDA has not determined whether significantly different conditions of use would be GRAS. The FDA has sought fully up-to-date toxicology information.

Ref.: AR 3

Benzoic acid is used in oral medicines up to 0.15%, in parenteral medicines up to 0.17% and in topical drugs up to 0.2%. Benzoic acid is used as an active ingredient in anti-fungal cream with salicylic acid (3.0%) up to 6%.
Sodium Benzoate, expressed as benzoic acid, is permitted in oral medicines up to 0.5%, in parenterally administered up to 0.5%.

Ref.: AR 4, AR 5

Benzoic acid is also an intermediate in the synthesis of phenol and caprolactam. Other end products include sodium and other benzoates, benzoyl chloride, and diethylene and dipropylene glycol dibenzoate plasticizers. Sodium benzoate is primarily a preservative and corrosion inhibitor (e.g., in technical systems as an additive to automotive engine antifreeze coolants).

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Acute oral toxicity information is based on summary information, no dossiers were provided. The indication is that the substances can be considered to possess low acute oral toxicity. The calculated LD_{50} values taken from the available experiments on the acute oral toxicity of Benzoic Acid and Sodium Benzoate are listed in the following tables:

Benzoic Acid

Species	LD_{50} [mg/kg]	Reference
Rats	1700	
	2530	4
Mice	1940	5
	2370	6

Sodium Benzoate

Species	LD_{50} [mg/kg]	Reference
Rats	1714	7
	2100 fasting	8
	3140	9
	3450 not fasted	8
	4070	10

3.3.1.2. Acute dermal toxicity

Benzoic acid

Rabbit $LD_{50} > 5\,000$ mg/kg
$LD_{50} > 10\,000$ mg/kg

Ref.: 14, citation only

3.3.1.3. Acute inhalation toxicity

No data submitted with Submission II (2004)

Benzoic acid

Rats $LC_{50} > 0.026$ mg/l/h

Ref.: 14, citation only

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Animal studies
Benzoic Acid

Guideline : OECD 404 (1981), EEC test method B.4, Annex V of EEC Directive 84/449/EEC (September 1984)
Species/strain : New Zealand albino rabbit
Group size : 3 females
Test substance : 0.5 g of benzoic acid moistened with 0.25 ml Milli-RO water
Batch : /
Purity : 99.5%
GLP : in compliance

A paste, (0.5 g benzoic acid and 0.25 ml water), was applied evenly to 6 cm^2 Metalline on a permeable tape (Micropore). This was applied to the right flank of the rabbit. A control patch without the test substance was applied to the left flank. These were left in place for 4 hours. The test site was cleaned first with a dry tissue and then by swabbed with a dampened tissue. The skin was examined for erythema, eschar formation and oedema at 1, 24, 48 and 72 hours after removal of the patches.

Results
Two animals showed slight erythema initially. 1 of these also showed slight oedema up to 24 h. This was resolved by 48 h. There was no indication of a systemic effect.
The results of this study indicate that the test item, benzoic acid, was minimally irritating (modified primary irritation index (PII) of 0.5) when applied to the intact rabbit skin under semi-occlusive patch conditions. The test substance does not need to be labelled as a skin irritant.
Ref.: 12

In submission II, three additional animal tests confirmed the low irritation potential of Benzoic Acid were summarized in a table. These were also provided in Submission I, but are only citations or summary information.

Species	***Exposure***	***Skin irritation***	***Reference***
rabbits	neat Benzoic acid/occluded / 24 h	mild	13
rabbits	500 mg dry powder/ 24 and 72h	PII = 1.66/8.00	14
rabbits	500 mg dry powder/ semiocclusive / 24 h	not irritating	15

Sodium Benzoate

Guideline : OECD 404 (1981)
Species/strain : New Zealand albino rabbit
Group size : 3 females
Test substance : 0.5 g sodium benzoate moistened with 0.25 ml Milli-RO water
Batch : /
Purity : > 99.5%

GLP : in compliance

Approximately 24 hours prior to the treatment, the dorsal fur was shaved. A paste, (0.5 g sodium benzoate and 0.25 ml water), was applied evenly to 6 cm² Metalline on a permeable tape (Micropore). The 6 cm² patch was removed four hours after semi-occlusive contact. The skin reactions were assessed approx. 1 hour, 24, 48 and 72 hours after termination of the exposure.
Under the conditions of the study, sodium benzoate was neither corrosive nor irritating (PII 0) when applied to the intact rabbit skin under semi-occlusive patch conditions for four hours. The test substance does not need to be labelled as a skin irritant. The animals did not show any symptoms of systemic intoxication.

Ref.: 16

Application of 500 mg sodium benzoate as a dry powder to rabbit for 24 h; responses were scored at end of treatment and after 48 h. It was considered not irritating.
There are no details of study.

Ref.: 17

Human Studies

Benzoic Acid
The results, presented only in scientific papers, are from studies both on volunteers and patients from dermatological clinics. 2% benzoic acid in petrolatum over 46h did not irritate intact skin of healthy volunteers. 24h application of 30% benzoic acid in ethanol was found to be the lowest irritating concentration.

Ref. : Gad et al, 1986, Kligman, 1977, Frosch & Kligman, 1976 cited in 13

Chamber test (72 h/occlusive): 0.1 ml of 7.5 % and 15 % benzoic acid in ethanol on scarified skin, 30 % benzoic acid in ethanol on normal skin (6 volunteers).

Results
Scarified skin : 7.5 % in ethanol, moderate irritant
15.0 % in ethanol : marked irritant with erosions
Normal skin : 30 % in ethanol, lowest irritant concentration

Ref.: 19, 21

Chamber test (20 min/occlusive), open test (30 min): 15 µl of 5 % benzoic acid in petrolatum, 15 atopic and 16 non-atopic patients. The atopics showed redness in both the chamber test, (73 %) and the open test, (80 %). Non-atopics showed 80% redness in both the chamber test and in the open test. There was no statistical difference between atopics and non-atopics.

Ref.: 20

8 out of 627 patients (1.3%) from dermatological clinics showed positive reactions to 5 % benzoic acid, in petrolatum under an occlusive dressing for 24 or 48 h. At this concentration, the authors suggest that these results could be interpreted as marginally irritating, rather than allergic.

Ref.: 18

Non-immunologic contact urticaria

Benzoic acid (5.0 % in petrolatum) was tested in an open test on 29 atopic and 74 non-atopic persons.

Results
Contact urticarial reactions to benzoic acid were seen in 27/29 (93 %) of the atopics and in 64/74 (87 %) of the non-atopics.
In the chamber test, 20 min occlusion (recorded 10 min later) 0.1 % benzoic acid in petrolatum and 0.05 % benzoic acid in water elicited reactions. When water was the vehicle, the reactions were oedematous.

Ref.: AR6

Urticaria occurred in 1/10 and 5/12 healthy volunteers with 0.1 and 1.0% benzoic acid, following 20 min covered contact. It was not clear if this was an irritant or immune response.

Ref.: Clemmensen &Hjorth, 1982 cited in 13

Non-immune immediate contact reactions (NIICR), erythema and oedema, have been produced in 78/80 women tested with 2% benzoic acid petrolatum but there was no correlation between the susceptibility to NIICR and age, atopic status or tanning ability. In another study, it was demonstrated that benzoic acid induced non-immune immediate contact reactions in the majority of 200 volunteers with no specific skin condition. Approximately 10% of the volunteers appeared particularly sensitive, reacting fairly strongly. The response, erythema or oedema, was specific to an individual with no significant correlation with age or sex on the degree of NIICR.

Ref.: 54

The Concise International Chemical Assessment Document (CICAD) No. 26 conclusion on benzoic acid and sodium benzoate was: “However, both substances are known to cause non-immunologic immediate contact reactions. This effect is scarce in healthy subjects, while in patients with frequent urticaria or asthma, symptoms or exacerbation of the symptoms were observed”.

