Scientific Committee on Consumer Safety

SCCS

OPINION ON

β-arbutin

The SCCS adopted this Opinion at its 9th plenary meeting

on 25 March 2015

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 Safety (SCCS), 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 Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

Scientific Committee members

Ulrike Bernauer, Qasim Chaudhry, Pieter Coenraads, Gisela Degen, Maria Dusinska, Werner Lilienblum, Elsa Nielsen, Thomas Platzek, Suresh Chandra Rastogi, Christophe Rousselle, Jan van Benthem.

Contact

European Commission
Health & Food safety
Directorate C: Public Health
Unit C2 – Health Information/ Secretariat of the Scientific Committee
Office: HTC 03/073
L-2920 Luxembourg
SANTE-C2-SCCS@ec.europa.eu

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SCCS Members

Dr. U. Bernauer
Prof. P.J. Coenraads
Prof. G. Degen (rapporteur)
Dr. M. Dusinska
Dr. W. Lilienblum
Dr. E. Nielsen
Prof. T. Platzek
Dr. S. Ch. Rastogi (chairman)
Dr. Ch. Rousselle
Dr. J. van Benthem

Former SCCS Member
Prof. A. Luch

External experts

Prof. A. Bernard
Prof. A.M. Giménez-Arnau
Prof. T. Vanhaecke

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1. BACKGROUND

Submission I for β-Arbutin (CAS 497-76-7) with the chemical name 4-hydroxy-phenyl-β-D-glucopyranoside and the INCI name Arbutin was submitted in July 2005 by COLIPA[1].
The effect of Arbutin seems to be due to the fact that it hydrolyses to Hydroquinone. However, Hydroquinone (CAS 123-31-9) is listed in Annex II/1339 of the Cosmetic Regulation No 1223/2009; it means it is banned as cosmetic ingredient with the exception of entry 14 in Annex III. Its permitted use is restricted to professional use in artificial nail systems in a concentration in the final product up to 0.02%. Since Hydroquinone could not be used as a skin whitener after introduction of a ban, other substances have been used for that purpose, including Arbutin.

The first opinion (SCCP/1158/08) on beta-arbutin was adopted the 15 April 2008 with the 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 ochronosis [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."

The substance is used as a chemical ingredient alone and as a component of plant extracts like Arctostaphylos Uva Ursi, Vaccinium Vitis-Idaea, Chimaphila Umbellata etc, all rich in content of arbutin, according to the information on skin lightening products available to the Commission Services.

The current submission II is a response to the first opinion on beta-Arbutin.

2. TERMS OF REFERENCE

(1) Does the SCCS consider on the basis of the provided scientific data, the use of β-arbutin to be safe for consumers in cosmetic products in a concentration up to 7% in face creams?

(2) Does the SCCS have any further scientific concerns with regard to the use of β-arbutin in cosmetic products?

[1] COLIPA – The European Cosmetics Association, now Cosmetics Europe

3. OPINION

The previous SCCP 2008 Opinion (SCCP/1158/08) was based on Submission I of the applicant (Shiseido via COLIPA) from July 2005. The present Opinion also includes Submission II from May 2009 and information provided thereafter (Cover Letter Shiseido to EU COM of April 2012 with "Final Expert statement SCCS Arbutin requests KS 26-04-2012")

3.1 Chemical and Physical Specifications

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

Arbutin (INCI name)

Ref.: 8, 9, 15

3.1.1.2 Chemical names

4-Hydroxyphenyl-β-D-Glucopyranoside

Ref.: 8, 9, 15

3.1.1.3 Trade names and abbreviations

Arbutin

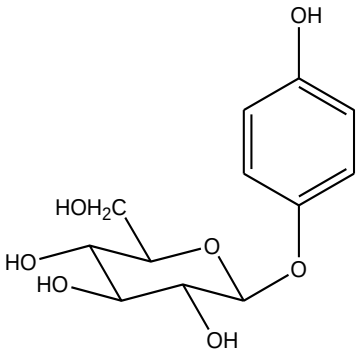
Ref.: 8, 9, 15

3.1.1.4 CAS / EC number

CAS: 497-76-7
EC: 207-850-3

Ref.: 9

3.1.1.5 Structural formula



Ref: Schindler et al. 2002

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 in Submission I

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 in Submission I

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 in Submission I

3.1.6 Solubility

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

Ref.: 15

Comment
The water solubility has not been measured by the EC method A.6

3.1.7 Partition coefficient (Log P_{ow})

- 1.35 (probably calculated, no details given).

Ref.: 8

Comment
The Log Pow has not been determined by the EC method A.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: (method not specified)	$[\alpha]_D^{25}$ = -62 to -68°:	
	Lot A: -64.2	Lot TDA-361: -65.8
	Lot B: -66.1	Lot TDD-422: -65.7
	Lot C: -64.8	Lot TPG-412: -65.0
		Lot TGJ-225: -65.4

3.1.9 Homogeneity and Stability

3.1.9.1 Stability of β-arbutin as a raw material

Data from submission II

Photostability test in aqueous solution (Test of 2006)

Sample preparation: Concentration of β-Arbutin (Lot TQI-771, TQI-772, TQI-773) were 3% and 6.3%; dissolved in 10% ethanol and water
Light Source - a white fluorescent lamp and near ultraviolet lamp
Procedure - Samples were kept in transparent glass bottle of 50mL. Protected samples wrapped in aluminium foil were used as controls. Samples were exposed to light providing an overall illumination of no less than 1.2 million lux hours and an integrated near ultraviolet energy of no less than 200 w·h/m^2 at 25°C then measured instantly in compliance with ICH guidelines, the analysis being repeated 3 times for each lot of product, using an HPLC assay with detection at 280 nm.

Test results are provided in the Summary Table below.
The quantitation limit of hydroquinone (HQ) in the samples was 1 ppm.

β-Arbutin		3%		6.3%	
		control	exposed	control	exposed
10%Ethanol	Lot.TQI-771	2.96(%)	3.00(%)	6.29(%)	6.33(%)
	Lot.TQI-772	2.98	2.99	6.34	6.38
	Lot.TQI-773	2.98	2.99	6.26	6.40
Water	Lot.TQI-771	2.94	2.95(%)	6.25	6.26
	Lot.TQI-772	2.96	2.96	6.21	6.31
	Lot.TQI-773	2.96	2.96	6.21	6.34

Hydroquinone		3%		6.3%	
		control	exposed	control	exposed
10%Ethanol	Lot.TQI-771	Less than 1ppm	Less than 1ppm	Less than 1ppm	1.6(ppm)
	Lot.TQI-772	Less than 1ppm	Less than 1ppm	Less than 1ppm	1.5
	Lot.TQI-773	Less than 1ppm	Less than 1ppm	Less than 1ppm	1.7
Water	Lot.TQI-771	Less than 1ppm	Less than 1ppm	1.0(ppm)	1.7
	Lot.TQI-772	Less than 1ppm	Less than 1ppm	Less than 1ppm	2.4
	Lot.TQI-773	Less than 1ppm	Less than 1ppm	Less than 1ppm	1.8

pH	3%	6.3%
10%Ethan	5.80	5.52
Water	5.60	5.43

(*)The pH values of each solution were reference values measured by using another samples later.

