Scientific Committee on Consumer Safety

SCCS

OPINION

on the safety of alpha-arbutin and beta-arbutin in cosmetic products

The SCCS adopted this document
by written procedure on 31 January 2023

ACKNOWLEDGMENTS

Members of the Working Group are acknowledged for their valuable contribution to this Opinion. The members of the Working Group are:

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This Opinion has been subject to a commenting period of eight weeks after its initial publication (from 25 March to 27 May 2022). Comments received during this period were considered by the SCCS. For this Opinion, minor changes of the content occurred in sections 3.1.9.1.2, 3.1.9.1.5 (B), and conclusion number 5.

All Declarations of Working Group members are available on the following webpage:
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1. ABSTRACT

The SCCS concludes the following:

1. *In light of the data provided, does the SCCS consider α-arbutin safe when used in face creams up to a maximum concentration of 2% and in body lotions up to a maximum concentration of 0.5 %?*

   The SCCS is of the opinion that alpha-arbutin used in face creams up to a maximum concentration of 2% and in body lotions up to a concentration of 0.5% is safe, also when used together.

2. *In the event that the estimated exposure to α-arbutin from cosmetic products is found to be of concern, SCCS is asked to recommend safe concentration limits.*

   Not applicable.

3. *In light of the data provided, does the SCCS consider β-arbutin safe when used in face creams up to a maximum concentration of 7%?*

   The SCCS is of the opinion that beta-arbutin used in face creams up to a maximum concentration of 7% is safe.

4. *In the event that the estimated exposure to β-arbutin from cosmetic products is found to be of concern, SCCS is asked to recommend safe concentration limits.*

   Not applicable.

5. *In light of the data provided, does the SCCS consider that the presence of hydroquinone in the cosmetic formulations must remain below 1 ppm for both α- and β-arbutin containing products?*

   Hydroquinone should remain as low as possible in formulations containing alpha-or beta-arbutin and should not be higher than the unavoidable traces in both arbutins. In the new studies, submitted by the applicant, 3ppm was the LOQ for hydroquinone and 1ppm for the LOD.

6. *Does the SCCS have any further scientific concerns regarding the use of a- and β-arbutin in cosmetic products in relation to aggregate exposure from such substances in cosmetics?*

   Aggregate exposure of alpha-arbutin (2% in face cream and 0.5% in body lotion) with beta-arbutin (7% in face cream) are considered safe.

Keywords: SCCS, scientific opinion, alpha-arbutin, beta-arbutin, Regulation 1223/2009

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on the safety of alpha- (CAS No. 84380-018, EC No. 617-561-8) and beta-arbutin (CAS No. 497-76-7, EC No. 207-8503) in cosmetic products, preliminary version of 15-16 March 2022, final version of 31 January 2023, SCCS/1642/22

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SCCS

The Committee shall provide Opinions on questions concerning 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.).

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SCCS - Opinions (europa.eu)

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2. MANDATE FROM THE EUROPEAN COMMISSION

Background

Alpha-arbutin (α-arbutin) (INCI name: Alpha-Arbutin, Chemical name: 4-Hydroxyphenyl-alpha-D-glucopyranoside, CAS No. 84380-01-8, EC No. 617-561-8) and the structurally related compound (optical isomer) beta-arbutin (β-arbutin) (INCI Name: Arbutin, Chemical name: 4-Hydroxyphenyl-b-D-glucopyranoside**,** CAS No. 497-76-7, EC No. 207-850-3) are similar cosmetic ingredients currently not regulated under the Cosmetic Regulation (EC) No. 1223/2009. Among the reported functions for both α- and β-arbutin are antioxidant, skin bleaching and skin conditioning.

The safety of α- and β-arbutin has been previously assessed in the SCCS/1552/15[1] [2] [3] and SCCS/1550/15, respectively. The effects of arbutins on the skin could be attributed to their gradual hydrolysis and release of hydroquinone (HQ). Hydroquinone (CAS No. 123-31-9. EC No. 204-617-8) is listed in entry 1339 of Annex II to the Cosmetic Regulation, which means that it is prohibited as cosmetic ingredient with the exception of entry 14 in Annex III (restricting its use to professionals in artificial nail systems with a maximum concentration up to 0.02% in the finished product).

In the relevant SCCS Opinions (SCCS/1552/15 and SCCS/1550/15), the SCCS concluded that

1. the use of α-arbutin is safe for consumers in cosmetic products in a concentration up to 2% in face creams and up to 0.5 % in body lotions.

2. the use of β-arbutin is 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 remains below 1 ppm.

Nevertheless, the SCCS highlighted in both Opinions that a potential combined use of hydroquinone releasing substances in cosmetic products has not been evaluated.

During previous discussions within the Working Group on Cosmetic Products, concerns have been raised on the HQ content, its release, as well as on the aggregate exposure from cosmetic products containing α-arbutin and/or β-arbutin. This led to additional consultation with the SCCS and resulted in the identification of a number of issues in the previous submissions. Notably, the stability and dermal absorption of α-arbutin and/or β-arbutin, the release rate of HQ and the aggregate exposure calculation from cosmetics exposure.

Following this, a call for data was launched from July 2020 to April 2021 (9 months). More specifically, interested parties were asked to contribute with data/information relevant to the stability of α- and β-arbutin, their dermal absorption, the HQ release rate (including biotransformation) and the aggregate exposure.

Terms of reference

1. In light of the data provided, does the SCCS consider α-arbutin safe when used in face creams up to a maximum concentration of 2% and in body lotions up to a maximum concentration of 0.5 %?

2. In the event that the estimated exposure to α-arbutin from cosmetic products is

[1] https://ec.europa.eu/health/scientific committees/consumer safety/docs/sccs o 176.pdf
[2] https://ec.europa.eu/health/scientific committees/consumer safety/docs/sccs o 169.pdf
[3] https://ec.europa.eu/newsroom/growth/items/684140

found to be of concern, SCCS is asked to recommend safe concentration limits.

3. In light of the data provided, does the SCCS consider β-arbutin safe when used in face creams up to a maximum concentration of 7%?

4. In the event that the estimated exposure to β-arbutin from cosmetic products is found to be of concern, SCCS is asked to recommend safe concentration limits.

5. In light of the data provided, does the SCCS consider that the presence of hydroquinone in the cosmetic formulations must remain below 1 ppm for both α- and β-arbutin containing products?

6. Does the SCCS have any further scientific concerns regarding the use of α- and β-arbutin in cosmetic products in relation to aggregate exposure from such substances in cosmetics?

3. OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

α-arbutin:
INCI Name: alpha-Arbutin

β-arbutin:
INCI Name: Arbutin

3.1.1.2 Chemical names

α-arbutin:
α-D-Glucopyranoside, 4-hydroxyphenyl
Hydroquinone O-α-D-glucopyranoside

4-hydroxyphenyl-alpha-D-glucopyranoside (ECHA-REACH)
Alpha-arbutin (ELINCS)
4-hydroxyphenyl-a-D-glucopyranoside (ELINCS)
A-arbutin (SWISS)

β-arbutin:
4-Hydroxyphenyl-β-D-Glucopyranoside

3.1.1.3 Trade names and abbreviations

α-arbutin:
ALPHA-ARBUTIN

β-arbutin:
Arbutin

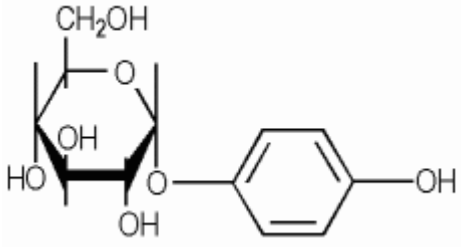
3.1.1.4 CAS / EC number

α-arbutin:
CAS No: 84380-01-8
EC: 617-561-8
EINECS/ELINCS: 440-470-8

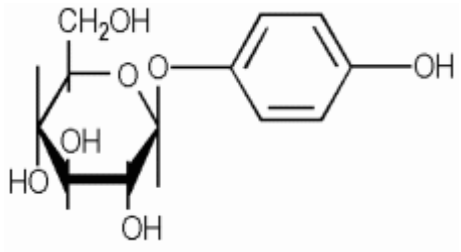
β-arbutin:
CAS No: 497-76-7
EC: 207-850-3

3.1.1.5 Structural formula

α-arbutin:



β-arbutin:



3.1.1.6 Empirical formula

α-arbutin: $C_{12}H_{16}O_7$

β-arbutin: $C_{12}H_{16}O_7$

3.1.2 Physical form

α-arbutin:
White to off-white powder

(DSM, 2010)

β-arbutin:
White to light grey powder

(Appendix 1, SCCS/1550/15)

3.1.3 Molecular weight

α-arbutin: 272.25 g/mol

β-arbutin: 272.25 g/mol

3.1.4 Purity, composition and substance codes

α-arbutin:
The purity is specified as ≥ 97% alpha-Arbutin. Various production batches gave results of 98.3%-100.6% as actually measured values for Lot numbers released into commerce (Table 3.1.4.1). Testing and characterisation were done mainly with pilot batches of alpha-Arbutin

as summarised in Table 3.1.4.2 below, section 3.1.10 (data from the manufacturer Ezaki Glico Co.)

	Lot No.	**Purity**
Pilot batches/ test batches	H12-10-N-160	99.7%
	H13-05-S-1	100.3%
Production batches (2002-2010)	020906	98.3%
	020423	>97% (measured value not given)
	21220	99.0%
	30326	99.2%
	41117	98.3%
	50210	99.8%
	50413	100.6%
	50624	98.3%
	100709	98.7%

(SCCS/1552/15)

Loss on drying ≤2%. According to EP 2.2.32, method b, 3h at 105 °C ± 2 °C sample 2g

(DSM Dossier, 2021)

Two commercial lots of DSM alpha-arbutin were used for the various studies to generate new data for the present opinion as shown in the following table:

Compound/Lot n°	**Physical form**	**Purity**	**Used in study**
Alpha-Arbutin			
Lot 190702	White powder	100%	Analytical method Stability Tape stripping
Lot 191108	White powder	99.2%	Analytical method Stability
Hydroquinone sigma Aldrich			
Lot BCBW1282	White powder		Analytical method

(DSM Dossier, 2021)

β-arbutin:
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%

(Appendix 1, SCCS/1550/15)

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

(SCCS/1550/15)

3.1.5 Impurities / accompanying contaminants

α-arbutin:

Hydroquinone
HQ: < 0.01% w/v solution specified

Others
Heavy metals: < 20 ppm
Arsenic: < 2 ppm

(Appendix 2 of SCCS/1552/15)

For the hydroquinone analysis by HPLC ≥0.15 µg of hydroquinone could be detected per 0.1 mg (or 100 µg) alpha-Arbutin powder, corresponding to 0.15% (Sugimoto K., 2010) (*3*). The representative Certificates of Analysis for each Lot number are included in Appendix 2.

By means of an HPLC method developed for analytical separation and quantitative determination of alpha-Arbutin and any potential degradation products such as hydroquinone ((*4*), the hydroquinone contents of purchased alpha-Arbutin was analysed by DSM only sporadically (Rosenberger V., 2010) (*5*) and hydroquinone content was not part of DSM product specifications (DSM, 2010) (*2*).

(SCCS/1552/15)

Newly submitted data
The hydroquinone content of alpha-arbutin was analysed at the release of batches by quality control. This was done using an HPLC method for analytical separation and quantitative determination of alpha-arbutin and any potential degradation products such as hydroquinone. Two alpha-arbutin lots are considered representative for all lots of alpha-arbutin on the

market by DSM. Measured values of hydroquinone are below LOQ (3 ppm). The impurity analysis was as follows:

Impurities	**Lot No 190702**	**Lot No 191108**
Hydroquinone	≤ LOQ (3ppm)	≤ LOQ (3ppm)
Heavy metals	≤ 20 ppm	≤ 20 ppm
Arsenic	≤ 2 ppm	≤ 2 ppm

As the content of hydroquinone was below the LOQ (3 ppm), the applicant developed and validated an analytical method to quantify hydroquinone in cosmetic formulations. Commercial hydroquinone with specified purity of 99.5% was used for the analytical method validation and as a reference substance (as described in the appendix 1).

(DSM dossier, 2021)

β-arbutin:
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

(Appendix 1 of SCCS/1550/15)

SCCS comment

The applicant developed an improved methodology for the determination of hydroquinone for which the LOQ is 3ppm and the LOD 1ppm. In most studies before the new submission, a lower LOQ than 3 ppm was mentioned, but this was not meant for the determination of alpha- or beta-arbutin as bulk material. A LOQ of 1 ppm could be reached for beta-arbutin when dissolved in water or in 10% ethanol/water.

3.1.6 Solubility

α-arbutin:
Alpha-arbutin has solubility in water of 151 g/L at 20 ± 5 °C as determined using the flask method A6 of Commission Directive 92/69/EEC.
Data on solubility, for example in DMSO, ethanol or other solvents, were not available.

(Woolley S., 2001; DSM Dossier, 2021)

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

(Nishimura T., 1995)

SCCS comment
The water solubility has not been measured by the EC method A.6 for β-arbutin

3.1.7 Partition coefficient (Log P_{ow})

α-arbutin:
Log $P_{o/w}$: 2.05 x10^{-2} at 21±5°C, $\log_{10}$ Po/w ~1.69 using the shake-flask method A8 of Commission Directive 92/69/EEC.

(Woolley S., 2001; DSM Dossier, 2021)

β-arbutin:
- 1.35

(Sugimoto K., 2008)

SCCS comment
The Log Pow has not been determined by the EC method A.8. for β-arbutin.

3.1.8 Additional physical and chemical specifications

α-arbutin:

melting point	201 ± 0.5°C, method A1 of Commission Directive 92/69/EEC	Ref.: Woolley S. & Mullee D., 2001
boiling point	~285°C at 102.17 kPa, method A2 of Commission Directive 92/69/EEC	Ref.: Woolley S. & Mullee D., 2001
flash point	Not highly flammable, method A10 of Commission Directive 92/69/EEC	Ref.: Woolley S. & Mullee D., 2001
UV/VIS absorption spectrum	λmax. ~280 nm	Appendix 2 of Submission I

(SCCS/1552/15)

β-arbutin:

λ_{max} = 285 nm (value present in summary report, no spectrum present)

Melting point: 197-201°C:

Lot A: 198.3°C	Lot TDA-361: 201.2°C
Lot B: 198.0°C	Lot TDD-422: 200.0°C
Lot C: 198.7°C	Lot TPG-412: 201.0°C
	Lot TGJ-225: 201.0°C

pH: 5-7:

Lot A: 5.78	Lot TDA-361: 6.00
Lot B: 5.63	Lot TDD-422: 6.10
Lot C: 5.75	Lot TPG-412: 6.10
	Lot TGJ-225: 6.10

Specific rotation: $[\alpha]_D^{25}$ = -62 to -68°:

(method not specified)	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

(SCCS/1550/15)

SCCS comment
UV-spectrum of B-arbutin: spectrum not available

3.1.9 Homogeneity and Stability

3.1.9.1 Stability of α-arbutin

3.1.9.1.1. <u>Stability data of alpha-Arbutin as a raw material from SCCS/1552/15</u>

A. Stability of alpha-Arbutin at pH 4.5, 5.0 and 6.0 including hydroquinone measurement

Alpha-Arbutin (Lot no. 061110) was dissolved at concentrations of 2% or 3% (w/v) in 20 mM acetic acid-Na buffer and adjusted to pH 4.5, 5.0 and 6.0. Each of the 15 mL samples were stored for 28 days at 50°C. At study time points 0, 14, 21 and 28 days, a 10-µL aliquot was analysed for alpha-Arbutin and hydroquinone by HPLC.
The results showed that Alpha-Arbutin at 2% and 3% each in solution at pH 4.5, 5.0, and 6.0 and stored 28 days at 50 °C remained stable (100% recovery) and hydroquinone was not quantifiable (LOD was given to be 50 µg hydroquinone per mL solution containing 2% or 3% alpha-Arbutin).

(Sugimoto K., 2008)

B. Stability of alpha-Arbutin at pH 3.5-6.5 and extrapolated half-life times

An experiment on stability available from laboratory record books is summarised as follows.
A series of 5 g aliquots of alpha-Arbutin (Lot/Batch not given) dissolved in 150 mL Na citrate-phosphate buffer (corresponding to 3.3% or 122 mM) were each adjusted to pH 3.5, 4.5, 5.5 or 6.5 and stored at 40°C. The ultimate measured pH was 4.9 instead of 4.5. Alpha-Arbutin content was measured every week over a period of 3 months by HPLC. Hydroquinone levels were not determined.
The half-life times of alpha-Arbutin in aqueous buffered solution at 40°C are shown in the table below.

pH	**3.5**	**4.9**	**5.5**	**6.5**
T1/2 (months)	~29	~77	~37	~28

It was concluded that the stability of alpha-Arbutin in buffered aqueous solution is pH-dependent, showing highest stability at about pH 5.0, which is within the range of the pH of finished cosmetic product formulations.

