Scientific Committee on Consumer Safety

SCCS

OPINION ON

α-arbutin

The SCCS adopted this Opinion by written procedure on

on 27 May 2015

About the Scientific Committees

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

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

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

SCCS

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

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http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

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1. BACKGROUND

α-Arbutin INCI name Alpha-Arbutin (CAS 84380-01-8; EC 283-934-3) with the chemical name 4-Hydroxyphenyl-alpha-D-glucopyranoside is a cosmetic ingredient that is not regulated under Cosmetic Regulation No 1223/2009. It is used as antioxidant, for skin bleaching and skin conditioning.

The structurally related compound beta-Arbutin (CAS 497-76-7) is also used as a depigmentating agent in cosmetic products. The safety of beta-Arbutin has been assessed previously (SCCP/1158/08); its effect seems to be due to the fact that it hydrolyses to Hydroquinone. However, Hydroquinone (CAS 123-31-9) is listed in Annex II/1339 of the Cosmetic Regulation No 1223/2009; it means it is banned as cosmetic ingredient with the exception of entry 14 in Annex III. Its permitted use is restricted to professional use in artificial nail systems in a concentration in the final product up to 0.02%. Since Hydroquinone could not be used as a skin whitener after introduction of a ban, other substances have been used for that purpose, including alpha-Arbutin and beta-Arbutin.

As the SCCP opinion (SCCP/1158/08) stated concerns with other substances resulting in the release and/or formation of hydroquinone, a safety assessment of Alpha-Arbutin (CAS 84380-01-8; EC 283-934-3), has been considered as necessary. A Dossier on alpha-Arbutin was submitted in January 2011 in response to the EU Commission's Call for Scientific data on alpha-Arbutin published in November 2010.

2. TERMS OF REFERENCE

(1) *Does the SCCS consider on the basis of the provided scientific data, the use of α-Arbutin to be safe for consumers in cosmetic products in a concentration up to 2% in face creams and up to 0.5 % in body lotions?*

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

3. OPINION

α-ARBUTIN

The present Opinion is based on information provided with the first submission of January 2011 and an Addendum submitted in December 2012 by the applicant (DSM). In response to an informal request from DG SANCO (of 26.11.2014), the applicant confirmed that additional information on alpha-Arbutin, to augment that submitted in their two previous dossiers of 2011 and 2012 is not available, in particular with regard to clinical testing or repeated toxicity tests in animals (Letter from DSM of 10.12.2014). In light of similarities in metabolism of alpha- and beta-arbutin (with limited release of hydroquinone in skin compartments), the applicant suggests a read across/combined consideration of results from toxicity tests for both forms for an assessment of systemic hazards.
DSM further notes "that topical cosmetics considered likely to contain alpha-Arbutin would not produce systemic exposures above 400 µg alpha-Arbutin/person/day. This is well below the TTC Cramer class I threshold." Yet, according to the terms of reference, the SCCS has to consider not only exposure resulting from the application of 2% α-Arbutin creams to the face, neck and hands, but also a scenario where body lotions containing 0.5% α-Arbutin are applied (also included in the first submission).

3.1 Chemical and Physical Specifications

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

INCI Name: α-Arbutin

3.1.1.2 Synonyms

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

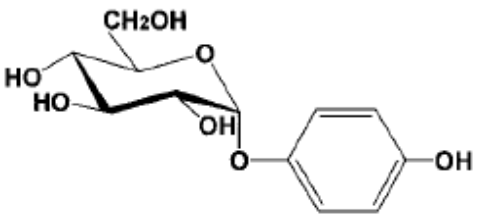
3.1.1.3 Trade names and abbreviations

ALPHA-ARBUTIN

3.1.1.4 CAS / EC number

CAS No: 84380-01-8
EC: 440-470-8

3.1.1.5 Structural formula



3.1.1.6 Empirical formula

$C_{12}H_{16}O_7$

3.1.2 Physical form

White to off-white powder.

Ref.: (*7*)

3.1.3 Molecular weight

Molecular weight: 272.25 g/mol

3.1.4 Purity, composition and substance codes

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.)

Table 3.1.4.1	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%

Submission I

3.1.5 Impurities / accompanying contaminants

Hydroquinone

HQ: < 0.01% w/v solution specified

Others

Heavy metals: < 20 ppm

Arsenic: < 2 ppm

Ref.: Appendix 2, Appendix 2.1 (submission I)

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% (*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 (*5*) and hydroquinone content was not part of DSM product specifications (*2*).

3.1.6 Solubility

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.

Ref.: (*6*) in Submission I

3.1.7 Partition coefficient (Log P_{ow})

Log Po/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.

Ref.: (*6*) in Submission I

3.1.8 Additional physical and chemical specifications

– melting point	201 ± 0.5°C, method A1 of Commission Directive 92/69/EEC	Ref.: (*6*)
– boiling point	~285°C at 102.17 kPa, method A2 of Commission Directive 92/69/EEC	Ref.: (*6*)
– flash point	Not highly flammable, method A10 of Commission Directive 92/69/EEC	Ref.: (*6*)
– UV/VIS absorption spectrum	$\lambda_{max.}$ ~280 nm	Appendix 2 of Submission I

3.1.9 Stability

3.1.9.1 Stability of alpha-Arbutin as a raw material

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 was 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.
Result: 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).

Alpha-Arbutin content in 2% or 3% alpha-Arbutin solution, respectively			
pH	**4.5**	**5.0**	**6.0**
Day 0	20 mg/mL or 30 mg/mL	20 mg/mL or 30 mg/mL	20 mg/mL or 30 mg/mL
Day 28	20 mg/mL or 30 mg/mL	20 mg/mL or 30 mg/mL	20 mg/mL or 30 mg/mL

Hydroquinone content for 2% and 3% sample, respectively			
Day 0	0 mg/mL	0 mg/mL	0 mg/mL
Day 28	0 mg/mL	0 mg/mL	0 mg/mL

Ref.: (*8*) submission I

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. Alpha-Arbutin content was measured every week over a period of 3 months by HPLC. Hydroquinone levels were not determined.

Result: 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**
$T_{1/2}$ (months)	~29	~77	~37	~28

Conclusion: 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.

Ref.: (*9*) submission I

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.

Result: 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 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.

Ref.: (*10*) submission I

3.1.9.2 Stability of alpha-Arbutin in a cosmetic formulation

submission II (Dec. 2012)

The stability of alpha-arbutin (aA) in representative cosmetic formulations was studied with respect to the formation of hydroquinone (HQ) and benzoquinone (BQ). A Steareth-2/Steareth-21-based O/W emulsion was chosen as cosmetic formulation. This "base formulation" was claimed to be robust against possible additional ingredients. For analytical

method validation, the base formulation (termed "nonstabilised formulation") was supplemented with different concentrations of aA, HQ and BQ and then measured.
In the first formulation series, 2% aA was tested at pH 5 and pH 6 according to the following criteria: **formulation stability, colour, presence of crystals, viscosity, change of pH, degradation of aA and formation of HQ**. Testing was performed right after preparing the formulation and after 2 and 6 weeks and 3 months at 5°C, 25°C (room temperature) and 40°C. aA and HQ were determined by using an RP-HPLC-DAD methodology which had been in-house validated by the applicant. Validation results were provided and appeared plausible. The solvent used for the extraction of HQ contained ascorbic acid (reduction of autoxidatively formed BQ into HQ).
In the second formulation series, PARSOL 1789, PARSOL 340, PARSOL SLX, Na_2EDTA, sodium metabisulfite and citrate buffer were added to **stabilise against pH shift**, which was observed in the first formulation series over time (during the period of 3 months the pH of 6 shifted toward pH 5.4). The pH values of the second series were adjusted to 4.5 and 5.5 (stabilised formulation).