Ref.: 1)

Conclusion

The photostability of β-arbutin (3% and 6.3% solutions) was investigated in 10% ethanol and pure water. Following the ICH guideline for the photostability testing, the solutions were exposed to light providing an overall illumination of no less than 1.2 million lux hours and an integrated near-ultraviolet energy of no less than 200 w·h/m^2 at 25°C. No apparent change in the amount of β-arbutin was observed between light-exposed and control samples. A slight increase of hydroquinone, on the other hand, was observed in light-exposed 6.3% solutions although those levels were always below 2.5 ppm after light exposure.

Ref. 1 in subm II

Photostability at different pH values and temperatures (Test date 2008-9)

Sample type: 7% β-arbutin in phosphate buffers ranging from pH 2.0-11.0

Samples prepared from Lot TTG-305 were subdivided and kept in transparent glass bottles (30ml). Protected samples wrapped in aluminium foil were used as dark controls. Samples were exposed to light (white fluorescent lamp) providing an overall illumination of no less than 1.2 million lux hours and an integrated near ultraviolet energy of no less than 200 w·h/m^2 at 25° (ICH guidelines). The effects of temperature were studied at 50°C for 1 month and 40°C for 3 months. The analyses were repeated 3 times for each lot of product.

Results (for pH of solutions, parent compound and hydroquinone content) at various conditions are summarised below:

		initial(control)	exposed	50℃1month	40℃3months
pH	pH2	1.93	1.95	1.97	1.94
	pH3	2.97	3.00	3.00	2.96
	pH4	3.98	4.00	4.01	3.97
	pH6	5.99	5.99	6.00	5.97
	pH8	7.55	7.50	7.35	7.31
	pH10	8.78	8.71	8.66	8.59
	pH11	9.10	9.04	9.03	9.00
β-Arbutin	pH2	6.94(%)	7.04(%)	5.86(%)	6.16(%)
	pH3	6.98	7.05	6.83	6.89
	pH4	6.95	7.02	6.94	6.96
	pH6	6.94	7.04	6.91	6.86
	pH8	7.00	7.08	6.99	6.85
	pH10	6.97	7.06	6.95	6.82
	pH11	6.96	7.06	6.93	6.80
Hydroquinone	pH2	Less than 1ppm	25.3(ppm)	4273.3(ppm)	3747.9(ppm)
	pH3	Less than 1ppm	5.7	474.6	388.8
	pH4	Less than 1ppm	2.5	68.0	49.6
	pH6	Less than 1ppm	2.5	15.3	Less than 1ppm
	pH8	Less than 1ppm	Less than 1ppm	Less than 1ppm	Less than 1ppm
	pH10	Less than 1ppm	Less than 1ppm	2.0	2.6
	pH11	Less than 1ppm	Less than 1ppm	1.2	1.4

* The quantitation limit of HQ in these samples was 1 ppm.

The authors concluded that for both the heat and light conditions, no apparent change on the amount of β-arbutin was observed at pH 4-11. β-Arbutin levels were slightly decreased at pH ≤3 accompanied by a rapid increase of hydroquinone. The degree of hydroquinone generation was higher at low pH (~4,300ppm at 50°C 1 month and 3,700ppm at 40°C 3 months at pH 2); however only trace levels of hydroquinone were detected in neutral to basic solutions (pH 6-11).

Ref. 2 in subm II

3.1.9.2 Stability of β-arbutin in a cosmetic formulation

Data from subm II

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

Date of study:	2008-2009
Method:	Internal protocol Shiseido Co, Japan.
Test substance:	Shiseido Product C (Lot 0804A, B, C) containing 5.67-6.93% β-arbutin at pH 5.0-7.0
Temperature:	40°C
Relative Humidity	75%
Analytical method:	HPLC

Samples 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 (for pH, parent compound and hydroquinone) at various conditions are summarised below:

		initial	3 months	6months
pH	Lot.0804A	6.07	5.89	5.82
	Lot.0804B	5.97	5.84	5.86
	Lot.0804C	6.00	5.86	5.88
β-Arbutin	Lot.0804A	6.15(%)	6.20(%)	6.11(%)
	Lot.0804B	6.16	6.21	6.13
	Lot.0804C	6.17	6.20	6.12
Hydroquinone	Lot.0804A	Less than 1ppm	Less than 1ppm	Less than 1ppm
	Lot.0804B	Less than 1ppm	Less than 1ppm	Less than 1ppm
	Lot.0804C	Less than 1ppm	Less than 1ppm	Less than 1ppm

Conclusions

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

Ref.: 20 *Ref. 3 in subm II*

B. β-arbutin in a finished cosmetic product (ambient test conditions for 36 months, i.e., long-term testing)

Date of study: 2003-6
Method: Internal protocol Shiseido Co, Japan.
Test substance: Shiseido Product C (lot A, B, C) containing 5.67-6.93% β-arbutin (pH 5.0-7.0),
Temperature: 25°C
Relative Humidity: 60%
Analytical method: HPLC

Samples of Shiseido Product C were kept in a transparent glass bottle at ambient conditions for 3 years. β-Arbutin and hydroquinone concentrations were measured in threefold by HPLC at the start and at the end of the study.

Results

		initial	12months	24months	36months
pH(*)	Lot.A	5.73	5.65	5.67	5.66
	Lot.B	5.73	5.67	5.68	5.68
	Lot.C	5.66	5.58	5.58	5.58
β-Arbutin	Lot.A	6.20(%)	6.35(%)	6.32(%)	6.41(%)
	Lot.B	6.22	6.33	6.37	6.38
	Lot.C	6.17	6.36	6.29	6.39
Hydroquinone	Lot.A	Less than 1ppm	Less than 1ppm	Less than 1ppm	Less than 1ppm
	Lot.B	Less than 1ppm	Less than 1ppm	Less than 1ppm	Less than 1ppm
	Lot.C	Less than 1ppm	Less than 1ppm	Less than 1ppm	Less than 1ppm

Since the concentrations of β-arbutin were stable and those of hydroquinone remained below 1 ppm (detection limit), the study authors conclude that β-arbutin is stable in the usual cosmetic formulation and that no hydroquinone is released under normal storage conditions for 3 years.