(Heidl & Ziegler, 2011)

C. Comparison of stability of alpha-Arbutin, beta-Arbutin and hydroquinone at pH 5.5, 6.5 and 7.5

Solutions containing alpha-Arbutin, beta-Arbutin and hydroquinone (0.037 M in 50 mM Na Acetate buffer, pH 5.5, P-Na buffer, pH 6.5 and Tris-HCl, pH 7.5) were stored at 37°C and absorbance was measured at different time points (1, 4, 23, 32 and 52 h) at 427 nm, 460 nm and 500 nm. The solutions became coloured due to the oxidation of hydroquinone and stability was estimated based on the absorption.
The stability indicated by absorption was higher for the glucosides (alpha-and beta-Arbutin) compared to hydroquinone, while alpha- and beta-Arbutin were comparable in stability. In

contrast to the expectation that a higher pH value would result in faster oxidation and higher absorbance, pH 6.5 gave higher absorptions than pH 7.5 which might indicate that alpha- and beta-arbutin are less stable in phosphate buffers pH 6.5.

(Nishimura, 2008)

3.1.9.1.2. Newly submitted stability data of alpha-arbutin as a raw material

Samples of alpha-arbutin were stored at the recommended storage temperature (25°C) and under accelerated condition (40°C) for up to 36 months. The samples were filled in the same packaging type and material as used for the commercial goods: polyethylene (PE) bags inside of aluminium bags. Room temperature storage condition was: 25°C ± 2°C / 60% RH + 5% RH, storage under accelerated condition was: 40°C + 2°C / 75% RH ± 5% RH. The Hydroquinone content and purity of Alpha-Arbutin were measured at different timepoints. Data are summarised in Tables 1 and 2.

Table 1: Stability of alpha-arbutin at the recommended storage temperature; pure powder stored in PE/Alu packaging

Stability at 25° C	Purity (%) at different times						Hydroquinone content (ppm) at different times LOQ is 3ppm					
Lot number	Months						Months					
	T0	3	6	12	24	36	T0	3	6	12	24	36
160309	99.1	98.8	98.7	98.0	98.0	98.5	< 3 ppm	< 3 ppm	< 3 ppm	< 3 ppm	< 3 ppm	< 3 ppm
160316	99.1	99.3	98.5	98.4	99.2	98.6	< 3 ppm	< 3 ppm	< 3 ppm	< 3 ppm	< 3 ppm	< 3 ppm
160318	99.3	99.5	98.6	98.8	99.3	98.0	< 3 ppm	< 3 ppm	< 3 ppm	< 3 ppm	< 3 ppm	< 3 ppm
180102	100.0	100.0	100.0	100.0	100.0	100.0	< 3 ppm	< 3 ppm	< 3 ppm	< 3 ppm	< 3 ppm	< 3 ppm
180104	100.0	100.0	100.0	100.0	99.7	100.0	< 3 ppm	< 3 ppm	< 3 ppm	< 3 ppm	< 3 ppm	< 3 ppm

Alpha-arbutin remained stable, with Hydroquinone content below LOQ (3ppm) at 25 °C for all time points analysed.

Table 2: Stability of alpha-arbutin at accelerated conditions 40 °C; pure powder stored in PE/Alu packaging

Stability at 40°C	Purity (%) at different times			Hydroquinone content (ppm) at different times LOQ is 3ppm		
Lot number	Months			Months		
	T0	3	6	T0	3	6
160309	99.1	98.9	98.1	< 3 ppm	< 3 ppm	< 3 ppm
160316	99.1	99.2	98.6	< 3 ppm	< 3 ppm	< 3 ppm
160318	99.3	98.9	98.6	< 3 ppm	< 3 ppm	< 3 ppm
180102	100.0	100.0	100.0	< 3 ppm	< 3 ppm	< 3 ppm
180104	100.0	100.0	100.0	< 3 ppm	< 3 ppm	< 3 ppm

Alpha-arbutin remained stable, with Hydroquinone content below LOQ (3ppm) at 40 °C for all time points analysed.

Overall, the applicant concluded that alpha-arbutin shows long-term stability, with Hydroquinone content below LOQ, no degradation is observed at 25°C and 40°C, up to 36 months for the samples of the various lots.

(DSM Dossier, 2021)

3.1.9.1.3. Open literature data of alpha-arbutin as a raw material

The photostability under normal daylight of alpha- (and beta-) arbutin in water or methanol was evaluated for at least 8 hours per day over 12 months. At the end of the storage period, no hydroquinone was detected in the samples, suggesting no photo-catalysed degradation took place.

(Avonto *et al.*, 2016)

3.1.9.1.4. Stability of alpha-Arbutin in a cosmetic formulation from SCCS/1552/15

A. Stability test of submission II December 2012

The stability of 2% alpha-arbutin (Lot 41174901) in a Steareth-2/Steareth-21-based O/W emulsion was studied with respect to the formation of hydroquinone and benzoquinone.

The levels of hydroquinone in the freshly prepared formulations (time point = 0) could be reduced from 13 – 18 ppm to about 1 – 3 ppm by including the citrate buffer (and other ingredients, cf. above). Yet, hydroquinone levels in these "stabilised" formulations were not prevented from significantly increasing to levels characteristic for freshly prepared unbuffered ("unstabilised") formulations (i.e.12 – 13 ppm). No measurable decrease of the concentration of alpha-arbutin was noted in any of the formulations, even after 13 weeks at 40°C. All the formulations contained measurable amounts of hydroquinone. The concentration of hydroquinone in 'non-stabilised formulations' was between 13 and 30 ppm at the beginning of the experiments (in the freshly prepared formulations) and increased to about 50 ppm (with a maximum of 79 ppm) after 13 weeks. Higher temperature and increased length of time appeared to favour the formation of hydroquinone, although this trend was not consistent in all formulations. Notably, the concentration of hydroquinone in 'stabilised formulations' was lower, i.e. from <1 to 3 ppm at the beginning and around 13 ppm after 13 weeks at 40°C (Ref A). The SCCS commented that several errors were noticed (e.g. mixing up of the UV spectra of hydroquinone and BQ, erroneous dilution factors) in the reports of the HPLC-DAD methods, as well as unintelligible reporting (*e.g.* in the determination of linearity) and poor analytical methodology (*e.g.* HPLC solvent gradient used for the analysis of alpha-arbutin, choice of wavelength for the detection of hydroquinone).

(*DSM, 2012c*); (DSM, 2012a); (DSM, 2012b)

3.1.9.1.5. Newly submitted stability data of alpha-arbutin in formulation

A. Stability of alpha-arbutin in cosmetic serum formulation

A study performed in 2016 was submitted by the applicant, examining the stability of alpha-arbutin (Lot No 41829801) over 3 months at 0.5 and 2% alpha-arbutin in a cosmetic aqueous serum. Composition of the aqueous serum was provided with a detailed description of the procedure. All samples were stored for 3 months at 4 °C, room temperature, 40 °C, 50 °C or

at daylight with room temperature. HPLC analysis was performed for the detection of alpha-arbutin and hydroquinone content after three months of storage at the different conditions. A validation report of the analytical method was furthermore provided. Visual inspection, odour, pH (5.8), viscosity and colour (L*a*b*) was determined. The results of the test showed that all samples appeared clear and odourless. In the 0.5% alpha-arbutin serum, a slight colour change to light yellow was observed at 50 °C and at daylight (room temperature) conditions. There was no visible colour change at 4°C, 40 °C and room temperature. In the 2% alpha-arbutin serum, a slight colour change to light yellow at 40 °C and with daylight (room temperature). At 50 °C a yellowish colour was observed. There was no visible colour change at 4 °C and room temperature conditions. The pH values remained stable at all conditions (shift <±0.2). At t0, alpha-arbutin did not impact the viscosity of the serum compared to the placebo. After 3 months of storage a slight decrease in viscosity was observed in all samples. Recovery rates of alpha-arbutin were minimum 98.5% after three months of storage at all conditions. The concentration of hydroquinone was below 3 ppm (LOQ) in all samples.

(Kohler L., 2016)

B. Stability of alpha-arbutin in different cosmetic formulations

A more recent stability study performed in 2021 was additionally submitted by the applicant. The study consisted of one 3-month stability test of alpha-arbutin at 1 and 2% in three different formulations and a 3-month stability test of 2% alpha-arbutin in aqueous solution tested in the pH range from 3.5 to 7.5.
The first part of the study examined the stability of alpha-arbutin in a O/W emulsion, aqueous serum and O/W sunscreen formulation with SPF 15. A detailed description of each formulation and its preparation was provided. The test was performed with two different lots of alpha-arbutin (Lot No 190702 and Lot No 191108). HPLC analysis was performed for the detection of alpha-arbutin and hydroquinone after three months of storage at either 4 degrees Celsius, room temperature or 40 degrees Celsius. A validation report of the analytical method was provided. Further parameters measured were: the visual aspects, odour, pH, viscosity, colour changes (L*a*b*) and microscopy (emulsions only). The second part of the study tested the compound's stability at different pH levels of 3.5, 4.5, 5.5, 6.5 and 7.5. Further determination of both alpha-arbutin and hydroquinone in the samples at the different pH levels was determined. For the latter test only alpha-arbutin at 2% (w/w) from Lot 190702 was used.

(i) O/W emulsion formulation stability test
All samples appeared almost white, odourless and stable without phase separation. All pH values remained stable at all conditions (shift < ±0.2). A slight decrease of viscosity of -10 to -17% was observed after 3 months in all samples, with alpha-arbutin slightly more than in the placebo (regardless of the concentration). No crystal was detected in any of the samples. All L*a*b* values (indicating colour changes) were very stable over 3 months (ΔE < 2 in all samples). The recovery of alpha-arbutin was minimum 98.1% for all samples, whilst hydroquinone was below 3 ppm (LOQ) in all samples at t0 and after 3 months of storage at the different conditions.

(ii) Aqueous serum formulation stability test
At 4 degrees Celsius and room temperature, all samples were almost colour- and odourless, clear and stable. At 40 degrees Celsius a slight colour change was observed, appearing very light yellow (1% alpha-arbutin concentration), or light yellow (2% alpha-arbutin). All pH values remained very stable at all conditions (shift <±0.1). At t0, alpha-arbutin did not impact the viscosity compared to the placebo. After three months a slight decrease in viscosity was observed in all samples of about -10%. The colour changes (L*a*b*) quantified as ΔE were above 2, but the visual impact was low. Recovery of alpha-arbutin was minimum 99.1% for all samples. Hydroquinone in all samples at t0 and after 3 months of storage at the different conditions was below 3 ppm.

(iii)O/W sunscreen with SPF 15 formulation stability test
At 4 degrees Celsius and room temperature, all samples are almost white, odourless and stable without any phase separation. At 40 degrees Celsius, all samples are odourless and stable without any phase separation but samples containing alpha-arbutin did appear very light beige in colouration. pH values remained very stable at all conditions (shift <±0.1). At t0 alpha-arbutin appeared to decrease viscosity by about 10% compared to the placebo. After three months the viscosity is comparable in all samples. Placebo decreased with about 10%, samples with alpha-arbutin by about 3%. No crystals were detected in all samples. ΔE remained below 2 in all samples, indicating a minor or no change in colour. Alpha-arbutin recovery was minimum 96.2% for all samples, whilst hydroquinone was below 3ppm in all samples at t0 and after 3 months at the different conditions.

Based on the data above, the applicant concluded that alpha-arbutin remains stable in typical cosmetic formulations at standard conditions of temperature and pH, which is in line with independent studies conducted by Jeon *et al.* (2015) and Avonto *et al.* (2016). They observed long term stability of alpha-arbutin in different cosmetic formulations.

(iv)Aqueous solution in the pH range 3.5 – 7.5 formulation stability test
The results showed that all solutions were clear and odourless. At 4 degrees Celsius the solution that was initially adjusted to pH 7.5 showed a slight colour change to very light yellow, whilst all other samples appeared colourless. At room temperature the solutions adjusted to pH 6.5 and 7.5 showed a slight colour change to very light yellow (pH 6.5) or to light yellow (7.5), all other samples are colourless. At 40 degrees Celsius all samples, except the solution adjusted to pH 4.5, showed a slight colour change to yellowish-brown, or to reddish-brown. The pH measurements showed that samples of pH 3.5 remained stable over time (shift <±0.1), whilst samples initially at pH 4.5 and 5.5 shifted to neutral pH. Samples at initial pH levels of 6.5 and 7.5 remained quite stable. Colour changes (L*a*b) at
4 degrees Celsius were above 2 (ΔE) but the visual impact was low. At 40 degrees Celsius, the visual impact was dependent on the initial pH. alpha-arbutin recovery was minimum 97.1% for all samples. Hydroquinone content in all samples at t0 and after 3 months of storage at the different conditions was below 3 ppm, except for one sample at pH 3.5. This sample, initially at pH 3.5, contained 22.7 ppm hydroquinone after 3 months of storage at 40 degrees Celsius. The alpha-arbutin recovery in the later sample was 100%.

The applicant concluded that alpha-arbutin remains stable in aqueous solutions at typical pH levels (between 4.5 to 7.5) at room temperature.

(Kohler L., 2021)

3.1.9.1.6. Open literature stability data of alpha-arbutin in formulation

The stability of alpha- (and beta-) arbutin was determined in nine cosmetic formulations present on the market (4 creams, 3 sera, 1 gel, 1 lotion) with varying pH (between 3.3 and 8.8). After a storage period of 16 months, 1 of the 3 sera samples showed a loss of 7.6%. The other formulations remained stable.

(Avonto *et al.*, 2016)

Both O/W and W/O emulsion bases containing 7% (w/w) alpha-arbutin (Lot AT60104) were evaluated for their stability under varying storage conditions. Cream bases were formulated at both pH 4.0 and 5.5 and evaluated for 60 days at either 2°C, 8°C, 30°C or 40°C. Results showed that more than 90% of alpha-arbutin could be recovered after 60 days of storage at all test conditions. pH was found to be of minimal influence on the formulation's stability. A distinct colour change was detected in both O/W (28 days) and W/O (14 days) formulations, in particular at 40°C. The colour change is suspected to be due to hydroquinone formation, however this was not measured.

(Teeranachaideekul *et al.*, 2021)

3.1.9.1.7. Release rate of hydroquinone from alpha-arbutin by skin microbiome: first submission

A. in vitro test with Yucatan micro-pig skin from SCCS/1552/15 (Masaki J., 2006)

A combination study which determined the *in vitro* percutaneous absorption with alpha-arbutin as well as the release rate of hydroquinone from alpha-arbutin was described. The study was performed with a cream formulation containing either 1% or 2% alpha-arbutin applied to thawed split-thickness skin taken from the right side of one 5 months old female Yucatan micro-pig provided by Charles River Laboratories, Japan, Inc. To each skin sample was applied "0.4-0.5 g" of 1% cream formulation on six Franz cells and similar amounts of the 2% formulation on the second set of 6 cells. The receptor fluid was stirred by magnetic stirrer and held for 24 hours when the experiment was terminated and samples were collected. Samples included residual material wiped from the surface skin and apparatus; the stratum corneum was collected by 20-times tape-stripping (D-Squame tape). Epidermis and dermis were separated by heat-treatment and analysed separately; each was finely minced with scissors, sonicated in HPLC mobile phase, 0.18% perchloric acid in water, centrifuged, and then filtered (0.45 µm). Receptor fluid was collected and passed through a 0.45 µm filter. The filtrates were analysed for alpha-Arbutin or hydroquinone by HPLC analysis and UV detection at 282 and 289 nm. The cream formulations (pH values were not reported) were also analysed in triplicate samples from each concentration for alpha-arbutin after addition of tetrahydrofuran and dilution with HPLC mobile phase followed by filtration and with the same the instrumental parameters. Recovery rate for the two experiments was 91% to 93% of applied test substance. Hydroquinone measurements did not reveal quantifiable amounts in any of the fractions analysed, although absorption peaks at the hydroquinone retention time were reported for several of the fractions based on the limit of determination (0.53 ppm) for the analytical system. The SCCS commented that the study is of limited value with regard to hydrolysis and degradation of alpha-arbutin in skin based on the study design. However, the results appear to be in line with other studies where about 2% was recovered from pig skin.