Ref.: 55

3.3.2.2. Mucous membrane irritation

Guideline	:	EEC test method B.5, Annex V of EEC Directive 84/449/EEC
Species/strain	:	New Zealand albino rabbit
Group size	:	3 females
Test substance	:	Benzoic acid
Purity	:	> 99.5%
Batch no	:	/
Dose	:	77 mg as a fine powder
GLP	:	in compliance

The test substance was instilled in a single application. The product was poured into the right conjunctival sac. After application, the lids of the treated eye were held closed for approximately two seconds. The untreated left eye was used as a control. The degree of eye irritation was

evaluated at immediately after dosing, 1, 24, 48 and 72 hours and 7, 14 and 21 days after treatment.
After 60 min, Animal 1 showed no reaction to light, with translucent corneal opacity and iridial injection. The other 2 also had slight corneal opacity. All 3 had moderate chemosis and slight conjunctival redness.
The corneal opacity noted in the first animal increased to nacreous areas. This persisted for 72 h. The translucent areas of opacity persisted up to Day 21. In Animal 2, the slight corneal opacity also persisted up to Day 21, but was resolved in Animal 3 by Day 7. By Day 14, in Animal 1, the iridial injection was resolved but no reaction to light. Animal 2 showed iridial injection on Day 2. The slight conjunctival redness in all animals increased to severe with a white/grey discolouration. It persisted in Animals 1 and 2 up to Day 21 and in Animal 3 to Day 7. The chemosis decreased slowly. In Animals 1 and 2, it was not completely resolved by Day 21 but it was resolved in Animal 3 by Day 14. The animals did not show any symptoms of systemic intoxication.

The estimated Draize score of 35 (60 min) is classed as severely irritating according to Kay and Calandra. Under the EU criteria, it should be labelled as an eye irritant.

Comment
This experiment was conducted in 1988, but the information was not provided in Submission 1.

Ref.: 22

Guideline	:	OECD 405
Species/strain	:	New Zealand albino rabbit
Group size	:	3 males
Test substance	:	Benzoic acid DAB 8
Batch no	:	/
Dose	:	/
GLP	:	/

The test substance was instilled in a single application. The product was poured into the right conjunctival sac. After application, the lids of the treated eye were held closed for approximately two seconds. The untreated left eye was used as a control. The degree of eye irritation was evaluated at immediately after dosing, 1, 24, 48 and 72 hours and 7, 14 and 21 days after treatment.
The authors considered benzoic acid was considered as a mild irritant, but the data provided is inadequate.

Ref.: 23, cited in BUA (14)

Instillation of 50 mg benzoic acid into the conjunctival sac of 2 rabbits.

Results
Moderate irritation. This is just a summary.

Ref.: 15, cited in BUA (14)

Additional information that was not mentioned but quoted in the BUA reference, was a citation of a single application of 100 mg dry powder to the rabbit eye. The irritation score was 65.0/110. It was scored at 24, 48, and 72 h.

Ref.: cited in BUA (14)

Comment
The data provided is inadequate.

Sodium benzoate

Guideline	:	OECD 405
Species/strain	:	New Zealand albino rabbit
Group size	:	3 females
Test substance	:	Sodium Benzoate
Purity	:	≥ 99.5%
Batch no	:	/
Dose	:	60 mg ground as a fine powder
GLP	:	in compliance

The test substance was instilled in a single application. The product was poured into the right conjunctival sac. After application, the lids of the treated eye were held closed for approximately two seconds. The untreated left eye was used as a control. The degree of eye irritation was evaluated at immediately after dosing, 1, 24, 48 and 72 hours and 7, 14 and 21 days after treatment.
Under the conditions of this study, sodium benzoate produced irritation of the conjunctiva that was reversed within 14 days. There was no effect on the cornea or iris. It was considered mildly irritating (Kay and Calendra score 9.3) and in the EC classification.

Ref.: 24

Application of 50 mg sodium benzoate/rabbit for 24 h; responses were scored at 24 h, 48 h and 72 h; post-exposure observation time: 7 d.
Results: not irritating.

Ref.: 17

3.3.3. Skin sensitisation

Benzoic acid
Animal tests
The results of two *in vivo* guinea pig assays, a Magnusson-Kligman test and a Buehler test were reported. These were in-house tests with no further details. Benzoic Acid did not induce sensitization in either test and was thus considered to have low skin sensitization potential.

Ref.: 25

Human volunteer studies

Benzoic Acid at 2 % and 5 % in petrolatum did not induce sensitization in two maximization tests.
25 human volunteers were given five 48 h patch tests (over a 10 d period) with 2 % benzoic acid in petrolatum. None gave positive reactions when challenged 10-14 d after the induction phase with a final 48 h closed patch test with 2 % benzoic acid in petrolatum.
This information is as a summary only.

Ref.: 26 and cited in BUA (14)

10 persons allergic to benzoyl peroxide were tested by a 48 h patch test with 5 % benzoic acid in a hydrophilic petrolatum. No reactions at 48, 72 and 96 h.

Ref.: 27

In a cosmetic intolerance assay, 5202 patients with suspected allergic contact dermatitis were patch tested (537 of the patients had a history of "intolerance" or allergy). Patch test conditions were not specified, but allergic reactions was noted in 34 patients (0.7% incidence) to benzoic acid.

Ref.: 28

The OECD SIDS report found both benzoic acid and sodium benzoate were non-sensitizing in animal test but showed a very low incidence in humans (patients) tested by the patch test.
This report included 2 additional human patch test references that were not in the submissions. The results showed that benzoic acid was an occasional or positive sensitizer (Rademaker & Forsyth, 1989; Forsbeck & Skog, 1977)

Ref.: 56

Sodium benzoate
Human
There were 2 additional human patch test references that were not included with any of the submissions. The first was a large cohort of 2045 patients, 5 gave positive results. These gave occasional positive results (Brasch, J. et al., 1993). In the second test, patch tests gave positive nonimmunologic contact urticaria (Nethercott, J.R., 1984).

Ref.: 56

3.3.4. Dermal / percutaneous absorption

^{14}C-labelled benzoic acid (4 - 40 µg/cm^2 dissolved in acetone) was applied to excised human skin in a static diffusion chamber. Samples of penetrated amounts were measured in 1- 2 hr interval over the first 24 hrs and 3-6 hrs interval over the remaining days. No occlusion or washing were applied and the investigation was determined at least in duplicate. A median of 45% of the applied benzoic acid was found in the receptor phase 48 hrs after application.

Ref.: 40

Percutaneous absorption of benzoic acid was studied in the Mexican hairless dog and in man. ^{14}C-labelled benzoic acid was injected subcutaneously and applied dermally to the neck skin of dogs. Human data were obtained from earlier investigations. Excretion of benzoic acid in man

was rapid, almost complete by day 3. In the dog, excretion was less extensive and greatly prolonged. This was accounted for by the persistence of benzoic acid in the skin.

Maximum absorption rate in man was 3.0 %/h, total absorption was 42.6 % of applied dose.
This *in vivo* result is comparable with *in vitro* test results obtained. The total penetration of radio-labelled benzoic acid was found to be approximately 43 % in a 5-day period. In this human study, 6 test subjects received a dose of 4 µg/cm² Benzoic Acid dissolved in acetone. They were allowed to wash their skin 24 hours after application of Benzoic Acid. It should be noted that the exposure of the *in vitro* and *in vivo* studies were extremely long (2 days and 5 days, respectively).

Ref.: 41

3.3.5. Repeated dose toxicity

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

Oral
Benzoic Acid and sodium benzoate show a low toxic potential in rodents in repeat dose studies.