Ref.: 20-1 *Ref. 3 in subm II*

Overall conclusions (applicant)
β-arbutin was relatively stable to light when dissolved in a 10% ethanol aqueous solution. No significant change in the amount of β-arbutin was detected and only trace levels of hydroquinone (<0.25 ppm) were detected under the conditions of the ICH photostability test. β-arbutin was found to be unstable in low pH solutions, decrease of β-arbutin and significant increase of hydroquinone was observed, especially at pH 2.
On the other hand, β-arbutin was stable in the finished product: hydroquinone content in the finished product was no more than 1 ppm (LLOQ) throughout the accelerated and long-term storage conditions (40°C, 75% relative humidity for 6 months and (25°C, 60% relative humidity for 36 months).

A recently published study tested ß-Arbutin (and deoxy-arbutin) at 10^{-4} M (<0.003 % w/v) in aqueous solution with 10% propylene glycol and UV irradiation (narrow band UVB light unit at 312 nm) at 2.65 mW/cm^2: under these conditions (pH not given), ß-arbutin was reduced to 43.5% of the initial level after 2 h of irradiation, and hydroquinone was detected at significant levels (up to 20%) over the test period (up to 12 h).

Add. Ref.: Yang CH et al. 2013

SCCS remark
As the test concentration is far below that used in cosmetic products, this study is considered to be of limited relevance.

SCCS comment
The results presented in the respective studies regarding photostability testing (according to ICH guideline) and pH- or temperature-dependent stabilities of β-arbutin sound conclusive and reliable. In cosmetic formulations hydroquinone as impurity remained below 1 ppm (detection limit). Yet, aside from reduced photostability under certain UV irradiation conditions (Yang et al. 2013), it is to be considered that β-arbutin may also undergo cutaneous hydrolysis into hydroquinone due to enzymatic activity through β-glucosidases in skin (Mier & van den Hurk 1976; Redoules et al. 2005) or via the activity of microbes residing on the surface of the skin (Bang et al. 2008). This is further discussed in section 3.3.4.2.

3.2 Function and uses

The naturally occurring compound β-arbutin (4-hydroxyphenyl-β-D-glucopyranoside, hydroquinone-β-glucopyranoside, CAS 497-76-7) is a β-D-glucopyranoside derivative of hydroquinone that can be found in the leaves and bark of many plants. It has been proposed for use as a skin-lightening agent, inhibiting production of the skin pigment melanin which is formed through a series of oxidative reactions involving the amino acid tyrosine and the enzyme tyrosinase. This enzyme catalyses the hydroxylation of tyrosine to L-DOPA (dihydroxyphenylalanine) and the oxidation of DOPA to dopaquinone which is then further metabolised to melanin. β-Arbutin is thought to act by inhibition of the activity of melanosomal tyrosinase, where the inhibition may be due to structural similarities to the substrate tyrosine (Zhu and Gao, 2008). β-Arbutin can be hydrolysed both in acid solution and by enzymic action to hydroquinone which also acts as a skin lightening agent, again by inhibiting tyrosinase. Extended use of ≥2% hydroquinone, topically applied, has been associated with adverse effects including exogenous ochronosis in dark-skinned people [27, 38]. Hydroquinone, like β-arbutin, is present in many dietary components in trace amounts and is metabolised *in vivo* to its glucuronide and sulphate conjugates.
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

From SCCP/1158/08 with minor corrections

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:	/

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 summarised 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 oral 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:	/

For both rats and mice, the test substance was applied evenly on the back after fur clipping (treatment 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 technically applicable maximal dose).

Ref.: 2

SCCS comment
The SCCS noted that there is no justification in relation to the 'the maximum technically feasible' dose volume of 3 ml/kg (recalculated to a dose of 928 mg/kg bw. According to OECD TG 402 (Acute dermal toxicity) a dose level of 2000 mg/kg is the cut-off for the limit test.

3.3.1.3 Acute inhalation toxicity

No data submitted

3.3.2 Irritation and corrosivity

From SCCP/1158/08 (with minor modifications)

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 distilled 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 (2.5 cm diameter) patch test plaster with 300 µl test material (10% β-arbutin in distilled water) was placed on shaved intact and abraded (by needle scratching not deep enough to cause bleeding) 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 for erythema and oedema 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 having 'little primary irritation potential'.

Ref.: 3

SCCS comment
A 10% aqueous solution of β-arbutin was slightly irritating to the skin.

3.3.2.2 Mucous membrane irritation / Eye irritation

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:	/

100 µl of test substance (10% β-arbutin in distilled water) was instilled to the right eye 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
Under the conditions of the study, a 10% aqueous solution of β-arbutin was not irritating to the rabbit eye.

3.3.3 Skin sensitisation

Taken from SCCP/1158/08 (with minor modifications)

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:	/		

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. All the animals challenged with 3 or 10% hydroquinone and 9 of 10 tested with 1% hydroquinone showed allergic reactions on challenge. Five out of five guinea pigs sensitised to DCNB showed an allergic reaction when challenged with 0.01 and 0.1% DNCB. 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 sensitising potential under the test conditions.

Ref.: 7

SCCS comment
Apparently no preliminary study for induction and challenge concentration determination has been performed. Challenge with 3 different concentrations is unusual. 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 (batchS-1601): 6.3% [^{14}C] β-arbutin Cream-CBP-L (batch S-1602): 3.0% [^{14}C] β-arbutin Cream-BOP-H (batch S-1603): 6.3% [^{14}C] β-arbutin Cream-BOP-L (batch S-1604): 3.0% [^{14}C] β-arbutin Gel-H (batch S-1605): 6.3% [^{14}C] β-arbutin Gel-L (batch S-1606): 3.0% [^{14}C] β-arbutin
Purity:	97.6% (ring labelled ß-arbutin)
Applied amount:	2.2-5.5 mg/cm²
Reference compound:	Testosterone
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 °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, a 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 using a 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** ± 0.255	**0.126** ± 0.060	**0.143** ± 0.083	**0.214** ± 0.114	**0.135** ± 0.066	**0.164** ± 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

TNO (2003) TNO Report V4768, *In vitro* percutaneous absorption of [^{14}C]-Arbutin using human skin membranes, TNO Chemistry, Zeist, The Netherlands for Shiseido Research Center, Safety Research Laboratories, Fukuura, Japan, 11 February 2003

SCCS comment
The study has some limitations: exact composition of the test formulations is not given and no information is provided on the pH and impurities (hydroquinone). The tested concentrations of 3.0 and 6.3% β-arbutin are below the requested maximum concentration of 7%, and the number of human skin samples (2 replicates from 3 donors) is lower than that usually required (triplicate samples from 3 donors). The variability of the results is high. All the tape strips were discarded. Thus the measured dermal absorption (0.126 - 0.214 % in 24 h) may be underestimated. For calculation of SED the upper value of 0.214% of the dose or 0.339 μg/cm^2 will be used.
This is in line with similar values for *in vivo* dermal absorption of β-arbutin as calculated by the SCCS based on the analysis of human biopsy samples from a study (ref. 9, below) with volunteers after repeated application.