(Masaki J., 2006)

B. Stability of Alpha-Arbutin in Aqueous Solutions and with Skin Homogenates from SCCS/1552/15

(Ruembeli *et al.*, 2012b)

In a non-GLP study, a series of *in vitro* experiments were conducted to evaluate alpha-Arbutin stability in buffered solutions at various physiological pH values and also when mixed into homogenates of frozen or fresh pig skin or human skin that had been stored frozen.

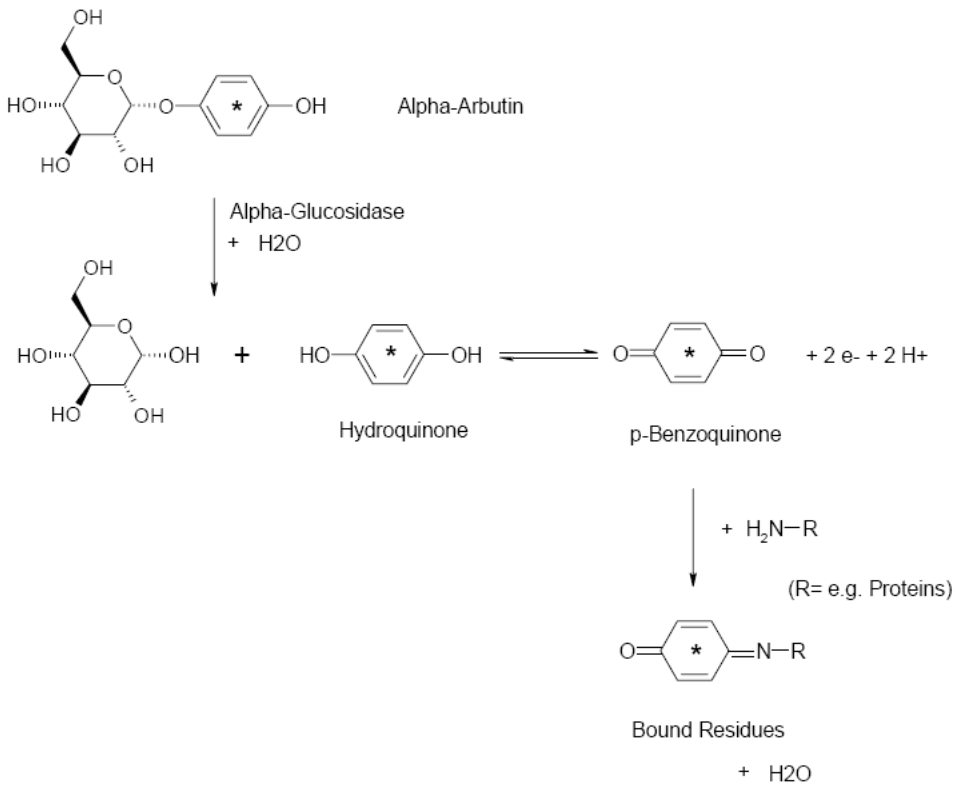
Analysis of buffer solutions (pH 5.4, 6.4 and 7.4) without skin showed alpha-Arbutin remained stable in the respective solutions and pH for up to one week. In addition, a stock solution of alpha-Arbutin stored at 4°C remained stable for 1 month.
Preliminary incubation experiments with pig skin homogenates without antibiotics showed that at 37°C the alpha-arbutin concentrations decreased rapidly with subsequent rapid increases in non-extractable radioactivity. All further experiments therefore included the antibiotic mixture to minimize the impact of microbial degradation on the experimental outcomes. Pig ear skin stored frozen was compared with fresh pig ear skin to evaluate an impact of freezing on skin enzyme activity.
The alpha-arbutin concentrations used in this study were 32 – 41 µM with the exception of one series (a-Hj) where a 100-fold higher concentration was used.
It was concluded that the incubation of ^{14}C-alpha-Arbutin with buffers and homogenates of pig skin and human skin at pH 5.4, 6.4 and 7.4 and 37°C showed the following results:

- Alpha-Arbutin was chemically stable in buffers in the pH range from 5.4 to 7.4 up to one week.

- With pig skin homogenate alpha-Arbutin was metabolised with a half-life between 5 and 11 hours depending on the pH.
- The reaction was slightly faster in pig skin homogenate and increased with the pH from 5.4 to 7.4.
- The half-life was not dependent on the concentration of alpha-Arbutin between 40 and 4000 µM.
- Freezing and storing pig skin had no negative effect on the glucosidase activity compared to fresh pig skin. By extension, this can be inferred for the human skin used in these experiments.
- The hydrolysis of alpha-Arbutin was much slower at pH 7.4 with human skin homogenate than with pig skin homogenate. At pH 5.4 the reaction kinetics were similar between human and pig skin homogenates.
- In skin homogenates alpha-Arbutin is hydrolysed enzymatically to hydroquinone, which is readily oxidized to benzoquinone. Benzoquinone is highly reactive with nucleophiles and binds covalently to high molecular weight compounds such as proteins.

The SCCS commented that the metabolism of alpha-arbutin in pig and human skin homogenate appears similar. In pig skin, the hydrolysis is higher with increasing pH. Incubation of alpha-arbutin at pH 7.4 with pig skin or human skin homogenate showed alpha-arbutin to be more stable in human than in pig skin homogenate. The same comparison performed at pH 5.4 showed an alpha-arbutin decrease with similar reaction kinetics for pig skin as for human skin homogenate. It cannot be concluded if the differences are due to a higher dependence of the human skin glucosidases on pH than the pig skin glucosidases or if the difference is subject specific since the skin sample was only from one human subject.

A metabolic pathway of alpha-Arbutin in skin homogenates is proposed to be as shown in Figure 1.



(Ruembeli, 2012b)

C. *In vitro* test with pig and human skin from SCCS/1552/15 -Ruembeli *et al.* 2012c

One part of an *in vitro* dermal absorption, distribution and metabolism study with pig and human skin, determined the influence of skin microflora on the stability of alpha-arbutin. The skin surface wash revealed alpha-arbutin and two additional small peaks in the chromatograms of which one was characterized as hydroquinone and the second was not identified. Alpha-arbutin represented 93.4 %, hydroquinone 3.0 %, and the unknown product 3.5 % of the total radioactivity recovered. In pig and human skin, treated with the antibiotic mixture and then 2% alpha-arbutin (details provided in report), the radioactivity recovered in the surface wash was 100% alpha-arbutin; most notable is that hydroquinone, benzoquinone, or other unknown substances, did not occur in measurable amounts on either pig or human skin. Comparison to the results for (pig) skin not pre-treated with antibiotics indicates an apparent effect of skin resident microbes on the stability of alpha-arbutin in that about 6% of applied alpha-Arbutin radioactivity was present as related degradation products. The SCCS commented that the use of antibiotics (1% penicillin/streptomycin solution) appeared to reduce degradation/hydrolysis of alpha-arbutin by skin resident microbes on pig skin, but for human skin, information on the effect of skin resident microbes on the stability of alpha-arbutin is lacking as all incubations with human skin contained antibiotics.

(Ruembeli *et al.* 2012c)

D. Submitted data: on human volunteers: submission 2022

The potential formation of Hydroquinone on the skin by the skin microbiome was evaluated in a tape-stripping study with human volunteers. In this placebo-controlled study, 15 healthy female volunteers (age 20-55) applied 2 mg/cm^2 alpha-arbutin-containing cream (2% ALPHA-ARBUTIN (100%), Lot 190702) on the inner forearms on four consecutive days. Each volunteer applied both the test item and the placebo on either forearm according to a randomisation scheme. The size of the application area was 5x5cm. The study design is given in Table 4 DSquame® discs were used for the tape stripping.

Table 3: Study design of the tape stripping study

Time	
2 days prior to beginning of the study (t0)	Wash-out phase No application of any cosmetic product on the inner forearms. No sanitation of the skin on the forearms
Beginning of the study (t0)	Tape stripping sampling on either left or right forearm. First product application at the institute
Between t0 and t1(4 days)	Application of the test products (test item and placebo) on inner forearms on a 5x5cm^2 area twice daily by the volunteers at home. Volunteers were instructed not to apply hand sanitizer on test areas during the entire study
t1 (t0 + 4 days)	Last application of the products 1 hour before the tape stripping of both inner forearms.

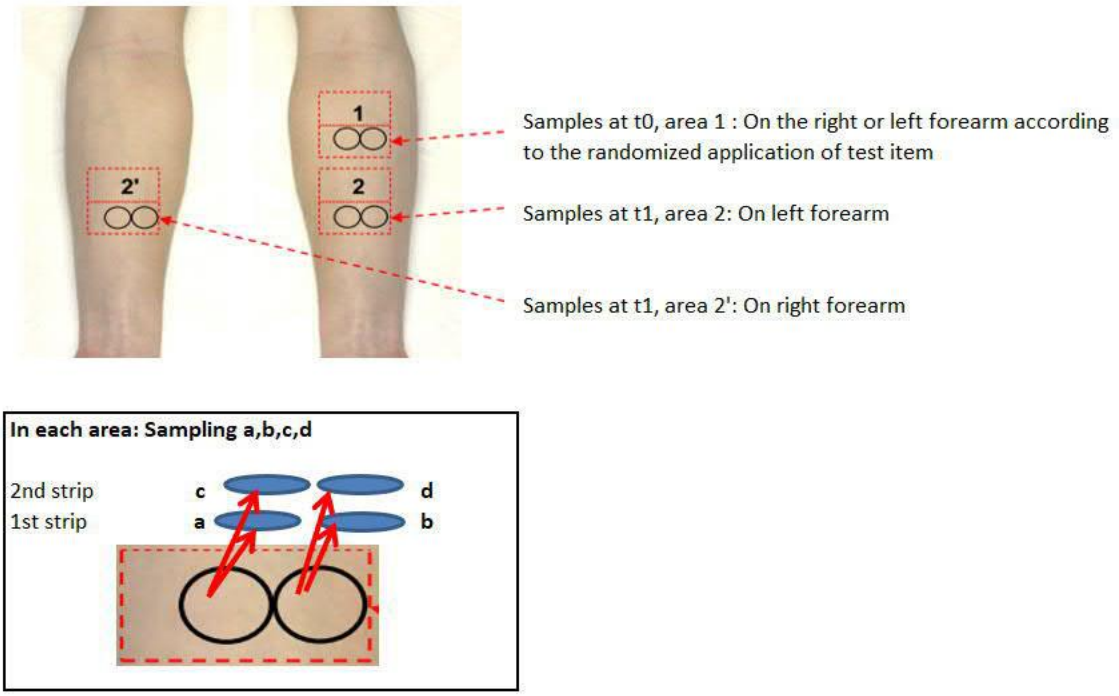


Figure 1: Illustration of the tape strip sampling.

The tape strip samples were stored at -80 °C until the analysis. After thawing, the samples were extracted with extraction solvent (water/methanol 80/20) for 15 min in ultrasonic bath. The samples were centrifugated and the supernatant was analysed by HPLC. Neither alpha-arbutin (LOD 0.02 µg) nor hydroquinone (LOD 0.01 µg) was detected in the t0 samples. In the t1 tape strips, 63.1 + 34.7 µg (mean + SD) of Alpha-Arbutin was measured, which corresponds to a recovery of 42 ± 23% (Table 4). 0.045 + 0.040 µg (mean + SD) Hydroquinone was detected in the t1 samples. Based on this study, it can be concluded that only a very low amount of alpha-arbutin (0.073 ± 0.065 mol%) is degraded to Hydroquinone by the skin microbiome/enzymes. In the t1 placebo samples, very low Alpha-Arbutin and Hydroquinone amounts were detected. Since the analytical method did not show any carry over, it was discussed that these low concentrations are likely due to a contamination during the application of the creams.

Table 4: Summary table with mean amounts of alpha-arbutin and hydroquinone quantified in tape strips (1st + 2nd strip).

	Test item		Placebo	
	AA[3]	HQ[4]	AA[3]	HQ[4]
	mean (n=15)		mean (n=15)	
Amount stripped off [µg] (mean ± SD)	63.1 + 34.7	0.045 + 0.040	1.8 + 4.2	0.024+0.021
Range [µg]	16.9-141	0.02 - 0.177	0.1 -19.1	0.02 - 0.131

Recovery by amount AA measured/AA applied[1] [%] (mean ± SD) AA degraded to HQ[2] (HQ measured/AA applied) [mol %]	42% ± 23%			
(mean ± SD)		0.073% ± 0.065%		0.040% ± 0.033%

Alpha-Arbutin (AA) recovered was calculated by ratio of measured AA to the amount of AA applied on the last (fourth) day (2 mg/cm^2 of 2% ALPHA-ARBUTIN formulation stripped with 3.8 cm^2 D-Squame tapes = 152 µg Alpha-Arbutin (≙ 100% recovered)).
Percentage of Alpha-Arbutin degraded to Hydroquinone (HQ) calculated by molar quantities as follows (HQ amount measured/molecular weight of HQ) / (AA applied / molecular weight of AA).
[3] LOD / LOQ (Alpha-Arbutin): 0.02 µg / 0.06 µg
[4] LOD / LOQ (Hydroquinone): 0.01 µg / 0.03 µg

Based on this study, the applicant concluded that only low percentages of alpha-arbutin (0.073 ± 0.065%) are degraded to hydroquinone by the skin microbiome.
(Waldemar E., 2021)

SCCS comment for first submission
The applicant submitted a study on human volunteers in which the potential release of hydroquinone from alpha-arbutin by the skin microbiome was evaluated through tape-stripping.
The SCCS is of the opinion that this study is not acceptable because of the lack of a mass balance of the alpha-arbutin applied on the skin during 4 consecutive days. Alpha-arbutin and hydroquinone were only measured on the 4th day in skin strips of the stratum corneum. As such, the fate of alpha-arbutin and hydroquinone are not known. Furthermore, the amount of alpha-arbutin applied during the 4 consecutive days was not taken into consideration for expressing the recovery of alpha-arbutin. On the contrary, the recovery of alpha-arbutin, measured 1 hour after the last application, was expressed as function of the last applied amount and was then only 42%, showing that already after 1 hour, the fate of 58% of the applied alpha-arbutin was unknown. The methodology of the study was also insufficiently described.
Alpha-arbutin can be converted by main skin microflora (Ruembeli *et al.* 2012b, 2012c) to hydroquinone. Staphylococcus epidermidis and Staphylococcus aureus, major members of the human skin microbiome (Byrd *et al.*, 2018), have been shown to play a role in this conversion (Bang *et al.* 2008). Furthermore, as hydroquinone might be converted to p-benzoquinone and quickly bind to macromolecules (Ruembeli *et al.* 2012b, Fig 1), the stability of alpha-arbutin on human skin in the presence of its microbiome is important in the safety assessment of alpha-arbutin present in face cream and body lotion as a skin bleaching agent.

E. Open literature data

The stability of alpha- arbutin was determined using pear peels as a *surrogate in vivo* model containing β-glucosidases and peroxidases. The pear peels were first depleted from their naturally present beta-arbutin. Alpha (or beta-) arbutin were added subsequently at 0.5 mg/g. After 8 hours of exposure, a loss of 62.4% alpha-arbutin was measured. During degradation no hydroquinone could, however, be measured. It was suggested that hydroquinone was not detectable due to chemical (hydroquinone degraded at pH >5 with a loss of 20% at pH 7.6 after 24h) or enzymatic instability (hydroquinone and alpha-arbutin are also a substrate of peroxidases). Thus, hydroquinone might be generated *in vivo* and rapidly degrade due to the cellular environment or rapidly convert into more stable end products.

(Avonto *et al.*, 2016)

General SCCS comments on the stability of alpha arbutin: first submission

The SCCS is of the opinion that:

- Alpha-arbutin can be stably kept for 36 months **as raw material** when appropriately packed and kept < 40°C.
- Alpha-arbutin is **stable *in vitro* at 2% in aqueous solutions and in cosmetic products** (o/w cream, sunscreen, serum) for at least 3 months when **temp < 40°C and 4.5 < pH < 7.5 under daylight**; attention must be paid for the use of buffers. It is not clear how they might affect the stability of alpha-arbutin.
- **Alpha arbutin is not stable in the presence of skin microbiome/enzymes;** the newly submitted data does not adequately answer the question about the effect of the skin microbiome/enzymes on the stability of alpha-arbutin. Literature data (Bang *et al.* 2008, Ruembeli *et al.* 212b, Avonto *et al.* 2016) and the newly submitted *in vivo* study point to significant degradation/skin metabolism of alpha-arbutin on the skin by enzymes and bacteria with the potential formation of hydroquinone. The latter could quickly disappear, possibly with adduct formation with cellular macromolecules. This could possibly play a role in adversity, in particular when chronic exposure occurs, which is the case for skin bleaching agents.
- The SCCS is of the opinion that **additional human-relevant data are necessary to exclude adversity due to the formation of hydroquinone and potentially benzoquinone by skin microbiome and skin metabolism.**

3.1.9.1.8 New data provided on the release rate of hydroquinone from alpha-arbutin by skin microbiome after concerns raised during the commenting period

To fill the gaps in the mass balance and to demonstrate the stability of alpha-arbutin on the skin (SCCS/1642/22), the applicant performed a new tape-stripping study with optimized protocol on human volunteers to evaluate the potential release of hydroquinone from alpha-arbutin by the skin microbiome.
To achieve a higher recovery, a single product application under standardized laboratory conditions was done. The incubation time for the applied product was set to one hour to evaluate the stability of alpha-arbutin *in vivo* taking the results of Bang *et al.* (2008) (nearly complete degradation of alpha-arbutin in one hour in the presence of microbes *in vitro*) into account. The number of tape strips was increased to 14 to include deeper skin layers. Each pair of two sequentially taken tape strips was analysed to receive seven data points. Furthermore, a calibrated pipette was used for product dosing and application, and the glove fingertip that was used for product distribution on the defined skin area was analysed for alpha-arbutin as well to be included in the mass balance calculation.