Benzoic Acid

Guideline	:	Directive 84/449/EEC, B.1 "Acute toxicity (oral)"
Species/strain	:	50 Spartan rat
Group size	:	5 male/ female
Test substance	:	technical grade benzoic acid
Purity	:	/
Batch no	:	/
Dose	:	500, 1250, 1984, 3150, and 5000 mg/kg.
Vehicle	:	corn oil
GLP	:	/

The test compound was suspended in corn oil and administered orally. Volumes of 10 ml/kg bw were administered at all dosage levels.
All surviving rats, males and females, exhibited normal body weight gains during the 14 day observation period. The acute oral LD50 of benzoic acid in male albino rats was calculated to be 2742 mg/kg (2279-3299 mg/kg).
The acute oral LD50 of benzoic acid in female albino rats was calculated to be 2360 mg/kg (2042-2726 mg/kg).
A combined acute oral LD50 for benzoic acid in male and female albino rats was calculated to be 2565 mg/kg (2292-2870 mg/kg).
Ref : IUCLID, Unpublished study (IRDC#163-282). Acute Toxicity Studies in Rats and Rabbits. (1974)
Comment: This study is available on IUCLID but was not submitted.

In rats, 648 mg/kg bw/d in feed for 28 days, has no effects.

Ref.: 14

Sodium benzoate
0.5, 1, 2, 4, and 8 % sodium benzoate in drinking water were administered for 35 days to groups of four female and four male Swiss albino mice.
In the 8 % dose level (approx. 24 g/kg bw/d) all mice died within 3 weeks. In the 4 % dose level (approx. 12 g/kg bw/d) 3 male and 3 female mice died within the 35-day observation period. The bodyweight of the surviving mice was substantially reduced. The 2 % dose level was chosen for a chronic toxicity (carcinogenicity) study.

Ref.: 42

In another study the body weight was slightly decreased at 2200 mg/kg bw/d. Groups of 6 male and 6 female Sherman rats were given 2 % (approx. 2.0 to 2.4 g/kg bw) or 5 % (5.7 g/kg bw for females and 7.8 g/kg bw for males) sodium benzoate in the diet for 28 days. In the 5 % dose group, all female rats died by day 11 and males by day 13. In the 2 % dose group a slight significant body weight depression was observed in male rats.

Ref.: 43

Dose levels from 16 to 1090 mg sodium benzoate/kg bw were given to groups of 10 rats (5 male and 5 female) for 30 days with the diet. No dose related adverse effects were observed.

Ref.: 10

Inhalation

Guideline	:	similar to OECD guideline 403
Species/strain	:	Rat, Sprague-Dawley (Charles River CD).
Group size	:	10 males + 10 females per dose
Test substance	:	benzoic acid
Batch no	:	59230350
Purity	:	/
Dose	:	0, 0.025, 0.25 and 1.2 mg/l
Exposure	:	dust aerosol exposure, (diameter 4.7 μ) 6 h/day for 5 days/week
GLP	:	in compliance

At 1.2 mg/l two animals (out of 20) died, exhibiting irritation of their upper respiratory tract.
These animals did not gain as much weight as controls, exhibited decreased organ weights and multifocal to generalized pulmonary fibrosis and inflammatory cell infiltrate. Exposure to 0.25 mg/l also resulted in an irritation of the upper respiratory tract. All treated animals survived and their body weight gain did not differ from controls. Neither significant effects on organ weights, nor significant effects on hematologic or biochemical parameters in the 0.25, 0.025 or 0 mg/l treatment groups were found. Exposure to the test material at all levels resulted in an increase in the frequency of pulmonary fibrosis and inflammatory cell infiltrate.
No compound related gross lesions were seen in any of the rats from the test groups that were terminally sacrificed or died during the study.
Compound-related microscopic lesions, consisting of an increase in the intensity and extent of inflammatory cell infiltrate and an increase in the incidence, intensity and extent of interstitial fibrosis in lungs of rats from all test groups, were observed.

Ref.: 44

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

Benzoic acid
50 male and 50 female mice received 80 mg benzoic acid/kg bw/d by gavage for 90 days. 14 surviving mice were subjected to a restricted dietary intake (90 % restriction) for up to 5 days. 10 mice were tested on their tolerance to CCl_4 (test on detoxifying capacity of liver) by giving 0.1 ml CCl_4 in an single dose by oral intubation at the end of the test.
Reduced body weight gain was observed and the mortality was higher in connection with reduced tolerance to CCl4 or food restriction than in the control group. The authors did not mention whether this finding was statistically significant. The relevance of the study will be compared to other long-term and carcinogenicity studies.
Data are not sufficient to justify conclusion.

Ref.: 45

Sodium benzoate
Groups of 4-5 male and 4-5 female rats received 0, 1, 2, 4, and 8 % sodium benzoate equivalent to 640, 1320, 2620, and 6290 mg/kg bw/d in the diet for 90 days.
4/8 animals died (average 13 days to death) in the 8 % dose level group, the average weight gain of the surviving rats was reduced and the relative liver and kidney weight was significantly increased with histopathological changes in liver and kidney (7/16).
Data are insufficient to justify a NOAEL of 4 % in the diet (2.6 g/kg bw/d).

Ref.: 8

3.3.5.3. Chronic (> 12 months) toxicity

Benzoic acid
25 male and 25 female mice were given 40 mg/kg bw/d for 17 months. Benzoic acid was fed in a paste prior to the main feed.
The weight of liver, kidney and testes relative to body weight in mice sacrificed at the end of the test period were lower in the group receiving sorbic acid (40 mg/kg bw/d) than in the group treated with benzoic acid. No further details were given.

Ref.: 45

10 male and 10 female rats received 40 mg benzoic acid/kg bw/d for 18 months. Benzoic acid was fed in a paste prior to the main feed.
The rats developed some tolerance to a lethal dose of benzoic acid given terminally (25 % mortality after 4000 mg/kg bw compared to 100 % mortality on the control group given one dose of 3600 mg/kg bw). No further details were given.

Ref.: 45

3.3.6. Mutagenicity / Genotoxicity

Bacterial Reverse Mutation Test

Benzoic acid
Benzoic acid (up to 10 mg/plate) was tested in the Salmonella/microsome test using *S. typhimurium* TA 92, TA 94, TA 98, TA 100, TA 1535 and TA 1537. No significant increases in the numbers of revertant colonies were detected in any *S. typhimurium* strains at the maximum dose.

Ref.: 29

Benzoic acid (up to 10 mg/plate) was tested in the Salmonella/microsome test using *S. typhimurium* TA 97, TA 98, TA 100, TA 1535, and TA 1537 with and without metabolic activation. Benzoic acid was non-mutagenic in this test.

Ref.: 30

Benzoic acid was tested in the Salmonella/microsome test using *S. typhimurium* TA 98, TA 100, TA 1535, and TA 1537 with and without metabolic activation. Benzoic acid was nonmutagenic in this test (< 0.0099 revertants/nmol).

Ref.: 31

Sodium benzoate

Sodium benzoate (up to 3.0 mg/plate) was tested in the Salmonella/microsome test using *S. typhimurium* TA 92, TA 94, TA 98, TA 100, TA 1535 and TA 1537. No significant increases in the numbers of revertant colonies were detected in any *S. typhimurium* strains at the maximum dose.

Ref.: 29

Sodium benzoate was tested in the *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 in the presence and absence of S9 mix. Sodium benzoate was non-mutagenic in this test.

Ref.: 32

***In vitro* mammalian chromosome aberration test in human lymphocytes**

Benzoic acid

Chromosome aberration test was carried out on benzoic acid (up to 1.5 mg/ml) using Chinese hamster lung fibroblasts. No metabolic activation system was applied. 8 % of the cells treated with benzoic acid (1.5 mg/ml) showed structural aberrations at 48 hrs after treatment.

Ref.: 33

Benzoic acid (1.5 mg/ml in DMSO) gave equivocal results using Chinese hamster lung fibroblasts. 8% chromosome aberration and 1% polypoidy were reported.

