EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

Scientific Committee on Consumer Products

SCCP

OPINION ON

β-ARBUTIN

Scientific Committees
on consumer products
on emerging and newly identified health risks
on health and environmental risks

The SCCP adopted this opinion at its 15th plenary of 15 April 2008

About the Scientific Committees
Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Products (SCCP), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Evaluation Agency (EMEA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCP
Questions concerning the safety of consumer products (non-food products intended for the consumer).
In particular, the Committee addresses questions related to the safety and allergenic properties of cosmetic products and ingredients with respect to their impact on consumer health, toys, textiles, clothing, personal care products, domestic products such as detergents and consumer services such as tattooing.

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ACKNOWLEDGMENTS

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Keywords: SCCP, scientific opinion, β-arbutin, directive 76/768/ECC, CAS 497-76-7, EINECS 207-850-3

Opinion to be cited as: SCCP (Scientific Committee on Consumer Products), Opinion on β-arbutin, 15 April 2008

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1. BACKGROUND

Submission I for β-Arbutin (CAS 497-76-7) (chemical name 4-hydroxyphenyl-β-glucopyranoside, INCI name Arbutin) was submitted in July 2005 by COLIPA [1].

β-Arbutin releases a limited amount of hydroquinone. Hydroquinone is listed in Annex III (entry 14) of the Cosmetics Directive 76/768/EEC. Its permitted use is restricted to hair-dye products and artificial fingernails. Since the banning of hydroquinone as a skin whitener, other substances have been used for this purpose, including Arbutin.

β-Arbutin is being used as an ingredient in skin lightening products.

2. TERMS OF REFERENCE

1. *Does the SCCP consider the use of ß-arbutin to be safe for consumers in cosmetic products in a concentration up to 7% with the provided scientific data?*

2. *Does the SCCP recommend any restrictions with regard to the use of ß-arbutin 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(s) and/or INCI name(s)

β-Arbutin (INCI name)

Ref.: 8, 9, 15

3.1.1.2 Chemical name(s)

4-Hydroxyphenyl-β-D-Glucopyranoside

Ref.: 8, 9, 15

3.1.1.3 Trade name(s) and abbreviation(s)

Arbutin

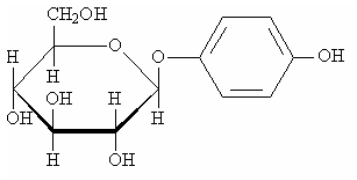
Ref.: 8, 9, 15

3.1.1.4 CAS / EINECS number

CAS N°: 497-76-7
EINECS N°: 207-850-3

Ref.: 9

3.1.1.5 Structural formula



Ref.: 15

3.1.1.6 Empirical formula

$C_{12}H_{16}O_7$

3.1.2 Physical form

White to light grey powder

Ref.: Appendix 1

3.1.3 Molecular weight

272.25 g/mol

Ref.: 8, 9, 15

3.1.4 Purity, composition and substance codes

Purity: 97.0 - 102.0 % β-arbutin:

Lot A:	99.7%	Lot TDA-361:	100.3%
Lot B:	97.8%	Lot TDD-422:	100.0%
Lot C:	99.8%	Lot TPG-412:	99.8%
		Lot TGJ-225:	100.4%

Ref.: Appendix 1

Batches used:	Lot A, B, C:	stability testing
	Lot A:	acute oral and dermal toxicity test
		skin and eye irritation in the rabbit
		skin sensitisation assay
		28 day oral & 90 day dermal toxicity test
		mutagenicity testing
		1-generation reproduction toxicity test
		phototoxicity & photosensitisation assay
		human patch test
	Lot TDD-422:	carcinogenicity study
	Lot TPG-412:	human skin metabolism (repeated topical application)
	Lot TDA-361:	human (patch) tests with 7-10% β-arbutin formulations

3.1.5 Impurities / accompanying contaminants

Hydroquinone: ≤ 0.030%:

Lot A:	0.0129%	Lot TDA-361:	0.0064%
Lot B:	0.0208%	Lot TDD-422:	0.0033%
Lot C:	0.0094%	Lot TPG-412:	0.0019%
		Lot TGJ-225:	0.0043%

Chloride:	≤ 0.036%
Heavy metals:	≤ 20 ppm
Arsenic:	≤ 2 ppm
Iron:	≤ 20 ppm

Ref.: Appendix 1

3.1.6 Solubility

Water, propylene glycol:	≥ 10g/100g
Ethanol, glycerine:	1-10g/100g
Squalane, olive oil:	≤ 1g/100g

Ref.: 15

3.1.7 Partition coefficient (Log P_{ow})

- 1.35 (not measured, but mentioned in *in vitro* dermal absorption study).

Ref.: 8

3.1.8 Additional physical and chemical specifications

UV light absorption spectrum:	spectrum not available		
	λ_{max} = 285 nm (in summary report)		
Melting point:	197-201°C:		
	Lot A: 198.3°C	Lot TDA-361:	201.2°C
	Lot B: 198.0°C	Lot TDD-422:	200.0°C

	Lot C: 198.7°C	Lot TPG-412: 201.0°C
		Lot TGJ-225: 201.0°C
pH:	5-7:	
	Lot A: 5.78	Lot TDA-361: 6.00
	Lot B: 5.63	Lot TDD-422: 6.10
	Lot C: 5.75	Lot TPG-412: 6.10
		Lot TGJ-225: 6.10
Specific rotation:	$[\alpha]_D^{25}$ = -62 to -68°:	
(method not	Lot A: -64.2	Lot TDA-361: -65.8
specified)	Lot B: -66.1	Lot TDD-422: -65.7
	Lot C: -64.8	Lot TPG-412: -65.0
		Lot TGJ-225: -65.4

Ref.: Appendix 1

3.1.9 Stability

3.1.9.1 Stability of β-arbutin as a raw material

A. Stability test of β-arbutin under sunlight

Date of study: Mar-May 1986
Method: Internal protocol Shiseido Co, Japan.
Test substance: β-arbutin (Lot A, B, C), dissolved at 3% in ethanol
Analytical method: HPLC

3% β-arbutin solutions in ethanol were kept in a transparent glass bottle under sunlight (outdoors) for 30 days. The β-arbutin concentration was measured in threefold by HPLC for each batch at the start and at the end of the study.

Results

Lot n°	Starting time	After 30 days
A	100.00%	100.9%
B	100.00%	99.9%
C	100.00%	100.5%

Conclusions
The study authors conclude that, since the concentration of β-arbutin did not change over the test period, the test substance can be considered very stable under sunlight.

Ref.: 21

B. Stability test of β-arbutin at several pH conditions

Date of study: Mar-Apr 1986
Method: Internal protocol Shiseido Co, Japan.
Test substance: β-arbutin (Lot A, B, C), dissolved at 3% in ethanol with phosphate buffer to obtain pH 2.0, 3.0 or 11.0
Analytical method: HPLC

3% β-arbutin solutions in ethanol and phosphate buffer were kept in a transparent glass bottle at 50°C for 30 days. The β-arbutin concentration was measured in threefold by HPLC for each batch at the start and at the end of the study.

Results

Lot n°	Starting time	pH 2.0	pH 3.0	pH 11.0
A	100.00%	90.5%	97.6%	98.0%
B	100.00%	89.0%	96.2%	95.6%
C	100.00%	80.0%	97.6%	99.3%

Conclusions
At pH 2.0, a partial decrease in stability is observed. Considering that cosmetics are usually formulated at pH 4-8 from a safety point of view, the study authors conclude that β-arbutin has an acceptable level of stability.

Ref.: 21, 22

3.1.9.2 Stability of β-arbutin in a cosmetic formulation

A. β-arbutin in a finished cosmetic product (accelerated test conditions for 6 months)

Date of study: May-Nov 2003
Method: Internal protocol Shiseido Co, Japan.
Test substance: CPB Serum Eclaircissant n (Lot A, B, C) (composition/pH not stated), containing 5.67-6.93% β-arbutin
Analytical method: HPLC

Samples of CPB Serum Eclaircissant n were kept in a transparent glass bottle at 40°C and 75% relative humidity for 6 months. β-arbutin and hydroquinone concentrations were measured in threefold by HPLC for each batch at the start of the study, after 3 months and after 6 months.

Results

Lot n°	Constituent	Starting time	After 3 months	After 6 months
A	β-arbutin	6.20%	6.38%	6.34%
	hydroquinone	< 1ppm	< 1ppm	< 1ppm
B	β-arbutin	6.22%	6.34%	6.34%
	hydroquinone	< 1ppm	< 1ppm	< 1ppm
C	β-arbutin	6.17%	6.31%	6.32%
	hydroquinone	< 1ppm	< 1ppm	< 1ppm

Conclusions
Since the concentrations of β-arbutin were very stable and those of hydroquinone remained below 1 ppm (detection limit), the study authors conclude that β-arbutin is very stable in final cosmetic formulations and that no hydroquinone is released under accelerated aging conditions for 6 months.

Ref.: 20

B. β-arbutin in a finished cosmetic product (ambient test conditions for 4 years)

Date of study: Nov 1998 - May 2003
Method: Internal protocol Shiseido Co, Japan.
Test substance: CPB Serum Eclaircissant n, containing 5.67-6.93% β-arbutin (Lot n° not mentioned, exact composition/pH unknown),
Analytical method: HPLC

Samples of CPB Serum Eclaircissant n were kept in a transparent glass bottle at ambient conditions for 4 years. β-arbutin and hydroquinone concentrations were measured in threefold by HPLC at the start and at the end of the study.

Results

Lot n°	Constituent	Starting time	After 4 years
unknown	β-arbutin	6.63%	6.56%
	hydroquinone	< 1ppm	< 1ppm

Since the concentrations of β-arbutin were very stable and those of hydroquinone remained below 1 ppm (detection limit), the study authors conclude that β-arbutin is very stable in usual cosmetics and that no hydroquinone is released under normal storage conditions for 4 years.