Methodology:
-17 female volunteers participated (20-60years);
-application of 2mg/cm², 15µl 2% alpha-arbutin on 5X5cm at inner forearm (12.45mg)
Tape stripping was done, 1 hour after application, with 3.8 cm² SquameScan® 850A strips taking 60.8% of the application surface.
Tapes were analysed for alpha-arbutin and hydroquinone content using HPLC-UV according to an internal method developed by the applicant and validated for cosmetic formulations containing alpha-arbutin and hydroquinone. The LOD / LOQ for hydroquinone were 0.01 µg/mL and 0.03 µg/mL, respectively. For alpha-arbutin, the LOD / LOQ were 0.02 µg/mL and 0.06 µg/mL, respectively.

Conclusion by applicant: alpha-arbutin is stable *in vivo* in the presence of the skin microbiome and this result is consistent with the findings of 2021 (2022: 0.079% (SD 0,0034) hydroquinone formed and 2021: 0.073%).
In addition, a high level of mass balance recovery of 93.7% (SD 14.0) for alpha-arbutin closed the gap observed in the 2021 study where the mass balance question was not considered

entirely. In this new study, deeper layers of the skin were analysed by collecting 14 tape strips. As such, a low penetration rate for alpha-arbutin was shown.
Hydroquinone levels were low or below LOD, demonstrating the stability of alpha-arbutin both on skin surface and on deeper layers.

SCCS comment
The SCCS agrees with the outcome of the study.

3.1.10 Stability of β-arbutin

3.1.10.1. Stability data with β-arbutin as a raw material from SCCS/1550/15

A. Photostability test in aqueous solution (Test of 2006) – Submission I

The photostability of β-arbutin (3% and 6.3% solutions of Lot No TQI-771, TQI-772, TQI-773) was investigated in 10% ethanol or 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. Protected samples wrapped in aluminium foil were used as controls. 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. The quantitation limit of hydroquinone in the samples was 1 ppm.

(In house data, 2006)

B. Photostability at different pH values and temperatures (Test date 2008-9) – Submission I

A sample was tested with 7% β-arbutin in phosphate buffers ranging from pH 2.0-11.0. The samples prepared from Lot No 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 degrees (ICH guidelines). The effects of temperature were studied at 50°C for 1 month and 40 degrees Celsius for 3 months. The analyses were repeated 3 times for each lot of product. The LOQ for the detection of hydroquinone 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. At pH 2 a hydroquinone was detected in samples at 4,273.3 ppm at 50°C after 1 month and 3747.9 ppm at 40°C after 3 months. Only trace levels of hydroquinone were detected in neutral to basic solutions (pH 6-11).

(In house data, 2008)

C. Open literature data

In a thermal decomposition experiment, beta-arbutin (arbutin) started breaking down at 200°C and ends at 288°C. Non-volatile decomposition products detected are mainly hydroquinone, 1,6-anhydro-B-D-glucopyranose, 1,6-B-D-glucofuranose and alpha- and beta-D-glucose.

(Tanaka & Nakamura, 1981)

Arbutin (CIT Arbutin, batch number 96007, CAS 497-76-7) was tested in a thermo-degradability experiment. The experiment revealed an activation energy of 7.6 kcal/mol and a $t_{90\%}$ (time necessary to obtain a decrease of 10% of the initial concentration) of 15.4 days at 20°C. Half times were determined at several time intervals and pH 7, showing an increase in degradation rate with increasing temperature.

(Coiffard, Coiffard & De Roeck-Holtzhauer, 1999)

Arbutin was used in a photodegradation experiment in buffer solution at pH 7 and 9. The degradation rate was calculated from the slope of the line of area of the peak versus time. The photodegradation of arbutin in diluted aqueous solution apparently follows first-order kinetics. The pH of the solution was found to influence the photostability of arbutin. Shelf-life was impacted significantly at pH 5, 7 and 9, showing significant impact on the stability of arbutin. The authors concluded that overall, arbutin is a photostable compound.

(Couteau & Coiffard, 2000)

A photodegradation experiment was undertaken with arbutin at different conditions in aqueous or O/W microemulsions. Photostability was at its best in stabilised aqueous solutions at pH 7.0. At lower concentrations of $5.0x10^{-3}$, arbutin was reported to be still fairly photostable. At higher arbutin concentration ($9.2x10^{-3}$), corresponding to 0.25% w/w, arbutin appeared less stable. A slight increase in stability was observed when microemulsion components were added. Across all test conditions, the best stability was observed with arbutin dissolved in the microemulsions, suggested by the authors to be due to the complex interfacial structure. The further addition of perfumes to the microemulsions did not impact the photostability of arbutin.

(Gallarate *et al.*, 2004)

ß-Arbutin was tested (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).

(Yang CH *et al.* 2013)

SCCS comment
The test concentration is far below that used in cosmetic products; pH not given; the study of Yang *et al.* (2013) is considered to be of limited relevance. The Gallarate *et al.* (2014) study gave too low a concentration.

The photostability of (alpha- and) beta-arbutin in water or methanol was evaluated under normal light irradiation for at least 8 hours per day over 12 months. At the end of the storage period, no hydroquinone was detected in the samples, suggesting that no photo-catalysed degradation at daylight took place.

(Avonto *et al.*, 2016)

A forced degradation study was performed with commercially available arbutin. Arbutin appeared stable to alkaline hydrolysis but degraded significantly under acidic conditions (1 M HCl at 40°C) with 24.5% reduction in 48 hours. Arbutin was found stable in neutral conditions up to 72 h at 40°C. A further oxidative degradation experiment (0.3% H_2O_2, 23°C) demonstrated a gradual decrease in arbutin peak area (28.1% in 48h) accompanied by an increase in hydroquinone. LOQ values were 36.5 µg/mL and 1.4 µg/mL for arbutin and hydroquinone, respectively. The authors report a smaller hydroquinone peak area with oxidative degradation (1.4 ppm; 48h) compared to acidic hydrolysis (11.5ppm; 48h), which may be due to the formation of additional degradation products besides hydroquinone. It was furthermore shown that arbutin remains stable at 105°C up to 24 h, indicating a good thermal stability. Exposure to light neither impacted the stability of arbutin when exposed for 5 days. Arbutin degraded more easily when tested in its natural matrix compared to the isolated form. The authors note that bearberry leaves contain more than one phenolic compound that may release hydroquinone upon degradation.

(Braga *et al.*, 2020)

3.1.10.2. Stability data with β-arbutin in a cosmetic formulation from SCCS/1550/15

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

A study performed in 2008-2009 with Shiseido Product C (Lot 0804A, B, C) containing 5.67-6.93% β-arbutin at pH 5.0-7.0 determined the stability of beta-arbutin in finished cosmetic product at 40 degrees Celsius and a relative humidity of 75%. HPLC analysis was used to determine both beta-arbutin and hydroquinone concentrations in threefold for each batch at the start of the study, after 3 months and 6 months of storage. 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.

(In house data with Shiseido Product C, 2008)

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

A study performed in 2003 using the Shiseido Product C (lot A, B, C) containing 5.67-6.93% β-arbutin (pH 5.0-7.0) describes the long-term stability test of beta-arbutin in finished cosmetic product at a temperature of 25◦C and relative humidity of 60%. HPLC was used to determine beta-arbutin recovery and hydroquinone concentrations. This was performed in threefold at the start and end of the study. Samples of Shiseido Product C were kept in a transparent glass bottle at ambient conditions for 3 years. 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.

(In house data with Shiseido Product C, 2008)

C. Open literature data
The stability of both (alpha- and) beta-arbutin was determined in nine different commercially available cosmetic formulations (4 creams, 3 sera, 1 lotion, 1 gel). After a storage period of 16 months, sera samples showed losses of beta-arbutin ranging from 5.8% to 20.7%. Major losses were seen in the presence of basic solutions. Samples with a pH value above 4 did not affect the stability of beta-arbutin.

(Avonto *et al.*, 2016)

3.1.10.2. Release rate of hydroquinone from β-arbutin by skin microbiome

A. Skin metabolism after repeated topical application of β-arbutin in human volunteers – from SCCS/1550/15

A multiple topically dosed open study performed in 2005 determined the release rate of hydroquinone from beta-arbutin (Lot A, B, C) by the skin microbiome. Healthy volunteers (9 females and 9 males) aged 18-45, were chosen according to set in- and exclusion criteria, pre-study check-up and physical examination results. They were treated once 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 (open application). The application period was determined based on the excreting profile of hydroquinone in prior human studies. 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 not occluded 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. β-arbutin and hydroquinone were extracted from the dismembrated skin and analysed by high-resolution GC-MS. To describe the possible changes in urinary hydroquinone 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 hydroquinone levels of acid-hydrolysed urine samples, a total of 90 samples, were determined quantitatively by an LC system equipped with electrochemical detector (LC-ECD).
In all control skin biopsy samples, very low levels of hydroquinone (<1.1 ng quantitative limit) and β-arbutin (<8.9 ng quantitative limit) were present. In all treated skin samples, average hydroquinone 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 hydroquinone levels, corrected by creatinine for diuresis (< 4.19 – 9.16 mmol/mol), were established. The study authors concluded that repeated topical application of gel containing 6.3% (w/w) β-arbutin leads to detectable amounts of β-arbutin and hydroquinone 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 hydroquinone as well as for β-arbutin. Based on the large variation in the established urinary total hydroquinone results, changes in urinary hydroquinone levels due to topical treatment of β-arbutin could not be established. When the hydroquinone content in the skin samples is taken relative, on a weight-to-weight basis, to the β-arbutin + hydroquinone content, on average 4.6% (± 2.9) (range: 1.69-11.77) of hydroquinone is present in these skin samples. The actual levels of hydroquinone in treated skin amounted on average to 0.018 ± 0.016 µg/cm² (range 0.003 – 0.072).

(Ziegler H. & Heidl M., 2011)

SCCS comment

Under the in-use conditions described in human volunteers, hydroquinone is released in the skin to a relative level of 4.6% (compared to the β-arbutin + hydroquinone 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 hydroquinone. It may result from absorption of hydroquinone across the skin, together with a component resulting from dietary intake, since many foodstuffs contain hydroquinone or its precursor β-arbutin, resulting in mean urinary hydroquinone concentrations of 1.2±0.54 mg/L (Deisinger *et al.* 1996). In any individual, there is poor correlation between skin hydroquinone and urinary hydroquinone measured in spot urine samples; the volunteers kept a food diary but the contribution of dietary hydroquinone intake to urinary hydroquinone excretion was not assessed so that it is not possible to determine from the data provided which part of the excreted hydroquinone was derived from food or from dermal application of β-arbutin. Moreover, the method used for hydroquinone determination (LC-ECD) 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 (eg. Schindler *et al.* 2002; Quintus *et al.* 2005). Because of this lack of sensitivity, no conclusion can be drawn from the measurement of urinary hydroquinone in this experiment. Another study assessed the relative amounts of hydroquinone 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 β-arbutin concentration tested (6.3%) is slightly lower than the required 7%.

B. Open literature data

The stability of beta-arbutin was determined using pear peels as a surrogate *in vivo* model containing β-glucosidases and peroxidases. The pear peels were first depleted from their naturally present beta-arbutin. Alpha or beta-arbutin were added subsequently at 0.5 mg/g. After 8 hours of exposure, a loss of 62.4% (beta-arbutin) was measured. During degradation no hydroquinone could be measured. It was suggested that hydroquinone was not detectable due to chemical (hydroquinone degraded at pH>5 with a loss of 20% at pH 7.6 after 24h) or enzymatic instability (hydroquinone and arbutin are also a potential substrate of peroxidases). Thus, hydroquinone might be generated *in vivo* and rapidly degrade due to the cellular environment or rapidly convert into more stable end products.

(Avonto *et al.*, 2016).

General SCCS Comment on the stability of beta-arbutin

In the 2015 opinion (SCCS/1550/15), the SCCS came to the following conclusions with respect to:

- The stability of beta-arbutin as a **raw material:**

Beta-arbutin is photostable (ICH guideline) in H_2O or 10% ethanol for 3 months at 40°C and for 1 month at 50°C when pH>3. The pH is important, as an acidic pH lower or equal to 3 gives high and quick hydroquinone formation. Beta-arbutin is thermostable as shown by 24h stability at 105°C.

- Stability of beta-arbutin **in formulations**:

Beta-arbutin is stable at pH 5-7 for 6 months at 40°C and for 36 months at 25°C. Again, the pH is a crucial factor as in the open literature high instability is shown at pH < 4 with major loss of beta-arbutin.

- Stability *in vivo*, **on human skin:**

The data provided are inconclusive.

As no new data were submitted, it is not known how the human skin microbiome/metabolism affects the stability of beta-arbutin when present in creams applied on the skin.
Literature data points to possible effects of skin enzymes (Mier & van den Hurk1976, Redoules *et al.* 2005, Avonto *et al.* 2016) and bacteria (Bang *et al.* 2008) on the degradation/ metabolism of topically applied beta-arbutin with hydroquinone formation that quickly disappears.

3.2 FUNCTIONS AND USES

3.2.1 Functions 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 the 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.

On the contrary, alpha-arbutin (DSM product code 5033934; CAS No. 84380-01-8) is a synthetic substance. It is produced by enzymatic glycosylation of hydroquinone in the presence of α-Amylase and dextrin (Nishimura T., 1994; Sugimoto K., 2007). Structurally, it is therefore the *α*-isomer of arbutin (beta-arbutin), and like the β-form it is a competitive inhibitor of human tyrosinase with a K_i of 0.2 mM (Sugimoto *et al.*, 2003). α-Arbutin is used with laser therapy to treat refractory melasma (Polnikorn, 2010), and it has been suggested as an alternative to β-arbutin as a skin-lightening agent in topical preparations (Zhu and Gao, 2008). As the compound has the α-glycosidic configuration, it would not be expected to be a substrate for the β-glucosidases and hydrolysis would be sterically hindered (Redoules *et al.*, 2005). However, release of hydroquinone could also occur as α-glucosidases can be found in human skin fibroblasts and lysosomes (Tai *et al.*, 2010) and in Candida strains (Ciebada-Adamiec *et al.*, 2010). Extended use of ≥2% hydroquinone, topically applied, has been associated with adverse effects including exogenous ochronosis in dark-skinned people (Levin & Maibach, 2001; Levitt, 2007). 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%. Alpha-Arbutin is used in cosmetic formulations to lighten skin pigmentation. For this purpose, up to 2% of alpha-Arbutin is used in finished cosmetic products for face/neck care and up to 0.5% for body lotions. In DSM's internal Monitoring of New Product Trends and Innovations, a number of examples of finished cosmetic products containing alpha-Arbutin are given, also including cosmetics applied to the body.

(SCCS/1552/15; SCCS/1550/15)

Newly submitted information
According to the applicant, the use of alpha-Arbutin - mainly in facial creams to reduce the signs of ageing (e.g. age spots) or to brighten the skin tone - is very limited: only three new products with alpha-arbutin were launched in the EU (including UK) in 2019, which represents 0.004% of all new cosmetic product placements on the EU market (Mintel research tool). In 2020, only two products were launched in Europe containing alpha-arbutin. Amongst 69875 new products launched in the EU (including UK (United Kingdom)) in 2019, only twenty-four contained beta-Arbutin, representing less than 0.04% of all launched products. Around 50% of alpha- or beta-Arbutin containing products were launched on the UK market. In addition, there is no market overlap between alpha-Arbutin and beta-Arbutin in the EU. The main market of alpha-Arbutin and beta-Arbutin is Asia, with ten to fifteen times more new product launches containing either ingredient compared to the EU.