3.3.4.2 *In vivo* dermal / percutaneous absorption - human

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:	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)
GCP/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 the performing facility. 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 testing 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 analysed 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-hydrolysed urine samples, a total of 90 samples, were determined quantitatively by an LC system equipped with electrochemical 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

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)**
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 1 and Day 5 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 (range 0.003 – 0.072).

Ref.: 9

SCCS comment

Under the in-use conditions described in human volunteers[2], hydroquinone is released in the skin to a relative level of 4.6% (compared to the β-arbutin + HQ content) and as high as 11.8% (w/w). This wide variation may reflect enzymatic action from skin bacteria (Bang et al., 2008) as well as the action of β-glucosidases in skin fibroblasts (Mier & van den Hurk, 1976; Redoules et al., 2005).

There is wide variation in urinary excretion of HQ. It may result from absorption of HQ across the skin, together with a component resulting from dietary intake, since many foodstuffs contain HQ or its precursor β-arbutin, resulting in mean urinary HQ concentrations of 1.2±0.54 mg/L (Deisinger et al. 1996). In any individual, there is poor correlation between skin HQ and urinary HQ measured in spot urine samples; the volunteers kept a food diary but the contribution of dietary HQ intake to urinary HQ excretion is not assessed so that it is not possible from the data provided to determine which part of the excreted HQ is derived from food or from dermal application of β-arbutin.

Moreover, the method used for HQ determination was not sensitive enough to quantify this analyte in the urine samples, since a large proportion (70%) of samples had concentrations below the detection limit of 0.974 mg/l. Much lower detection limits are reported in other studies (e.g. Schindler et al. 2002; Quintus et al. 2005). Because of this lack of sensitivity, no conclusion can be drawn from the measurement of urinary HQ in this experiment.

Another study assessed the relative amounts of HQ in tape strips from the skin of volunteers after application of a gel with either 3% alpha-arbutin or 3% beta-arbutin or 1.9% hydroquinone (Mamabolo et al., unpublished data). The results, discussed in more

[2] The β-arbutin concentration tested (6.3%) is slightly lower than the required 7%.

depth in the alpha-arbutin Opinion (SCCS/1552/15[3] section 3.3.11.3), indicate low conversion of arbutins to HQ, but this data allows no conclusions on percutaneous absorption.

3.3.5 Repeated dose toxicity

3.3.5.1 Repeated Dose (28 days) oral toxicity

Taken from SCCP/1158/08 (with minor corrections)

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:	/

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, phosphorus, 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 differ between control and treated animals during the dosing period.

[3] Planned to be published later

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, this can be considered as the NOEL value.

Ref.: 10

3.3.5.2 Sub-chronic (90 days) dermal toxicity

Guideline:	OECD Guideline 411: Repeated Dose 90-Day Dermal Toxicity Study in Rodents
Date of test:	February - May 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 in 2.0 mL/kg bw
Observation period:	90 days
GLP/QAU:	/

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 phosphorus;
- 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^{2+} 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), this can be considered as the NOEL value.

Ref.: 11

SCCS comment
The change in 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.

SCCS conclusion on repeated dose toxicity
A repeated dose 28-day oral study and 90-day dermal study with the rat only revealed some sporadic observations that 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.

3.3.5.3 Chronic (≥ 12 months) toxicity

No data.

3.3.6 Mutagenicity / Genotoxicity

Taken from SCCP/1158/08 (with minor modifications)

3.3.6.1 Mutagenicity/Genotoxicity *in vitro*

Gene mutation test in bacteria

Guideline:	OECD 471 (1997)
Species/strain:	*Salmonella typhimurium*, TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2 *uvr*A
Replicates:	Triplicates per test concentration
Test substance:	β-arbutin
Batch:	Lot A
Purity:	99.7%

Vehicle:	distilled water
Concentrations:	0, 156.25, 312.5, 625.0, 2500 and 5000 µg/plate, with and without metabolic activation (rat S9-mix)
GLP:	/
Date of study:	9 February 1987 -19 February 1987

Beta-arbutin was investigated for the induction of gene mutations in strains of *Salmonella typhimurium* and *Escherichia coli* (Ames test). Concentrations were based on the results of a concentration determination test; no toxicity of β-arbutin was observed in any of the test strains at 5000 µg/plate. Therefore, the maximum concentration was set at 5000 µg/plate and 6 concentrations were set at a common ratio of 2. In the main study, the strains were exposed to β-arbutin in the presence and absence of rat liver metabolic activating system. Liver S9 fraction was prepared from livers of male Sprague-Dawley rats that had received the intraperitoneal injections of sodium phenobarbital and 5,6-benzoflavone. Distilled water alone served as negative control. As a positive standard requiring metabolic activation 2-aminoanthracene was used. N-ethyl-N'-nitro-N-nitrosoguanidine, ICR-191 and 2-(2-furyl)-3-(5-nitro-2-furyl) acrylic amide were used as positive standards without metabolic activation.

Results
A biologically relevant increase in the number of revertant colonies was not observed in 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
Under the experimental conditions used, β-arbutin can be considered non-mutagenic in all tested strains under all tested conditions.

Ref.: 12

***In vitro* mammalian chromosome aberration test**

Guideline:	OECD 473 (1997)
Cells:	Chinese hamster lung (CHL) fibroblast cells
Replicates:	duplicate cultures
Test substance:	β -arbutin
Batch:	Lot A
Purity:	99.7%
Solvent:	physiological saline
Concentrations:	0, 0.34, 0.68, 1.36 and 2.72 mg/ml with and without metabolic activation (rat S9-mix)
Treatment:	6 h treatment without and with S9-mix; harvest time 24 h after the start of treatment 24 and 48 h treatment without S9-mix; harvest time immediately after the end of treatment
GLP/QAU:	/
Date of study:	11 April 1986 - 5 September 1986

Beta-arbutin has been investigated for the induction of chromosomal aberrations in CHL cells in the absence and presence of metabolic activation. As exogenous metabolic activation system, liver S9 fraction from sodium phenobarbital and 5,6-benzoflavone induced rats was used. In the main study, cells were incubated with β-arbutin at concentrations of 0, 0.34, 0.68, 1.36 and 2.72 mg/ml without and with S9-mix for 6 h and a harvest time of 24 h after the start of treatment. Additionally, cells were treated with the same concentrations for 24 and 48 h without S9-mix and harvested immediately after the end of treatment. Two hours before the end of the incubation period, colcemid was added to the cultures. N-Methyl-N'-nitro-N-nitrosoguanidine and benzo[a]pyrene were used as positive controls for the direct and the metabolic activation methods, respectively. Per

culture, 100 metaphases were scored for structural chromosomal aberrations and polyploidy.