Ref.: 20-1

General comments with regard to section 3.1

- More extended solubility data are mentioned in e.g. the 1-generation reproduction toxicity test:
  in water: 7% by weight at 0°C, 16% at 25°C, 37% at 50°C;
  in hydroalcoholic solution (1:1): 13% by weight at 0°C, 25% at 25°C, 32% at 50°C;
  in DMSO: readily soluble.
- The octanol/water partition coefficient was not measured, but simply mentioned in the *in vitro* dermal absorption study report.
- pH measurements: it is not stated at which concentration in which solvent the pH was measured. This is crucial as it has been reported [36] that β-arbutin is hydrolyzed in the presence of weak acids. Also during absorption through the skin hydroquinone can be formed and absorbed.
- Stability test of β-arbutin under sunlight: the test description is very brief and not standardised. The temperature of the test is not stated. It is not clear whether the measurements at the start of the study are actual measurements (3 times exactly 100.0%) or whether the starting values were arbitrarily set to 100.00%, towards which all other measurements were relatively expressed. No controls are included of the intensity and duration of the sunlight to which the samples have been exposed. It is only mentioned that they were kept "outdoors" for 30 days.
- Stability test of β-arbutin at several pH conditions: the test description is very brief. It is not clear whether the measurements at the start of the study are actual measurements (3 times exactly 100.0%) or whether the starting values were arbitrarily set to 100.00%. Based upon a partial decrease in stability at pH 2.0, which was not observed at pH 3.0 or 11.0, the authors conclude that stability at pH 4-8 is guaranteed. However, the actual stability in this relevant pH range was not measured.
- Stability of β-arbutin in a finished cosmetic product (accelerated test conditions for 6 months): the test description is very brief. Neither the qualitative nor the quantitative composition of the tested formulation is stated. Also, the pH of the formulation is not given and this is crucial for potential hydrolysis.
- Stability of β-arbutin in a finished cosmetic product (ambient test conditions for 4 years): the test description is very brief. Neither the batch number nor the qualitative or quantitative composition of the tested formulation is stated. Also, the pH of the formulation is not given and this is crucial for potential hydrolysis.
- β-arbutin Lot TPG-412 is not taken up in the list under 3.1.4, thus its purity is not stated.

3.2 FUNCTION AND USES

β-arbutin is proposed to be used as a skin lightening agent in cosmetic face creams or face lotions at concentrations up to 7%.

3.3 TOXICOLOGICAL EVALUATION

3.3.1 Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline: OECD TG 401 (1981)
Date of test: 3-17 December 1985
Species/strain: Sprague Dawley (Crj:CD, SPF) rats and ICR (Crj:CD-1, SPF) mice
Group size: 5 rats or mice/sex/dose
Test substance: β-arbutin
Batch: Lot A
Purity: 99.7%
Dosages: 1792, 2509, 3513, 4919, 6886, 9641, 13496, 18895 mg/kg bw
Observation period: 14 days
GLP/QAU: before introduction of GLP (1987), thus not applicable.

The test substance was applied by oral gavage at dosages of 1792, 2509, 3513, 4919, 6886, 9641, 13496, 18895 mg/kg bw to groups of 5 male and/or 5 female rats and mice. The animals were checked daily for mortality and clinical signs. Body weights were recorded on days 1 to 5, 7, 8, 11 and 15. Animals were observed for 14 days. Animals that died during the test and all surviving animals at the end of the observation period were submitted to gross necropsy.

Results
Lethality is summarized in the following table:

Dosage (mg/kg bw)	Lethality male rats	Lethality female rats	Lethality male mice	Lethality female mice
1792	0/5	0/5	0/5	0/5
2509	0/5	0/5	0/5	0/5
3513	0/5	0/5	0/5	0/5
4919	0/5	0/5	1/5	0/5
6886	2/5	1/5	1/5	1/4*
9641	3/5	3/5	1/5	3/5
13496	4/5	4/5	3/5	3/5
18895	5/5	5/5	5/5	5/5

* one of the five 6886 mg/kg bw male mice died by gavage accident

Liver changes associated with the test substance were observed at 4149 mg/kg bw in mice and at 9641 mg/kg bw in rats, though not at lower dosage levels.
There were no deaths in mice given ≤ 3513 mg/kg bw or in rats given ≤ 4919 mg/kg bw. Few toxic signs were observed in either species at dosage levels up to 3513 mg/kg bw.

Conclusion
The study authors conclude that the LD_{50}-value for β-arbutin is 9804 mg/kg bw for the mouse and 8715 mg/kg bw for the rat. These values indicate a low level of acute oral toxicity.

Ref.: 1

3.3.1.2. Acute dermal toxicity

Guideline: OECD TG 402 (1981)
Date of test: January-April 1986
Species/strain: Sprague Dawley (Crj:CD, SPF) rats and ICR (Crj:CD-1, SPF) mice
Group size: 10 rats or mice/sex
Test substance: β-arbutin, 30% (w/w) in a 50:50 ethanol:water solution

Batch:	Lot A
Purity:	99.7%
Dosages:	928 mg/kg bw (technically applicable maximal dosage)
Observation period:	14 days
GLP/QAU:	before introduction of GLP (1987), thus not applicable.

For both rats and mice, the test substance was applied evenly on the back after fur clipping (treated skin surface area not mentioned). The applied volume was 3 ml/kg, the maximum technically achieved. 30% β-arbutin in 50% ethanol aqueous solution led to a dosage of 928 mg β-arbutin/kg bw.
For mice, clinical signs were recorded for 14 days except for holidays, and body weight was measured on day 1 to 3, 5 to 8, 12 and 15. For rats, clinical signs were recorded for 14 days, and body weight was measured on day 1 to 3, 5 to 8 and 15. Since no animal died during the observation period, all animals were sacrificed with chloroform at the end the observation period and subjected to necropsy.

Results
No lethality occurred during the test and no abnormalities in clinical signs were seen. Body weight gain was normal throughout the observation period. There were no remarkable findings at necropsy for either mice or rats.

Conclusion
For rats and mice, the LD_{50} value via the dermal route showed to be greater than 928 mg/kg bw, the maximum practically applicable dosage. The study authors therefore conclude that the acute dermal toxicity of β-arbutin is low.

Ref.: 2

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

Guideline:	Draize method (1959), no official guideline
Date of test:	April 1986
Species/strain:	Japanese white rabbits
Group size:	6 males
Test substance:	β-arbutin, dissolved as a 10% (w/w) solution in water
Batch:	Lot A
Purity:	99.7%
Dosages:	300 µl of the 10% solution was applied on patch of 4.9 cm²
Observation period:	72 hours
GLP/QAU:	before introduction of GLP (1987), thus not applicable.

A 4.9 cm² patch with 300 µl test material (10% β-arbutin in distilled water) was placed on shaved intact and abraded dorsal skin sections of six male rabbits for 24 hours. After the 24 hour application time, the patch was removed. No rinsing was done. Skin reactions were evaluated after 48 and 72 hours.

Results
Slight erythema (score 1) was observed in one rabbit after 24 and 72 hours on intact and abraded skin. No oedema was observed during the test period.

Conclusion
The study authors conclude that a 10% aqueous solution of β-arbutin can be considered as non-irritating to the skin.

Ref.: 3

Comment
According to current guidelines (Annex V B.4 and OECD 404), 500 µl of test substance should be applied on an intact skin surface area of 6 cm² for 4 hours. Subsequently the skin should be rinsed and readings should be performed after 24h, 48h and 72h. In this test, one can assume that 300 µl was applied on 4.9 cm² during 24 hours. There is no mention whether the applied patch was occlusive or semi-occlusive. There was no rinsing step after patch removal and the 48h readings are lacking.

3.3.2.3 Mucous membrane irritation - rabbit

Guideline:	Internal protocol performing laboratory (Japan), no official guideline
Date of test:	March-April 1986
Species/strain:	Japanese white rabbits
Group size:	3 males
Test substance:	β-arbutin, dissolved as a 10% (w/w) solution in water
Batch:	Lot A
Purity:	99.7%
Dosages:	100 µl of the 10% solution
Observation period:	72 hours
GLP/QAU:	before introduction of GLP (1987), thus not applicable.

100 µl of test substance (10% β-arbutin in distilled water) was instilled to the right eyes of three rabbits. The eyes were left unrinsed and the untreated left eyes served as control. Ocular reactions were measured for one week according to the Draize method. Reading times were at 1, 4, and 24 hours, 2, 3, 6, and 7 days after instillation. Parameters checked included cornea (opacity, area of cornea involved), iris (morbidity value) and conjunctiva (redness, chemosis, discharge)

Results
No reactions were observed in the cornea, iris or conjunctiva during the test period on any animal treated with test substance.

Conclusion
The study authors conclude that a 10% aqueous solution of β-arbutin has little potential for eye irritation.

Ref.: 6

3.3.3 Skin sensitisation

Guideline:	Magnusson Kligman Guinea Pig Maximisation Test (1970)		
Date of test:	May-June 1986		
Species/strain:	Hartley albino guinea pig		
Group size:	10 females treated with β-arbutin 10 females treated with hydroquinone 5 females treated with positive control (DNCB) 10 females treated with distilled water (control group)		
Test substance:	β-arbutin, dissolved as a 10% (w/w) solution in water		
Batch:	Lot A		
Purity:	99.7%		
Dosages:	dermal induction:	β-arbutin	10% in water:ethanol
		hydroquinone:	5% in water:ethanol

		DNCB:	0.1% in liquid paraffin
	dermal challenge:	β-arbutin	1, 3, 10% in water:ethanol
		hydroquinone:	1, 3, 10% in water:ethanol
		DNCB:	0.001%, 0.1% in acetone
Observation period:	72 hours		
GLP/QAU:	before introduction of GLP (1987), thus not applicable.		