(DSM dossier, 2021)

3.2.2 Dermal / percutaneous absorption

3.2.2.1 Dermal / percutaneous absorption of α-arbutin:

Data from SCCS/1552/15

A. The *in vitro* percutaneous absorption of alpha-arbutin (submission I)

The *in vitro* percutaneous absorption of alpha-arbutin and the release of hydroquinone were determined with a cream formulation containing either 1% or 2% alpha-arbutin applied to thawed split-thickness skin taken from the right side of one 5 months old female Yucatan micropig. The analytical results of the cream formulation showed good agreement with the labelled concentrations. The percutaneous absorption and distribution of alpha-arbutin after 24-hours revealed only limited amounts in the epidermis (0.3% to 0.47% of applied dose) and not detectable amounts in the dermis and receptor chamber from both concentrations applied as cream. The surface wipe and apparatus accounted for 90% to 92% of the applied

dose. Recovery rate for the two experiments was 91% to 93% of applied test substance. The study results suggest that alpha-arbutin has only **low percutaneous absorption with less than 1% of applied substance** being found in the epidermis and nothing in the receptor chamber. The SCCS commented that the study is of limited value with regard to hydrolysis and degradation of alpha-arbutin in skin based on the study design. However, the results appear to be in line with other studies where **about 2% was recovered from pig skin**.

(Masaki J., 2006)

B. Pig ear skin *in vitro* penetration & distribution of alpha-arbutin (submission II)

In a non-GLP study, [Phenyl-U-^{14}C]-alpha-arbutin plus non-radio-labelled alpha-arbutin was added into o/w cosmetic-type formulations, in sufficient quantity to give 0.5% and 2.0% (w/w) concentrations in the dosing formulations. Fresh pig ears obtained from a local slaughterhouse were dermatomed at 400 µm thickness and the prepared fresh pieces applied to individual cells arranged in a series of flow through diffusion cells each having a 0.64 cm^2 treatable skin area. The formulations were applied at a target dose level of 2 mg formulation per cm^2 at a temperature of 32 °C under non-occluded conditions.

The overall recovery of radioactivity was 91.5 and 95.1 % of the dose applied for the formulations with 2 % and 0.5 % alpha-arbutin, respectively. Most of the radioactivity could be washed off and be removed by tape stripping of the stratum corneum. Minor amounts of radioactivity could be recovered from epidermis, dermis and receptor fluid.
The dosing formulations remained stable and did not show degradation after 10 and 17 days storage at room temperature. Based on the radioactivity recovered from epidermis, dermis and receptor fluid the amount of radioactivity absorbed **represented 2.1 % of the alpha-arbutin dose applied within 24 h**. This corresponds to 0.83 and 0.21 µg alpha-arbutin equivalents/cm^2 in 24 h **for the 2 % and 0.5 % formulations, respectively**. Differences in the percentage of radioactivity penetrated were not apparent when comparing results for the two concentrations used.

(Ruembeli *et al.* 2012a)

C. *In vitro* dermal absorption, distribution and metabolism in pig and human skin (submission II)

The three objectives of this non-GLP study (Ruembeli *et al.* 2012c) were to:
1. determine the *in vitro* dermal absorption and distribution of ^{14}C-α-arbutin in fresh pig ear skin and human upper leg skin (stored frozen);
2. determine the influence of skin microflora on the stability of alpha-arbutin; and
3. quantify alpha-arbutin and metabolites, including hydroquinone and non-extractables in each skin compartment after 24 hours of incubation in a diffusion cell system.

1. In vitro dermal absorption & distribution
The skin from 6 pig ears and 3 human donors was dermatomed to 600 µm thickness and the penetration of [phenyl-U-^{14}C]-alpha-arbutin through skin was investigated in a static system with diffusion cells having 5 cm^2 treatable skin area; the dosing form was an o/w formulation containing 2 % (w/w) alpha-arbutin and Euxyl PE 9010 as preservative system. Skin pieces were treated with antibiotic mixture of penicillin/streptomycin before mounting on the static cells. The doses were applied at a target dose level of 2 mg formulation per cm^2 and incubated at a temperature of 32 °C under non-occluded conditions.
The overall recovery of radioactivity was 93.6 % and 102.2 % of the dose applied for the series with pig and human skin, respectively. Most of the radioactivity could be washed off and be removed by tape stripping of the stratum corneum. Minor amounts of radioactivity could be recovered from epidermis, dermis and receptor fluid.

Based on the radioactivity recovered from epidermis, dermis and receptor fluid the amount of alpha-arbutin absorbed **within 24 h represented 2.13 % and 0.27 % of the dose applied**. This corresponds to 0.85 and 0.11 µg alpha-arbutin equivalents/cm² in 24 h **for pig and human skin**, respectively. Therefore, the penetration with human skin was about 8 times lower than with pig skin. The applicant concluded that a comparison of the *in vitro* penetration results across the two studies reveals a very high concordance for pig skin in the smaller surface area flow-through system and the static system despite the larger surface area. Further, the different doses of alpha-arbutin gave similar penetration results. These findings substantiate the relevance of the human skin results from the static system and the 8-fold lower penetration rate.

(Ruembeli *et al.*, 2012c)

2. Skin surface Stability to Microbes
See section 3.1.9.1.7 Stability of alpha-arbutin.

(Ruembeli *et al.*, 2012c)

3. Distribution of Metabolites in the Test System
From the 24h penetration experiments with pig and human skin with antibiotic pre-treatment described above, the recovered radioactivity from the individual skin compartments was characterized by radio-HPLC of the extracted samples. Previous experimental work revealed the importance of deactivating the intra-tissue enzymes immediately after skin separation and including ascorbic acid in the extraction process in order to retain the relative proportions of alpha-arbutin, hydroquinone and unknowns in the skin at termination.
As summarized in the previous section for antibiotic treated skin, after 24 h the skin surface for both pig and human skin did not show measurable degradation of the applied alpha-arbutin. Analysis of the human skin compartments showed that the extractable radioactivity was found mainly in the stratum corneum and epidermis and was predominantly alpha-Arbutin plus a small amount of hydroquinone and unknown. Non-extractable radioactivity was also measured at low amounts in each compartment. Pig skin compartments showed a similar relative distribution of the recovered radioactivity that reflected the about 8-fold higher penetration than human skin. These results, expressed as % of applied dose, are shown in the summary table. A species difference in the pattern of metabolites was not apparent with this test system.

(Ruembeli *et al.*, 2012c)

SCCS comment from SCCS/1552/15 on (Ruembeli et al., 2012c)
Although a different Franz cell set-up is used (static system with a diameter of 5 cm versus a dynamic system with a diameter of 9 mm in the previous study), a similar 2% dermal absorption of alpha-arbutin is found for pig skin. Total dermal absorption in human skin is 0.27 ± 0.13 %, i.e. much lower than that in fresh pig skin. In the human skin experiments, 8-day frozen skin was used, and samples from three donors only. In light of this, **the average value + 2 SD, i.e. 0.53 % is taken for further assessments of dermal absorption**.

Overall conclusion on in vitro dermal percutaneous absorption
No new data could be identified. As a result, the previous conclusion on alpha-arbutin from SCCS/1552/15 remains valid:
'The results indicate that human skin *in vitro* is about 8-fold less permeable to alpha-arbutin than pig skin, about 80% of recoverable material remains as alpha-arbutin with some increase in hydroquinone in the lower skin layers, and intact skin *in vitro* incubated with alpha-arbutin shows only limited release of hydroquinone. Homogenised pig skin and human skin each showed similar metabolism pathway, but with somewhat different kinetics related to the surrounding pH.
The use of antibiotics (1% penicillin/streptomycin solution) in the *in vitro* dermal penetration studies apparently reduced degradation/hydrolysis of alpha-arbutin by skin resident microbes on pig skin. But for human skin, information on the effect of skin resident microbes on the stability of alpha-Arbutin is lacking, as all incubations with human skin contained antibiotics.'

Percutaneous absorption *in vivo* from SCCS/1552/15
In a study with human volunteers who applied a gel with either 3% alpha-arbutin or 3% beta-arbutin or 1.9% hydroquinone, the (relative) amounts present in tape strips were assessed (Mamabolo *et al.*, unpublished results). The results provided could indicate a low conversion of arbutins, but this data allows no conclusions on dermal absorption.

3.2.2.2 Dermal / percutaneous absorption of β-arbutin

Data from SCCS/1550/15

A. *In vitro* percutaneous absorption

An *in vitro* dermal absorption study with ^{14}C β-arbutin (97.6% purity) from 2002 (GLP and guideline compliant) was described using human skin membranes. Either cream or gel formulations were applied to the skin membranes at doses ranging between 2.2-5.5 mg/cm², corresponding to 3.0% or 6.3% in formulation. 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. 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 24h exposure period. The SCCS commented that the study has some limitations: the exact composition of the test formulations was not given and no information was provided on the pH and impurities (hydroquinone). The tested concentrations of 3.0 and 6.3% β-arbutin were below the requested maximum concentration of 7%, and the number of human skin samples (2 replicates from 3 donors) was lower than that usually required (triplicate samples from 3 donors). Furthermore, the variability of the results was 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/cm² was used. This was said to be 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 (Meuling W.J.A., 2003) with volunteers after repeated application.

B. *In vivo* dermal / percutaneous absorption

A human study from 2005 was described with repeated topical application of β-arbutin during 4 days. Nine female and male subjects received 6.3% β-arbutin in a gel formulation. The test substance (141.5 mg of β-arbutin gel) was applied once per day, not occluded for 30 minutes on a delineated area of 50cm² on the right side of the upper buttock. The applied dose corresponds to about 2.83 mg of test formulation per cm². 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. Food and drink intake was monitored. Total hydroquinone levels of acid-hydrolysed urine samples, a total of 90 samples, were determined quantitatively by an LC system equipped with electrochemical detector (LC-ECD). The study authors concluded that repeated topical application of gel containing 6.3% (w/w) β-arbutin leads to detectable amounts of β-arbutin and hydroquinone 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 hydroquinone as well as for β-arbutin. Based on the large variation in the established urinary total hydroquinone results, changes in urinary hydroquinone levels due to topical treatment of β-arbutin could not be established. When the hydroquinone content in the skin samples is taken relative, on a weight-to-weight basis, to the β-arbutin + hydroquinone content, on average 4.6% (± 2.9) (range: 1.69-11.77) of hydroquinone is present in these skin samples. Actual levels of hydroquinone in treated skin amounted on average to 0.018 ± 0.016 µg/cm² (range 0.003 – 0.072).

(Meuling W.J.A., 2003)

SCCS comment on *in vivo* study by Meuling W.J.A. (2003)
Under the in-use conditions described in human volunteers, hydroquinone is released in the skin to a relative level of 4.6% (compared to the β-arbutin + hydroquinone 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 hydroquinone. It may result from absorption of hydroquinone across the skin, together with a component resulting from dietary intake, since many foodstuffs contain hydroquinone or its precursor β-arbutin, resulting in mean urinary hydroquinone concentrations of 1.2±0.54 mg/L (Deisinger *et al.* 1996). In any individual, there is poor correlation between skin hydroquinone and urinary hydroquinone measured in spot urine samples; the volunteers kept a food diary but the contribution of dietary hydroquinone intake to urinary hydroquinone excretion was not assessed so that it is not possible from the data provided to determine which part of the excreted hydroquinone is derived from food or from dermal application of β-arbutin. Moreover, the method used for hydroquinone 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 were reported in other studies (eg. Schindler *et al.* 2002; Quintus *et al.* 2005). Because of this lack of sensitivity, no conclusion can be drawn from the measurement of urinary hydroquinone in this experiment.
Another study assessed the relative amounts of hydroquinone 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.*, 2008). The results, discussed in more depth in Section 3.3.9., indicate low conversion of arbutins to hydroquinone, but this data allows no conclusions on percutaneous absorption.

SCCS comment
No new data was submitted for dermal absorption of beta-arbutin.

General SCCS comments on dermal absorption of alpha-and beta-arbutin
As no new dermal absorption studies have been submitted for alpha- and beta-arbutin, the value of 0.53% (0.27% +2SD), proposed in SCCS/1552/15 for the dermal absorption of alpha-arbutin, will be used. For beta-arbutin also the 2015 opinion data will be applied.

3.3 TOXICOLOGICAL EVALUATION

3.3.1. Irritation and corrosivity

As no new data were submitted, the data from SCCS/1550/15 and SCCS/1552/15 Opinions was taken over.

3.3.1.1 Skin irritation

Alpha-arbutin: non-irritant to rabbit skin (OECD 404)

Beta-arbutin: a 10% aqueous solution of beta-arbutin was slightly irritating to the skin.

(SCCS/1552/15 and SCCS/1550/15)

3.3.1.2 Mucous membrane irritation / eye irritation

Alpha-arbutin: only minimally irritant to rabbit eye (OECD 405).

Beta-arbutin: a 10% aqueous solution of beta-arbutin was not irritating to the rabbit eye.

(SCCS/1552/15 and SCCS/1550/15)

3.3.2 Skin sensitisation

Alpha-arbutin: (OECD 406): considered to be a mild sensitiser in guinea pigs.

Beta-arbutin: the study authors concluded that beta-arbutin did not possess skin sensitising potential under the test conditions used.

(SCCS/1552/15 and SCCS/1550/15)

New from open literature:

-No new data for alpha-arbutin

-New human data for beta-arbutin

(Matsuo *et al.*, 2015; Numata *et al.*, 2016; Oiso *et al.*, 2017)

SCCS conclusion
Alpha-arbutin is a weak skin sensitiser in animal studies; human data are not available. Beta-arbutin is not a skin sensitiser in animal studies. A limited number of human case studies have been reported for beta-arbutin between 2015 and now, demonstrating that, in humans, beta-arbutin is a rare cause of allergic contact dermatitis.

3.3.3 Acute toxicity

3.3.3.1 Acute oral toxicity

Alpha-arbutin: no new data was submitted.
From SCCS/1552/15 it is noted that acute oral toxicity tests with neat compound or with skin-whitening cream containing 5% or 10% alpha-Arbutin in rats (both Sprague-Dawley strains) provided LD50 estimates of 300-500 mg/kg bw, and of >125 or >250 mg/kg/bw

Beta-arbutin: no new data was submitted.
From SCCS/1550/1, it is noted 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.

(SCCS/1552/15 and SCCS/1550/15)

3.3.3.2 Acute dermal toxicity

Alpha-arbutin: no data available

Beta-arbutin: for rats and mice, the LD_{50} value via the dermal route showed to be greater than 928 mg/kg bw.

(SCCS/1552/15 and SCCS/1550/15)

3.3.3.3 Acute inhalation toxicity

Alpha-arbutin: no data available

Beta-arbutin: no data available

(SCCS/1552/15 and SCCS/1550/15)

3.3.4 Repeated dose toxicity

Alpha-arbutin: no conclusive data was available in the SCCS/1552/15 Opinion; no new data was submitted, and no open literature data are available.

Overall conclusion on repeated dose toxicity for alpha-arbutin
Only one study with repeated dermal application of alpha-arbutin was available in SCCS/1552/15. Due to its focus on irritation and the limited number of animals (3 guinea pigs of each sex), it is not suitable for conclusions on systemic effects or derivation of a NOAEL. No new data are available, therefore a MoS for alpha-arbutin cannot be calculated.

(SCCS/1552/15)

Beta-arbutin: In SCCS/1550/15 only limited data was present; no new data was submitted, and no open literature data is available.

Overall conclusion on repeated dose toxicity for beta-arbutin
As no new data is available and only a repeated dose 28-day oral study and 90-day dermal study with the rat revealed some sporadic observations that could not be related to beta-arbutin, the highest dosage tested of these studies was 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.

(SCCS/1550/15)

3.3.5 genotoxicity/mutagenicity

Alpha-arbutin

The genotoxicity of alpha-arbutin has been investigated in genotoxicity tests for gene mutations in bacteria and for structural and numerical chromosomal aberrations in an *in vivo* micronucleus test. Alpha-arbutin did not induce gene mutations in bacteria nor an increase in cells with micronuclei in bone marrow cells of mice. Based on this limited number of tests which, however, cover the 3 endpoints of genotoxicity, alpha-arbutin can be considered to have no *in vivo* genotoxic potential.
Arbutins (alpha-, beta- and deoxy-arbutin) are considered to be metabolised differently by different glycosidases, yet they all form hydroquinone upon hydrolysis. The latter is considered as the genotoxic moiety; therefore, a read across approach using hydroquinone release is a possibility to assess the conclusion on the genotoxic potential of the arbutins as a group.

(SCCS/1552/15)

New data from the open literature:

No new genotoxicity data on alpha-arbutin has been submitted or identified from the open literature.