Ref.: 29

Sodium benzoate

Human embryonic lung cells (WI-38) were used. Dose levels for sodium benzoate were 0, 2, 20, 200 mg/ml. 200 mg/l, previously established as the lowest toxic level. The positive control was 0.1 mg/ml triethylene melamin. The test compound was added to three culture bottles for each dose level, 24 hours after plating. When sufficient mitoses were observed, usually after 24-48 hours, the cells were harvested and prepared for the analysis. 100 anaphases per dose were analysed. For the mitotic index at least 500 cells were counted.
Sodium benzoate produced no significant chromosomal aberrations in human tissue culture cells when tested at any dose level tested. No substance related effects were observed. The chromosome abnormalities as well as the mitotic indices were within normal values.

Ref.: 34

Chromosome aberration test was carried out on sodium benzoate (up to 2.0 mg/ml) using a Chinese hamster fibroblast cell line. No metabolic activation system was applied. 38 % of the cells treated with sodium benzoate showed chromosome aberrations at 48 h.

Ref.: 29, 35,

Chromosome aberration test was carried out on sodium benzoate using a pseudodiploid Chinese hamster cell line (DON). No metabolic activation system was applied.
Concentration above 0.002 mol/l showed twofold background effects of chromosome aberrations; but no increase in the frequency of sister chromatid exchange was observed.

Ref.: 36

Chromosome aberration test was carried out on sodium benzoate using human embryonic lung culture cells. Sodium benzoate produced no significant increase in the aberration frequency in the anaphase chromosomes when tested at the dosage levels 0, 2.0 µg/ml, 20 µg/ml and 200 µg/ml.

Ref.: 33

***In vitro* Sister Chromatid Exchange (SCE)**
Benzoic acid
Benzoic acid, without metabolic activation, was reported to be negative for SCEs *in vitro* using 3 different cell lines. These were literature citations with no other information.

Ref.: 37, 38, 39

***In vivo* Host-Mediated Assay**
0, 50, 500 and 5000 mg sodium benzoate/kg bw was given orally to mice (single dose or once a day for 5 days) in an Host-Mediated Assay. Elevated mutant frequencies were seen with Salmonella TA 1530 in the acute intermediate dose level. The subacute and the other acute dose levels showed no increase in mutant frequencies. Tests with Salmonella G 46 were negative while giving slightly elevated mutant frequencies. Tests with Saccharomyces D 3 produced no increases in recombinant frequencies.

Ref.: 33

***In vivo* chromosome aberration test**
Chromosome aberration of sodium benzoate was investigated *in vivo* in rats. Five rats per group were dosed with 0, 50, 500 and 5000 mg/kg bw by gavage in an single dose or once a day for 5 days. The animals were killed at 6, 24 and 48 h after dosing in the acute study and 6 h after dosing in the subacute study. Bone marrow metaphase chromosomes were checked for aberrations.
A low incidence of treatment related breaks was observed in the acute study but within the control range. In the subacute study, only the mid dose showed breaks (1%) but were not considered to be significant. The mitotic indices were not different from controls. Sodium benzoate did not induce chromosomal aberrations in this test system.

Ref.: 34

***In vivo* dominant lethal assay**
A dominant lethal assay was conducted in rats. Following dosing with sodium benzoate by gavage (0, 50, 500, 5000 mg/kg bw, single dose or once a day for 5 days) treated male rats, 5 per group, were mated with two females per week for 8 weeks (acute study) or 7 weeks (subacute study). Fertility Index, number of implantations, corpora lutea, pre-implantation losses, resorptions/pregnant female and proportions of females with one or more dead, and two and more and overall dead implants were monitored.

In the acute study all three doses showed at week 8 significant, dose-related decreases in corpora lutea, at week 7 significant dose-related increases of average pre-implantation losses. Average resorptions were significant, dose-related increased at the low and high doses of week 2 and the low and intermediate doses of week 7 were significantly increased over the control group. Overall dead implants were significant increased at week 7 for the low and intermediate doses and week 2 for the low dose.
In the subacute study significant increases of average pre-implantation losses were observed at a number of weeks but no significant increases of average resorptions were observed. Overall dead implants were not increased.
The authors considered sodium benzoate to be non-mutagenic in rats in this test system although positive results were obtained.

Ref.: Submission 1, 33

Remark
IPCS CICAD 26 (2000) mentioned this dominant lethal assay as a positive result, however evaluation of the raw data in the original report (by experts of the industry consortium and a recent independent review by Prof. R. Kroes) gives no support for this. In addition the authors of the study clearly conclude negative. FDA also evaluated this study as negative. In addition sodium benzoate doesn't contain a structural alert for genotoxicity.

Ref.: 55

Other recent Evaluations of Genotoxicity

OECD SIDS Initial Assessment Report on **Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol**

They concluded that Benzoic acid and Sodium benzoate, (also Potassium benzoate and benzyl alcohol) showed no mutagenic activity in *in vitro* Ames tests. Various results were obtained with other *in vitro* genotoxicity assays. Sodium benzoate (and benzyl alcohol) showed no genotoxicity *in vivo*. While some mixed and/or equivocal *in vitro* chromosomal/chromatid responses have been observed, no genotoxicity was observed in the *in vivo* cytogenetic, micronucleus, or other assays. The weight of the evidence of the *in vitro* and *in vivo* genotoxicity data indicates that these chemicals are not mutagenic or clastogenic. They also are not carcinogenic in long-term carcinogenicity studies.
They also remarked since the sodium salt of benzoic acid instantaneously dissociates to the benzoic acid, the studies with sodium benzoate are also representative for benzoic acid and potassium benzoate.

Ref.: 56

JECFA report, 1998
In addition data from *in vivo* genotoxicity studies on benzyl acetate and benzaldehyde are supportive evidence for the non-genotoxicity of benzoic acid and sodium benzoate.

Ref.: 52

Scientific Committee on Food Opinion: Re-evaluation of genotoxicity
In view of the additional genotoxicity data now available, the Committee has compiled its own summary of the studies (Annex 2). The Committee agrees that since benzylacetate,

benzylalcohol and benzylaldehyde are all metabolised to benzoic acid, the results of *in vivo* tests performed with these compounds as well as with sodium benzoate may be applied also to benzoic acid itself. Considering the database as a whole, weak genotoxic effects have been reported mainly at chromosome level in some *in vitro* systems. However, all the in vivo genotoxicity tests were negative at somatic or germ cell level. The essentially negative results obtained in three carcinogenicity studies (one in mice, two in rats) on sodium benzoate, nothwithstanding some limitations, give further reassurance. On this basis, it is very unlikely that benzoic acid would interfere with chromosomes *in vivo*.

Ref.: 3

3.3.7. Carcinogenicity

Benzoic Acid:
20 male and 20 female rats were fed 1 % Benzoic Acid (approx. 700 mg/kg bw/d) in diet for a maximum of 1000 days. No evidence of carcinogenicity was seen upon microscopic examination of all analysed tissues.

Ref.: 48

Sodium benzoate
Sodium benzoate was given in 2 % in drinking water to 50 female and 50 male Swiss albino mice from weeks 5 on for lifespan. The average daily intake of sodium benzoate was 119.2 mg for a female and 124.0 mg for a male (approx. 5.95 - 6.2 g/kg bw/d).
There was no effect of the survival of the treated mice when compared with the untreated control. There were no significant differences between the tumour distribution in sodium benzoate-treated and untreated control mice.

Ref.: 42

Groups of 50 males and 52 female Fischer 344 rats were fed sodium benzoate at 1 or 2 % in the diet (approx. 0.5 or 1.0 g/kg bw/d) for 18 to 24 months. The control group consisted of 25 males and 43 females.
No clinical signs directly attributed to sodium benzoate were observed in treated animals. Differences in the average body weight between the treated and control groups were negligible. 40 rats died during the first 16 months, except for myeloproliferative disorder developed in one female control rat, all other dead animals showed pneumonia with abscess. Around 100 rats including those of the control group died after 16 months of hemorrhagic pneumonia with oedema.
The poor survival in all groups does limit the value of this study, although the type of tumours was similar between test and control rats of each sex.