On the first day, three samples were intradermally injected on the upper dorsal region:
1) 0.1 ml of Freund's complete adjuvant (FCA):water (1:1)
2) 0.1 ml of 10% β- Arbutin in distilled water
3) 0.1 ml of 10% β-arbutin emulsified in FCA

One week after the injections, 50 mg of 10% sodium lauryl sulfate in petrolatum was applied to the upper dorsal region. On the next day, 0.2 ml of 10% β-arbutin was occlusively applied for 48 hours. Distilled water was used as negative control, 5% HQ was used as reference control, and 0.2% 2,4-dinitrochlorobenzene (DNCB) was used as positive control.
Three weeks after the first induction, 0.01 ml of 10, 3, and 1% β-arbutin in aqueous/ethanol (50/50) solution were topically applied on the flank of animals. 0.01 ml of 10, 3, and 1% HQ in aqueous/ethanol (50/50) solution were applied as reference control, and 0.01 mL of 0.01 and 0.1% DNCB in acetone were applied as positive control. The reaction was evaluated 24 and 48 hours after challenge application.

Results
No positive reactions were observed at any reading time in animals in either the treated or control groups challenged with 10, 3, or 1% β-arbutin in aqueous/ethanol (50/50) solution. As well the animals challenged with 1, 3 or 10% hydroquinone as the ones challenged with 0.01 and 0.1% DNCB showed positive reactions at all tested concentrations.
No positive reactions were observed with the control group at any challenge concentration.

Conclusion
The study authors conclude that β-arbutin does not possess skin sensitizing potential under the test conditions.

Ref.: 7

Comment
- No preliminary study for induction and challenge concentration determination appears to have been performed.
- Challenge with 3 different concentrations is unconventional.
- The scores after induction are not stated, thus the adequacy of the concentrations used cannot be checked.
- The question can be raised whether an aqueous/ethanol (50/50) solution is an appropriate solvent. An aqueous solution with slightly acidic pH would be more suitable.

3.3.4 Dermal / percutaneous absorption

3.3.4.1 *In vitro* dermal / percutaneous absorption

Guideline:	Draft OECD TG 428: Percutaneous Absorption: *in vitro* Method (2000) and SCCNFP Notes of Guidance (SCCNFP/0321/00)
Date of test:	August-September 2002
Test system:	Excised dermatomed human skin (combination of freshly isolated and frozen skin) from 3 donors flow-through diffusion cells, exposure area 0.64cm².
N° of samples:	6 per test substance (each formulation was applied to 2 skin samples of 3 different donors)

Receptor fluid:	mixture of 2 culture media (DMEM & Ham F12, 3:1), supplemented with epidermal growth factor (10µg/l), hydrocortisone (400µg/l), gentamycin (50mg/l) and foetal calf serum (10%, w/w)
Test substances:	Cream-CBP-H (Batch nr. S-1601): 6.3% [^{14}C] β-arbutin Cream-CBP-L (Batch nr. S-1602): 3.0% [^{14}C] β-arbutin Cream-BOP-H (Batch nr. S-1603): 6.3% [^{14}C] β-arbutin Cream-BOP-L (Batch nr. S-1604): 3.0% [^{14}C] β-arbutin Gel-H (Batch nr. S-1605): 6.3% [^{14}C] β-arbutin Gel-L (Batch nr. S-1606): 3.0% [^{14}C] β-arbutin
Purity:	Not stated
Applied amount:	2.2-5.5 mg/cm²
Duration of study:	24 hours
GLP/QAU:	In compliance.

Human skin membranes, 875 µm (experiment 1), 793 µm (experiment 2) and 848 µm (experiment 3) in thickness, were mounted in 9 mm flow-through type diffusion cells. The exposure area of the skin membranes in these cells was 0.64 cm^2. The temperature of the cells was approximately 32 ^{o}C, at ambient humidity. The receptor fluid (culture medium continuously gassed with 95% O_2 and 5% CO_2) was pumped at a speed of approximately 1.5 ml/h. Prior to each experiment, skin integrity test was conducted using the marker substance tritiated water. Only skin membranes with a permeability coefficient (Kp) of less than 1.98 x 10^{-3} cm.h^{-1} for tritiated water were used. The test formulation was applied to the skin membranes at doses ranging between 1.4 and 3.5 mg/membrane; (2.2-5.5 mg/cm²) for an exposure period of 24 h. In all experiments, the receptor fluid was collected at 1, 2, 4, 6, 8, 12, 16, 20, and 24 h after application. Each skin membrane was separated into the following compartments 24 h after application: donor cell, skin wash area (exposed and non-exposed), tape strips (stratum corneum), epidermis and dermis. The radioactivity in all samples was determined by liquid scintillation counter. Total relative absorption (% of dose applied) was calculated as the sum of radioactivity in epidermis, dermis and the receptor fluid.

Results

The following table shows the tissue distribution of β-arbutin, the mean flux constants and the mean lag times as measured:

Test formulation **β-arbutin concentration**	**Cream-CPB-H** **6.3%**	**Cream-CPB-L** **3%**	**Cream-BOP-H** **6.3%**	**Cream-BOP-L** **3%**	**Gel-H** **6.3%**	**Gel-L** **3%**
Donor cell (%)	1.842 ± 2.324	0.901 ± 0.745	1.807 ± 3.496	3.041 ± 3.454	8.240 ± 8.408	10.394 ± 8.336
Skin wash exposed area (%)	93.9 ± 6.3	99.4 ± 4.4	97.3 ± 6.2	95.2 ± 6.9	89.1 ± 10.9	88.3 ± 3.7
Tape strips exposed area (%)	0.071 ± 0.096	0.154 ± 0.132	0.162 ± 0.206	0.217 ± 0.175	0.187 ± 0.266	0.106 ± 0.039
Skin wash non-exposed area (%)	0.109 ± 0.042	0.162 ± 0.109	0.172 ± 0.084	0.162 ± 0.076	0.493 ± 0.504	0.464 ± 0.330
Tape strips non-exposed area (%)	0.023 ± 0.042	0.005 ± 0.004	0.004 ± 0.003	0.005 ± 0.003	0.006 ± 0.003	0.007 ± 0.006
Epidermis (%)	0.057 ± 0.093	0.070 ± 0.036	0.077 ± 0.077	0.109 ± 0.067	0.050 ± 0.041	0.042 ± 0.021
Dermis (%)	0.034 ± 0.058	0.027 ± 0.026	0.029 ± 0.018	0.050 ± 0.029	0.034 ± 0.021	0.044 ± 0.029
Rest skin (%)	0.052 ± 0.106	0.011 ± 0.009	0.014 ± 0.005	0.015 ± 0.010	0.028 ± 0.015	0.040 ± 0.016
Receptor fluid samples (%)	0.015 ± 0.019	0.015 ± 0.014	0.020 ± 0.016	0.036 ± 0.036	0.020 ± 0.011	0.034 ± 0.020
Receptor compartment (%)	0.002 ± 0.004	0.002 ± 0.002	0.002 ± 0.001	0.003 ± 0.002	0.003 ± 0.002	0.005 ± 0.004
Total recovery (%)	96.1 ± 6.2	100.8 ± 4.3	99.6 ± 3.0	98.9 ± 7.2	98.2 ± 7.7	99.4 ± 8.8
Total absorption (%)	0.160	0.126	0.143	0.214	0.135	0.164

Test formulation β-arbutin concentration	Cream-CPB-H 6.3%	Cream-CPB-L 3%	Cream-BOP-H 6.3%	Cream-BOP-L 3%	Gel-H 6.3%	Gel-L 3%
	± 0.255	± 0.060	± 0.083	± 0.114	± 0.066	± 0.016
Mean flux constant (µg/cm².h)	0.0016	0.0009	0.0025	0.0019	0.0022	0.0017
Mean lag time (hours)	0.8	1.2	0.9	1.3	0.7	1.4

With respect to the reference compound (testosterone), no considerable differences were observed based on flux constants and Kp value between freshly isolated skin and frozen skin samples.

Conclusion
The study authors conclude that the mean total absorption of radioactivity from the three formulation types used in the present study was very low, ranging from 0.126 to 0.214% of the applied dose over a 24-h exposure period.

Ref.: 8

Comment
- The exact composition of the test formulations is unknown and no information is available on the pH and the impurities (hydroquinone).
- The tested concentrations of 3.0 and 6.3% β-arbutin are below the requested maximum concentration of 7%.
- The number of skin samples is lower than the required 6 samples from 3 donors each.
- There are no data on the solubility of the test substance in the receptor fluid. β-arbutin is only reported to be "highly" soluble in water.
- The variability of the results is high.

3.3.4.2 *In vivo* dermal / percutaneous absorption - human

See section 3.3.9: Toxicokinetics.

3.3.5 Repeated dose toxicity

3.3.5.1 Repeated dose (28 days) oral toxicity

Guideline:	Not stated, though mainly according to Annex V to Dir. 67/548/EEC, Method B.7: Repeated dose (28 days) toxicity (oral), OECD Guideline 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents
Date of test:	September - November 1986
Species/strain:	Sprague Dawley rat (SPF Crj:CD)
Group size:	16 animals/sex/dosage group, out of which 6/sex were scheduled for the 28-day recovery measurements
Test substance:	β-arbutin
Batch:	Lot A
Purity:	99.7%
Dosages:	0 - 40 - 200 - 1000 mg/kg bw/day
Observation period:	56 days
GLP/QAU:	before introduction of GLP (1987), thus not applicable.

Groups of 10 male and 10 female rats received 0, 40, 200 and 1000 mg/kg bw/day of β-arbutin by oral gavage for 28 days (7 days per week). Control and high dose groups were supplemented with 6 rats/sex in order to study the reversibility of treatment-related effects after a subsequent 28-day treatment-free period. Clinical signs were monitored at every dosing. Body weights and food consumption were recorded weekly.