Beta-arbutin

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 hydroquinone release is a possibility to assess the genotoxic potential of the arbutins as a group.

(SCCS/1550/15)

New data from the open literature:

Open literature data identified for beta-arbutin are summarised in Annex 1, tables 1-5.

Discussion:
Arbutin was tested ***in vitro*** using a mammalian cell micronucleus test in 3 different cell types. Using either isolated peripheral blood leukocytes (Jurica *et al.*, 2018; Benkovic *et al.*, 2021) or MCF-7 human breast cancer cells (Hazman *et al.*, 2021), the results had limited or low relevance and were either negative or inconclusive. In one of the 3 studies, Chinese hamster V79 cells were used and a *Hprt* gene mutation assay was carried out (Blaut *et al.*, 2006). Arbutin induced a weak genotoxic effect (-S9 fraction) at the high concentration of 10 mM. Application of bacterial extract considerably increased the mutagenic effect. The SCCS, however, considered the results of low relevance as the use of a bacterial extract is not relevant for the safety assessment of cosmetics.
Arbutin was also tested in an *in vitro* comet assay also using isolated human peripheral blood leukocytes (Jurica *et al.*, 2018), MCF-7 human breast cancer cells (Hazman *et al.*, 2021) or human peripheral blood leukocytes (Benkovic *et al.*, 2021). The results were of low relevance and inconclusive for the first two cell types and of limited relevance and negative for the MCF-7 cancer cell line.

One ***in vivo*** **study** was found in the open literature. A micronucleus test was done in arbutin-treated mice and the results were of limited relevance and negative (Nadi *et al.*, 2016).
Three comet assay studies in rats, exposed to arbutin, were inconclusive and of low relevance as well in hepatocytes (Jurica *et al.*, 2020), kidney cells (Jurica *et al.*, 2018) as in whole blood leukocytes (Jurica *et al.*, 2018).

After considering all new data from the open literature, the SCCS is of the opinion that the conclusions of Opinion SCCS/1552/15 are still valid.

3.3.6 Carcinogenicity

Information added in the commenting period by the applicant

As hydroquinone is classified as carcinogen cat 2 (H351) and mutagen cat 2 (H341), Life-time-risk (LTR) was calculated in the earlier published opinion. Now another reasoning is followed:
ECHA published an evaluation of hydroquinone, based on newly submitted genotoxic data: a transgenic rodent mutagenicity study and an *in vivo* comet assay, which both were negative. ECHA (2017) discussed the mechanisms of carcinogenicity and pointed out the higher susceptibility of male Fischer F344 rats to chronic progressive nephropathy, higher kidney exposure to toxic metabolites of hydroquinone in that rat strain, and the lack of direct DNA damage as well as lack of DNA adducts. It was said that negative responses in the transgenic rodent gene mutation assay and in the *in vivo* comet assay, would support aneugenic mode of action. Therefore, ECHA considered hydroquinone as a threshold carcinogen and derived

a BMDL10 of 15 mg/kg bw/day based on combined incidence of renal tubule hyperplasia and adenomas in a 2-year oral carcinogenicity study (ECHA 2017 and detailed in the hydroquinone REACH Dossier). The applicant followed that reasoning and also calculated MoS. The oral BMDL10 of 15 mg/kg bw/day is based on a chronic toxicity study, where the animals were dosed five days a week for 2 years. To correct from 5-days/week dosing to 7 days/week, a factor of 5/7 was used. No correction for bioavailability was needed, since kinetic studies showed nearly 100% bioavailability of hydroquinone (REACH dossier hydroquinone, section endpoint summary – toxicokinetics, metabolism and distribution). These results in **PODsys of 15 mg/kg bw/day x 5/7 = 10.7 mg/kg bw/day.**

According to the applicant, the MoS for hydroquinone in various exposure scenarios is as follows:

exposure from	SED	MoS
alpha-arbutin	0.00211 mg/kg bw/day	10.7 /0.00211=5100*
beta-arbutin	0.000763 mg/kg bw/day	14000*
aggregate	0.00287 mg/kg bw/ day	3700*

- All numbers have been rounded
- For beta-arbutin, no data were submitted and the values shown here are based on the SCCS 2015 opinion.

SCCS comment

The two *in vivo* studies on hydroquinone by Matsumoto *et al.* (2014) on TGR MutaMouse gene mutations and O'Donoghue *et al.* (2021) on the comet assay in rats' duodenum, kidney, liver and testes indicate that hydroquinone does not induce gene mutations or DNA strand breaks. Analysis of available literature data indicates that hydroquinone is not a directly acting DNA damaging agent and its genotoxicity is expressed predominately through an aneugenic mechanism when tested *in vitro* and following intra peritoneal administration. Testing by oral route in micronucleus test gives in overall negative results (McGregor *et al.*, 2007).

The SCCS agrees with ECHAs conclusion on hydroquinone that there is a potential threshold for inducing aneugenic effect by hydroquinone, and it would be above the chronic dose level for nephrotoxicity. Therefore, SCCS agrees with the calculation, performed by the applicant, of MoS for the carcinogenic effect resulting from induction of chronic progressive nephropathy by hydroquinone in chronically exposed F344 male rats as described in Hard, (1997).

Beta-arbutin

No new data was submitted

From SCCS/1550/15: A dermal carcinogenicity study in mice revealed that dosages up to 400 mg/kg bw/day of β-arbutin failed to induce dose-related tumour formation. No rationale was 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) revealed that, whether or not caused by aging alone, the general condition of the animals appeared to be poor.
No conclusion with regard to carcinogenicity could be drawn from this study.

(SCCS/1550/15)

3.3.7 Reproductive toxicity

Alpha-arbutin
no data available and no newly submitted data

Beta-arbutin
no data available and no newly submitted data

3.3.8 Special investigations

Beta-arbutin was shown to induce apoptosis in cancer cell lines (Safari *et al.* 2020, Hazman *et al.* 2021, Jiang *et al.* 2018, Yang *et al.* 2021, Ebadollahi *et al.*, 2021). However, in ARPE-19 cells in a high glucose environment, exposure to beta-arbutin appeared to reduce cell apoptosis (Ma *et al.* 2021). The remaining mechanistic data presents conflicting results related to inflammation and generation of cellular stress by arbutin. All the studies were considered of low relevance for the SCCS Opinion.
Detailed analysis of the data in a tabularised format is provided in Annex II which shows an overview of newly identified mechanistic studies from the open literature.

3.3.8 Photo-induced toxicity

Alpha-arbutin:
No new data was submitted
In studies conducted in guinea pigs neither phototoxicity nor photoallergy occurred with alpha-arbutin in doses up to 10% w/v in distilled water.

(SCCS/1552/15)

Beta-arbutin
No new data was submitted.
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.

(SCCS/1550/15)

3.4 SAFETY EVALUATION (including calculation of the MoS)

3.4.1 Alpha-arbutin

3.4.1.1. SCCS comments on the safety evaluation of alpha-arbutin by the applicant at the first publication

In the 2015 Opinions on alpha- and beta-arbutin (SCCS/1552/15, SCCS/1550/15), the effect of the human skin microbiome on the stability of the arbutins was not taken into consideration for the calculation of the margin of safety, although in these Opinions serious concern was expressed that these *in vivo* human stability data were missing.
During the last years, the general knowledge on the human microbiome has substantially increased, also with respect to the skin microbiome (Byrd *et al.*, 2018). Consequently, if

arbutins are significantly metabolised by the skin microbiome, hydroquinone could be generated which could give rise to benzoquinone. The latter is very reactive and could bind to different macromolecules. As such, the skin microbiome/enzymes could play an important role in the safety assessment of alpha-and beta-arbutin. Hydroquinone itself may cause exogenous ochronosis from a concentration of 1% and higher and leukomelanoderma. It has been banned for this reason in cosmetic products.

The release of hydroquinone from alpha-arbutin by the skin microbiome has been recently studied and submitted by the applicant (see 3.1.9.1.7).
The SCCS is of the opinion that this study is not acceptable for several reasons:
-the recovery of the parent compound is very low
-no data is provided on the fate of the substantial 'lost' fraction of alpha-arbutin
-no data is available on the total amount of hydroquinone released
-no general mass balance has been presented
-no data is presented on the metabolism of alpha- arbutin in the presence of the skin microbiome/enzymes (transformation into benzoquinone and binding to macromolecules).
Consequently, because of the existing uncertainty, the safety assessment and MoS calculation cannot be done and can only be considered if the formation of benzoquinone, possibly resulting from the conversion of alpha-arbutin into hydroquinone, could be excluded through experimental evidence.

3.4.1.2. Safety evaluation of alpha-arbutin submitted by applicant during commenting period

-exposure to face cream: 1.54 g (NoG) -exposure to body lotion: 7.84 g (NoG).	DA	0.53 %
Body weight	bw	60 kg
	Face cream	
Exposure to product	E product	1.54 g/day
Concentration substance	C	2 %
Systemic exposure dose	SED = (Eproduct X C/100 X DA/100 x 1000) / bw	0.00272 mg/kg bw/day
	Body lotion	
Exposure to product	E product	7.82 g/day
Concentration substance	C	0.5 %
Systemic exposure dose	SED = (Eproduct x C/100 x DA/100 x1000) / bw	0.00345 mg/kg bw/day

Aggregate exposure	
SED face cream	2.72 µg/kg bw/day
SED body lotion	3.45 µg/kg bw/day
SED aggregate	6.17 µg/kg bw/day

Repeated dose toxicity data for alpha-arbutin is not available. In the SCCS 2015 opinion it was discussed that systemic toxicity of alpha-arbutin would likely be due to hydroquinone formation.

Assuming the worst-case scenario, where all systemically available alpha-arbutin is cleaved to hydroquinone, the hydroquinone exposure is calculated by correcting the aggregated SED of 6.17 µg/kg bw/day with the respective molecular weight of alpha-arbutin (272.25 g/mol) and hydroquinone (110.11 g/mol) which is 2.50 µg/kg bw/day

→ **MoS**= 20mg/kg bw/d / 0.0025mg/kg bw/d = **8000 → safe**

3.4.1.3. SCCS comments after the commenting period

The SCCS is of the opinion that **alpha-arbutin used separately in a concentration of 2% in face cream and 0.5% in body lotion or used simultaneously are safe for human health.**
All calculations by the applicant used the amount of product per day assuming a default body weight of 60 kg. In the NoG, the consumptions per kg body weight per day are present for which the real body weights have been determined. The difference is limited and has as such no effect on the outcome.

3.4.2 Beta-arbutin

3.4.2.1. SCCS comments on the safety evaluation by the applicant at the first publication

No new data was submitted for beta-arbutin and no *in vivo* studies are available in the open literature that take the metabolism of beta-arbutin in the presence of the human skin microbiome/enzymes into consideration.

3.4.2.2. SCCS comments on the safety evaluation of beta-arbutin by the applicant after the commenting period

As no new data have been submitted by the applicant for beta-arbutin, **calculations can only be done for beta-arbutin and for aggregate exposure of both arbutins,** using for beta-arbutin the numbers as present in the SCCS opinion 2015.

The maximum exposure to beta-arbutin is 6.8 µg/kg bw/day (SCCS 2015).
In the reproduction toxicity study with subcutaneous administration of beta-arbutin, the conservative NOAEL was 100 mg/kg bw/day (SCCS 2015). If this NOAEL is applied, **the MoS is 14700.**

3.4.3 Hydroquinone

3.4.3.1. Safety evaluation of hydroquinone submitted by applicant during the commenting period

ALPHA-ARBUTIN

The exposure to hydroquinone via alpha- and/or beta-arbutin can occur via:
1. Cleavage of alpha- or beta-arbutin to hydroquinone in skin upon dermal penetration;
2. Hydroquinone as an impurity of alpha- or beta-arbutin;
3. Formation of hydroquinone from alpha- or beta-arbutin by skin microbiome on the skin.

The exposure to hydroquinone from each of these sources for alpha-arbutin is calculated below:
1. The aggregate exposure to alpha-arbutin from the use of face cream and body lotion containing 2% and 0.5% alpha-arbutin, respectively, is 6.17 µg/kg bw/day (see 3.4.1.2). As proposed before by the SCCS (2015), it is reasonable conservative to assume that 20% of the absorbed alpha-arbutin can be cleaved to hydroquinone or related products. Assuming

20% cleavage, the exposure to HQ is 20/100 X 6.17 µg/kg bw/day = **1.23 µg/kg bw/day** for a 60-kg person.

2. As reported in the stability studies provided by the applicant, hydroquinone was not detected (< LOQ 3 ppm) in the product at any time during the storage of up to three years. Therefore, the LOQ of 3 ppm is taken as a worst-case scenario for the exposure estimate. Based on the use of 1.54 g/day face cream containing 2% alpha-arbutin and the use of 7.82 g/day body cream containing 0.5% alpha-arbutin, the external hydroquinone exposure is: (1.54 g/day X 2/100 + 7.82 g/day X 5/100) X 0.000003 X 1000 = 0.00021 mg/day = 0.21 µg/day. Using a conservative 50% dermal absorption for hydroquinone, the SED is 0.105 µg/day or for a 60-kg person **0.00175 µg/kg bw/day.**

3. Based on the results of the new tape stripping study, on average 0.079 ± 0.034% of alpha-arbutin was degraded to hydroquinone on the human skin. To cover the upper range for the exposure calculation, 2SD are added to the mean, resulting in 0.15% formation of hydroquinone from the applied alpha-arbutin. The external exposure to alpha-arbutin from the use of 1.54 g/day of face cream containing 2% and the use of 7.82 g/day body cream containing 0.5% is 69.9 mg/day. Thus, the external hydroquinone exposure is 69.9 mg/day x 0.15/100 = 0.105 mg/day. With a dermal penetration of 50% the result is in line with the recent studies (Desmedt 2016) and the internal exposure to hydroquinone is 53 µg/day **or 0.883 µg/kg bw/day for a 60-kg person.**

total exposure to hydroquinone: exposure from sources 1-3 is added up.	**SED Hydroquinone**
1 (cleavage from ALPHA-ARBUTIN upon absorption)	1.23 µg/kg bw/day
2 (impurity of ALPHA-ARBUTIN)	0.00175 µg/kg bw/day
3 (formation from ALPHA-ARBUTIN via skin microbiome)	0.883 µg/kg bw/day
Total	**2.11 µg/kg bw/day**

BETA-ARBUTIN

No new data are available for Beta-Arbutin, and therefore the exposure to hydroquinone is **taken from the SCCS 2015 opinion**. **SED hydroquinone** from beta-arbutin exposure is 45.8 µg /day or **0.763 µg /kg bw/day for a 60-kg person**

MoS calculations

	HQ-SED	**MoS**	**SED** conservative	**MoS** conservative
Repeated dose toxicity NOAEL 20 mg/kg bw/d				
Alpha-arbutin	0.00211	20/0.00211 =**9500***	0.00211	**9500***
Beta-arbutin	0.000763 (2015opinion)	**26200***	0.003853 (+microbiome)	**5200***
Aggregate deterministic exposure	0.00287	**7000***	0.005963	**3300***
Reproduction toxicity NOAEL 15 mg/kg bw/d				
Alpha-arbutin	0.00211	15/0.00211 =**7100***	0.00211	**9500***
Beta-arbutin	0.000763	**19700***	0.003853	**3900***

(2015 opinion)	(2015opinion)		(+microbiome)	
Aggregate deterministic exposure	0.00287	**5200***	0.005963	**2500***

- All numbers have been rounded; SED$_{conservative}$ and MoS $_{conservative}$ take the worst cases of release of hydroquinone into consideration (by dermal absorption of the arbutins, impurities and contact with skin microbiome)

3.4.3.2. Safety evaluation of hydroquinone: induction of ochronosis

Lowest dose to cause ochronosis is 8 mg/day (2015 opinion).	SED	**SED ratio**
Alpha-arbutin	0.127 mg/day	8/ 0.127=**63**
Beta-arbutin	0.046 mg/day	8/0.046=**174**
(2015 opinion) Aggregate deterministic exposure	0.173 mg/day	8/0.173=**46**

3.4.3.3 Comments by the SCCS after commenting period

The SCCS is of the opinion that the use of **alpha-arbutin** in face cream and body lotion at a concentration of 2% and 0.5%, respectively, is safe.
The hydroquinone that could become available in the worst-case scenario from alpha-arbutin, namely the sum of the amounts formed when cleavage of alpha-arbutin to hydroquinone in skin upon dermal penetration is considered together with the amount present as impurity and the formation coming from alpha-arbutin by the action of the skin microbiome, is considered safe.