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

Conclusion
Under the experimental conditions used, β-arbutin was not genotoxic (clastogenic) in this chromosome aberration test in CHL cells.

Ref.: 13

Overall conclusion on mutagenicity
The genotoxicity of β-arbutin is sufficiently investigated in valid genotoxicity tests for gene mutations in bacteria and structural chromosomal aberrations and polyploidy in mammalian cells. β-arbutin did not induce gene mutations in bacteria nor an increase in cells with chromosomal aberrations or polyploidy in CHL cells. Although a gene mutation test in mammalian cells is lacking, based on the present reports β-arbutin can be considered to have no *in vivo* genotoxic potential and additional tests are unnecessary.

Arbutins are considered to be metabolised differently by different glycosidases or by spontaneous hydrolysis, yet they all form hydroquinone. The latter is considered as the genotoxic moiety; therefore a read across approach based on HQ release is a possibility to assess the genotoxic potential of the arbutins as a group.

3.3.7 Carcinogenicity

Dermal study

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 in 2 mL/kg bw
Observation period:	78 weeks (18 months)
GLP/QAU:	reported to be present, but not included in the 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 was 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 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. Haematology 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 haematopoiesis in the spleen, calcification in brain thalamus, subcapsular hyperplasia in the adrenal gland, ovary cysts, cystic 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

SCCS comment
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. 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. As the incidence of tumour and non-tumour lesions did not show an increasing dose-relationship, and since they are frequently observed in aging mice, they are regarded as spontaneous lesions. 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.
No conclusion with regard to carcinogenicity can be drawn from this study.

3.3.8 Reproductive toxicity

Taken from SCCP/1158/08

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 in saline
Route:	s.c. injection (of 2 mL/kg bw)
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 and 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. Absolute and relative organ weights of the left ovary of the 400 mg/kg group were significantly lower, but no significant differences were observed with the total absolute and relative weight of the right ovary.

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

SCCS comment
This is the lowest NOEL for β-arbutin; yet, subcutaneous injection is an uncommon application route in one-generation reproductive toxicity tests.

3.3.8.2 Teratogenicity

No data submitted.

3.3.9 Toxicokinetics

ß-Arbutin is hydrolysed into hydroquinone and glucose in the presence of weak acids. Cosmetics with ß-arbutin may contain small amounts of hydroquinone (HQ) as impurity. Hydrolysis of dermally applied arbutin may also occur to some extent on the skin surface (by microflora) and in the skin (by hydrolases). Data on skin metabolism, however, are scarce.

Dermal route:
See section 3.3.4.2 for a study in human volunteers with repeated topical application of a gel with 6.3 % ß-arbutin (Ref. 9). The results show some release of hydroquinone from ß-arbutin in the skin.

Oral Route:
Many foodstuffs contain HQ or its precursor β-arbutin, resulting in mean urinary HQ concentrations of 1.2±0.54 mg/L (Deisinger et al. 1996; Ref 28, subm. I). The metabolism of ß-arbutin was also assessed in studies which measured urinary excretion in human volunteers after oral administration of herbal preparations which are used to treat urinary tract infections.

(A) Three human volunteers were dosed with bearberry leaf extract (containing 150 mg ß-arbutin), and the metabolites of arbutin were measured in urine; >50% of the arbutin was excreted within 4 hours as HQ glucuronide and HQ sulphate while >75% of the arbutin (as HQ metabolites) was excreted in 24 hours. In one volunteer only, 5.6% of the total arbutin was found as free HQ (Quintus et al. 2005).

(B) Sixteen human volunteers were dosed with bearberry leaf extract as film-coated tablet or as aqueous solution containing equal amounts of ß-arbutin (210 mg). HQ glucuronide and HQ sulphate were the main urinary metabolites; free HQ was also detected, but represented only 0.1 % of the applied dose (Schindler et al. 2002).

(C) An earlier study on the metabolism of arbutin in 12 subjects after ingestion of 420 mg arbutin, reported that 70% of the administered dose was excreted as conjugates of glucuronide and sulphuric acid and only 0.6% as free hydroquinone (Siegers et al., 1997).

SCCS comment
Despite intersubject variability observed in these studies with oral application, the main metabolic route is apparently phase II metabolism of hydroquinone (HQ). This implies that any ß-arbutin absorbed across the skin when hydrolysed to HQ is then also predominantly excreted by systemic conjugation as glucuronide and sulphate.

3.3.10 Photo-induced toxicity

Taken from SCCP/1158/08 (with minor modifications)

3.3.10.1 Phototoxicity / photo-irritation and photosensitisation

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:	ß-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:	/

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% ß-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}=360 nm) 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 ß-arbutin. Conversely, a strong phototoxicity reaction was observed with the positive control substance 8-MOP.

Conclusion
The study authors conclude that ß--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:	ß-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% ß-arbutin in 50% ethanol 100 µl of 5% 6-MC in ethanol challenge: 20 µl of 10% ß-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 µl of 10% ß-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, λ_{max}=360 nm) had a glass filter to eliminate 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% ß-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 unirradiated 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 ß-arbutin. Conversely, strong photosensitisation reactions were observed with the positive control substance 6-MC.

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

Ref.: 16

3.3.11 Human data

Taken from SCCP/1158/08 (with minor modifications and updated)

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

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

50 µl of 10% ß-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% ß-arbutin solution.

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

Ref.: 4

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

Method:	24-hour 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% ß-arbutin), 2) AR-91 SWT Essence A10 (containing 10% ß-arbutin), 3) AR-91 SWT Essence AC (containing 0% ß-arbutin). No details on qualitative and/or quantitative compositions.
Batch:	For ß-arbutin: Lot TDA-361
Purity:	100.3%
Doses:	30 µl of above-mentioned formulations (0%, 7% or 10% ß-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)
Date of study:	29 Jun - 01 Jul 1992

The irritation potential was assessed in a closed patch test with Finn chambers: 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 immobilised 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% ß -arbutin products and the positive rate was 0% in all cases.