Haematology, serum chemistry, and urine measurements were performed at the end of the dosage period (day 28) and after the recovery period (day 56), and included:
- for haematology: red blood cell count, haemoglobin, haematocrit, platelet count, white blood cell count, mean red blood cell volume, mean red blood cell haemoglobin amount, mean red blood cell haemoglobin concentration, basophils, eosinophils, neutrophils, lymphocytes, monocytes, reticulocytes;
- for serum chemistry: alkaline phosphatase, calcium, total cholesterol, creatinine, glucose, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, phosphorous, total protein, triglyceride, blood urea nitrogen, sodium, potassium, chloride, albumin/globulin;
- for urine: pH, protein, glucose, ketone bodies, bilirubin, occult blood, nitrite, urobilinogen.

Absolute and relative weights of brain, pituitary gland, salivary gland, thymus, heart, liver, spleen, kidney, adrenal gland, testis, prostate and ovary were determined at the end of the dosage period (day 28) and after the recovery period (day 56). In addition tissues were fixed for histopathological examination.

Results
No test substance-related clinical signs or deaths were observed. Neither did body weight gain and food consumption differed between control and treated animals during the dosing period.
Some slight changes in haematological, serum chemistry and urine parameters were observed, though no dose-dependency could be observed and the observed changes were small and reported to be within the normal ranges for the tested species.
Necropsy at the end of the dosing period revealed some spontaneous anomalies, but no test compound-related changes. There were no histopathological findings related to administration of the test substance.

Conclusion
The study authors conclude that, since no changes attributed to β-arbutin were observed up to a dosage of 1000 mg/kg bw/day, the latter can be considered as the NOEL value.

Ref.: 10

3.3.5.2 Sub-chronic (90 days) dermal toxicity

Guideline:	Not stated, though mainly according to Annex V to Dir. 67/548/EEC, Method B.28: Repeated dose (90 days) toxicity (dermal), OECD Guideline 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents
Date of test:	September - November 1986
Species/strain:	Sprague Dawley rat (SPF Crj:CD)
Group size:	10 animals/sex/dosage group
Test substance:	β-arbutin dissolved in 50% aqueous ethanol solution (vehicle)
Batch:	Lot A
Purity:	99.7%
Dosages:	0 (untreated) - 0 (vehicle) - 56 - 294 - 618 mg/kg bw/day
Observation period:	90 days
GLP/QAU:	before introduction of GLP (1987), thus not applicable.

Prepared test substance or vehicle was applied to the dorsal skin (clipped of fur) of 10 male and 10 female rats per dosage group, 6 days per week for 90 days. Fur clipping was performed once per week. Clinical signs were monitored at the time the test substance or vehicle was applied.

Body weights and food consumption were recorded weekly. Haematology, serum chemistry, and urine measurements were performed at the end of the dosage period (day 90) and included:

- for haematology: red blood cell count, white blood cell count, platelet count, haemoglobin, mean red blood cell volume, mean red blood cell haemoglobin, mean red blood cell haemoglobin concentration, haematocrit, reticulocyte count and white blood cell differential count;
- for serum chemistry: total protein, albumin/globulin ratio, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase, total cholesterol, triglyceride, blood urea nitrogen, creatinine, glucose, chloride, calcium, sodium, potassium, inorganic phosphorous;
- for urine: pH, protein, glucose, ketone bodies, bilirubin, occult blood, nitrite, urobilinogen.

Absolute and relative brain, pituitary gland, salivary gland, thymus, heart, liver, spleen, kidney, adrenal gland, testis, prostate and ovary weights were determined at the end of the dosage period (day 90) and after the recovery period (day 56). In addition tissues were fixed for histopathological examination.

Results
All animals survived the test. Observations:

56 mg/kg bw/day:	reduced body weight in males week 6-8; increased relative weights for spleen, thymus and adrenal gland
294 mg/kg bw/day:	decrease in Ca^{++} in females; decrease in absolute pituitary gland weight in males; decreased absolute and relative pituitary gland and thymus weights in females;
618 mg/kg bw/day:	increase in monocyte ratio in females; decreased relative thymus weight.

No substance-related abnormalities were observed in clinical signs and in the urinanalysis. There were no remarkable findings at necropsy in either sex at any dose level. No histopathological changes were observed.

The study authors consider the reduced body weight at 56 mg/kg/day within the normal range of variation (no dose-dependency observed). The same is claimed for the increased monocyte ratio (618 mg/kg bw/day) and the decreased Ca^{++} level in females (294 mg/kg bw/day), which are regarded as being within normal physiological variation.

Conclusion
The study authors conclude that, since no test substance-related changes attributed to β-arbutin were observed up to a dosage of 618 mg/kg bw/day (the maximum technically applicable dosage), the latter can be considered as the NOEL value.

Ref.: 11

Remark
The evolution of the relative thymus weight (increase at lower dosage level and decrease at higher dosage levels) did not occur in the 28 day oral study, supporting the authors' finding that the observed variations were within the normal range of variation and not substance-related.

3.3.5.3 Chronic (≥ 12 months) toxicity

No data.

3.3.6 Mutagenicity / genotoxicity

3.3.6.1 Mutagenicity/Genotoxicity *in vitro*

A. Reverse mutation test in bacteria

Date of study:	9-19 Feb 1987
Guideline:	National official protocol (Japan), mainly according to Annex V to Dir. 67/548/EEC, Method B.13/14: Mutagenicity: reverse mutation test using bacteria, OECD Guideline 471: Bacterial Reverse Mutation Test
Species/strain:	*Salmonella typhimurium*, TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2 *uvr*A
Replicates:	3
Test substance:	β-arbutin
Batch:	Lot A
Purity:	99.7%
Concentrations:	0 - 156.25 - 312.5 - 625.0 - 2500 - 5000 µg/plate, with and without metabolic activation (rat S9 mix)
GLP/QAU:	Study performed before issue of GLP guidelines.

In a concentration determination test no toxicity of β-arbutin was observed with all test strains in 5000 µg/plate. Therefore, the maximum concentration was set at 5000 µg/plate and 6 levels of concentration were set at common ratio of 2.
In the main study, the strains were exposed to β-arbutin dissolved in distilled water on plates containing histidine deficient agar in the presence and absence of rat liver metabolic activating system (S9 mix prepared from livers of male Sprague-Dawley rats that had received the intraperitoneal injections of sodium phenobarbital and 5,6-benzoflavone). The concentrations tested ranged from 156.25 to 5000 µg/plate. Distilled water alone served as negative control. As a positive standard requiring metabolic activation 2-aminoanthracene was used. N-ethyl-N'-nitro-N-nitrosoguanidine (for TA 1535 and WP2 uvr A), ICR-191 (for TA 1537) and 2-(2-furyl) -3-(5-nitro-2-furyl) acrylic amide (for TA100 and TA 98) were used as positive standards without metabolic activation.

Results
No increase in the number of revertant colonies was observed with any of the test strains at any concentration, irrespective of metabolic activation. The number of revertant colonies was similar to that for the solvent control. Positive control substances induced mutagenic responses in respective test strains.

Conclusion
The study authors conclude that β-arbutin can be considered nonmutagenic in all tested strains under all tested conditions.

Ref.: 12

B. *In vitro* mammalian chromosome aberration test

Date of study:	11 Apr - 5 Sep 1986
Guideline:	National official protocol (Japan), mainly according to Annex V to Dir. 67/548/EEC, Method B.10: Mutagenicity: *in vitro* mammalian chromosome aberration test, OECD Guideline 473: *In vitro* Mammalian Chromosomal Aberration Test.
Test system:	Chinese hamster lung (CHL) fibroblast cells
Replicates:	2
Test substance:	β-arbutin
Batch:	Lot A

Purity:	99.7%
Concentrations:	0 - 2.72 - 1.36 - 0.68 - 0.34 mg/ml in physiological saline, with and without metabolic activation (rat S9 mix)
GLP/QAU:	Study performed before issue of GLP guidelines.

A cytostatic preliminary test with β-arbutin revealed growth ratios of 51% for 2.72 mg/ml, 80% for 1.36 mg/ml, 90% for 0.68 mg/ml and 101% for 0.34 mg/ml.
In the main study, logarithmically growing cells were incubated in Eagle MEM culture medium containing 10% calf serum with β-arbutin at concentrations of 2.72, 1.36, 0.68 and 0.34 mg/ml with/without S9 mix for 6 hours. Medium was subsequently changed and the cells were further cultured in normal medium for 18 hours. As exogenous metabolic activation system, liver S9 fraction from sodium phenobarbital and 5,6-benzoflavone induced rats was used.
In addition, logarithmically growing cells were incubated in Eagle MEM culture medium containing 10% calf serum with β-arbutin at concentrations of 0.34 to 2.72 mg/ml without S9 mix for 24 and 48 hours.
In each experimental group two parallel cultures were set up.
N-Methyl-N'-nitro-N-nitrosoguanidine and Benzo[a]pyrene were used as positive controls for direct and metabolic activation method, respectively. 2 hours before the end of the incubation period, colcemid was added to the cultures. The cells were put onto glass slides, treated with hypotonic potassium chloride solution, fixed in methanol and acetic acid and stained with Giemsa solution. Per culture 100 metaphases were scored for structural chromosomal aberrations (breaks, exchanges and others) and polyploids. Gaps were recorded separately.

Results
There were no biologically relevant and statistically significant increases in cells with structural aberrations and polyploids after treatment with β-arbutin at any concentration or incubation time, irrespective of the presence of metabolic activation system. The reference mutagens used as positive controls showed distinct increases in cells with structural chromosome aberrations.

Conclusion
The study authors concluded that β-arbutin did not induce chromosomal aberrations at concentrations up to 0.34 mg/ml in Chinese hamster lung fibroblasts, irrespective of metabolic activation.