Results of first formulation series: No measurable decrease of the concentration of aA was noted in any of the formulations, even after 3 months at 40°C irrespective of the pH (all values in the expected range of RSD% ± 2.6%). All the formulations contained measurable amounts of HQ (starting with about 13 – 18 ppm in freshly prepared solutions and reaching levels of 43 – 48 ppm after 3 months at 40°C. HQ formation increased temperature dependently, but was unaffected by pH. At 5°C and room temperature (25°C) HQ levels reached a plateau after 6 weeks and did not further increase with time. Despite the increases in HQ levels, no decreases in aA levels were measurable.

Results of second formulation series: pH alterations over time were slightly reduced but not completely prevented in the formulations by adding citrate buffer. The small changes (increases) of aA levels recognized over time were comparable in both buffer-stabilised or unstabilised formulations. However, although the initial concentrations of HQ were much lower in stabilised formulations (around detection limits of 1 ppm – 3 ppm) when compared to unstabilised ones (16 – 30 ppm), relative increases were much stronger in the case of buffered formulations (from 1 ppm up to >12 ppm). Still, the overall levels of HQ in unbuffered formulations were much higher, irrespective of the time point of analysis: starting with 16 – 30 ppm and reaching levels of about 50 ppm (room temperature) and 80 ppm (40°C) over time with no clear dependence on time.

So, altogether it seems obvious that the levels of HQ 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, HQ 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).

References in Submission II: Ref A (*Gutzwiller 2012*);
Ref B (Düsterloh 2012); Ref C (Düsterloh 2012)

SCCS comment

The SCCS noted several errors (e.g. mixing up of the UV spectra of HQ 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 HQ).

In addition to studies on the stability in cosmetic formulations, Submission II also provided data on the *in vitro* skin penetration of alpha-arbutin and its stability with skin samples (slices, homogenate) from pig or human. These results are discussed in sections 3.3.4. and 3.3.12, respectively.

3.1.10 Characterization of alpha-Arbutin used in current toxicity studies

(Table 3.1.4.2 from submission I)

Lot/Batch No.	Description	Purity	Used in Study
H12-10, N-160 (received at Safepharm Lab on 16 October 2000)	White powder	>97% (see Appendix 2)	1461-001 acute oral gavage; 1461-002 acute dermal tox; 1461/003 eye irritation; 1461/004 skin sensitisation; 1461-005 14-d dermal repeated 1461-006 Phototox-sensitisation 1461/007 Ames mutation 1461/009 MNT *in vivo*
AWT1722/201 (received at Safepharm Lab on 17 June 2002)	Skin whitening cream, 10% alpha-Arbutin: "white paste"	A cream formulation was used; the alpha-Arbutin Lot No. was not specified, but is expected to be within the specification (>97%)	1461/010 Acute oral toxicity, rat
061110	Alpha-Arbutin	Was not specified but is expected to be within the specification (>97%)	Stability of alpha-Arbutin (see 3.2.9.1)
5725515	Cosmetic formulation with 1% Alpha-Arbutin	Was not specified but is expected to be within the specification (>97%)	Short time study of the tolerance and the efficacy of a skin-lightening cosmetic product: alpha-Arbutin (Ref. R01/07/420, 25.6.2002)
EX-03-42/ FA	Cosmetic formulation with 1% alpha-Arbutin	A cream formulation was used; the alpha-Arbutin Lot No. was not specified but is expected to be within the specification (>97%)	Application study with follow-up of 12 weeks with volunteers of Asia under Sun Light Exposition. Final Report Date: 21.5.2004
H13-04, 5-1000 (received on 13 August 2001)	Powder	Was not specified but is expected to be within the specification (>97%)	Determination of general physico-chemical properties SafePharm study no. 208/225

3.2 Function and uses

Alpha-Arbutin (DSM product code 5033934) is a synthetic substance produced by enzymatic glycosylation of hydroquinone in the presence of α-Amylase and dextrin (*11, 12*). Structurally, it is therefore the *α*□isomer of arbutin (β-arbutin), and like the β-form it is a competitive inhibitor of human tyrosinase with a K_i of 0.2 mM ((*13*) = Sugimoto et al.,

2005). 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).

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 Appendix 1 (ref. D submission II), a number of examples of finished cosmetic products containing alpha-Arbutin are given, also including cosmetics applied to the body (pages 3, 8, 13, 16, 30).

3.3 Toxicological Evaluation

3.3.1 Acute toxicity

3.3.1.1 Acute oral toxicity

1) neat compound

Guideline/method:	OECD 423 (March 1996), EEC Directive 96/54 Method B1
Species/strain:	Rat/Sprague-Dawley CD
Group size:	3 rats/sex
Test substance:	alpha-Arbutin
Batch:	H12-10, N-160 (purity: >99%)
Dose levels:	2000 and 200 mg/kg bw
Vehicle:	water
Application volume:	10 mL/kg bw
Route:	Oral (gavage)
Exposure:	Single application
Observation period:	14 days
GLP:	Yes
Study period	Nov – Dec 2000
Date of report:	2001

Method: Body weights were recorded individually on day 0, before dosing and then on days 7 and 14, or at death. Clinical signs were monitored 30 minutes, 1, 2, and 4 hours and then once daily for 14 days after dosing; inspection for mortality/morbidity was twice daily on normal workdays and once daily on holidays and each weekend day. At death, each animal was examined for gross morphological changes during necropsy.

The test substance was prepared in distilled water at concentrations of 200 mg/ml or 20 mg/ml and dosed in a single gavage dose at 10 ml/kg body weight once via intubation canula. Doses were 2000 mg test substance/kg body wt. or 200 mg/kg; the high dose group consisted of 3 females and the lower dose group of 3 animals of each sex. Food was withheld during the night before dosing for each group of animals.

Results. The high dose group of 3 females given 2000 mg/kg body wt. were all dead within four hours of dosing. Clinical signs observed included hunched posture, increase salivation, and occasional body tremors. Gross observations at necropsy revealed abnormally red lungs, dark liver, dark kidneys, and slight haemorrhage on the gastric mucosa.

In the 200 mg/kg group, all 3 males and 3 females survived and showed no clinical signs of toxicity until study termination at 14 days after dosing. Body weight and body weight gain

was not adversely affected in any of the surviving animals. Necropsy did not reveal changes associated with the test material.

Conclusion: Estimated LD50 is between 300-500 mg/kg bodyweight. Mortalities were noted at a dose level of 2000 mg/kg bw; no mortalities were noted at 200 mg/kg bw.

Ref.: (*18*) in submission I

2) 5% alpha-Arbutin skin whitening cream

Guideline/method:	OECD 423, "Acute Toxic Class Method"
Species/strain:	Rat/Sprague-Dawley CD
Group size:	2 groups of 3 rats (females) each
Test substance:	alpha-Arbutin 5% in cream
Batch:	AWT 1722/2.01
Dose levels:	2000 mg/kg bw
Vehicle:	cream in water (200 mg/mL)
Application volume:	10 mL/kg bw
Route:	Oral (gavage)
Exposure:	Single application
Observation period:	14 days
GLP:	Yes
Study period	July – Aug 2002
Date of report:	2002

Methods: Body weights were recorded individually on day 0, before dosing and then on days 7 and 14, or at death. Clinical signs were monitored 30 minutes, 1, 2, and 4 hours and then once daily for 14 days after dosing; inspection for mortality/morbidity was twice daily on normal workdays and once daily on holidays and each weekend day. At death or terminal sacrifice, each animal was examined for gross morphological changes during necropsy.