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

Ref.: 5

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

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% ß-arbutin), 2) AR-91 SWT Essence A10 (containing 10% ß-arbutin), 3) AR-91 SWT Essence AC (containing 0% ß-arbutin). No details on pH or qualitative and/or quantitative compositions.
Batch:	For ß-arbutin: Lot TDA-361
Purity:	100.3%
Doses:	20 mg of above-mentioned formulations (0%, 7% or 10% ß-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
Date of study:	30 Jul 1992 - 20 Jan 1993

Test samples were applied at least three times daily for 24 consecutive weeks. The 0% ß-arbutin product was applied randomly to either the upper or lower area of each arm, while the 7 and 10% ß-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 analysed 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% ß-arbutin products and no pigmentation/discoloration was 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% ß-arbutin product.

Ref.: 18

<u>D. Repeated open application test with a 10% ß-arbutin-containing finished product, with exposure to sunlight on human volunteers</u>

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% ß-arbutin), 2) AR-91 SWT Essence AC (containing 0% ß-arbutin). No details on qualitative and/or quantitative compositions.
Batch:	For ß-arbutin: Lot TDA-361
Purity:	100.3%
Doses:	50 mg of above-mentioned formulations (0% or 10% ß-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% ß-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 analysed.
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% ß-arbutin products.

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

Ref.: 19

E. Post marketing surveillance

Some side effects from the use of Shiseido Product A or Shiseido Product B were reported by 30 out of 1,382 users surveyed. However, based on the results of subsequent investigations, the majority of these people were able to continue using the product with no trouble. In addition, the skin symptoms experienced by a user with a sensitive skin were probably not caused by allergic contact but may have been an irritation response.
Based on the results above, it was concluded that Shiseido Product A and Shiseido Product B are safe and in particular with no reactions attributable to arbutin.

Ref. 5 (PMS Shiseido in house data) in subm. II files.

A case report on contact allergy to ß-arbutin could be identified (Matsuo et al. 2015).
Contact allergy to hydroquinone has been described, notably in hairdressers and their clients reacting to hair-dye (Uter et al. 2014).

SCCS comment
The sensitising potential of ß-arbutin is negligible. It is not an irritant.

3.3.12 Special investigations

In vitro studies

ß-Arbutin inhibits melanin production in guinea pig B16 cells and decreases tyrosinase activity in a cell-free system (Lim et al. 2005). Using A375 human malignant melanoma cells in a proteomic approach, ß-arbutin was shown to have a role in tumour suppression, as found by regulation of p53 and cell apoptosis (Nawarak et al. 2005).

3.3.13 Information on the toxicity of hydroquinone

Taken in part from the SCCP/1158/08 Opinion and updated

LD_{50}-oral-rat = 298 mg/kg
Slightly irritating to the eye
Sensitising to the skin

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. Oxidised 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, Ref. 37). In the EU, hydroquinone is classified as Carc Cat 2 H351 (suspected of causing cancer) based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI[4].

[4] The SCCS is aware that HQ is presently evaluated under REACH, and further data is requested on its *in vivo* oral genotoxicity (http://echa.europa.eu/documents/10162/7e36b2f6-80c6-42fb-9d67-7f7c7a86ee10)

A more recent literature review on the carcinogenicity of hydroquinone concludes that indeed renal tumours were observed in male 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

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

The Cosmetic Ingredient Review (CIR) Expert Panel conducted a safety assessment of hydroquinone as used in cosmetics and concluded that it is safe at concentrations ≤ 1% in hair dyes and is safe for use in nail adhesives. Hydroquinone should not be used in other leave-on cosmetics. With regard to the use of HQ in topical formulations as skin bleaching and depigmenting agent, the CIR report (2010) points out that this use is considered in the USA as drug use and thus falls under the purview of the FDA. Prescription and over-the-counter-products range in concentration from 0.4 to 5% (Anderson et al. 2010 new Ref.?#)

Studies on the kinetics (ADME) of hydroquinone (HQ) in humans and rodents (reviewed by McGregor 2007 and CIR/Anderson et al. 2010 new Ref.?#) indicate rather high bioavailability and rapid clearance after oral or dermal administration. The dermal penetration in humans was examined for 2% HQ in cream *in vitro* and *in vivo* with similar results [ref.24, Wester et al. 1998]: Total permeation of HQ after 24h was 43.3% of the dose *in vitro*; flux was 2.93 µg/h-cm^2. An average of 45.3±11.2% of the dose was recovered in urines of volunteers after application of HQ to forehead skin. It is worth noting that dermal penetration of HQ is considerably higher than that of ß-Arbutin.
For the safety evaluation of HQ exposure resulting from the use of skin bleaching products in comparison to internal HQ doses which may induce ochronosis, the SCCS will use 50% dermal absorption for HQ.

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

Safety evaluation for ß-arbutin (7% a.i. in face creams, applied 2 times per day)

The systemic exposure dose (SED) was calculated for µg/cm^2 derived from the *in vitro* dermal penetration study (ref. 8). The no observed effect level (NOEL) from the 28-day oral toxicity rat study (ref. 10) of 1000 mg/ kg bw was adjusted by a factor of 3 for time.

Absorption through the skin	A	=	0.339 µg/cm²
Skin Area surface	SAS	=	565 cm^2
Dermal absorption per treatment	SAS x A x 0.001	=	0.192 mg (x 2)
Typical body weight of human		=	60 kg
Systemic exposure dose (SED)	SAS x A x 0.001/bw	=	0.0064 mg/kg bw

No observed effect level for 28-day, oral, rat : 3	NOEL	= 333 mg/kg bw/d
Margin of Safety	**NOEL/SED**	**= 52082**

Additional MOS calculations for the NOAEL of 100 mg/kg bw from the rat reprotoxicity study with subcutaneous administration (ref. 15) also arrive at very high value (MOS=15625).
An SED calculation based on a dermal absorption of 0.214% of dose (instead of µg/cm^2) arrives at 0.004 mg/kg bw (lower than the 0.0064 mg/kg bw above). Accordingly, the MoS values for ß-arbutin would be even higher (83250 and 25000).

Further safety considerations will focus on ingredient-related exposure to hydroquinone as resulting from topical application of β-arbutin.

Safety evaluation for the Hydroquinone (HQ) formed

Below the exposure to hydroquinone from application of β-arbutin containing products is assessed and compared to risks related to 1.) repeated dose toxicity, 2.) induction of ochronosis and 3.) carcinogenicity.
Dermal penetration of β-arbutin, either expressed in % of dose (A) or in µg/cm^2 (B), are used as starting points in the following calculation of internal amounts of HQ.