Ref.: 13

3.3.7 Carcinogenicity

Guideline:	National official protocol (Japan), partly according to Annex V to Dir. 67/548/EEC, Method B.32: Carcinogenicity test, OECD Guideline 451: Carcinogenicity Studies.
Date of test:	Apr 1993 - Oct 1994
Species/strain:	Crj:CD1 (ICR) mice
Group size:	50 animals/sex/dosage group
Test substance:	β-arbutin, dissolved in 50% ethanol solution in water
Batch:	Lot TDD-422
Purity:	100.0%
Dosages:	0 - 45 - 135 - 400 mg/kg bw/day
Observation period:	78 weeks (18 months)
GLP/QAU:	reported to be present, but not included in the current submission.

Groups of 50 male and 50 female mice were treated with 0, 45, 135 and 400 mg/kg bw/day of β-arbutin through dermal application for 78 weeks (6 days per week). The application site was the interscapular skin (+ 2 x 2 cm), clipped with an electrical clipper once every one or two weeks. Control animals received 10 ml of distilled water. Clinical signs and presence of

dead or moribund animals were checked twice per day. In addition, body surface were palpated for masses once per week. Documentation of detected masses included date, size and progression. Body weights and food consumption were recorded once per week the until week 26 and once every two weeks thereafter.
Haematology was performed at the end of the dosage period (week 78) and after the recovery period (day 56), and included red blood cell count, white blood cell count, differential count, eosinophil, basophil, monocyte, band neutrophil and segment neutrophil count. Hematology was also performed whenever possible on moribund animals.
All surviving animals were sacrificed and body surfaces, intracranial tissues, and internal organs were examined. Observed masses were documented as to site, shape, size and number. Absolute and relative brain, heart, lung, liver, kidney, spleen, testis and ovary weights were determined. Histopathology was performed on organs and tissues of all animals.

Results
Mortality rates, clinical signs and food consumption did not show significant differences between control and treated animals. Red blood cell count, white blood cell count, white blood cell differential count, absolute and relative organ weight measurements and necropsy and microscopic examination did not reveal substance-related differences.
Non-tumour lesions included hyperplasia of mucosal epithelium in the glandular stomach, myocardial degeneration and necrosis in the heart, excessive extramedullary hematopoiesis in the spleen, calcification in brain thalamus, subcapsular hyperplasia in the adrenal gland, ovary cysts, cystoic endometrial hyperplasia in the uterus and detachment or hypertrophy of articular chondrocytes in the joint.
Tumour lesions included hepatocellular adenoma, bronchiolar/alveolar adenoma and malignant lymphoma. These lesions occurred in all groups (also in the control group).

Conclusion
The study authors conclude that, since the observed (non-)tumour lesions are the ones frequently observed in aging mice, the NOEL value for β-arbutin in the present study is estimated to be 400 mg/kg bw/day in male and female mice. It is also concluded that the test substance is not carcinogenic under the conditions of the performed study.

Ref.: 14

Remarks
No rationale is given for the choice of another species than the one used in the 28 day or 90 day repeated dose study (mice instead of rats). In addition, the weight variation in the animals at the beginning of the study exceeded 20% (± 50%) and the intermediate haematological examination (after 12 months) was not performed. Finally, the non-tumour and the tumour lesions observed in all animals (including the control group) reveal that, whether or not caused by aging alone, the general condition of the animals appeared to be poor.
No positive control group has been included. This is not necessary, but historical data from the testing facility to detect carcinogens with the method used, are lacking.

3.3.8 Reproductive toxicity

3.3.8.1 1-Generation reproduction toxicity

Guideline:	Not stated, though partly according to Annex V to Dir. 67/548/EEC, Method B.34: One-generation reproduction toxicity test.
Date of test:	Not specified, around April 1986
Species/strain:	Sprague Dawley rat (SPF Crj:CD)
Group size:	35 animals/sex/dosage group
Test substance:	β-arbutin

Batch:	Lot A
Purity:	99.7%
Dosages:	0 - 25 - 100 - 400 mg/kg bw/day
Observation period:	up to 10 weeks
GLP/QAU:	probably before introduction of GLP (1987), thus not applicable.

β-arbutin or vehicle was injected subcutaneously into male rats for 9 weeks prior to mating, from 6 to 15 weeks of age. Dosing continued until the rats were observed to have successfully copulated. For female rats, dosing was performed for 2 weeks before mating, i.e. from 8 to 10 weeks of age, after which it continued during the mating period. A group of 20 pregnant rats were subjected to a caesarean section after daily dosages from days 0 to 19 of pregnancy. The remaining animals that were scheduled to go to term (+ 10) were dosed daily from day 0 of pregnancy till day 21 after parturition.
Observations for the parent (P) rats included clinical signs, mortality, body weight and food intake on a daily basis. To examine the oestrous cycle, vaginal smears were examined. For the males, testis, epididymis, and prostate were weighed. Ovary and uterus from female rats that did not successfully mate and from non-pregnant female rats were examined histopathologically.
In the caesarean section group, foetuses were fixed in alcohol for either skeletal examination or visceral examination. Pups from the delivery groups were lactated and clinical signs, body weights, physical and behavioural development were observed up to day 21 after parturition. One male and female pair from each litter was mated at 10 weeks of age to evaluate reproductive function. One rat/sex from each litter was sacrificed at 7 and 10 weeks of age and the organs were weighed. The number of corpora lutea, implantations, live and dead foetuses, and the number of resolved embryos were counted. Male F1 rats used for mating were sacrificed for *post-mortem* examination to measure the weight of testis, epididymis and prostate.

Results
No clinical or body weight abnormalities related to the test substance were observed in the parent rats. Food intakes were slightly lower on Days 51 and 58 for the 100 mg/kg group and on Days 2 and 58 for the 400 mg/kg group, though only for the male rats.
Oestrous cycles, copulation indices, and fertility indices in the treatment groups were similar to the control group.
For ovulation, implantation, foetal development, number of ovulations (number of corpora lutea), implants, live foetuses, implantation rate, embryo-lethality rate, placental weights, and sex ratios, the measurements in the treatment groups were similar to the ones in the control group. Body weights of female foetuses in the 400 mg/kg bw/day group were significantly lower than the control group. Gross findings, organ weight checks, histopathological examinations and examination of female parent rats at delivery, revealed no abnormalities related to the test substance.
The live foetuses showed no abnormalities in external, visceral, and skeletal examination. The degrees of ossification and the incidences of skeletal variations of the treatment groups were similar to the control group. Mean numbers of F1 pups, viability indices at 4 days of age, weaning indices at 21 days of age, body weights of F1 pups during the lactation period, behavioural & physical development parameters did not differ between treated and control animals. After weaning, the F1 rats showed no adverse clinical signs or altered body weight gain and food intake values. With regard to absolute and relative organ weights (7 or 10 weeks of age), no significant differences were observed in the 25 and 100 mg/kg groups. The left ovary weight of the 400 mg/kg group was significantly lower, but no significant differences were observed with the total absolute and relative organ weights of the left and right ovaries.

Conclusion
It is concluded that 400 mg/kg/day of β-arbutin does not affect reproductive functions of the parent animals and F1 rats, but caused body weight decrease in female foetuses,

decreased organ weights of the unilateral ovary of female F1 rats. Therefore the study authors estimate the no observable effect dose of β-arbutin to be 100 mg/kg/day.

Ref.: 15

3.3.8.2 Teratogenicity

No data submitted.

3.3.9 Toxicokinetics

Skin metabolism after repeated topical application of β-arbutin in human volunteers

Date of study:	09-13 Jan 2005
Method:	Multiple topically dosed open study (4 days application)
Subjects:	Healthy volunteers, age 18-45 years (mean: 30 years)
Group size:	9 female and 9 male subjects
Test substance:	CP-SEN, a 6.3% β-arbutin-containing gel (full quantitative composition available)
Batch numbers:	CP-SEN: Lot 043 β-arbutin: Lot A, B, C
Purity of β-arbutin:	Lot A: 99.7%, Lot B: 97.8%, Lot C: 99.8%
Hydroquinone content:	Lot A: 0.0129%, Lot B: 0.0208%, Lot C: 0.0094% CP-SEN Lot 043: 0.1 ppm
Doses:	2.8 mg/cm² of 6.3% β-arbutin-containing gel
Exposure time:	Open application, once/day, unoccluded for 30 minutes
Volunteers:	18 healthy volunteers (9 females and 9 males)
GLP/QAU:	Undersigned statements available, including ethical approvals

Volunteers (9 females and 9 males) were chosen according to set in- and exclusion criteria, pre-study check-up and physical examination results. They were treated daily for 4 consecutive days with 141.5 mg of a 6.3 %(w/w) β-arbutin-containing gel on a delineated area of 50 cm² (10 x 5 cm) on the right side of the upper part of the buttock. Their diet was not restricted; they only needed to keep a diary of their daily food and drink intake. The use of hair dyes was prohibited from 3 days prior to the study till the end of the study. During the whole topical treatment period, showering and bathing was only allowed in the morning just prior to visiting TNO. The application period was determined based on the excreting profile of hydroquinone in human studies ref. 23 and 24.
The applied dose of 141.5 mg on 50 cm² corresponds to about 2.83 mg of test formulation per cm². This results in a daily application of ~8.9 mg of β-arbutin. The subjects were instructed to leave the application area unoccluded for 60 minutes after each application and were therefore confined to the TNO facility.
Prior to the first application (day 1), an area at the upper left buttock was tape-stripped and three skin biopsies were obtained as controls from each volunteer. Approximately 24-30 hours after the last application (Day 5), three treated skin biopsies were taken from the right buttock as well. From either the untreated or treated skin area 4 mm punch biopsies (n=3) were taken.
Control and treated skin samples taken from each day (Day 1 and Day 5) were frozen in liquid nitrogen. β-arbutin and hydroquinone (HQ) were extracted from the dismembrated skin and analyzed by high- resolution GC-MS.
Within each study day (Day 1 and Day 5), calibration line (CAL) and quality control (QC) samples for HQ and β-arbutin were prepared and analysed separately.
It is well known that human subjects take HQ from food and excrete it in urine. To describe the possible changes in urinary HQ levels, spot urine samples were collected daily during the study. Urine samples were collected each morning (prior to application until approximately 24-30 hours after the last application). Total HQ levels of acid-hydrolyzed urine samples, total 90 samples, were determined quantitatively by an LC system equipped

with electro colorimetric detector (LC-ECD). For measuring HQ, CAL and QC samples were prepared and analysed.