The test substance cream was prepared in distilled water at a concentration of 200 mg cream/ml water and administered in a single gavage dose at 10 ml/kg body weight once via intubation cannula. Doses were 2000 mg test substance/kg body wt. in the first dose group consisting of 3 females; a second group of 3 females was dosed subsequently with 2000 mg/kg body wt. Food was withheld during the night before dosing for each group of animals.

Results: Death did not occur during the study in either group of females dosed at 2000 mg/kg bodyweight; clinical signs of toxicity did not occur, body weight and body weight gain were not affected adversely during the study period, and examination at necropsy did not reveal changes or abnormalities associated with the test substance.

Conclusion (of Applicant): Estimated LD50 is >2500 mg/kg bodyweight for the cream preparation or >500 mg alpha-Arbutin/kg bodyweight.

Ref.: (*19*)

SCCS comment

The SCCS noted that the LD50 value >2500 mg/kg bw corresponds to >125 mg/kg of alpha-Arbutin as the test substance is 5% alpha-Arbutin in the cream.

3) 10% alpha-Arbutin skin whitening cream

Guideline/method:	OECD 423, "Acute Toxic Class Method"
Species/strain:	Rat/Sprague-Dawley CD
Group size:	2 groups of 3 rats (females) each

Test substance:	alpha-Arbutin 10% in cream
Batch:	AWT 1722/2.01
Dose levels:	2000 mg/kg bw
Vehicle:	cream in water (200 mg/mL)
Application volume:	10 mL/kg bw
Route:	Oral (gavage)
Exposure:	Single application
Observation period:	14 days
GLP:	Yes
Study period	July – August 2002
Date of report:	2002

Method: Body weights were recorded individually on day 0, before dosing and then on days 7 and 14, or at death. Clinical signs were monitored 30 minutes, 1, 2, and 4 hours and then once daily for 14 days after dosing; inspection for mortality/morbidity was twice daily on normal workdays and once daily on holidays and each weekend day. At death or terminal sacrifice each animal was examined for gross morphological changes during necropsy.

The test substance cream was prepared in distilled water at a concentration of 200 mg cream/ml water and administered in a single gavage dose at 10 ml/kg body weight once via intubation canula. Doses were 2000 mg test substance/kg body wt. in the first dose group consisting of 3 females; a second group of 3 females were dosed subsequently with 2000 mg/kg body wt. Food was withheld during the night before dosing for each group of animals.

Results: Death did not occur during the study in either group of females dosed at 2000 mg/kg bodyweight; clinical signs of toxicity did not occur, body weight and body weight gain were not affected adversely during the study period, and examination at necropsy did not reveal changes or abnormalities associated with the test substance.

Conclusion: Estimated LD50 is >2500 mg/kg bodyweight for the cream preparation or >250 mg alpha-Arbutin/kg bodyweight.

Ref.: (*20*)

SCCS comment
Acute oral toxicity tests with neat compound or with skin-whitening cream containing 5% or 10% alpha-Arbutin in rats (two SD strains) provided LD50 estimates 300-500 mg/kg bw, and of >125 or >250 mg/kg bodyweight.

3.3.1.2 Acute dermal toxicity

Data are not available.

3.3.1.3 Acute inhalation toxicity

Data are not available.

3.3.1.4 Acute intraperitoneal toxicity

Data are not available.

3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

In vitro (no data / test results provided)

In vivo

Guideline/method:	OECD 404, EEC Method B4
Species/strain:	New Zealand White rabbits
Group size:	3 animals (males)
Test substance:	alpha-Arbutin
Batch:	H12-10,N-160 (purity > 99%)
Dose levels:	0.5 g at dose site
Vehicle:	moistened with 0.5 mL water
Application volume:	1 mL under 2.5x2.5 cm cotton gauze patch
Route:	dermal
Exposure:	Single application
Observation period:	72 hours
GLP:	Yes
Study period	Oct 2000
Date of report:	Feb 2001

Method: On the day before dosing, each animal was prepared by using a veterinary clipper to remove the fur from dorsal flank area; only animals with intact epidermis, based on visual examination, were assigned to the test group. The test substance at 10% w/v in distilled water had pH 6.8. For dermal exposure, 0.5 g of test substance as received was moistened with 0.5 ml distilled water and applied to the dose site and then covered with 2.5 x 2.5 cm gauze patch that was then secured by an elastic wrapping around the animal's trunk.

The wrap and patch were removed after 4 hours exposure and residual test substance removed by gentle wiping of the dose site with cotton-wool soaked in distilled water. The dose site was evaluated visually for changes and scored by Draize Scheme about 1 hour after patch removal and then 24, 48, and 72 hours later. The primary irritation index was calculated on a scale up to 8 (severe irritant).

Results: All scores at the dosing sites were zero (no erythema; no edema) in each rabbit at each observation time point. Other changes or clinical signs related to the test substance did not occur during the study.

Conclusion: The test substance is concluded to be non-irritant to skin.

Ref.: (*21*)

3.3.2.2 Mucous membrane irritation / Eye irritation

Guideline/method:	OECD 405
Species/strain:	New Zealand White rabbits
Group size:	3 animals (2 males, 1 female)
Test substance:	alpha-Arbutin
Batch:	H12-10,N-160 (purity > 99%)
Dose levels:	10% solution
Vehicle:	water
Application volume:	0.1 mL
Route:	conjunctival sac of the eye
Exposure:	Single application
Observation period:	1, 24, 48 and 72 hours
GLP:	Yes
Study period	Nov 2000

Date of report: Feb 2001

Method: The eyes of each rabbit were examined with aid of a light source; only those free of irritation and damage were assigned to the test. The first rabbit tested received 0.1 ml, equivalent to 0.53 g, of test substance placed into the conjunctival sac of the right eye; rinsing was not performed. The left eye served as the comparative control. The ocular response supported the similar treatment of 2 additional rabbits.

Observations were recorded 1 hour and then 24, 48, and 72 hours after dosing. The changes observed were scored according to Draize criteria and the numerical values for conjunctiva, iris, and cornea were combined for a group mean score calculated similarly to the method of Kay and Calandra (1962).

Results: The iris and cornea of all animals did not show any adverse effects from the test substance (all scores = 0). The conjunctivae of all 3 rabbits showed erythema, chemosis, and discharge at 1 hour (total group mean score = 6.7) and in one rabbit at 24 hours after dosing (group mean score = 2.0); all scores were zero in all rabbits at the 48-hour observation point. Other changes or clinical signs related to the test substance did not occur during the study.

Conclusion: The test substance is concluded to be only minimally irritant to rabbit eye.

Ref.: (*22*) in submission I

SCCS comment
Alpha-Arbutin when tested as 10% solution was non-irritant to rabbit skin and only minimally irritant to rabbit eye.