Ref.: 42

3.3.8. Reproductive toxicity

3.3.8.1 4-Generation reproductive toxicity

Benzoic acid
A four generation study with benzoic acid was conducted in rats. Males and females of the first and second generation were fed 0.5 or 1.0 % benzoic acid in the diet (approximately 0.25 or 0.5 g/kg bw d). The third generation was treated for 16 weeks and generation 4 was treated until breeding.

There were no unfavourable side-effects on growth, food utilisation, duration of life, procreation, feeding of the offspring, weight of organs and histological pattern of organs in the 1 % dose group. In the 0.5 % group there was a significant prolongation of lifetime.
NOAEL: 500 mg/kg bw.

Ref.: 41

3.3.8.2. Teratogenicity

Benzoic acid

In a study to determine the teratologic effects, benzoic acid was administered in a single dose of 510 mg/kg bw to 7 on pregnant Wistar albino rats at day 9 of gestation. The malformations and resorption rates were comparable to those in control animals. The data was considered inadequate, since only a single dose was used.

Ref.: 46

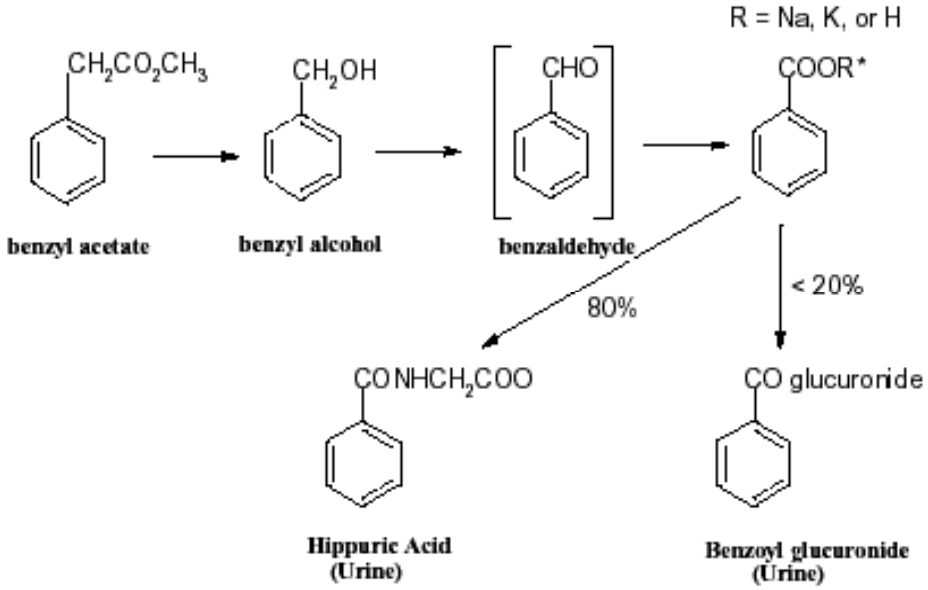
Sodium benzoate
Sodium benzoate at doses of 0, 1.75, 8, 38 and 175 mg/kg bw was administered by gavage to groups of at least 20 pregnant albino CD outbred mice and White albino rats on gestation day 6 to 15. Groups of 21 to 22 pregnant hamsters were dosed with 0, 3, 14, 65 or 300 mg/kg bw on gestation days 6 to 10. Groups of 10 Dutch-belted rabbits were artificially inseminated and then dosed by oral intubation with 0, 2.5, 12, 54 or 250 mg/kg bw on gestation days 6 to 18. Caesareans were performed on mice, rats, hamsters and rabbits on days 17, 20, 14 and 29, respectively.
There was no clearly discernible effect on nidation or on maternal or foetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls.
The NOEL in these studies was in all species identical with the highest dose tested:

mice and rats	NOEL : 175 mg/kg bw
hamsters	NOEL : 300 mg/kg bw
rabbits	NOEL : 250 mg/kg bw

Ref.: 47

3.3.9. Toxicokinetics

Benzoic acid and its sodium and potassium salt are considered with benzylacetate, benzylalcohol, benzaldehyde as a single category from the human health view by JECFA, as they are all rapidly metabolized and excreted via a common pathway within 24hrs.



Sodium benzoate is expected to immediately dissociate and form benzoic acid in an aqueous environment.

Ref.: 52

3.3.10. Photo-induced toxicity

No data was submitted.
In the JECFA report, there was a summary of an *in vitro* test by Eberlein-Konig et al, 1993. Suspensions of human erythrocytes were incubated with Benzoic Acid and Sodium Benzoate. Each material was tested at 10^{-5}, 10^{-4} and 10^{-3} mol/l. Erythrocyte-free samples were also incubated with the test material and used as controls. Following incubation, suspensions and samples were exposed to varying amounts of UVA Iight from one of three sources. Hemolysis was measured as a function of absorbance of 550 nm light.
The substances did not produce significant photohaemolysis.

Ref.: 52

3.3.11. Human data

Included in the appropriate sections

3.3.12. Special investigations

3.3.13. Safety evaluation (including calculation of the MoS)

100% skin absorption was assumed since the dermal absorption studies were old and not to modern standards.

CALCULATION OF THE MARGIN OF SAFETY

Rinse off-products (ro):		
Maximum content of active ingredient	(a. I.) [%]	2.5
Exposure to rinse-off-products	E [g/day]	0.72
Maximum amount of a. I. applied	I_{ro} [mg/day]	18.00
Oral hygiene-products (oh):		
Maximum content of active ingredient	(a. I.) [%]	1.7
Exposure to oral hygiene-products	E [g/day]	3.52
Maximum amount of a. I. applied	I_{oh} [mg/day]	59.84
Eye-products (ep):		
Maximum content of active ingredient	(a. I.) [%]	0.5
Exposure to eye-products	E [g/day]	0.05
Maximum amount of a. I. applied	I_{ep} [mg/day]	0.25
Non rinse off-products (nro):		
Maximum content of active ingredient	(a. I.) [%]	0.5
Exposure to non rinse-off-products	E [g/day]	13.50
Maximum amount of a. I. applied	I_{nro} [mg/day]	67.50
Typical body weight (human)	TBW [kg]	60 kg

Exposure from all product groups:
Systemic exposure dose SED [mg/kg bw/d] = [Ioh + Iro + Iep + Inro x A]/TBW = 2.43

No observed adverse effect level (NOAEL) from a 4-generation study (selected by SCF) 500 mg/kg bw/d

MARGIN OF SAFETY (MOS):

MOS (NOAEL/SED)	500 mg/kg bw/d / 2.43 mg/kg bw/d	=	206

3.3.14. Discussion

Since the adoption of the previous opinion of the SCCNFP on 4 June 2002 on Benzoic acid and Sodium benzoate (SCCNFP/0532/01, final), there have been 2 major re-evaluations of the data on Benzoic acid and sodium benzoate.
The SIDS report agrees that many toxicological studies on benzoic acid and its salts are old and "do not always fulfil for 100% present-day guidelines". They did appear to be acceptable studies for evaluation, since 'well-known' research groups and/or test laboratories ran the studies according to scientific standards and or accepted protocols at that time. Also, all were peer-reviewed and published in high quality scientific literature. Most of them have been reviewed and accepted by other fora like FDA, JECFA, and IPCS as acceptable studies.
In addition, there is good consistency in the individual data for a substance in the group as well as between members of the group (benzyl acetate and benzaldehyde data inclusive). Therefore, taken as a whole, the available studies give a robust database for hazard assessment and hazard evaluation of these compounds and further studies are not indicated. The JECFA Committee (1997) concluded that the data reviewed for compounds in this group were sufficient to demonstrate lack of teratogenic, reproductive or carcinogenic potential. Consequently, the Committee concluded that further studies were not required'

Ref.: 56

The conclusions of the SCF in 2002 were:

"The database is much more extensive than that considered by the Committee in 1994, both for developmental toxicity and for genotoxicity.
There appear to be sufficient studies to conclude absence of teratogenic potential, with an overall NOAEL for developmental toxicity of 500 mg/kg bw/day, based on effects on foetal weight. The fact that this overall NOAEL takes into account gavage as well as dietary studies gives further reassurance. It is therefore concluded that a further teratogenicity study on benzoic acid should no longer be required.
Similarly for genotoxicity, while some of the *in vitro* tests have been positive or equivocal, all the results from *in vivo* studies have been negative. It is therefore concluded that an *in vivo* study for clastogenic activity on benzoic acid should no longer be required.
On the basis of these data and the other types of study previously evaluated by the Committee, the Committee can establish a full Group ADI of 0 - 5 mg/kg bw for benzoic acid and its salts including benzyl alcohol and related benzyl derivatives used as flavourings."