1. Based on repeated toxicity study and reproductive toxicity tests for HQ

A) Exposure to hydroquinone (HQ) from application of products containing up to 7% β-arbutin (and its dermal penetration in % of dose) is derived as follows:

- The amount of cream applied is 1.6 g with 7% a.i. = 112 mg ß-arbutin (external);
- then 0.214 % of β-arbutin (ref. 8) is absorbed or 240 µg/person/day (internal);
- the fraction of HQ in skin, relative to total absorbed, was up 12 parts (11.8% ref. 9) or in total 29 µg HQ.
- For a possible presence of HQ as impurity (<1 ppm) in the applied product, one may add another 1.6 µg HQ (if 1 ppm in 1.6 g product as worst case) and use 50% dermal absorption for HQ, resulting in an extra 0.8 µg HQ per day.

The internal HQ exposure amounts to ≤**31 µg per day**; divided by 60 kg body weight
= (SED) 0.517 µg/kg bw/d or 0.0005 mg/kg bw/d

NOAEL reported for HQ repeated toxicity (section 3.3.13) are 15 or 20 mg/kg bw/d
NOAEL / SED results in **MOS of 25000 or 33333**

B) Exposure to hydroquinone (HQ) from application of products containing up to 7% β-arbutin (and its dermal absorption in µg/cm^2 instead of % of dose) is derived as follows:

- For ß-arbutin (see above) SED = 0.0064 mg/kg bw/d or 6.4 µg/kg bw/d
- the fraction of HQ in skin, relative to total absorbed, is up to 11.8% (ref. 9) which is equivalent to 0.755 µg/kg bw HQ (x 60 kg = **45 µg HQ per day**)
- For a possible presence of HQ as impurity (below 1 ppm) in the applied product (i.e. 1.6 g creme), one may add 1.6 µg HQ (external) and use 50% dermal absorption for HQ, resulting in an **extra 0.8 µg HQ per day**

The internal HQ exposure amounts to **45.8 µg per day**; divided by 60 kg body weight
SED = 0.763 µg/kg bw/d or 0.00076 mg/kg bw/d

NOAEL reported for HQ repeated toxicity (section 3.3.13) are 15 or 20 mg/kg bw/d
NOAEL / SED results in **MOS of 19737 or 26315**

2. For exogenous ochronosis

HQ is suspected to cause exogenous ochronosis. As a NOAEL has not been established for exogenous ochronosis (EO), the lowest effect level described in a case report was used to calculate the Exposure Dose of HQ that might cause ochronosis: 1% has been adopted as the minimum exposure level of HQ causing EO since no publication available suggests EO with products formulated with 1% or less HQ.

Exposure Dose of HQ that may cause ochronosis was calculated as **8 mg/day**
[1 x 0.8 x 1000 x 2 x 50 / 100 x 100]

- Lowest concentration in product 1%
- Maximum quantity of application 0.8 g
- Frequency of application per day 2
- Average absorption of HQ through skin 50%

Based on dermal absorption from the *in vitro* percutaneous absorption study and the *in vivo* conversion ratio from β-arbutin to HQ (11.8 % as individual maximum value) plus possible extra exposure from HQ impurities in the formulation, the total HQ exposure when using 7% β-arbutin formulation was **0.046 mg/day** (section 1. above)

The Exposure Dose of HQ that might cause ochronosis (8 mg/day) and the Exposure Dose of HQ formed on and in skin when using cosmetic product with 7% ß-arbutin (0.048 mg/day) is calculated as **Ratio: 8 / 0.046 = 174**

The minimum exposure level of HQ causing exogenous ochronosis is calculated as being 174 times higher than the maximum HQ exposure level formed from β-arbutin. Therefore it can be concluded that ß-arbutin does not pose a risk with regard to induction of exogenous ochronosis in humans (under the conditions of use specified in the terms of reference).

3. For carcinogenicity

According to IARC (1999) HQ is not classifiable as to its carcinogenicity for humans (group 3). In more recent reviews on the carcinogenicity of HQ (McGregor 2007; CIR 2010) a nongenotoxic mode of action that involves exacerbation of a spontaneously occurring rodent renal disease, chronic progressive nephropathy (CPN), has been proposed. CPN is particularly prominent in male rats and the evidence is consistent with an absence of a human counterpart.
In the EU, hydroquinone is classified as Carc Cat 2 H351 (suspected human carcinogen) Muta Cat 2 H341 (suspected of causing genetic defects) based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI.
The SCCS has carried a calculation to determine lifetime cancer risk (as in section 3-7.4 of the SCCS Notes of Guidance). First an animal dose descriptor (T25) for carcinogenic potency is determined and then converted to a human dose descriptor (HT25) based on comparative metabolic rates by using the following formula:

$$HT25 = \frac{T25}{(\text{body weight}_{human}/\text{body weight}_{animal})^{0.25}}$$

For the critical effect (renal tubular cell adenomas in male F344 rats; NTP study 1989), the **T25 dose descriptor** for hydroquinone is **61.4 mg/kg bw/day**.
From this a **HT25 value of 18.2 mg/kg bw/d** was obtained.

$$\text{Lifetime cancer risk} = \frac{SED}{HT25 / 0.25}$$

For a systemic HQ exposure dose of 0.0005 mg/kg bw/d (section 1 A above), the risk is calculated to be 6.8 x 10^{-6}.

For a systemic HQ exposure dose of 0.00076 mg/kg bw/d (section 1 B above), the risk is calculated to be 1 x 10^{-5}.

However, it has to be taken into account that this tumour type may be considered rodent specific and may have little relevance for humans and risk has been calculated by linear extrapolation (in this case a highly conservative approach). Therefore, hydroquinone (HQ) exposure resulting from use of cosmetic products which can release HQ is not of concern with regard to carcinogenicity.

HQ exposure from dermal application as compared with oral intake:

The applicant argues that systemic exposure to HQ that is released from ß-arbutin containing cosmetic products is low/negligible in relation to HQ amounts ingested with diet. The SCCS considered the following values for such a comparison:

Dermal route (see above)

45.8 µg HQ per day / 60 kg bw = 0.76 µg/kg bw or ≤0.0008 mg/kg bw/day

Oral intake of HQ (from ß-Arbutin) with food (coffee, wheat products, fruits) (from *Deisinger et al. Ref. 28 subm. I,* value range 783.9 – 1278.7 µg):

average 1020 µg HQ day / 60 kg bw = 17 µg/kg bw or 0.017 mg/kg bw/day

Comparing these HQ exposures, HQ derived from ß-arbutin in cosmetic products is about 22-fold lower than the estimated dietary intake of HQ. Therefore, the use of ß-arbutin in cosmetics would not substantially increase the plasma concentrations of HQ.

3.4 Discussion

Physico-chemical properties

In acidic medium, β-arbutin is easily hydrolysed into hydroquinone [42, 43]. This may be of relevance if β-arbutin is incorporated in aqueous lotions with a slightly acidic pH, facilitating hydrolysis into hydroquinone within the formulation. Data are now presented on the stability of the product, which appears to be photostable and temperature stable in the pH range of the formulation. The concentrations of hydroquinone in the formulations studied remain below 1 ppm.