3.3.3 Skin sensitisation

Guideline/method:	OECD 406
Species/strain:	Dunkin Hartley guinea pigs
Group size:	15 animals (10 test, 5 controls)
Test substance:	alpha-Arbutin
Batch:	H12-10,N-160 (purity > 99%)
Dose levels:	25% solution for intradermal induction and 50% for topical induction; 25 and 50% for topical challenge
Vehicle:	water
Application volume:	0.1 mL
Route:	intradermal; topical
Exposure:	Single application
Observation period:	24 and 48 hours
GLP:	Yes
Study period	Nov 2000
Date of report:	Feb 2001

Method: On test day 0, the intradermal injections of 0.1 ml each, paired across the mid-line of each test group animal's shoulder region that was clipped of hair, were Freund's Complete Adjuvant, 1:1 in distilled water; 25% (w/w) alpha-Arbutin in distilled water; and 25% (w/w) test substance in the Freund's Complete Adjuvant solution. Skin reactions were observed and graded 24 and 48 hours after injection.

The test group's induction exposures started on study day 7: a 50% (w/w) solution of alpha-Arbutin in distilled water was applied as a thick, even layer of test substance to a 40cm x 20cm piece of filter paper and placed over the intradermal injection sites on shoulder region of each animal. The dose area was occluded by aluminum foil and surgical

tape with a full-torso elastic wrap held in place for 48 hours. Skin reactions were observed and recorded after removal of each occlusive dressing.

The control group animals each received intradermal injections and topical dressings similar to test group animals but without test material included.
Challenge exposures were given on test day 21 after clipping fur from the left and right flanks of each test and control group animal. The right flank was exposed to the 50% test substance applied as a thick, even layer with a 20mm x 20mm filter paper and held in place with surgical tape. A 25% (w/w) test material dilution was applied to the left flank. Both dose sites were occluded with aluminium foil and an elastic wrapping about the torso; the patches were removed after 24-hours' exposure and residual test material removed by swabbing with distilled water on cotton-wool. The fur was clipped from the dosed sites and skin reactions observed and recorded at 24-hours and 48-hours after dose-patch removals.

Results: Induction exposures resulted in discrete or patchy to moderate and confluent erythema after both the intradermal injections and topical doses.
Control group animals did not show any skin responses to either of the challenge doses at the 24- or 48-hour observation times.
At 50% challenge 24 hours after patch removal, six animals showed erythema: grade 1 in four animals and grade 2 in two animals; edema (grade 1) occurred in one animal (animal number 6) that showed grade 2 erythema. At the 48 hour reading, one animal (number 6) still showed grade 2 erythema and grade 1 oedema with desquamation; all other animals' scores had resolved to zeros.
At the 25% challenge site's 24-hour reading, three animals showed only grade 1 erythema, including animal number 6, whose erythema score remained unchanged at the 48-hour reading time. All other sites did not show erythema or edema.
Body weight and body weight gain was not adversely affected by the treatments applied during the study. Other clinical signs of toxicity did not occur.
Overall, the 24-hour readout (with 6/10 animals responding at 50% test substance and 3/10 at 25% test substance) would imply a "moderate sensitiser" categorisation. At the 48-hour readout, 1 of 10 animals showed skin responses considered indicative of skin sensitisation at 50% and 25% test substance (same animal); this 10% response rate implied a "mild sensitiser" categorisation.

Conclusion (of applicant)
The test substance up to 50% in this study is not a skin contact sensitiser.

Ref.: (*23*)

SCCS comment
Considering the definitions used in the study report (p.36) for defining degrees of allergenicity, since 1 in 10 of the guinea pigs was undoubtedly sensitised (i.e. 10%), the test substance should, also by the definitions provided in a SCCP memorandum (SCCP/0919/05) for this test, be considered as a 'mild sensitiser'.

3.3.3.1 Skin Sensitisation – cross reactivity to hydroquinone

A publication in 2010 by To-o et al. has reported the results of skin sensitisation testing of alpha-Arbutin by cross-reactivity to hydroquinone (HQ) induction conducted in guinea pigs. This brief summary is focused on that portion of the experimentation done with HQ and alpha-Arbutin. A Magnusson-Kligman maximisation test approach was used for this experiment, in which two sets of four groups each of 4 female Hartley-strain guinea pigs were given HQ induction doses as follows:

On day 0, a row of three injections was administrated as follows: (i) 0.05 mL emulsified mixture of FCA and saline (1:1); (ii) 0.05 mL of 2% HQ in saline; and (iii) 0.05 mL of 2% HQ in emulsified mixture of FCA and saline (1:1). On day 7, 0.5 g of 1% HQ in petrolatum was applied for 48 h under occlusion.

One set of the 4 groups induced with HQ were given challenge doses of alpha-Arbutin in acetone-water (80:20) at concentrations of 0 (vehicle), 0.2% (0.1 mg alpha-Arbutin), 2% (1 mg alpha-Arbutin), and 20% (10 mg alpha-Arbutin). The second set of four groups received challenge doses with the same concentrations of HQ: 0 (vehicle), 0.2% (0.1 mg HQ), 2% (1 mg HQ), and 20% (10 mg HQ). Challenge doses were applied without occlusion and were evaluated after 24 hours. Animal husbandry were not reported in full detail by To-o et al. (2010), but were not expected to influence adversely the study outcomes.

Results. Strong sensitisation responses were seen with each of HQ doses applied. In contrast, skin sensitisation responses to alpha-Arbutin did not occur in any of the animals in any of the challenge groups after induction with HQ. The results are shown in the tables (below) as copied from the publication.

Summary results from To-o et al. (2010)

Table 5. Cross-reactivity of α-arbutin to hydroquinone

α-Arbutin challenge	24 h	48 h	72 h
Vehicle	0/4	0/4	0/4
0.2% (0.1 mg)	0/4	0/4	0/4
2% (1 mg)	0/4	0/4	0/4
20% (10 mg)	0/4	0/4	0/4

Table 6. Sensitization potency of HQ

HQ challenge	24 h	48 h	72 h
Vehicle	0/4	0/4	0/4
0.2% (0.1 mg)	3/4	3/4	2/4
2% (1 mg)	4/4	3/4	2/4
20% (10 mg)	4/4	4/4	4/4

HQ, hydroquinone.

Conclusion

The study authors concluded that after induction with 1% HQ (5 mg HQ), alpha-Arbutin did not show cross-reactivity sensitisation reactions, even at the highest exposure concentration of 20% alpha-Arbutin. Comparison to HQ responses positive at 0.2% HQ, inferred that the alpha-Arbutin on skin was not hydrolyzed by resident glucosidases or skin-resident flora and at 20% alpha-Arbutin, less than 0.2% HQ, if any, was released during the experiment.

Ref.: (*24*)

SCCS comment

In a guinea pig maximisation test with up to 50% alpha-arbutin, the test agent was found to be a mild skin contact sensitiser. No skin contact sensitisation occurred in another study with alpha-arbutin up to 20% which tested cross-reactivity to hydroquinone (HQ) induction. Since HQ challenge (0.2% or more) caused strong sensitisation reactions, the negative results for alpha-arbutin imply that HQ formation in skin was below the level that elicits sensitisation in the guinea pig.

3.3.4 Dermal / percutaneous absorption

Percutaneous absorption *in vitro*

Submission I

Method: 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-month old female Yucatan micropig provided by Charles River Laboratories, Japan, Inc. Two sets of 6 Franz cells each were filled with phosphate buffer solution (not described further) as receptor fluid and "defatted" porcine skin fitted to the cells, which were then allowed to equilibrate (duration not stated) while the receptor fluid was maintained at 32 °C. 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.

Results. The cream formulation analytical results (Table 1 in the final report), 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 tables below, taken from the final report, summarise the analytical results as µg alpha-Arbutin or % of applied alpha-Arbutin.