Ref.: 3

This consensus of opinion for teratogenic, reproductive or carcinogenic potential of benzoic acid and sodium benzoate is welcome.
Benzoic acid and sodium benzoate rapidly metabolize and excrete via a common pathway within 24hrs. Systemic toxic effects on liver and kidney were observed.
Benzoic acid and sodium benzoate have low acute oral and dermal toxicity with LD_{50} values >2000 mg/kg bw. The 4 hrs inhalation exposure of benzoic acid at 0.026mg/l/h also show low acute inhalation toxicity.
Benzoic acid is a mild skin irritant, but sodium benzoate was not a skin irritant. Neither benzoic acid nor benzoate gave indication of a sensitizing effect in animals, but occasionally very low positive reactions were recorded with humans in patch tests with benzoic acid. It has been suggested that these positive reactions are a non-immunologic contact urticaria.

In the CICAD report, the conclusion on benzoic acid and sodium benzoate was: "However, both substances are known to cause non-immunologic immediate contact reactions. This effect is scarce in healthy subjects, while in patients with frequent urticaria or asthma, symptoms or exacerbation of the symptoms were observed". This was considered to be a possible cholinergic mechanism. This should be borne in mind for products used on children.

Benzoic acid should be labelled as an eye irritant under the EU criteria for classification.

The percutaneous absorption study of ^{14}C-labelled benzoic acid *in vitro* with excised human skin was old, not conforming to modern guidelines.. Total absorption was found to be approximately 45 %. These data were comparable to *in vivo* data in human, in which the total absorption of labelled benzoic acid was approximately 43 %.
Data is only given for sodium benzoate, but not the other salts or esters. It is thought that the loss of acidity due to the salt (sodium, potassium) should decrease toxicity. Since the loss of acidity is not uniform, this could be a problem. No data was provided for the esters.

4. CONCLUSION

Adequate data was provided only for benzoic acid and sodium benzoate, but not the other salts or esters.

On the basis of provided data, the SCCP is of the opinion that benzoic acid and sodium benzoate are safe for use for preservative and non-preservative purposes in cosmetic rinse-off products at a maximum concentration of 2.5 % and in cosmetic oral-care products at a maximum concentration of 1.7 %, and in leave-on products up to 0.5%. The possible non-preservative functions have not been stated.

However, in the interest of consumer safety, generation of data on irritation and sensitisation of the other salts and esters is required if they are to be used in cosmetic products.

Comment: Inclusion of all relevant data should have been made available, particularly if from the grey literature as required by the SCCNFP Notes of Guidance (SCCNFP/0690/03). In the IUCLID database, more modern percutaneous absorption studies were summarised. The study for eye irritation was not provided with the Submission I. These should all have been provided with Submission I.

5. MINORITY OPINION

Not applicable

6. REFERENCES

1. Washkuhn R.J., Patel V.K., Robinson J.R. (1971) *J. Pharm. Sci*. **60(5)**, 736-744.
2. Wright J.L. and Carstensen J.T. (1986) *J. Pharm. Sci.* **75(6)**, 546-551.
3. SCF/CS/ADD/CONS/48 Final, 17 Sept 2002, expressed on 24 September 2002.
4. Marhold, J.V. Personal communication to the editor of RTECS, VUOS 539-18, Cincinnati (1977). Cited in Henschler, D. (ed) Toxikologisch-arbeitsmedizinische Begründung von

MAK-Werten. Benzoesäure. VCH VerlagsGmbH, Weinheim (1985).
5. Abe S. et al. (1984), *Iyakuhin Kenyuku* **15**, 359-370.
6. Mc Cormick G.C. and Speaker T.J. (1973) *Toxicol Appl Pharmacol* **25**, 478.
7. Hager G.P. et al. (1942) *J. Am. Pharm. Assoc.* **31**, 253-255.
8. Deuel H.J. Jr et al. (1954) *Food Res* **19**, 1-12.
9. Loeser, E. Bayer AG data. Akute orale Toxizität (1977). R0300478
10. Smyth H.F. Jr and Carpenter C.P. (1948) *J. Ind. Hyg. Toxicol*. **30**, 63.
11. Draize J.H. (1959), Appraisal of the safety of chemicals in foods, drugs, and cosmetics, Association of Food and Drug Officials of the US, p. 46-59.
12. RCC NOTOX, Primary skin irritation/corrosion study of Benzoic Acid in the rabbit (study no. 0847/1083). RCC NOTOX B. V., DD 's-Hertogenbosch (1988).
13. Moreno O.M. (1977) Report to RIFM, 22 August. Cited in BIBRA Report Toxicity Profile – Benzoid Acid, Bibra Toxicology International, 1st ed. (1989), 1-11.
14. Bio-Fax, Benzoid acid, Industrial Bio-Test Laboratories, Inc., Northbrook, Ill., Data Sheet No. 28-4/73 (1973). Cited in BUA-Stoffbericht 145, S. Hirzel, Wissenschaftliche Verlagsgesellschaft, December 1993.
15. Bayer AG, Untersuchung zur Haut- und Schleimhautverträglichkeit von Benzoesäure, Bayer AG Wuppertal (1978).
16. RCC NOTOX, Primary skin irritation/corrosion study with natrium benzoate in rabbits (study no. 014658). RCC NOTOX B. V., DD's-Hertogenbosch, The Netherlands (1989).
17. Loeser E., Bayer AG data, Untersuchungen zur Haut- und Schleimhautverträglichkeit (1977).
18. De Groot A.C. et al. (1986) *Contact Dermatitis* **14**, 120.
19. Frosch P.J. and Kligman A.M. (1976) *Contact Dermatitis* **2**, 314
20. Lathi A. (1978) *Contact Dermatitis* **4**, 302-303.
21. Frosch P.J. and Kligman A.M., cited in Drill, V. A. & Lazar, P. (ed.) Cutaneous toxicity, Academic Press Inc., New York, 127-154 (1977).
22. RCC NOTOX, Acute eye irritation/corrosion study with Benzoic Acid in rabbits (study no. 0847/1084). RCC NOTOX B. V., DD 's-Hertogenbosch, The Netherlands (1988).
23. Suberg H., Bayer AG data, Benzoesäure DAB 8, Prüfung auf primär reizende/ätzende Wirkung am Kaninchenauge (1986).
24. RCC NOTOX, Acute eye irritation/corrosion study with Sodium Benzoate in rabbits (study no. 014669). RCC NOTOX B. V., DD 's-Hertogenbosch, The Netherlands (1989).
25. Gad S.C. et al. (1986) *Toxicol Appl Pharm* **84**, 93-114.
26. Kligman A.M. (1977) Report to RIFM, 24 May. Cited in Food Cosmet Toxicol 17 Special Issue V (1979) Monographs on Fragrance Raw Materials, 695-923.
27. Leyden J.J. and Kligman A.M. (1977) *Contact Dermatitis* **3**, 273-275.
28. Broeckx W. et al. (1987) *Contact Dermatitis* **16**, 189. S
29. Ishidate M. Jr et al. (1984) *Food Chem Toxicol* **22**, 623.
30. Zeiger E. et al. (1988) *Environ Mol Mutagen* **11** Suppl 12, 1.
31. Mc Cann J. et al. (1975) *Proc Nat Acad Sci USA* **72**, 5135.
32. Prival M.J. et al. (1991) *Mutat Res* **260**, 321.
33. Ishidate M. Jr et al. (1988) *Mutat Res* **195**, 151-213.
34. Litton Bionetics, Inc. (1974) Mutagenic evaluation of compound FDA 71-37, Sodium Benzoate. NTIS Report.
35. Ishidate M. Jr and Odashima S. (1977) *Mutat Res* **48**, 337-354.
36. Abe S. and Sasaki M. (1977) *J Nat Cancer Inst* **58**, 1635-1641.
37. Tohda H., Horaguchi K., Takahashi K., Oikawa A., Matsushima T. (1980), Epstein-Barr virus-transformed human lymphoblastoid cells for study of sister chromatid exchanges.