For **beta-arbutin**, no new data were submitted. Only the data from the 2015 opinion were available.
The SCCS is of the opinion that the use of **beta-arbutin** in face cream at a concentration of 7% is safe.
The hydroquinone that could become available in the worst-case scenario from beta-arbutin, namely the sum of the amounts formed when cleavage of beta-arbutin to hydroquinone in skin upon dermal penetration is considered together with the amount coming from beta-arbutin by the action of the skin microbiome, is considered safe. Reasoning is as follows:
- Alpha-and beta-arbutin are both glucosides and stereoisomers that only differ in a beta- or alpha-anomer of D-glucose for beta- or alpha-arbutin, respectively. When hydrolysed, both release hydroquinone.
- The conservative assumption is made that beta-arbutin, in contact with the skin microbiome, releases hydroquinone in a similar way as observed for alpha-arbutin, taking into consideration that 7% beta-arbutin is present in face cream instead of 2% of alpha-arbutin → 0.00309 mg/kg bw/day hydroquinone is maximally released.

Aggregate exposure of alpha- and beta-arbutin is considered safe. It should be noted that no new data were made available for beta-arbutin and the real interaction with the skin microbiome is not known. Therefore, a worst-case scenario has been applied for the potential maximal release of hydroquinone by beta-arbutin upon skin contact.

When the possibility of occurrence of ochronosis is considered, all exposures considered are safe.

DISCUSSION

Physicochemical properties and stability

From the previous Opinion (SCCS/1552/15), open literature data and newly submitted data for alpha-arbutin, it can be concluded that:

- Alpha-arbutin is stable for 36 months as raw material when appropriately packed and kept < 40°C.
- Alpha-arbutin is stable ***in vitro*** at 2% in aqueous solutions and in cosmetic products (o/w cream, sunscreen, serum) for at least 3 months when temp < 40°C and 4.5 < pH < 7.5 under daylight.
- Alpha arbutin is not stable ***in vivo***, in the presence of skin microbiome/enzymes; the newly submitted data does not appropriately answer the question about the effect of the skin microbiome/enzymes on the stability of alpha-arbutin. Literature data and the newly submitted *in vivo* study point to significant degradation/metabolism of alpha-arbutin on the skin by enzymes and bacteria with the potential formation of hydroquinone. The latter could quickly disappear, possibly with adduct formation with cellular macromolecules. This could play a role in adversity, in particular when chronic exposure occurs, which is the case for skin bleaching agents.

Already in the 2015 opinion, it was clearly mentioned that there was uncertainty about whether the conversion of alpha-arbutin topically applied as cutaneous hydrolysis into hydroquinone could be present due to enzymatic activity through alpha-glucosidases (Mier & van den Hurk 1976) or via the activity of microbes residing in or on the surface of the skin (Bang *et al.* 2008).

Exposure assessment & toxicokinetics

Hydroquinone is an Annex II substance that is not allowed in cosmetics. It is classified as Carc Cat 2 H351 (suspected of causing cancer) based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI1.
As the mechanism of action of alpha-arbutin is for the most part based on the release of hydroquinone, which inhibits the tyrosinase activity in the skin, it is important to get reliable exposure data which takes the *in vivo* formation of hydroquinone on the skin and its further fate into consideration.
During the commenting period, a new tape-strip study on human volunteers was submitted by the applicant in which the release of hydroquinone from alpha-arbutin (with mass balance) was measured when the compound was in contact with the skin microbiome. The amounts of hydroquinone thus released were taken into consideration in the safety evaluation of alpha-arbutin when present as 2% and 0.5% in face cream and body lotion, respectively.

Toxicological Evaluation

Irritation and corrosivity: the conclusions of 2015 Opinion are taken over, namely Alpha-arbutin is non-irritant to rabbit skin and a 10% aqueous solution of beta-arbutin is slightly irritating to the skin.

Skin sensitisation: conclusions of 2015 Opinion are taken over, namely alpha-arbutin is a weak skin sensitiser in animal studies; human data are not available.

Beta-arbutin is not a skin sensitiser in animal studies. As a limited number of human case studies have been reported for beta-arbutin between 2015 and now, it is confirmed that for humans, beta-arbutin is a rare cause of allergic contact dermatitis.

Repeated dose toxicity*:* no new data and previous data are inconclusive for both arbutins.

Reproductive toxicity: no data available

Mutagenicity / genotoxicity

For alpha-arbutin, no new data was submitted by the applicant and no recent publications were available in the open literature. Therefore, the SCCS considers that the conclusion of the SCCS/1550/15 Opinion is still valid and consequently considers alpha-arbutin to be non-mutagenic.

For beta-arbutin, no new data was submitted by the applicant, but a number of recent *in vitro* and *in vivo* studies were present in the open literature (Annex I, tables 1-5). After analysis, the SCCS is of the opinion that the previous Opinion (SCCS/1552/15) is still valid and consequently considers beta-arbutin to be non-mutagenic.

Carcinogenicity

As no new data was submitted, the reasoning of ECHA for arbutin (ECHA 2017) was followed. Namely, hydroquinone was considered by ECHA as a threshold carcinogen and a BMDL10 of 15 mg/kg bw/day could be derived by the applicant, based on combined incidence of renal tubule hyperplasia and adenomas in a 2-year oral carcinogenicity study (ECHA 2017).

Photo-induced toxicity: as no new data were submitted, the conclusions of previous Opinion were taken over: neither arbutin has any phototoxic or photoallergic potential.

Human data: the importance of knowing the effects of the human skin microbiome is discussed under 'discussed under exposure assessment & toxicokinetics'.

4. CONCLUSION

1. *In light of the data provided, does the SCCS consider α-arbutin safe when used in face creams up to a maximum concentration of 2% and in body lotions up to a maximum concentration of 0.5 %?*

   The SCCS is of the opinion that alpha-arbutin used in face creams up to a maximum concentration of 2% and in body lotions up to a concentration of 0.5% is safe, also when used together.

2. *In the event that the estimated exposure to α-arbutin from cosmetic products is found to be of concern, SCCS is asked to recommend safe concentration limits.*

   Not applicable

3. *In light of the data provided, does the SCCS consider β-arbutin safe when used in face creams up to a maximum concentration of 7%?*

   The SCCS is of the opinion that beta-arbutin used in face creams up to a maximum concentration of 7% is safe.

4. *In the event that the estimated exposure to β-arbutin from cosmetic products is found to be of concern, SCCS is asked to recommend safe concentration limits.*

   Not applicable

5. *In light of the data provided, does the SCCS consider that the presence of hydroquinone in the cosmetic formulations must remain below 1 ppm for both α- and β-arbutin containing products?*

   Hydroquinone should remain as low as possible in formulations containing alpha-or beta-arbutin and should not be higher than the unavoidable traces in both arbutins. In the new studies, submitted by the applicant, 3ppm was the LOQ for hydroquinone and 1ppm for the LOD.

6. *Does the SCCS have any further scientific concerns regarding the use of a- and β-arbutin in cosmetic products in relation to aggregate exposure from such substances in cosmetics?*

   Aggregate exposure of alpha-arbutin (2% in face cream and 0.5% in body lotion) with beta-arbutin (7% in face cream) are considered safe.

5. MINORITY OPINION

/

6. REFERENCES

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7. GLOSSARY OF TERMS

See SCCS/1628/21, 11th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation.

8. LIST OF ABBREVIATIONS

See SCCS/1628/21, 11th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation

9. ANNEX I: Overview of newly identified genotoxicity studies from the open literature

Alpha-arbutin mutagenicity/genotoxicity
No new genotoxicity data on alpha-arbutin has been submitted or identified from open literature.

Beta-arbutin mutagenicity/genotoxicity
The open literature about beta-arbutin with regard to genotoxicity has been identified and summarised in tables below.
For bacterial gene mutation no new data could be retrieved.

In most articles beta-arbutin is indicated as arbutin without further explication, which is quite confusing

Table 1: *In vitro* mammalian cell micronucleus test/ chromosomal aberrations – 3 new studies

Test system/ Test object	Exposure conditions (concentration/duration/metabolic activation	Information on the characteristics of the test substance including source/manufacturer, CAS number, purity of the test material	Result as evaluated by SCCS	Reliability / Comments	Relevance of the result as evaluated by SCCS	Authors & year
Micronucelus test (CBMN) on isolated peripheral blood leukocytes (1 male donor; Histopaque 1077 reagent)	Arbutin concentrations: 11.4, 200, 400 µg/mL. Exposure for 24 h: 2 separate experiments. Bleomycin (1.25 µg/mL) was used as a positive control. A total of 2000 binucleated (BN) cells was examined for each treatment (1000 BN cells per each replica). The same slides were used to study the nuclear division index (NDI), by screening 1000 cells per sample (500 cells per slide).	Arbutin (4-hydroxyphenyl-b-D-glucopyranoside; CAS Number: 497-76-7) purchased from Sigma-Aldrich (Steinheim, Germany).	**Negative**	**2** Study not according to OECD TG or GLP. Only condition without metabolic activation was used. No data on historical controls were provided.	**Limited**	Jurica K, Brčić Karačonji I, Mikolić A, Milojković-Opsenica D, Benković V, Kopjar N. In vitro safety assessment of the strawberry tree (Arbutus unedo L.) water leaf extract and arbutin in human peripheral blood lymphocytes. Cytotechnology. 2018 Aug;70(4):1261-1278. doi: 10.1007/s10616-018-0218-4.
Micronucleus test	Human breast adenocarcinoma (MCF-7) cells. Exposure time: 24 h Exposure concentrations: 0.15 mM (LD0); 2.3 mM (0.63 mg/mL, LD10) and 69.9 mM (19 mg/mL, LD50). The cells in the samples in each group were spread on clean slides. The dried slides were fixed in pure ethanol for 20 min. The slides were then air dried. The	ß-arbutin (ab141906, Abcam, England)	**Inconclusive**	**3** No positive control was used. No data on historical controls. MN frequencies (up to 0.09%) can be well within normal	**Low**	Hazman, Ö., Sarıova, A., Bozkurt, M. F., & Ciğerci, İ. H. (2021). The anticarcinogen activity of β-arbutin on MCF-7 cells: Stimulation of apoptosis through estrogen receptor-α signal pathway, inflammation and genotoxicity. Molecular and Cellular Biochemistry, 476(1), 349-360.

Test system/ Test object	Exposure conditions (concentration/duration/metabolic activation	Information on the characteristics of the test substance including source/manufacturer, CAS number, purity of the test material	Result as evaluated by SCCS	Reliability / Comments	Relevance of the result as evaluated by SCCS	Authors & year
	dried slides were stored in 5% giemsa solution for 20 min to stain the cell nuclei. The analysis was conducted with three preparations of each sample. The counting was conducted for 1000 cells at 100X in each preparation. Micronucleus index was analyzed for an average of 1000 cells.			historical range. Validity of MCF-7 cell line not confirmed according to OECD 487. Basic details on methodology not been provided (e.g. the laconic description indicates using cytochalasin, but no). No data on CBPI, RI or %cytostatis Concentration of 19 mg/mL is very high (maximum allowable concentration by OECD 487 is 2 mg/mL).		

Test system/ Test object	Exposure conditions (concentration/duration/metabolic activation	Information on the characteristics of the test substance including source/manufacturer, CAS number, purity of the test material	Result as evaluated by SCCS	Reliability / Comments	Relevance of the result as evaluated by SCCS	Authors & year
				Considering high concentrations used, it is not clear if and how some changes of the culture medium (e.g. pH) could influence the results.		
Micronucleus cytome assay Human leucocytes 3 donors	Potential radioprotective properties of arbutin in concentrations of 11.4 µg/mL, 57 µg/mL, 200 µg/mL and 400 µg/mL administered alone and as a pre-treatment for one hour before exposing human leukocytes to ionising radiation at a therapeutic dose of 2 Gy Altogether 3000 binucleated (BN) cells were examined for each treatment (1000 BN cells per donor). Positive control – irradiated cells 2Gy	arbutin (4-hydroxyphenyl-β-D-glucopyranoside; CAS Number: 497-76-7)	**Negative**	**2** Study not according to OECD TG or GLP. Only condition without metabolic activation No data on historical controls	**Limited**	Benković V, Marčin N, Horvat Knežević A, Šikić D, Rajevac V, Milić M, Kopjar N. Potential radioprotective properties of arbutin against ionising radiation on human leukocytes in vitro. Mutat Res Genet Toxicol Environ Mutagen. 2021 Dec;872:503413. doi: 10.1016/j.mrgentox.2021.503413.

Table 2: *In vitro* mammalian cell gene mutation assays: 1 new study

Test system/ Test object	Exposure conditions (concentration/duration/metabolic activation)	Information on the characteristics of the test substance including source/manufacturer, CAS number, purity of test material	Result as evaluated by SCCS	Reliability/ Comments	Relevance of the result as evaluated by SCCS	Authors & year
Hypoxanthine guanine phosphoribosyltransferase (HPRT) assay Chinese hamster V79 lung fibroblasts	Arbutin (dissolved in water) and/or cytosolic fraction prepared from B. distasonis, E. ramulus or E. coli was added to $1.5x10^6$ V79 cells. Arbutin (0.1-10 mM) and the bacterial cell extracts for 3 days. Expression time: 12 days Mutation frequency represented by the number of 6-TG-resistant mutants per 10^6 viable cells.	Arbutin, no source indicated.	without metabolic activation system, negative(10mM) A slight increase in the mutant frequency only observed at highest concentration of 10 mM [(13.2 ± 0.5) $x10^6$, as compared to (3.5 ± 0.5) $x10^6$ in the negative control]. **Strongly positive with cytosolic preparations from a bacterial species showing high arbutin-glycosidase activity (B.**	**3** A trace of HQ, possibly present in the sample of arbutin used or its formation by V79 enzymes, could have led to the effect observed. Study not according to OECD TG and GLP. No historical control data. Use of bacterial extract has low relevance for use of	**Low**	Blaut, 2006

Test system/ Test object	Exposure conditions (concentration/duration/metabolic activation)	Information on the characteristics of the test substance including source/manufacturer, CAS number, purity of test material	Result as evaluated by SCCS	Reliability/ Comments	Relevance of the result as evaluated by SCCS	Authors & year
			distasonis, E. ramulus).	arbutin in cosmetics.		

Table 3: *In vitro* DNA damage (e.g. Comet assay) – 3 new studies

Test system/ Test object	Exposure conditions (concentration/duration/metabolic activation)	Information on the characteristics of the test substance including source/manufacturer, CAS number, purity of the test material	Result as evaluated by SCCS	Reliability / Comments	Relevance of the result as evaluated by SCCS	Authors &_year
Comet assay isolated peripheral blood leukocytes (1 male donor; Histopaque 1077 reagent)	Arbutin concentrations: 11.4, 200, 400 µg/mL. Exposure for 24 h: 2 separate experiments. H2O2 (100 µM for 10 min on ice) used as a positive control. Comet Assay IV software was used to measure comet parameters (tail length in micrometres and tail intensity, i.e. DNA % in tail). Duplicate slides were prepared and two hundred independent comet measurements per sample per experimental point were performed (2	Arbutin (4-hydroxyphenyl-b-D-glucopyranoside; CAS Number: 497-76-7) purchased from Sigma-Aldrich (Steinheim, Germany).	**Inconclusive**	**3** Very high range of min-max values for the comet parameters, especially for H2O2 results. This decreases reliability of the whole study. No data on historical controls	**Low**	Jurica K, Brčić Karačonji I, Mikolić A, Milojković-Opsenica D, Benković V, Kopjar N. In vitro safety assessment of the strawberry tree (Arbutus unedo L.) water leaf extract and arbutin in human peripheral blood lymphocytes. Cytotechnology. 2018 Aug;70(4):1261-1278. doi: 10.1007/s10616-018-0218-4.

Test system/ Test object	Exposure conditions (concentration/duration/metabolic activation)	Information on the characteristics of the test substance including source/manufacturer, CAS number, purity of the test material	Result as evaluated by SCCS	Reliability / Comments	Relevance of the result as evaluated by SCCS	Authors &_year
	independent experiments).					
Comet assay	Human breast adenocarcinoma (MCF-7) cells. Exposure time: 24 h Exposure concentrations: 0.15 mM (LD0); 2.3 mM (0.63 mg/mL, LD10) and 69.9 mM (19 mg/mL, LD50). Cellular suspension was mixed with 100 µL low melting agarose (LMA) in eppendorf tubes. All cell-LMA mixture in the eppendorf was prepared on the slides treated with normal melting agarose (NMA) one day before. After the lysis and	ß-arbutin (ab141906, Abcam, England)	**Inconclusive**	**3** Description of basic test parameters have not been provided. It is not clear how the comet slides were prepared and scored. No positive control substance was used. Considering high concentrations used, it is not clear if and how some	**Low**	Hazman, Ö., Sarıova, A., Bozkurt, M. F., & Ciğerci, İ. H. (2021). The anticarcinogen activity of β-arbutin on MCF-7 cells: Stimulation of apoptosis through estrogen receptor-α signal pathway, inflammation and genotoxicity. Molecular and Cellular Biochemistry, 476(1), 349-360.