Table below: Measured µg Alpha-Arbutin (data from 6 Franz cells per test concentration); LOD=0.26 ppm Alpha-Arbutin as % of dose (data for 6 Franz cells per test concentration)

Measured µg Alpha-Arbutin (results from 6 Franz cells per test concentration); LOD=0.26 ppm

Sample / Determination site	Cream containing 1% α-arbutin			Cream containing 2% α-arbutin		
	Mean (µg)	Standard deviation (±)	CV (%)	Mean (µg)	Standard deviation (±)	CV (%)
Unabsorbed samples/samples on apparatuses	4558.8	200.3	4.4	8464.7	332.2	3.9
Corneal layer	46.9	25.2	53.7	78.1	32.9	42.1
Epidermis	14.7	5.6	37.9	43.9	19.4	44.2
Dermis	0.0	0.0	-	0.0	0.0	-
Receptor fluid	0.0	0.0	-	0.0	0.0	-
Total amount absorbed percutaneously	61.6	27.0	43.9	122.0	44.3	36.3
Applied amount	4955.7	178.0	3.6	9381.6	361.1	3.8
Recovered amount	4620.4	197.7	4.3	8586.8	309.4	3.6
Recovery rate (%)	93.2	0.8	0.8	91.5	0.7	0.7

Alpha-Arbutin as % of applied dose (results for 6 Franz cells per test concentration)

Sample / Determination site	Cream containing 1% α-arbutin			Cream containing 2% α-arbutin		
	Mean (%)	Standard deviation (±)	CV (%)	Mean (%)	Standard deviation (±)	CV (%)
Corneal layer	0.94	0.49	51.96	0.83	0.35	41.76
Epidermis	0.30	0.12	39.01	0.47	0.23	48.72
Dermis	0.00	0.00	-	0.00	0.00	-
Receptor fluid	0.00	0.00	-	0.00	0.00	-
Percutaneous absorption rate (%)	1.24	0.53	42.62	1.31	0.49	37.57

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.

Conclusion

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. Hydroquinone could not be reliably measured from the samples.

Ref.: (25) submission I

SCCS comment

This study, with frozen/thawed skin from just one animal, is of limited values with regard to hydrolysis and degradation of alpha-arbutin in skin. By and large, the total absorption of about 1.3% is in line with other studies which find 2% in pig skin (see below).

Submission II

***In Vitro* skin Penetration and Stability with Skin**

In response to the 2011-2012 SCCS call for data on the stability of alpha-Arbutin on skin and the concern for release of hydroquinone (HQ) to skin from the use of skin bleaching cosmetic products, the applicant (DSM) has provided three *in vitro* studies to assess alpha-Arbutin skin penetration and distribution as well as stability and metabolism when associated with skin. These studies are summarized in the following section.

Percutaneous Penetration and Distribution Studies with Pig Skin and Human Skin *in vitro*

3.3.4.1. Pig ear skin *in vitro* penetration & distribution

Method: In a non-GLP study [Phenyl-U-^{14}C]-alpha-Arbutin plus non-radio-labelled alpha-Arbutin was added into oil-in-water cosmetic-type formulations, in sufficient quantity to give 0.5% and 2.0% (w/w) concentrations in the dosing formulations (Ruembeli et al. 2012a). 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 contained Euxyl PE 9010 as preservative system and 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 skin from 9 or 8 pig ears was used per formulation.
Skin integrity was tested in the flow-through cell system by means of ^3H_2O permeation measurement; skin samples were excluded if they had a permeability coefficient Kp > 3.5 x 10^{-3} cm/h or with an initial penetration rate > 4 % of dose/h. This resulted in 17 and 14 individual diffusion cells used for the 2 % and 0.5 % formulations, respectively.
The penetration of the test substance was determined over 24 h by collecting the receptor solution (0.9 % w/v NaCl) in intervals of 0-1 h, 1-3 h, 3-6 h, 6-12 h, 12-18 h and 18-24 h post-application. At 24 h after application of the test substance the skin was washed with mild soap solution, the stratum corneum was removed by tape stripping and the remaining skin was separated into epidermis and dermis. The total radioactivity was determined by liquid scintillation counting in all samples collected.

Results: 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. Mean levels of radioactivity expressed as % of applied dose are presented in the following table:

Summary of ^{14}C-alpha-Arbutin Dose Recovered From Pig Ear Skin (0.64 cm^2) *in Vitro*. (Ruembeli et al. 2012a)

	Radioactivity [% of dose]	
Formulation	2% α-Arbutin	0.5% α-Arbutin
	Mean (n=17)	Mean (n=14)
Surface Wash	86.98	90.61
Stratum Corneum	2.44	2.39
Epidermis	0.38	0.25
Dermis	1.23	1.50
Receptor 0-24h	0.47	0.31
Penetration	**2.07**	**2.06**
± SD	2.25	1.21

The dosing formulations remained stable and did not show degradation after 17 and 10 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.

Ref.: 4 submission II; Ruembeli 2012a

3.3.4.2. *In vitro* dermal absorption, distribution and metabolism in pig and human skin

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

In vitro dermal absorption & distribution

These endpoints were determined in the experimental for pig skin (Series F) and for human skin samples (Series G). 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 penetration of the test substance was determined over 24 h by collecting the receptor solution (0.9 % w/v NaCl with 1 % penicillin/-streptomycin solution) from 0 – 24 h. At 24 h after application of the test substance the skin was washed with mild soap solution, the stratum corneum was removed by tape stripping and the remaining skin was separated into epidermis and dermis. The skin samples were extracted with ethanol/0.5 % ascorbic acid 80/20 (v/v) to deactivate tissue enzymes and prevent further reaction of any hydroquinone. The total radioactivity was determined by liquid scintillation counting of the extracts and by liquid scintillation counting after treatment of the non-extractables with tissue solubiliser.

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. Mean levels of radioactivity expressed as % of applied dose are presented in the table below.

Results: 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/cm^2 in 24 h for pig and human skin, respectively. Therefore, the penetration with human skin was about 8 times lower than with pig skin.

Absorption and distribution of ^{14}C-alpha-Arbutin in pig (Series F) and human (Series G) skin (5 cm^2) under static conditions. (Ruembeli et al. 2012c)

	Radioactivity [% of dose]	
Skin	Pig	Human
	Mean (n=6)	Mean (n=3)
Surface Wash	89.79	101.52
Stratum Corneum	1.69	0.42
Epidermis	0.86	0.20
Dermis	1.15	0.07
Receptor 0-24h	0.13	0.01
Penetration	**2.13**	**0.27**
± SD	0.41	0.13

Conclusion: 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.
The data comparison is shown in the next table.

Comparative Summary of *in vitro* penetration results for pig and human skin dosed with either 0.5% or 2% alpha-Arbutin. (Ruembeli et al. 2012c)

	Radioactivity [% of dose]							
Skin	**Pig Skin**		**Pig Skin**		**Pig Skin**		**Human Skin**	
Series	ref. [1]		ref. [1]		F		G	
Cell Type	Flow through		Flow through		Static		Static	
Antibiotics	no		no		yes		yes	
Skin Area [cm²]	0.64		0.64		5		5	
Formulation	2% α-Arbutin		0.5% α-Arbutin		2% α-Arbutin		2% α-Arbutin	
	mean n=17	SD	**mean** n=14	SD	**mean** n=6	SD	**mean** n=3	SD
Surface Wash	**86.98**	7.76	**90.61**	14.44	**89.79**	5.77	**101.52**	1.33
Stratum Corneum	**2.44**	2.62	**2.39**	2.42	**1.69**	0.62	**0.42**	0.24
Epidermis	**0.40**	0.59	**0.25**	0.23	**0.86**	0.32	**0.20**	0.09
Dermis	**1.23**	1.71	**1.50**	1.13	**1.15**	0.38	**0.07**	0.04
Receptor 0-24h	**0.47**	0.49	**0.31**	0.31	**0.13**	0.07	**0.01**	0.03
Penetration *	**2.07**	2.25	**2.06**	1.21	**2.13**	0.41	**0.27**	0.13

* Penetration = sum of epidermis, dermis & receptor-fluid

Skin surface Stability to Microbes
In a preliminary experiment (Series E in final report), the dermal stability of ^{14}C-α-Arbutin in a formulation with 0.5 % alpha-Arbutin was tested in three static diffusion cells fitted with fresh pig skin each having a 5 cm^2 surface area. Antibiotics were not used either on the skin or in the receptor fluid during the 24 hours of the experiment.