Cancer Research 40: 4775-4780.
38. Jansson T., Curvall M., Hedin A. and Enzell C.R. (1988), In vitro studies of the biological effects of cigarette smoke condensate. Induction of SCE by some phenolic and related constituents derived from cigarette smoke condensate. A study of structure-activity relationship. Mutation Research 206: 17-24.
39. Oikawa A., Tohda H., Kanai M., Miwa M. and Sugimura T. (1980), Inhibitors of poly(adenosine diphosphate ribose)polymerase induce sister chromatid exchanges. Biochemical and Biophysical Research Communications 97: 1311-1316.
40. Franz T.J. (1975) *J Invest Derm* **64**, 190-195.
41. Hunziker N. et al. (1978) *Dermatologica* **156**, 79-88.
42. Toth B. (1984) *Fund Appl Toxicol* **4**, 494-496.
43. Fanelli G.M. and Halliday S.L. (1963) *Arch Int Pharmacodyn* **144**, 120-125.
44. International Research and Development Corporation, Four Week Subacute Inhalation Toxicity Study of Benzoic Acid in Rats (study no. 163-676). International Research and Development Corporation, Mattawan/Michigan USA (1981).
45. Shtenberg A.J. and Ignat'ev A.D. (1970) *Food Cosmet Toxicol* **8**, 369.
46. Kimmel C.A. et al. (1971) *Teratology* **4**, 15-24.
47. Food and Drug Research Labs., Inc. (1972) Teratologic evaluation of FDA 71-37 (Sodium Benzoate), NTIS Report No. PB-221-777. Cited in: Cosmetic Ingredient Review, Final Report, Safety Assessment of Benzyl Alcohol, Benzoic Acid and Sodium Benzoate, May 19, 1998.
48. Kieckebusch W. and Lang K. (1960) *Arzneim-Forsch* **10**, 1001-1003.
49. Sodemoto Y. and Enomoto M. (1980) *J Environ Pathol Toxicol* **4**, 87-95.
50. SCCNFP Notes of Guidance for Testing of Cosmetic Ingredients for their Safety Evaluation (SCCNFP/0321/00 Final, 2000)
51. Rothschild D.L. Jr. (1990) The Food Chemical News Guide. Washington, D.C., Cited in: Cosmetic Ingredient Review, Final Report, Safety Assessment of Benzyl Alcohol, Benzoic Acid and Sodium Benzoate, May 19, 1998.
52. Food and Agriculture Organization of the United Nations/World Health Organisation (FAO/WHO). 1994, Summary of the Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA): United States: International Life Sciences Institute. Cited in: Cosmetic Ingredient Review, Final Report, Safety Assessment of Benzyl Alcohol, Benzoic Acid and Sodium Benzoate, May 19, 1998.
53. Williams F.M. (1985) Clinical Significance of Esterases in Man, *Clinical Pharmacokinetics* **10**, 392-403.
54. Cosmetic Ingredient Review (1998) Final report: Safety Assessment of Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate, May 19, 1998, 35 pages. S
55. International Programme on Chemical Safety (2000) Concise International Chemical Assessment Document No. 26, Benzoic Acid and Sodium Benzoate, http://www.inchem.org/documents/cicads/cicads/cicad26.htm
56. SIDS Initial Assessment Report (2001) Benzoates: Benzoic acid, Sodium Benzoate, Potassium Benzoate, Benzyl Alcohol, final draft under: http://www.oecd.org/document/63/0,2340,en_2649_34379_1897983_1_1_1_1,00.htm

References from Submission 1

* Referred to but no documentation supplied.

1. Marhold JV (1977) Personal communication to the editor of the Registry of Toxic Effects of Chemical Substances, VUOS 539-18, Cincinnati , Ohio, USA, 29 March. Cited in Henschler D (ed) Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten. Benzoesäure. VCH VerlagsGmbH, Weinheim (1985)