Results:
- In all control skin biopsy samples very low levels of HQ (< 1.1 ng quantitative limit) and β-arbutin (< 8.9 ng quantitative limit) were present.
- In all treated skin samples average HQ content of 177 ± 149 ng/g (32.0 – 602 ng/g) and average β-arbutin content of 3735.8 ng/g ± 2137.5 (863.0-9809.0 ng/g) could be established.
- In a number of spot urine samples (24/90) detectable HQ levels, corrected by creatinine for diuresis (< 4.19 – 9.16 mmol/mol), were established.

The following table shows the amount of β-arbutin and HQ and the percentage of HQ present in skin, together with the urinary total HQ analysis results (detection limit urinanalysis = 0.974 mg/l).

Subject number	β-arbutin and HQ skin biopsy results			HQ urinanalysis results				
	β-arbutin (ng/g skin)	HQ (ng/g skin)	HQ/(Arb+HQ) (%)	Day 1 (mg/l)	Day 2 (mg/l)	Day 3 (mg/l)	Day 4 (mg/l)	Day 5 (mg/l)
01	3048.7	84	2.69	< 0.974	< 0.974	< 0.974	< 0.974	< 0.974
02	3185.4	89	2.73	< 0.974	< 0.974	1.32	< 0.974	2.37
03	4132.8	196	4.53	< 0.974	< 0.974	< 0.974	< 0.974	< 0.974
04	863.0	32	3.57	1.59	1.54	< 0.974	< 0.974	1.16
05	7744.6	133	1.69	1.51	1.19	2.54	< 0.974	< 0.974
06	1652.6	74	4.26	< 0.974	< 0.974	1.36	< 0.974	< 0.974
07	3321.2	90	2.64	< 0.974	< 0.974	< 0.974	< 0.974	< 0.974
08	4102.5	140	3.29	< 0.974	< 0.974	< 0.974	< 0.974	< 0.974
09	4140.6	134	3.13	< 0.974	< 0.974	< 0.974	1.46	< 0.974
10	3543.6	473	11.77	2.32	< 0.974	3.05	< 0.974	< 0.974
11	1851.9	35	1.86	< 0.974	< 0.974	< 0.974	1.22	< 0.974
12	4239.0	142	3.24	1.15	2.38	2.97	< 0.974	< 0.974
13	4865.1	602	11.01	1.77	2.01	1.32	< 0.974	< 0.974
14	2366.3	168	6.61	< 0.974	< 0.974	< 0.974	< 0.974	< 0.974
15	3486.5	162	4.43	1.09	1.32	< 0.974	< 0.974	< 0.974
16	2860.5	253	8.14	< 0.974	1.60	1.39	< 0.974	< 0.974
17	9809.0	286	2.83	< 0.974	< 0.974	< 0.974	< 0.974	< 0.974
18	2031.0	88	4.15	< 0.974	< 0.974	1.05	< 0.974	< 0.974
Mean ± S.D.	3735.8 ± 2137.5	177 ± 149	4.6 ± 2.9					

Conclusion
The study authors conclude that:
- Repeated topical application of gel containing 6.3% (w/w) β-arbutin leads to detectable amounts of β-arbutin and HQ in skin.
- The statistical tests showed significant differences ($p < 0.0001$) between Day 01 and Day 05 for the variables: analysed amount (ng) and corrected amount (ng/g) for HQ as well as for β-arbutin.
- Based on the large variation in the established urinary total HQ results, changes in urinary HQ levels due to topical treatment of β-arbutin could not be established.
- When the HQ content in the skin samples is taken relative, on a weight to weight basis, to the β-arbutin+HQ content, on average 4.6% (± 2.9) (range: 1.69-11.77) of HQ is present in these skin samples.
- Actual levels of HQ in treated skin amounted on average to 0.018 ± 0.016 µg/cm², ranging 0.003 – 0.072.

Ref.: 9

Comments
Under the in use conditions described in human volunteers, hydroquinone is released to a relative level (compared to the β-arbutin+HQ content) as high as 11.8% (w/w).

3.3.10 Photo-induced toxicity

3.3.10.1 Phototoxicity test

Guideline:	Morikawa et al. 1974 (Ref. 34)
Date of test:	24-28 Mar 1986
Species/strain:	Hartley male albino guinea pigs
Group size:	10 animals
Test substance:	β-arbutin, 10% solution in 50% v/v ethanol solution in water
Batch:	Lot A
Purity:	99.7%
Positive control:	8-Methoxypsoralen (8-MOP) at 0.02% in ethanol
Dose:	20 µl applied to a skin surface area of 1.5cm x 1.5cm
Observation period:	72 hours
GLP/QAU:	before introduction of GLP (1987), thus not applicable.

The fur of the back of 10 guinea pigs was clipped with an electric clipper and depilated with Shiseido hair remover. The test was carried out 24 hours after depilation. 20 µl of either 10% β-arbutin in 50% ethanol or 8-MOP were applied to two 1.5cm x 1.5cm skin areas. Immediately after application, one side was covered with aluminium foil and 30 minutes later, the other side was irradiated with six Toshiba model FL-40 BLB lamps (emission: 300-400 nm, λ_{max}=360nm) arranged in parallel and fitted with a window-glass filter to eliminate radiation below 320 nm. The distance from the light source to the skin was 10 cm and the energy used was 14.0 Joule/cm^2. Erythema and oedema were evaluated at 24, 48, and 72 hours after irradiation. Phototoxicity was evaluated by comparing scores of the irradiated and non-irradiated sections.

Results
No skin reactions were observed in either irradiated or non-irradiated sections treated with β-arbutin. Conversely, a strong phototoxicity reaction was observed with the positive control substance 8-MOP.

Conclusion
The study authors conclude that β-arbutin has little phototoxicity potential.

Ref.: 17

3.3.10.2 Photosensitisation test

Guideline:	Ichikawa et al. 1981 (Ref. 35)
Date of test:	10 Mar - 2 Apr 1986
Species/strain:	Hartley male albino guinea pigs
Group size:	10 animals (treatment & control), 5 animals (positive control)
Test substance:	β-arbutin, 10% solution in 50% v/v ethanol solution in water
Batch:	Lot A
Purity:	99.7%
Positive control:	6-methylcoumarin (6-MC) at 1.0% and 0.1% in ethanol
Dose:	induction: 100 µl of 10% β-arbutin in 50% ethanol 100 µl of 5% 6-MC in ethanol
	challenge: 20 µl of 10% β-arbutin in 50% ethanol 20 µl of 1 and 0.1% 6-MC in ethanol
Observation period:	24 days
GLP/QAU:	before introduction of GLP (1987), thus not applicable.

100 µl of emulsified Freund's Complete Adjuvant (FCA) was injected intradermally at the 4 corners of the clipped and shaved 2cm x 4cm nuchal area of the guinea pigs. 100 µm of 10% β-arbutin in 50% ethanol solution was then applied to the area defined by the injection sites. Subsequently the area was irradiated with 10.2 Joule/cm^2 of UVA. Whereas FCA was injected once at the start of the induction exposure, the remaining procedures were repeated for 5 consecutive days. The light source used composed of six tubes of Black Light Lamp (λ=300-400 nm, λmax=360 nm) eliminating radiation below 320 nm. The distance from the light source to the skin was 10 cm. Three weeks after first induction, 20 µl of 10% β-arbutin in 50% ethanol was applied to the dorsal site. One side was irradiated with 10.2 Joule/cm^2 of UVA, while the other side was covered with aluminium foil thus serving as unradiated control. 20 µl of 1% and 0.1% 6-Methylcoumarin (6-MC) in ethanol was applied as positive control. Erythema and oedema formation were evaluated 24 and 48 hours after irradiation.

Results
No positive reactions were observed in either irradiated or non-irradiated sections treated with β-arbutin. Conversely, strong photosensitisation reactions were observed with the positive control substance 6-MC.

Conclusion
The study authors conclude that β-arbutin does not possess photoallergic potential under the test conditions.

Ref.: 16

3.3.11 Human data

3.3.11.1 Single patch tests on human volunteers - skin irritation

A. Single patch test with β-arbutin on human volunteers

Date of study:	01-03 Apr 1986
Method:	48 hours closed patch test in human volunteers
Subjects:	Healthy volunteers, age 25-47 years (mean: 35 years)
Group size:	43 male subjects
Test substance:	β-arbutin, 10% solution in distilled water
Batch:	Lot A
Purity:	99.7%
Doses:	50 µl of 10% β-arbutin solution

Exposure time:	48 hours		
Scoring system:	-	negative:	no reaction
	±	pseudo-positive:	mild erythema
	+	positive, weak:	erythema
	++	positive, medium:	erythema + oedema
	+++	positive, strong:	erythema + oedema + (serious) papules or small vesicles
	++++	positive, strongest:	large vesicles
GCP:	No statement (performed before issue GCP guidelines)		

50 µl of 10% β-arbutin solution was placed on a piece of lint which was attached to the back of each subject. The application site was subsequently immobilized using Nichiban Keepsilk plasters (16 mm in diameter). After 48 hours, the plasters were removed.
The first reading was performed 30 minutes after the removal (48 hour reading), and the severity of skin reactions was scored. A second reading was performed 24 hours later (72 hour reading).