Only the skin surface wash was analysed by radio-HPLC, which besides alpha-Arbutin revealed 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 skin treated with the antibiotic mixture and then 2% alpha-Arbutin as described above (Series F and Series G), 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.

SCCS comment
The use of antibiotics (1% penicillin/streptomycin solution) 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.

Distribution of Metabolites in the Test System
From the 24 h penetration experiments with pig and human skin with antibiotic pretreatment 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.

Summary Table. Distribution of Radioactivity (% of applied dose) in Skin Compartments: static cells, 5 cm^2 skin area, after 24h. (Ruembeli et al. 2012c)

	Radioactivity [% of dose]			
Skin	**Pig**		**Human**	
	mean (n=6)	***SD***	**mean (n=3)**	***SD***
Surface Wash				
α-Arbutin	**89.79**	*5.77*	**101.52**	*0.68*
Hydroquinone	**0.00**	*0.00*	**0.00**	*0.00*
unknown	**0.00**	*0.00*	**0.00**	*0.65*
Stratum Corneum				
Extractable	***1.63***	*0.60*	***0.41***	*0.23*
α-Arbutin	**1.59**	*0.62*	**0.40**	*0.22*
Hydroquinone	**0.03**	*0.05*	**0.02**	*0.02*
unknown	**0.01**	*0.01*	**0.00**	*0.00*
Non-extractable	***0.06***	*0.02*	***0.01***	*0.00*
Epidermis				
Extractable	***0.82***	*0.30*	***0.18***	*0.09*
α-Arbutin	**0.81**	*0.31*	**0.16**	*0.08*
Hydroquinone	**0.01**	*0.01*	**0.00**	*0.00*
unknown	**0.00**	*0.00*	**0.02**	*0.02*
Non-extractable	***0.03***	*0.01*	***0.02***	*0.01*
Dermis				
Extractable	***1.01***	*0.36*	***0.05***	*0.03*
α-Arbutin	**0.94**	*0.34*	**0.05**	*0.03*
Hydroquinone	**0.04**	*0.04*	**0.00**	*0.00*
unknown	**0.03**	*0.04*	**0.00**	*0.00*
Non-extractable	***0.14***	*0.03*	***0.01***	*0.01*
Receptor				
α-Arbutin	**0.10**	*0.05*	**0.00**	*0.00*
Hydroquinone	**0.03**	*0.02*	**0.00**	*0.00*
unknown	**0.00**	*0.00*	**0.01**	*0.00*
Penetration*				
Extractable	***1.95***	*0.40*	***0.24***	*0.12*
α-Arbutin	**1.84**	*0.40*	**0.21**	*0.11*
Hydroquinone	**0.08**	*0.04*	**0.00**	*0.00*
unknown	**0.03**	*0.04*	**0.03**	*0.02*
Non-extractable	***0.18***	*0.03*	***0.03***	*0.01*
Total	**2.13**	*0.41*	**0.27**	*0.13*

Ref.: 6 submission II; Ruembeli 2012c

SCCS comment
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) again 2 % dermal absorption of alpha-arbutin is found for pig skin. Total dermal absorption with human skin is 0.27 ± 0.13 %, i.e. much lower than that of fresh pig skin.

In the human skin experiments skin was used that was frozen for 8 days, 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.

The SCCS further notes that the use of antibiotics (1% penicillin/streptomycin solution) to reduce effects on stability of alpha-arbutin by skin resident microbes may be justified for pig skin samples, but not for human skin.

3.3.4.3. Stability of Alpha-Arbutin in Aqueous Solutions and with Skin Homogenates

In a non-GLP study, a series of *in vitro* experiments were conducted to evaluate alpha-Arbutin stability in buffered solutions at various pH values and also when mixed into homogenates of frozen or fresh pig skin or human skin that had been stored frozen (Ruembeli 2012b).

Subsection 3.1 **Stability of alpha-Arbutin in aqueous solutions at various physiological pH values**

Method: Stock solutions of sodium and potassium phosphate buffers were prepared in distilled water and mixed in appropriate proportions to yield solutions with pH 5.4, 6.4 or 7.4; after mixing the buffers, an antibiotic mixture of penicillin/streptomycin and ascorbic acid was added before use to conserve the relative proportions of alpha-Arbutin, hydroquinone, benzoquinone, or unknowns at experiment termination. A separate stock solution of non-radiolabelled alpha-Arbutin and ^{14}C-alpha-Arbutin were prepared and stored at -18 °C until used. The pH values were selected to represent approximate conditions of stratum corneum, epidermis, and dermis, respectively.
Results: Analysis of each buffer solution 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. Based on these findings any loss of alpha-Arbutin from incubations with skin could be attributed to other experimental parameters or factors and not to an effect of the aqueous solutions or their pH value.

Subsection 3.2 **Incubation of alpha-Arbutin with skin homogenates**

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. Thus, all experiments included the antibiotic mixture to minimize the impact of microbial degradation on the experimental outcomes. In addition, it was concluded that skin homogenation without inhibition of skin protease enzymes would not be representative of an intact skin exposure environment where such enzymes are compartmentalized. The protease inhibitor phenylmethanesulfonyl fluoride (PMSF) in isopropanol was used in the majority of the incubation experiments.
Methods: Pig ear skin stored frozen was compared with fresh pig ear skin to evaluate an impact of freezing on skin enzyme activity. These results were also used to support the reliability or representativeness of human skin that had been stored frozen. At the time of these experiments, additional samples of human skin were not available so only a limited number of experiments could be completed.

Estimating the relevant concentration of alpha-Arbutin. The following assumptions were made:
Dose: usual topical application rate of 2 mg end formulation per cm^2 skin with 2% alpha-Arbutin corresponds to 40 µg alpha-Arbutin/cm^2 skin.
Percutaneous absorption: It was assumed that 10 % alpha-Arbutin applied would penetrate into stratum corneum, epidermis and dermis, corresponding to 4 µg alpha-Arbutin / cm^2.
A layer of 500 µm skin was used, giving a concentration of 4 µg alpha-Arbutin / 0.05 cm^3 skin or 0.08 mg alpha-Arbutin/g skin (using a skin density of 1g/cm^3).

Homogenate concentrations: a total of 1 g skin was homogenized in 4 g buffer. Therefore the concentration would be 0.08 mg alpha-Arbutin / 5 g incubation solution, giving a concentration of 60 µmol alpha-Arbutin/L incubation solution. 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.
Skin slices from dermatomed 500 µm thick top layers of pig or human skin were added into the appropriate pH-buffer solution with antibiotic mixture plus a protease inhibitor and then homogenized, centrifuged and aliquots of supernatant added into tubes for incubation over different lengths of time. Samples were analysed by liquid scintillation counting and radio-HPLC to quantitate distribution of analytes into soluble and non-extractable parts of the samples. Analytes included alpha-Arbutin, hydroquinone, benzoquinone, and unknowns.