2. Abe S et al. (1984) Studies on the Toxicity of Oxaprozin (1) Acute Toxicity of Oxaprozin, its Metabolites and Contaminants. IYAKUHIN KENKYU 15 359 - 70 (Japanese, only abstract and tables in English)
3. McCormick GC & Speaker TJ (1973) Comparison of the Acute Toxicity, Distribution, Fate and Some Pharmacological Properties of the Non-benzenoid Aromatic Compound Acid with those of Benzoid and Naphtoic Acids. Toxicol Appl Pharmacol 25 478 (only abstract)
*4. Fassett DW (1962) in Patty FA (ed): "Industrial Hygiene and Toxicology", 2 nd rev. Ed., Vol. II, p. 1858, Interscience Publishers, New York, USA. Cited in Henschler D (ed) Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten. Benzoesäure. VCH VerlagsGmbH, Weinheim (1985)
*5. Loeser E (1977) Bayer AG data. Akute orale Toxizität. Cited in BUA-Stoffbericht 145, December 1993. S. Hirzel, Wissenschaftliche Verlagsgesellschaft, (1995)
6. Deuel HJ et al. (1954) Sorbic Acid as a Fungistatic Agent for Foods. I. Harmlessness of Sorbic Acid as a Dietary Component. Food Res 19 1 - 12
7. Smyth HF Jr & Carpenter CP (1948) Further Experience with the Range Finding Test in the Industrial Toxicology Laboratory. J Ind Hyg Toxicol 30 63 - 68
*8. Moreno OM (1977) Report to RIFM, 22 August. Cited in BIBRA Report Toxicity Profile - Benzoic Acid, TNO BIBRA Toxicology International Ltd. (1989)
*9. Biofax (1973) Benzoic acid. Industrial Bio-Test laboratories, Inc. Northbrook, Illinois, Data Sheet No. 28-4/73. Cited in BUA-Stoffbericht 145, December 1993. S. Hirzel, Wissenschaftliche Verlagsgesellschaft, (1995)
*10. RCC NOTOX (1988) Primary skin irritation/corrosion study of benzoic acid in the rabbit (study no. 0847/1083). RCC NOTOX BV DD's-Hertogenbosch. Cited in BUA-Stoffbericht 145, December 1993. S. Hirzel, Wissenschaftliche Verlagsgesellschaft, (1995)
*11. RCC NOTOX Primary skin irritation/corrosion study of natrium benzoate in rabbits (study no. 014658). RCC NOTOX BV DD's-Hertogenbosch. Cited in BUA-Stoffbericht 145, December 1993. S. Hirzel, Wissenschaftliche Verlagsgesellschaft, (1995)
*12. Loeser E (1977) Untersuchungen zur Haut- und Schleimhautverträglichkeit, Bayer AG data. Cited in BUA-Stoffbericht 145, December 1993. S. Hirzel, Wissenschaftliche Verlagsgesellschaft, (1995)
13. Gad SC (1986) Development and Validation of an Alternative Dermal Sensitization Test: The Mouse Ear Swelling Test (MEST) Toxicol Appl Pharm 84 93 - 114
14. De Groot AC et al (1986) Contact allergy to preservatives (I) Contact Derm 14 120 – 122
15. Lahti A (1978) Skin reactions to some antimicrobial agents. Contact Derm 4 302 - 303
16. Frosch PJ & Kligman AM (1977) The Chamber-Scarification Test of Assessing Irritancy of Topically Applied Substances. In Drill VA & Lazar P (Ed.) Cutaneous Toxicity, Ac. Press Inc. New York, 127 - 154
17. Frosch PJ & Kligman AM (1976) The chamber-scarification test for irritancy. Contact Derm 2 314 - 324
18. Kremer (1999) Gebrauchstest, COLIPA: Pril 2 in 1 Spülmittel & antibakterielle Handseife. (Prüfbericht (9902786-0) Report Nr. R 9900872) unpublished data.
19. Tronnier H (1999) Anwendungs- und Verträglichkeitstest des Prüfpräparates Handschirrgeschirrspülmittel und antibakterielle Handseife 2 in 1 (Berichts-Nr.: R 9901179) unpublished data.
*20. Suberg H (1986) Benzoesäure DAB 8, Prüfung auf primär reizende/ätzende Wirkung am Kaninchenauge, Bayer AG data. Cited in BUA-Stoffbericht 145, December 1993. S. Hirzel, Wissenschaftliche Verlagsgesellschaft, (1995)
*21. Bayer AG (1978) Untersuchungen zur Haut- und Schleimhautverträglichkeit, Bayer AG Wuppertal. Cited in BUA-Stoffbericht 145, December 1993. S. Hirzel, Wissenschaftliche Verlagsgesellschaft, (1995)
*22. RCC NOTOX Acute eye irritation/corrosion study with natrium benzoate in rabbits (study no. 014669). RCC NOTOX BV DD's-Hertogenbosch. Cited in BUA-Stoffbericht 145, December 1993. S. Hirzel, Wissenschaftliche Verlagsgesellschaft, (1995)
*23. Kligman AM (1977) Report to RIFM, 14 May. Cited in BIBRA Report Toxicity Profile - Benzoic Acid (1989) TNO BIBRA Toxicology International Ltd.
24. Leyden JJ & Kligman AM (1977) Contact sensitization to benzoyl peroxide. Contact Derm 3 273 - 275
25. Broeckx W et al. (1987) Cosmetic intolerance. Contact Derm 16 189 – 194
26. Ishidate M JR et al. (1984) Primary mutagenicity screening of food additives currently used in Japan. Fd Chem Toxic 22 623 - 636
27. Zeiger E et al (1988) Salmonella Mutagenicity Test: IV. Results From the Testing of 300 Chemicals. Environ Mol Mutagen 11 Suppl. 12 1 - 18
28. McCann J et al (1975) Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc Nat Acad Sci 72 5135 - 5139
29. Prival MJ et al (1991) Bacterial mutagenicity testing of 49 food ingredients gives very few positive results. Mutat Res 260 321 - 329

30. Ishidate M JR et al (1988) A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. Mutat Res 195 151 - 213
31. Ishidate M JR & Odashima S (1977) Chromosome tests with 134 compounds on Chinese hamster cells in vitro - a screening for chemical carcinogens. Mutat Res 48 337 - 354
32. Abe S & Sasaki M (1977) Chromosome Aberration and Sister Chromatid Exchange in Chinese Hamster Cells Exposed to Various Chemicals. J Natl Cancer Inst 58 1635 - 1641
33. Fabrizio DBA (1974) Mutagenic Evaluation of Compound FDA 71-27 Sodium Benzoate, Litton Bionetics, Inc. (very bad copy!)
34. Frantz TJ (1975) Percutaneous absorption. On the relevance of in vitro data. J Invest Derm 64 190 - 195
35. Hunziker et al (1978) Animal Models of Percutaneous Penetration: Comparison between Mexican Hairless Dogs and Man. Dermatologica 156 79 - 88
36. Toth B (1984) Lack of Tumorigenicity of Sodium benzoate in Mice. Fundam Appl Toxicol 4 494 - 496
37. Fanelli GM & Halliday SL (1963) Relative toxicity of Chlortetracycline and Sodium benzoate after oral administration to rats. Arch Int Pharmacodyn 144 120 - 125
38. Shtenberg AJ & Ignat'ev AD (1970) Toxicological Evaluation of some Combinations of Food Preservatives. Fd Cosmet Toxicol 8 369 - 380
39. Food and Drug Research Labs., Inc. (1972) Teratologic evaluation of FDA 71-37 (sodium benzoate) in mice, rats, hamsters and rabbits. NTIS Report PB-221 777
40. Kimmel CA et al (1971) Studies on Metabolism and Identification of the Causative Agent in Aspirin Teratogenesis in Rats. Teratology 4 15 - 24
41. Kieckebusch W & Lang K (1960) Die Verträglichkeit der Benzoesäure im chronischen Fütterungsversuch. Arznei-Forsch 10 1001- 1004
42. Sodemoto Y & Enomoto M (1980) Report of carcinogenesis bioassay of Sodium benzoate in rats: Absence of carcinogenicity of sodium benzoate in rats. J Environ Pathol Toxicol 4 87 - 95
*43. Not given
*44. Not given
45. Beratergremium für umweltrelevante Altstoffe (BUA) der Gesellschaft Deutscher Chemiker (ed) BUA-Stoffbericht 145, December 1993. S. Hirzel, Wissenschaftliche Verlagsgesellschaft, (1995)
46. BIBRA Report Toxicity Profile - Benzoic Acid (1989) TNO BIBRA Toxicology International Ltd.

Additional references (provided by SCCNFP):

AR 1. European Parliament and Council Directive No 95/2/EC of 20 February 1995 on foodadditives other than colours and sweeteners. Official Journal L 061 , 18/03/1995 p. 0001-0040
AR 2. Commission Directive No 96/77/EC of 2 December 1996 laying down specific purity criteria on food additives other than colours and sweeteners. OJ No L339, 1-6.
AR 3. FDA, Code of Federal Regulations, Title 21- Food and Drugs, Volume 3 [Revised as of April 1, 2001], 184. 464-559.
AR 4. Handbook of pharmaceutical excipients 3rd ed 2000 editors: Kibbe, I, Arthur H, Pharmaceutical Society of Great Britain; American Pharmaceutical Association
AR 5. Martindale : The Complete Drug Reference 32nd ed. 1999 editors: Parfitt K, Sweetman, S C, Blake P S, Parsons, A V.
AR 6. Lahti A (1980) Non-immunologic contact urticaria. University of Oulu.
AR 7. Final report on the safety assessment of benzyl alcohol, benzoic acid, and sodium benzoate. Cosmetic Ingredient Review, Washington, DC, 20036, USA. Intern. Journal of Toxicology (2001), 20 (Suppl. 3), 23-50
AR 8. International Programme On Chemical Safety, (2000) Concise International Chemical Assessment Document No. 26 Benzoic Acid And Sodium Benzoate. http://www.inchem.org/documents/cicads/cicads/cicad26.htm
AR9. Feldmann RJ & Maibach HI (1970) Absorbtion of some organic compounds through the skin in man. J Invest Derm 54 399-404).
AR10. Edwards RC, Voegeli CJ (1984) Inadvisability of using caffeine and sodium benzoate in neonates. Am J Hosp Pharm 41, 658.
AR11. Schiff D et al (1971) Fixed drug combinations and the displacement of bilirubin from albumin. Pediatrics 48, 139 -41.

7. ACKNOWLEDGEMENTS

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