Test system/ Test object	Exposure conditions (concentration/duration/metabolic activation)	Information on the characteristics of the test substance including source/manufacturer, CAS number, purity of the test material	Result as evaluated by SCCS	Reliability / Comments	Relevance of the result as evaluated by SCCS	Authors &_year
	electrophoresis steps, each preparation was stained. The stained preparations were scored by counting 100 cells under a fluorescence microscope according to Singh *et al.* (1988).			changes of the culture medium (e.g. pH) could influence the results. No data on historical controls were provided.		
Comet assay Human leucocytes 3 donors	Potential radioprotective properties of arbutin in concentrations of 11.4 µg/mL, 57 µg/mL, 200 µg/mL and 400 µg/mL administered alone and as a pre-treatment for one hour before exposing human leukocytes to ionising radiation at a therapeutic dose of 2 Gy.	arbutin (4-hydroxyphenyl-β-D-glucopyranoside; CAS Number: 497-76-7)	**Negative**	***2*** High range of min-max values No data on historical controls were provided.	**Limited**	Benković V, Marčin N, Horvat Knežević A, Šikić D, Rajevac V, Milić M, Kopjar N. Potential radioprotective properties of arbutin against ionising radiation on human leukocytes in vitro. Mutat Res Genet Toxicol Environ Mutagen. 2021 Dec;872:503413. doi: 10.1016/j.mrgentox.2021.503413.

Test system/ Test object	Exposure conditions (concentration/duration/metabolic activation)	Information on the characteristics of the test substance including source/manufacturer, CAS number, purity of the test material	Result as evaluated by SCCS	Reliability / Comments	Relevance of the result as evaluated by SCCS	Authors &_year
	100 nuclei were measured per each slide. Using a computer image analysis system Comet Assay IV™ software comet descriptors- tail length, tail intensity (% DNA in tail) and tail moment. Agarose microgels 600 comets per sample H2O2 (100 µM for 10 min on ice) used as a positive control.					

Table 4: *In vivo* Micronucleus test – 1 new study

Test system/Test object	Exposure conditions (concentration/duration/ metabolic activation)	Information on the characteristics of the test substance including source/manufacturer, CAS number, purity of the test material	Result as evaluated by SCCS	Reliability/ Comments	Relevance of the result as evaluated by SCCS	Authors_year
NMRI mice	Arbutin at 200 mg/kg was intraperitoneally (ip) and the bone marrow collected after 24. Additionally, arbutin (50, 100, and 200 mg/kg) was intraperitoneally (ip) administered to NMRI mice 2 hours before gamma radiation at 2 and 4 gray (Gy). The frequency of micronuclei in 1000 PCEs (MnPCEs) and the ratio of PCE/PCE+NCE were calculated for each sample.	Pure (98%) arbutin powder from Sigma. No information which arbutin was used for the study.	Negative Arbutin significantly protected against the effects of gamma irradiation.	2 Only one dose was used which did not induce PCE/NCE change. Study not according to OECD TG or GLP status.	Limited	Nadi, S., Msc, A. S. M., Mozdarani, H., Mahmodzade, A., & Pouramir, M. (2016). Effects of arbutin on radiation-induced micronuclei in mice bone marrow cells and its definite dose reduction factor. Iranian journal of medical sciences, 41(3), 180.

Table 5: *In vivo* Comet assay – 3 new studies

Test system/Test object	Exposure conditions (concentration/duration)	Information on the characteristics of the test substance including source/manufacturer, CAS number, purity of the test material	Result as evaluated by SCCS	Reliability/ Comments	Relevance of the result as evaluated by SCCS	Authors, year
Comet assay on rat hepatocytes	Arbutin was orally administered at dose of 200 mg/kg bw (rat males and females N=4 per group) for 14 or 28 days. Animals were sacrificed 24 hours after the last dose administration. No details on preparation of single hepatocyte suspension was provided. DNA damage in individual cells was assessed with an image analysis system Comet Assay IV.	Arbutin (4-hydroxyphenyl-b-D-glucopyranoside; CAS Number: 497–76-7) from Sigma-Aldrich (Steinheim, Germany).	**Inconclusive**	**3** Very high range of min-max values noted for the comet parameters, especially for EMS results. This decreases reliability of the whole study. Only one dose used. No historical control values	**Low**	Jurica K, Benković V, Sikirić S, Kopjar N, Brčić Karačonji I. Liver function and DNA integrity in hepatocytes of rats evaluated after treatments with strawberry tree (Arbutus unedo L.) water leaf extract and arbutin. Drug Chem Toxicol. 2020 Mar;43(2):127-137. doi: 10.1080/01480545.2018.1477794.

Test system/Test object	Exposure conditions (concentration/duration)	Information on the characteristics of the test substance including source/manufacturer, CAS number, purity of the test material	Result as evaluated by SCCS	Reliability/ Comments	Relevance of the result as evaluated by SCCS	Authors, year
	EMS administered by oral gavage at 300 mg/kg for 24 h. Mean values of 200 independent comet measurements per each experimental group (50 comets/rat were scored on duplicate slides).					
Comet assay on rat kidney cells	Arbutin was orally administered at dose of 200 mg/kg bw (rat males and females N=4 per group) for 14 or 28 days. Animals were sacrificed 24 hours after the last dose administration. DNA damage in individual cells was assessed with an image analysis	Arbutin (4-hydroxyphenyl-b-D-glucopyranoside; CAS Number: 497–76-7) from Sigma-Aldrich (Steinheim, Germany).	**Inconclusive**	**3** Very high range of min-max values was noted for the comet parameters. This decreases reliability of the whole study. No information on a positive control substance was provided.	**Low**	Jurica K, Vesna Benković, Sunčana Sikirić, Irena Brčić Karačonji & Nevenka Kopjar (2018): The effects of strawberry tree (Arbutus unedo L.) water leaf extract and arbutin upon kidney function and primary DNA damage in renal cells of rats, Natural Product Research, DOI: 10.1080/14786419.2018.1534106

Test system/Test object	Exposure conditions (concentration/duration)	Information on the characteristics of the test substance including source/manufacturer, CAS number, purity of the test material	Result as evaluated by SCCS	Reliability/ Comments	Relevance of the result as evaluated by SCCS	Authors, year
	system Comet Assay IV. A total of 50 comets per rat (25 from each of two replicate slides) were scored.			Only one dose, two exposure times. No historical control values provided.		
Comet assay on whole blood leucocytes	Arbutin was orally administered at dose of 200 mg/kg bw (rat males and females N=4 per group) for 14 or 28 days. Animals were sacrificed 24 hours after the last dose administration. DNA damage in individual cells was assessed with an image analysis system Comet Assay IV. A total of 50 comets per rat (25 from each	Arbutin from Sigma-Aldrich (Steinheim, Germany).	**Inconclusive**	**3** Although Standard Error values were relatively small, very high range of min-max values was noted for the comet parameters. This decreases reliability of the whole study. No information on a positive control substance. No historical control values	**Low**	Jurica K, Brčić Karačonji I, Kopjar N, Shek-Vugrovečki A, Cikač T, Benković V. The effects of strawberry tree water leaf extract, arbutin and hydroquinone on haematological parameters and levels of primary DNA damage in white blood cells of rats. J Ethnopharmacol. 2018 Apr 6;215:83-90. doi: 10.1016/j.jep.2017.12.039.

Test system/Test object	Exposure conditions (concentration/duration)	Information on the characteristics of the test substance including source/manufacturer, CAS number, purity of the test material	Result as evaluated by SCCS	Reliability/ Comments	Relevance of the result as evaluated by SCCS	Authors, year
	of two replicate slides) were scored.			Only one dose used, 2 exposure times.		

ANNEX II: Overview of newly identified mechanistic studies from the open literature

Alpha-arbutin:

Test	Compound	Dose	Tissue	Result	Remark	Reference
MTT method (cytotoxicity)	Alpha-arbutin	0.5mM, 1mM, 10mM, 25mM, 50mM, 100mM 200mM	human breast adenocarcinoma (MCF-7) cells	At the lower concentrations (1–10 mM), α-arbutin appears to be more toxic than β-arbutin. At higher (5-200 mM) and LD50 (69.6 mM) concentrations β-arbutin toxicity appears to be higher than α-arbutin.		Hazman, Ö., Sarıova, A., Bozkurt, M. F., & Ciğerci, İ. H. (2021). The anticarcinogen activity of β-arbutin on MCF-7 cells: Stimulation of apoptosis through estrogen receptor-α signal pathway, inflammation and genotoxicity. Molecular and Cellular Biochemistry, 476(1), 349-360.

Beta-arbutin:

Test	Compound	Dose	Tissue	Result	Remark	Ref
Cell proliferation (MTT assay), cell cycle analysis and Western blotting	Arbutin (hydroquinone-O-d-glucopyranoside)	Cell viability: 0–500 μg/ml for 24 h cell proliferation: 0-100 μg/ml for 0–4 days	TCCSUP human urinary bladder carcinoma cells	Arbutin significantly decreased TCCSUP cell proliferation in a concentration- and time-dependent manner. Furthermore, cell cycle analysis revealed that arbutin strongly disrupted the cell cycle in a time-dependent manner. Western blot analysis demonstrated that arbutin led to the inactivation of extracellular signal-regulated kinase (ERK), which is known to critically regulate cell proliferation. In addition, arbutin markedly increased the expression of p21WAF1/CIP1		1

				(p21), which is known to be involved in cell cycle regulation.		
MTT assay (cell viability), quantification of 2,7-dichlorodihydrofluorescein diacetate (H2DCFDA), apoptosis via Annexin V-FITC kit and PI solution, IL-1β determination by ELISA, expression of IL-1β and TNF-α using real-time PCR	Arbutin	Cytotoxicity: 0 to 2000 µM ROS assay: 10, 100 and 1,000 µM IL-1β determination and expression of IL-1β and TNF-α genes: 100 and 1,000 µM for 24h	LNCaP cell line (androgen-responsive in vitro model of human prostate carcinoma)	Arbutin significantly decreased ROS levels in a dose-dependent manner. At 1000µM arbutin remarkably induced apoptosis, while tBHP as ROS inducer prompted necrosis. In addition, at 1000µM arbutin decreased expression of IL-1β levels.		2
MTT method (cytotoxicity), biochemical analysis of lysates for determination of total protein, total antioxidant status (TAS), total oxidant status (TOS) and proinfammatory cytokine (TNF-α, IFN-γ and IL-1β) levels, Bcl-2, GRP-78 and Caspase 3 mRNA expression levels by the Reverse Transcription Polymerase Chain Reaction (RT-PCR)	β-arbutin	MTT assay: 0,5 and 200 mM for 24 hours	human breast adenocarcinoma (MCF-7) cells	At low (1–10 mM) doses, α-arbutin appears to be more toxic than β-arbutin. At higher (5–200 mM) and LD50 (69.6 mM) doses beta arbutin toxicity appears to be higher than alpha arbutin. Thus, the study was continued with β-arbutin. The results demonstrated that the β-arbutin doses administered to MCF-7 cells did not affect oxidative and endoplasmic reticulum stress in the experimental groups. However, it was found that administration of LD50 dose β-arbutin induced inflammation in these cells via proinflammatory cytokine levels (TNF-α, IFN-γ and IL-1β). β-Arbutin at LD50 dose induced apoptosis in MCF-7 cells via p53 and Caspase 3. All β-arbutin doses inhibited estrogen receptor-α in MCF-7 cells. Arbutin increased the activation of apoptotic caspase-3 through p53, at LD50.		3

Cell viability assay (MTT), melanin content assay, tyrosinase activity assay, cell cycle and apoptosis analysis via FITC kit, migration assay (wound healing assay), mitochondrial membrane potential (MMP) assessment, analysis of total protein by Western blot	Arbutin and acetylated arbutin	0.3-5.4 mM	B16 murine melanoma cells	Acetylated arbutin was prepared in order to improve the biological effects of arbutin, and it was found to significantly inhibit the biosynthesis of melanin and tyrosinase activity compared with parent arbutin in B16 cells. Interestingly, only acetylated arbutin strongly inhibited B16 cell migration in a dose-dependent manner. Both arbutin and acetylated arbutin significantly reduced cell viability, promoted cell apoptosis, caused G1 cell cycle arrest and induced mitochondrial disruption in B16 cells. Furthermore, reduced expression of B-cell lymphoma-extra large (Bcl-xL) and Bcl-2 were observed in arbutin- and acetylated arbutin-treated cells. Therefore, arbutin and acetylated arbutin were found to exert pro-apoptotic effects on B16 cells, mediated through the mitochondrial pathway.		4
Analysis of pro-inflammatory cytokines (TNFα, IL-1β, IL-6 and p65) using ELISA, Western blot assay (NF-κB and COX-2, expression of Bcl2, BAX, cleaved caspase-3, cleaved PARP, LC3II, LC3I and beclin1), cell apoptosis by TUNEL assay, autophagy levels determined by LC3II immunofluorescence staining	Arbutin	0; 25; 50; 100 μM	Adult human retinal pigment epithelial (ARPE-19) cells	There was no statistically significant difference in the viability of APRE19 cells treated with 25 μM, 50 μM or 100 μM arbutin after 24 h or 48 h compared with the control group. When the cells were pretreated with 25 μM, 50 μM or 100 μM arbutin for 1 h and then treated with high glucose (HG, 30 mM), the activity of ARPE-19 cells was significantly higher than that of the HG group in a dose-dependent manner. Arbutin treatment markedly enhanced viability and autophagy mediators, decreased pro-inflammatory proteins and reduced apoptosis in ARPE cells under HG exposure, while increasing SIRT1 protein level. This could be blocked by Sirtinol treatment. Additionally, 3MA (3-methyladenine, an autophagy inhibitor) treatment significantly reduced the efficacy of arbutin against inflammatory		5

				markers and apoptosis in ARPE cells exposed to HG.		
qPCR to quantify MTHFDIL or miR-338-3p expression, cell viability (CCK-8), cell migration and invasion via transwell chamber, luciferase activity (binding site to miR-338-3p), Western blot analysis of cell lysates	Arbutin (cat. no HY-N0192; purity > 98%)	Proliferative ability by CCK-8 kit: 10-500 μM	Human osteosarcoma cells OS: MG-63 and SW1353	Arbutin suppressed OS cell viability in a dose and time-dependent manner. Arbutin decreased the protein levels of MTHFDIL, CCND1 and phosphorylated-protein kinase B (AKT)/phosporylated-mammalian target of rapamycin (mTOR). Knockdown of miR-338-3p promoted cell invasion, migration and invasion via miR-338-3p/MTHFDIL and by inactivating AKT/mTOR pathway.		6
Cell viability (MTT), ROS staining using DCFDA/H2DCFDA-Cellular ROS Assay Kit, mitochondrial membrane potential staining (MMP), AO/EB staining (cell viability), cell adhesion assay, qPCR	Arbutin	10-60μM (pre-screening cell viability), 30μM or 40μM in other tests	Rat C6 glioma cells	Arbutin effectively induced apoptosis in the cells and the IC50 dose was obtained at 30μM. Arbutin generated excessive ROS and disrupted the mitochondrial membrane, which induced apoptosis in cells. It also inhibited the cell adhesion property of C6 glioma cells. qPCR analysis clearly indicates arbutin increases the apoptotic genes and decreases the inflammatory and PI3K/mTOR signalling molecules.		7
Cells were pre-treated with arbutin (50, 250 and 1000 μM). After 24 hours, t-BHP (30 and 35 μM) was added to the cells. Viability was measured (at 24 and 48 hours) using MTT assay. The antioxidant effect of arbutin was measured by FRAP assay. The mRNA expression of P53 and BAX/BCL-2 ratio were	Arbutin	50, 250 and 1000 μM for 24 or 48 h	Human prostate cancer cells (LNCaP) and fibroblast cells isolated from human newborn foreskin	Arbutin pre-treatment increased the total antioxidative power and cell viability in the MTT assay and reduced BAX/BCL-2 ratio, P53 mRNA expression and necrosis in fibroblasts exposed to t-BHP. No data were provided for arbutin exposure as a single agent. Additionally, arbutin decreased cell viability, induced apoptosis and increased BAX/BCL-2 ratio in LNCaP cells at some concentrations. No data		8

measured using quantitative polymerase chain reaction (PCR).				were provided for arbutin exposure as a single agent.		

References of 9. ANNEX II

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