Results. Several experimental series covering various combinations and incubations conditions have been conducted, the results of which are summarized in Table 24 of the final report (Ruembeli et al. 2012b). Here only a summary of the various experiments (test series designation given) and key conclusions derived is provided.
Comparison of homogenate from fresh pig skin with homogenate from frozen pig skin indicates that freezing and storage at -18°C has no negative effect on the activity of the glucosidases in pig skin. By extension, this can be inferred for the human skin used in these experiments.
Incubation of alpha-Arbutin with homogenate from fresh pig skin showed that alpha-Arbutin was hydrolysed enzymatically to hydroquinone with a half-life of 11 hours at pH 5.4 (series a-Hc), 8 hours at pH 6.4 (series a-Hd and a-Hd+V), and 5 hours at pH 7.4 (series a-Hg). This indicates that the stability of alpha-Arbutin against enzymatic hydrolysis is lower at higher pH values.
Incubation of alpha-Arbutin with homogenate from frozen human skin at pH 5.4 and 37 °C for 24 hours showed that the added alpha-Arbutin was hydrolysed enzymatically to mainly hydroquinone (80% of dose) plus about 3% as non-extractable radioactivity; about 16% remained as alpha-Arbutin. A half-life of 10 hours was estimated for the reaction. In contrast to pig skin (above), at pH 7.4 the reaction kinetics with human skin homogenate was much slower in that about 85% alpha-Arbutin and 15% hydroquinone was found after 24 hours.
Comparison of incubations with the same amount of skin homogenate but with 100-fold different alpha-Arbutin concentrations (37 µM, series a-Hd) vs. (4035 µM, series a-Hj) showed only a minor difference in the reaction kinetics.
By comparing the incubation of alpha-Arbutin in pig skin homogenate without (series a-Hd) and with (series a-Hd+V) ascorbic acid, it was shown that the metabolism proceeds via hydroquinone, which is oxidized (in the absence of ascorbic acid) to benzoquinone. Benzoquinone is highly reactive to nucleophiles such as amino groups in proteins and therefore can form non-extractable residues.
Ascorbic acid had no effect on the first hydrolysis step from alpha-Arbutin to hydroquinone.
The comparison of results for the incubation of alpha-Arbutin in pig skin homogenate at pH 7.4 (series a-Hg) with human skin homogenate at pH 7.4 (a-Mh) showed alpha-Arbutin to be much more stable in human than in pig skin homogenates. The same comparison performed at pH 5.4 showed an alpha-Arbutin decrease with similar reaction kinetics for pig skin (series a-Hc) and for human skin homogenates (series a-Mk).

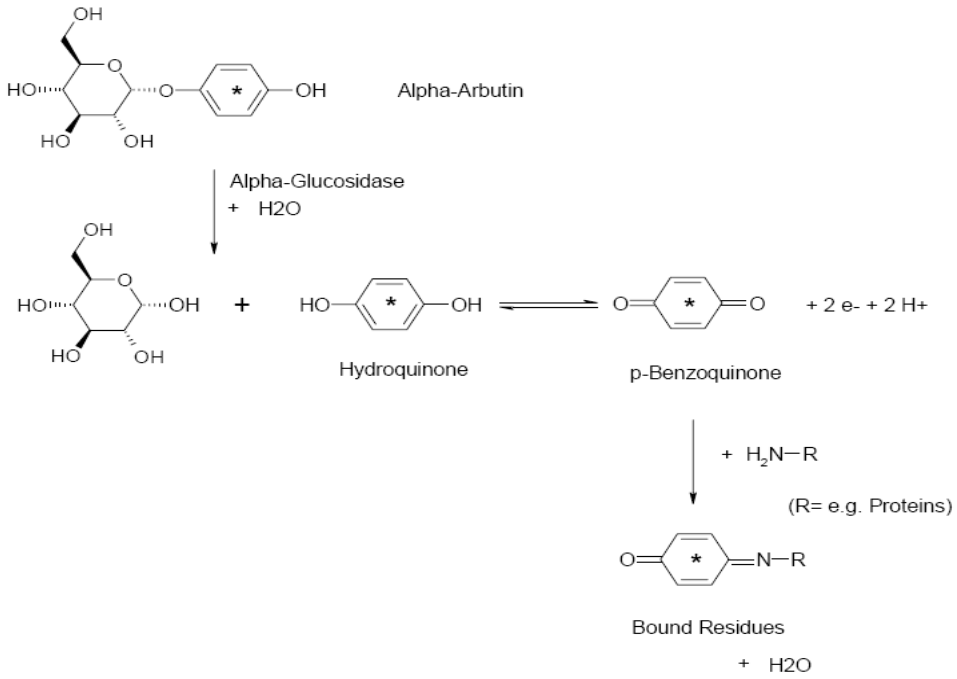
Conclusions from homogenate incubation experiments

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 metabolized with a half-life between 5 and 11 hours depending on the pH.
- The reaction was slightly faster in pig skin homogenate and increasing 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.
- A metabolic pathway of alpha-Arbutin in skin homogenates is proposed to be as shown in Figure 1.

Figure 1. Proposed metabolic pathway for alpha-Arbutin in skin homogenates.



Ref.: 5 submission II; Ruembeli 2012b

SCCS comment

The metabolism of alpha-arbutin in pig and human skin homogenate is 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.

Overall conclusion on in vitro dermal percutaneous absorption

Overall, 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 HQ in the lower skin layers, and intact skin *in vitro* incubated with

alpha-Arbutin shows only limited release of HQ. Homogenized pig skin and human skin each showed similar metabolism pathway, but with somewhat different kinetics related to 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*
In a study with human volunteers who applied a gel with either 3% alpha-arbutin or 3% beta-arbutin or 1.9% hydroquinone (HQ), the (relative) amounts of HQ present in tape strips were assessed (Mamabolo et al.). The results, discussed in section 3.3.11, indicate low conversion of arbutins to HQ, but this data allows no conclusions on percutaneous absorption.

3.3.5 Repeated dose toxicity

3.3.5.1 Repeated Dose (14 days) dermal irritation -> with irritation studies

Guideline/method:	– (*study design meets the requirements of the Japanese Ministry of Health and Welfare (1992)
Species/strain:	Dunkin Hartley guinea pigs
Group size:	6 animals (3 males, 3 females)
Test substance:	alpha-Arbutin
Batch:	H12-10,N-160 (purity > 99%)
Dose levels:	0.05 g moistened in water
Vehicle:	water (10% solution had pH 6.8)
Application volume:	0.1 mL
Route:	topical to 2 x 2 cm area, non-occluded
Exposure:	application on 14 consecutive days dose sites were re-shaved with an electric razor on days 3, 7, 10, and 14
Observation period:	immediately before each application and 24 hours after the last one (14 days)
GLP:	Yes
Study period	Nov 2000
Date of report:	Feb 2001

Method: Dose sites were evaluated for evidence of skin irritation and scored; observations occurred daily before the daily application and about 24 hours after the 14th application. Each animal was observed for clinical signs of toxicity each day and body weights recorded on days 0, 7, and 14. The daily scores for skin reactions (erythema and eschar formation, edema) at the dose sites were tabulated (Draize scale 0 to 4), and used to calculate daily and then weekly mean irritation indices, which were compared to a classification table for the degree of irritation.