Results
No positive reactions were seen in any subjects at 48 or 72 hours after application of 10% β-arbutin solution. The calculated positive rate was 0%.

Conclusion
The study authors conclude that β-arbutin displays low irritation potential.

Ref.: 4

B. Single patch test with β-arbutin-containing finished products on human volunteers

Date of study:	29 Jun - 01 Jul 1992		
Method:	24 hours closed patch test in human volunteers		
Subjects:	Healthy volunteers, age 19-59 years (mean: 32 years)		
Group size:	24 female and 23 male subjects		
Test substance:	1) AR-91 SWT Essence (containing 7% β-arbutin), 2) AR-91 SWT Essence A10 (containing 10% β-arbutin), 3) AR-91 SWT Essence AC (containing 0% β-arbutin). No details on qualitative and/or quantitative compositions.		
Batch:	For β-arbutin: Lot TDA-361		
Purity:	100.3%		
Doses:	30 µl of above-mentioned formulations (0%, 7% or 10% β-arbutin)		
Exposure time:	24 hours		
Scoring system:	-	negative:	no reaction
	±	pseudo-positive:	mild erythema
	+	positive, weak:	erythema
	++	positive, medium:	erythema + oedema
	+++	positive, strong:	erythema + oedema + (serious) papules or small vesicles
	++++	positive, strongest:	large vesicles
GCP:	No statement (performed before issue GCP guidelines)		

30 µl of each product was placed on a filter paper positioned on an aluminium plate with petroleum jelly, which was subsequently attached to the flexor side of the forearm of each volunteer. The application site was immobilized using an elastic bandage. Elastic and adhesive bandages were removed three hours before the first reading (24 hours) and the severity of skin reactions was scored. A second reading was performed 24 hours later (48 hour reading).

Results

No positive reactions were seen at 24 or 48 hours after application of the 0%, 7% or 10% β-arbutin products and the positive rate was 0% in all cases.

Conclusion
The study authors conclude that 0%, 7% and 10% β-arbutin-containing products display low irritation potential.

Ref.: 5

C. Repeated open application test with β-arbutin-containing finished products, without exposure to sunlight on human volunteers

Date of study:	30 Jul 1992 - 20 Jan 1993
Method:	24 week repeated open application test in human volunteers
Subjects:	Healthy volunteers, age 18-53 years (mean: 34 years) without skin diseases
Group size:	23 female and 23 male subjects
Test substance:	1) AR-91 SWT Essence (containing 7% β-arbutin), 2) AR-91 SWT Essence A10 (containing 10% β-arbutin), 3) AR-91 SWT Essence AC (containing 0% β-arbutin). No details on pH or qualitative and/or quantitative compositions.
Batch:	For β-arbutin: Lot TDA-361
Purity:	100.3%
Doses:	20 mg of above-mentioned formulations (0%, 7% or 10% β-arbutin)
Exposure time:	Open test, 3 applications/day, thus continuous exposure for 24 weeks
Scoring system:	Skin colour reading criteria: **0** no **1** slight **2** significant Adverse reactions reading criteria: **-** none ± slight **+** mild **++** moderate **+++** severe (indicates termination of the test)
GCP:	No statement

Test samples were applied at least three times daily for 24 consecutive weeks. The 0% β-arbutin product was applied randomly to either the upper or lower area of each arm, while the 7 and 10% β-arbutin product were randomly assigned to the left or right arm, thus ensuring that product application was left-right symmetric. Four tubes were labelled "upper right", "lower right", "upper left" and "lower left". For each application, subjects were instructed to apply about 20 mg of product (about the size of a rice grain) using their fingers. Readings were performed at 4, 8, 12, 16, 20 and 24 weeks after the start of application. Severity of pigmentation and discoloration were assessed in relation to skin colour of adjacent areas. Adverse reactions due to the application of test materials (for example irritation, itching, redness, oedema, papules/vesicles, and desquamation) were assessed for each area and graded.

Results
Of the 64 subjects, two women withdrew from the study (after 8 and 20 weeks, respectively). Data obtained from these subjects were analyzed until their last reading. Application of test products was not terminated due to the onset of adverse reactions in any of the subjects, and none of the subjects violated any of the restrictions.
For all reading periods after the start of application, there were no differences in relative skin colour for the 7%, 10%, and 0% β-arbutin products and no pigmentation/discoloration were observed. No adverse reactions caused by the application of test materials were seen in any subject at any reading period.

Conclusion
The study authors conclude that there were no safety-related problems with the tested 7% and 10% β-arbutin product.

Ref.: 18

D. Repeated open application test with a 10% β-arbutin-containing finished product, with exposure to sunlight on human volunteers

Date of study:	24 Sep 1992 - 09 Mar 1993
Method:	24 week repeated open application test in human volunteers, with exposure to sunlight
Subjects:	Healthy volunteers, age 32-60 years (mean: 43 years) without skin diseases
Group size:	59 female subjects
Test substance:	1) AR-91 SWT Essence A10 (containing 10% β-arbutin), 2) AR-91 SWT Essence AC (containing 0% β-arbutin). No details on qualitative and/or quantitative compositions.
Batch:	For β-arbutin: Lot TDA-361
Purity:	100.3%
Doses:	50 mg of above-mentioned formulations (0% or 10% β-arbutin)
Exposure time:	Open test, 4 applications/day, thus continuous exposure for 24 weeks
Scoring system:	Skin colour reading criteria: **0** no **1** slight **2** significant Adverse reactions reading criteria: **-** none ± slight **+** mild **++** moderate **+++** severe (indicates termination of the test)
GCP:	No statement

Test substances were applied at least four times daily for 24 consecutive weeks. The 10% and 0% β-arbutin products were randomly assigned so that one product was applied to the right hand while the other was applied to the left hand. In case the hands came in contact with water, products were reapplied. Subjects were instructed to apply about 50 mg (size of a soybean) of product to the back of the respective hand. Readings were performed at 4, 12 and 24 weeks after the start of application.
The presence and severity of adverse reactions at the application sites were scored and adverse reactions such as irritation, itching, flushing, swelling, papules/vesicles, dry skin, desquamation, discoloration and pigmentation were graded.

Results
None of the subjects experienced adverse reactions severe enough to terminate application of the products, and as a result, data obtained from all 59 subjects were analyzed.
Skin readings conducted at 4 and 12 weeks after the start of application revealed no skin reactions. Skin readings conducted at 24 weeks after the start of application revealed a few reactions in two subjects. However, it was determined that these reactions were not caused by the 10% or 0% β-arbutin products.

Conclusion
The study authors conclude that there were no safety-related problems in applying the 10% β-arbutin product to the backs of the hands of 59 healthy women for 24 consecutive weeks.

Ref.: 19

3.3.12 Special investigations

Additional information on the impurity hydroquinone (1,4-Benzenediol):

LD_{50}-oral-rat = 298 mg/kg
Slightly irritating to the eye
Sensitising to the skin.
Absorption rate (5% aqueous solution)-human skin: 0.522 µg/cm²/h
NOEL (28d/90d-oral-rat) = 20 mg/kg/day
NOAEL (28d/90d-dermal-rat) = 74 mg/kg/day
NOEL (developmental toxicity-rabbit) = 25 mg/kg/day (dams).
NOEL (developmental toxicity-rabbit) = 75 mg/kg/day (teratogenic effects).
NOEL (1-generation reproduction toxicity-rat) = 15 mg/kg/day (general toxicity).
NOEL (1-generation reproduction toxicity-rat) = 150 mg/kg/day (reproductive toxicity).
Negative in the Ames test, the dominant lethal assay and the mouse spot test.
Positive in the *in vitro* chromosome aberration test (+S9)
Positive (i.p.) and weakly positive (oral) in the *in vivo* micronucleus test.
Equivocal conclusions on potential carcinogenic effects at dosage levels ≥ 25 mg/kg/day.
Ref.: 26, 29

Hydroquinone has been used for many years in skin-bleaching preparations up to 2%. It does not directly bleach the skin, but acts through competitive inhibition of tyrosinase resulting in gradual fading of hyperpigmented spots by a reduction in the formation of new pigment.
With regard to potential adverse effects caused by hydroquinone, covalent binding and oxidative stress are mechanisms postulated to be induced by the molecule. Oxidized hydroquinone metabolites may covalently bind cellular macromolecules or alkylate low molecular weight nucleophiles (e.g. glutathione) resulting in enzyme inhibition, alterations in nucleic acids and oxidative stress. Cell proliferation associated with nephrotoxicity in a sensitive strain of animals (male F344 rat) has been postulated to be involved in the production of renal tumours in rats.

Ref.: 26

According to IARC, hydroquinone is not classifiable as to its carcinogenicity to humans. This conclusion was based upon limited evidence in experimental animals and inadequate evidence in humans (IARC 1999).
A more recent literature review on the carcinogenicity of hydroquinone concludes that indeed renal tumours were observed in rats, but that the mode of action (exacerbated chronic progressive nephropathy) appears to be a rat-specific disease that appears to lack a human counterpart. The available cohort studies (all involving occupational exposure) failed to show a clear causal relationship between exposure to hydroquinone and the development of several types of malignancies.

Ref.: 36, 37

A final side effect linked to the use of hydroquinone as a skin bleaching agent is ochronosis, the darkening of the skin accompanied by changes in the papillary dermis.
Recently a literature review from 1966 to 2007 on the topic of human exposure to topically applied pharmaceutical hydroquinone preparations was published. Data on more than 10,000 patients were screened. Applied hydroquinone concentrations ranged from 1 to 30% and the duration of exposure from 1 day to 20 years. More than 9,500 patients used hydroquinone for a period longer than one month. In total, 789 cases of ochronosis were reported, of which 756 arose from Africa.
When hydroquinone is used at relatively high concentrations (> 2%) in the medicinal world to treat for example dyschromia, a risk-benefit analysis is performed.