Yet, it is to be considered that ß-arbutin might be also undergo cutaneous hydrolysis into hydroquinone due to enzymatic activity (through ß-glucosidases: Mier & van den Hurk 1976; Redoules et al. 2005) or via the activity of microbes residing in or on the surface of the skin (Bang et al. 2008).

A study of the degradation by beta-glucosidase has not been performed.

Toxicity

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 test 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 sensitiser.

Since hydroquinone has been identified to be a skin sensitiser, its release from ß-arbutin containing products could cause sensitisation. Yet, no problems were reported in the study with human volunteers or in post-marketing surveillance with β-arbutin.

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, and an upper value for dermal absorption of 0.339 µg/cm². It was further noted that the number of skin samples was lower than that usually required and that the concentration of β-arbutin in the test formulation (6.3%) was slightly below the requested maximum concentration (7.0%).

Although the *in vitro* percutaneous penetration study has some (minor) limitations, it is considered as sufficiently valid. A low absorption of the applied ß-arbutin related radioactivity was measured over 24h. Although specific analysis of parent compound and its cleavage products was not performed, it can be considered likely that the great majority of the measured radioactivity relates to β-arbutin. This view is supported by results from an *in vivo* study in humans with repeated topical application and analysis of skin biopsies for β-arbutin and hydroquinone content (see below "Toxicokinetics").

Systemic toxicity

The acute oral toxicity profile of β-arbutin can be considered low, viewing the LD_{50}-oral-rat value of 8715 mg/kg/day whereas the LD_{50}-dermal-rat was >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

Hydrolysis to hydroquinone (HQ) has been described as significantly taking place in the case of oral intake of β-arbutin (stomach acids), but also to a lesser extent after dermal exposure [43, 38]. Subsequent enzymatic biotransformation (by phase II enzymes) of HQ may be expected in both cases [40, 42].

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 concentrations (yet determined by a rather insensitive analytical method with a high LOD), 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 total (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.
Arbutins (alpha-, beta- and deoxy-arbutin), are considered to be metabolised differently by different glycosidases or by spontaneous hydrolysis, yet they all form hydroquinone. The latter is considered as the genotoxic moiety; therefore a read across approach based on HQ release is a possibility to assess the genotoxic potential of the arbutins as a group.

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. Yet, 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 conclusion with regard to carcinogenicity can be drawn from this study.

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 phototoxic 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.

Issues related to hydroquinone (HQ) in skin lightening products
If HQ is released in relevant amounts either in the product or during the use of arbutins (β-arbutin or similar ingredients), the product could not be considered safe, since HQ has been assessed as being unsafe for use in skin lightening applications due to the danger of exogenous **ochronosis** and leukomelanoderma [41] and consequently is currently banned for this use in the EU. The HQ product concentrations at which ochronosis has been described are of 1% and higher [44]. A 2% formulation of ^{14}C–hydroquinone was shown to have 45.3% +/- 11.2% availability with a 24-hour application in human volunteers (6). The bioavailability was calculated as 43.3% of the dose with a flux of 2.85 µg/cm^2/h.
As no data is available on HQ 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 and largely confined to individuals with very dark skin, the occurring cases can be severe and irreversible.
The use of arbutins (alpha-, beta- and deoxy-arbutin) in skin bleaching products is a complex situation for which the application level and the local availability of hydroquinone cannot be generalised. The factors causing HQ release at the skin surface will depend on the activity of skin bacteria and enzymes (glycosidases) in any one individual and there is

insufficient information on their quantitative variation. Moreover, dermal penetration of the active ingredient and its stability in formulations can differ. Therefore, a **case-by-case evaluation** is needed.

Dermal absorption of β-arbutin is rather low (max. 0.214 % of the applied dose or 0.339 µg/cm^2), which is considerably lower than that of HQ itself. It is noted that the ratio hydroquinone/(β-arbutin + hydroquinone) in the skin averaged 4.6% and amounted up to 11.8% in the skin metabolism study in human volunteers which is clearly higher than the ratio of the two substances (<1 ppm HQ) in the applied product. If formation of hydroquinone from β-arbutin in the skin is taken with an upper value of 12%, then a 7% level of arbutin in cosmetic products, applied twice per day to face and neck (an area of 565 cm^2), could give rise to a systemically available dose of about 30 to 45 µg of hydroquinone per person and day, including also the HQ present as impurity in the cosmetic product.
This internal exposure value for HQ was used in safety assessments related to ochronosis and other endpoints.
In comparison, the internal exposure resulting from use of a product with 1% hydroquinone (taking a dermal penetration of about 50%) would amount to about 8 mg hydroquinone. This dose (which may elicit ochronosis) is 174-times higher than that resulting from the use of products with ß-arbutin. Therefore it can be concluded that ß-arbutin does not pose a risk with regard to induction of exogenous ochronosis in humans (under the conditions of use specified in the terms of reference).
The general potential toxicity of HQ has been reported (7; ref. xy) as being highly sex-, species- and strain- specific, with nephrotoxicity and carcinogenicity primarily limited to male F-344 rats. Epidemiology and occupational studies of workers with extensive exposure to HQ have not reported any evidence of adverse *systemic* health effects.
Since hydroquinone is classified in the EU as Carc Cat 2 H351 (suspected of causing cancer) based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, the SCCS has carried a calculation to determine a lifetime cancer risk. Risk was calculated to be 10^{-5}, based on a systemic exposure dose of 0.00076 mg/kg bw/day and a HT25 dose derived from the T25 dose descriptor for renal tubular cell adenomas in male F344 rats (NTP study 1989).
Taking into account that risk has been calculated by linear extrapolation (in this case a highly conservative approach), and that the tumour type considered may have little relevance for humans, hydroquinone (HQ) exposure resulting from ß-arbutin containing cosmetic products (under the conditions of use specified in the terms of reference) is not of concern with regard to carcinogenicity.

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4. CONCLUSION

(1) Does the SCCS consider on the basis of the provided scientific data, the use of β-arbutin to be safe for consumers in cosmetic products in a concentration up to 7% in face creams?

The SCCS considers the use of β-arbutin to be safe for consumers in cosmetic products in a concentration up to 7% in face creams provided that the contamination of hydroquinone in the cosmetic formulations remain below 1 ppm.

(2) Does the SCCS have any further scientific concerns with regard to the use of β-arbutin in cosmetic products?

A potential combined use of β-arbutin and other hydroquinone releasing substances in cosmetic products has not been evaluated in this Opinion.

5. MINORITY OPINION

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