Ref.: 38

3.3.13 Safety evaluation (including calculation of the Margin of Safety)

Not applicable.

3.3.14 Discussion

3.3.14.1 The physicochemical profile of β-arbutin

The submission lacks a number of data with respect to the identification, stability and physico-chemical data of compound under study. More specifically, the following remarks were made:

- More extended solubility data are mentioned in e.g. the 1-generation reproduction toxicity test than in the physicochemical section of the dossier:
  in water: 7% by weight at 0°C, 16% at 25°C, 37% at 50°C;
  in hydroalcoholic solution (1:1): 13% by weight at 0°C, 25% at 25°C, 32% at 50°C;
  in DMSO: readily soluble.
- The octanol/water partition coefficient was not measured, but simply mentioned in the *in vitro* dermal absorption study report.
- pH measurements: it is not stated at which concentration in which solvent the pH was measured. This is crucial, as it has been reported [36] that β-arbutin is hydrolyzed in the presence of weak acids. Also during absorption through the skin hydroquinone can be formed and absorbed.
- Stability test of β-arbutin under sunlight: the test description is very brief and not standardised. The temperature of the test is not stated. It is not clear whether the measurements at the start of the study are actual measurements (3 times exactly 100.0%) or whether the starting values were arbitrarily set to 100.00%, towards which all other measurements were relatively expressed. No controls are included of the intensity and duration of the sunlight to which the samples have been exposed. It is only mentioned that they were kept "outdoors" for 30 days.
- Stability test of β-arbutin at several pH conditions: the test description is very brief. It is not clear whether the measurements at the start of the study are actual measurements (3 times exactly 100.0%) or whether the starting values were arbitrarily set to 100.00%. Based upon a partial decrease in stability at pH 2.0, which was not observed at pH 3.0 or 11.0, the authors conclude that stability at pH 4-8 is guaranteed. However, the actual stability in this relevant pH range was not measured.
- Stability of β-arbutin in a finished cosmetic product (accelerated test conditions for 6 months): the test description is very brief. Neither the qualitative nor the quantitative composition of the tested formulation is stated. Also, the pH of the formulation is not given and this is crucial for potential hydrolysis.
- Stability of β-arbutin in a finished cosmetic product (ambient test conditions for 4 years): the test description is very brief. Neither the batch number nor the qualitative or quantitative composition of the tested formulation is stated. Also, the pH of the formulation is not given and this is crucial for potential hydrolysis.
- β-arbutin Lot TPG-412 is not taken up in the list under 3.1.4, thus its purity is not stated.

Therefore the identity/physicochemistry section of the dossier is considered incomplete. In particular, the stability of β-arbutin and the potential release of hydroquinone under in use conditions are important and cannot be retrieved from these data.

3.3.14.2 The toxicological profile of β-arbutin

A general remark is that the majority of the toxicological tests have been performed in Japan. In practical terms, this means that the presented corresponding test reports consist of translated summaries only. The company's toxicologist certifies that they truthfully represent the original Japanese duly-signed in-house test reports.

Local toxicity
The skin irritation study in the rabbit was not completely performed according to current standards. Nevertheless, since the skin was abraded and the 10% solution did not show any irritating properties, β-arbutin can be considered as non-irritating to the rabbit skin at dilutions up to 10%. That same solution moreover was shown to be non-irritating to the rabbit eye.
In a single patch tests on human volunteers with β-arbutin at 10% in water and at 7 and 10% in a cosmetic formulation, the substance revealed to be non-irritating. Repeated open application tests with and without sunlight exposure showed that the 10% β-arbutin-containing cosmetic formulation was well-tolerated by the volunteers. No adverse reactions related to the test substance were noted.

Skin sensitisation
Although the available Magnusson & Kligman test was not entirely performed according to current guidelines, it used β-arbutin concentrations up to 10%. The tested concentrations showed to be negative, whereas the positive control clearly elicited an allergic reaction. Therefore β-arbutin as such is not expected to be a skin sensitizer.

Dermal absorption
The study authors conclude for the *in vitro* dermal absorption test performed that the mean total absorption was very low, ranging from 0.126 to 0.214% of the applied dose over a 24 h exposure period. It was further noted that the number of skin samples was insufficient and that the concentration of β-arbutin in the test formulation (6.3%) was slightly below the requested maximum concentration (7.0%).

Systemic toxicity
The acute toxicity profile of β-arbutin can be considered low, viewing the LD_{50}-oral-rat value of 8715 mg/kg/day and the LD_{50}-dermal-rat of > 928 mg/kg (maximum practically applicable dosage).
A repeated dose 28 day oral study and 90 day dermal study with the rat only revealed some sporadic observations which could not be related to the test substance. Therefore in both tests, the highest dosage tested could be designated as NOEL, i.e. 1000 mg/kg bw/day for the repeated dose oral study and 618 mg/kg bw/day for the repeated dose dermal study.

Toxicokinetics
A human skin metabolism study revealed that daily topical application for 4 consecutive days of a gel containing 6.3% (w/w) β-arbutin led to detectable amounts of β-arbutin and HQ in skin. Based on a large variation in the established urinary total HQ results, changes in urinary HQ levels due to topical treatment of β-arbutin could not be established.
When the HQ content in the skin samples was taken relative to the (β-arbutin+HQ) content, on average 4.6% of HQ was present in these skin samples going up as high as 11% in two samples. Actual levels of HQ in treated skin amounted on average to 0.018 ± 0.016 µg/cm², with a top level of 0.060 µg/cm² in one sample. This exposure to HQ reflects a realistic in use situation in man and the release of HQ cannot be ignored.

Mutagenicity/genotoxicity
The Ames test did not reveal any increase in the number of revertant colonies with any of the test strains at any concentration, irrespective of metabolic activation.

Neither were there any biologically relevant and statistically significant increases in cells with structural aberrations and polyploids after treatment with β-arbutin at any concentration or incubation time, irrespective of the presence of metabolic activation system in the presented *in vitro* mammalian chromosome aberration test. Therefore β-arbutin is considered to be non-mutagenic.
Not all three tests as mentioned in the SCCP Notes of Guidance have been presented.

Carcinogenicity
A dermal carcinogenicity study in mice revealed that dosages up to 400 mg/kg bw/day failed to induce dose-related tumour formation due to the administration of β-arbutin.

Reproductive toxicity
A one-generation reproduction study with β-arbutin revealed a NOEL for reproduction toxicity of 100 mg/kg bw/day based upon the observation of body weight decrease in female foetuses and decreased organ weights of the unilateral ovary of female F1 rats at 400 mg/kg bw/day.

Photo-induced adverse effects
The presented summaries of a phototoxicity and photosensitisation assay with β-arbutin in the guinea pig conclude that the substance displays neither photototoxic nor photoallergic potential.
The human repeated open application test with so-called "exposure to sunlight" lacks standardisation of the intensity and duration of the sunlight exposure. The volunteers were only exposed to sunlight through their normal daily activities with the product applied on the back of their hands.

3.3.14.3 Issues to be considered

In acidic medium, β-arbutin is easily hydrolyzed into hydroquinone [42, 43]. This can be of relevance in case β-arbutin is incorporated in **aqueous lotions with a slightly acidic pH**, facilitating hydrolysis into hydroquinone within the formulation.
Hydrolysis has been described to significantly take place in the case of oral intake of β-arbutin (stomach acids), but also to a lesser extent after dermal exposure [43, 38]. In addition, enzymatic biotransformation may be expected in both cases [40, 42].
In light of the above, it also needs to be noted that the ratio hydroquinone/(β-arbutin + hydroquinone) in the skin amounted up to 11.77% in the skin metabolism study in human volunteers which is considerably higher than the ratio of the two substances in the applied product. Although this finding was not considered alarming by the performing laboratory because the absolute levels of β-arbutin and hydroquinone in the skin were considered relatively small and their contribution to the total body burden was considered negligible, it needs to be considered that this study was of limited size (18 volunteers) with only one type of formulation and that dermal absorption will be influenced by the vehicle used. Moreover, the dermal absorption of hydroquinone is reported to be 57% [44], which is much higher than the dermal absorption value observed here for β-arbutin.

These considerations raise questions as to the safety of the use of 7% β-arbutin in cosmetic products for skin bleaching purposes. If hydroquinone is released in relevant amounts either in the product or during the use of β-arbutin, the product could not be considered safe, since hydroquinone has been assessed as being unsafe for use in skin lightening applications due to the danger of ochronosis and leukomelanoderma [41] and consequently has been banned for this use in the EU. The hydroquinone levels at which ochronosis has been described are of 1% and higher [44]. As no data is available on concentration levels below 1%, a lower threshold for the occurrence of ochronosis is difficult to establish. Although the risk for ochronosis may be relatively low, the occurring cases can be severe and irreversible.

In the case of release of hydroquinone, the aspect of skin sensitisation has to be considered, since hydroquinone has been identified to be a skin sensitiser. Also, the concerns about cancer risks become then an issue [44, 39].

The above illustrates that the use of β-arbutin at 7% in skin bleaching products induces a complex situation for which the local application level and the bioavailability of hydroquinone cannot be generalized.

4. CONCLUSION

Although the general toxicological assessment of β-arbutin suggests that the substance may be safe, the bioavailability of hydroquinone under conditions of intended use of the substance is of concern. Whereas hydroquinone was initially permitted at a concentration of 2%, a 1998 opinion of the SCCNFP recommended that the substance should not be used any more as a depigmentating agent in cosmetic products due to observed clinical side effects, among which exogenous ochronisis [41].
Consequently, the SCCP considers the currently requested use of β-arbutin in cosmetic products unsafe.

In addition, it is the opinion of the SCCP that the same concern can be expressed for other products that result in the release and/or formation of hydroquinone before or upon application on the skin.

5. MINORITY OPINION

Not applicable

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