Results: One animal showed clinical signs of systemic toxicity on day 13 that included hunched posture, emaciation and laboured breathing where after it was sacrificed; the noted effects were not assigned to the test substance. All other animals did not show adverse effects or signs of toxicity. Body weight and weight gains for surviving animals were considered normal.

Skin erythema was noted at the dosed site of only one animal during each of the 14 days of the study. The control sites did not show signs of irritation. The scores (and a maximum weekly irritation index of 0.03*), showed that the test substance is non-irritating with daily dosing repeated for 14 days.

Conclusion
This study is designed to give mainly dermal irritation information and has very limited reliability for demonstrating more subtle and meaningful systemic effects to support definition of a NOAEL.

Ref.: (*26*)

SCCS comment
*Maximum weekly irritation index is not a category used by SCCS. Yet, the results are in line with the acute skin irritation study (section 3.3.2.1) and the conclusion that alpha-arbutin is not irritating to skin.

3.3.5.2 Sub-chronic (90 days) toxicity (oral)

3.3.5.3 Chronic (> 12 months) toxicity

No chronic toxicity study with alpha-Arbutin is available.

Overall conclusion on repeated dose toxicity
Only one study with repeated dermal application of alpha-arbutin is available. 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.
The data base for toxicity of alpha-arbutin is insufficient to calculate a MOS; yet the ban on animal studies, effective of 2013 in the EU, precludes requests for a repeated toxicity study.

3.3.6 Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial reverse mutation assay

Guideline:	OECD 471 (1997)
Species/strain:	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *Escherichia coli* strain WP2uvrA$^-$
Replicates:	Triplicates per test concentration in 2 independent experiments
Test substance:	alpha-arbutin
Batch:	H12-10,N-160
Purity:	98%
Vehicle:	water
Concentrations:	Experiment I: 50; 150; 500; and 5000 µg/plate Experiment II: 50, 150; 500; and 5000 µg/plate
Controls:	ENNG, 2AA, 4NQO as direct acting mutagens; 2-AA and B(a)P as positive controls requiring metabolic activation
Treatment:	direct plate incorporation method
GLP:	In compliance
Study period:	24 October 2000 –11 November 2000
Date of report:	12 March 2001

Alpha-arbutin was investigated for the induction of gene mutations in strains of *Salmonella typhimurium* and *Escherichia coli* (Ames test). Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. The test material was diluted in distilled water, with correction for the 98% purity, just before dosing of the bacterial-seeded agar plates. A preliminary toxicity study was conducted with strains TA100 and WP2uvrA$^-$ with and without S9-mix and showed the test substance as non-toxic up to 5000 µg/plate; a concentration-range test run subsequently substantiated this conclusion. Mutation testing by direct plate incorporation of the test substance, with and without S9-mix, was at concentrations of 50, 150, 500, 1500, and 5000 µg/plate. Positive control chemicals were dosed according to their specific strain.

Results
Each of the test strains showed the expected increase in revertants per plate for their respective positive control substance. Alpha-arbutin did not show reduction of bacterial growth background or precipitation at any of the concentrations, with or without S9 metabolic activation. Biologically relevant and statistically significant increases in the number of revertants did not occur, with and without S9-mix, at any of the concentrations used in any of the tested strains.

Conclusion
Under the experimental conditions used, alpha-arbutin was not mutagenic in this gene mutation tests in bacteria.

Ref.: (27)

3.3.6.2 Mutagenicity / Genotoxicity *in vivo*

Mouse Micronucleus Assay with intraperitoneal application

Guideline:	OECD 474 (1997)
Species/strain:	Crl:CD-1(ICR)BR mice
Group size:	Range finding test: 4 (2 male, 2 female) per dose Main study: 7 male mice per dose group
Test substance:	alpha-arbutin
Batch No.:	H13-05,S-1
Purity:	99.9%,
Dose levels:	500, 1000 and 2000 mg/kg bw as single doses
Route:	intraperitoneal (and p.o. in first study)
Vehicle:	water or arachis oil
Sacrifice times:	24 hours and 48 hours (high dose only) after start of the treatment
Positive control:	Cyclophosphamide
GLP:	In compliance
Study Period:	28 Feb – 8 April 2002
Date of report:	6 June 2001

Alpha-arbutin has been investigated for the induction of micronuclei in bone marrow cells of mice to assess chromosome damage and aneuploidy in bone marrow erythrocytes. Cyclophosphamide monohydrate in distilled water was used as the positive control substance. Test substance vehicles were water or arachis oil. Doses were based on dose range experiments with groups of 2 male and 2 female mice per dose with 2000 mg/kg bw by intraperitoneal injection or with 200 mg/ml by oral gavage. A reportedly insufficient solubility of alpha-arbutin in water led to selection of arachis oil as the dosing vehicle. In the main experiment, alpha-arbutin was administered in a single dose of 10 ml/kg body weight to one group each at 500 or 1000 mg/kg bw, or to two groups given 2000 mg/kg bw. The one positive control group was dosed at 50 mg/kg bw; two groups served as the vehicle control and received arachis oil. Bone marrow cells from both femurs were collected 24 hours and 48 hours after dosing. Toxicity and thus exposure of the target cells was determined by counting the number of normochromatic erythrocytes (NEC) among 1000

erythrocytes.

Results
In the dose range finding experiments, sex differences in toxicity did not occur; so the main study used male mice dosed once by intraperitoneal injection.
In the range finding test, 1 of 2 females dosed by gavage with test substance in water at 2000 mg/kg bw showed signs of systemic toxicity including tremors, tiptoe gait, ataxia, and hunched posture before its death within 1-hour of dosing. All other animals in each of the other test groups survived without signs of toxicity for the two days after dosing. In the main experiment, deaths or signs of adverse effects did not occur in any of the animals of any group. The test substance is considered to have been systemically absorbed based on the marked decrease at 48-hours in the PCE/NCE ratio in the high dose group animals compared to the solvent control group.
The test substance did not induce a biologically relevant and statistically significant increase in the number of bone marrow cells with micronuclei.

Conclusion
Under the experimental conditions used alpha-arbutin is not genotoxic (clastogenic and/or aneugenic) in this micronucleus test in bone marrow cells of mice.

Ref.: (28)

SCCS comment
The applicant claimed that there was a marked decrease in the PCE/NCE ratio and thus systemic toxicity for alpha-arbutin in bone marrow cells. Although this decrease was pronounced at the highest dose, it was not statistically significant. However, it is generally accepted that intraperitoneal administration guarantees systemic exposure.
In the report the applicant (DSM) mentioned: *"The study director/report author states that poor water solubility of the test substance precluded water as vehicle. We cannot verify the dosing solution preparations made for this test but point out to the reviewers that the same contract testing laboratory conducted several other studies of alpha-Arbutin with water as vehicle and a similar problem was not mentioned in their reports. We do not find this observation a sufficient basis to question the results of studies conducted with water as the dosing vehicle."* The SCCS accepts this statement. Moreover, the route dependent toxicity observed in the range finding study with gavage application of 2 g/kg bw alpha-arbutin may have been due to release of hydroquinone in the stomach (acidic pH).

Overall conclusion on mutagenicity
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 3 endpoints of genotoxicity, alpha-arbutin can be considered to have no *in vivo* genotoxic potential.
Arbutins are considered to be metabolised differently by different glycosidases or by spontaneous hydrolysis, yet they all form hydroquinone (HQ). The latter is considered as the genotoxic moiety; therefore a read across approach based on HQ release is a possibility to assess the genotoxic potential of the arbutins as a group.

3.3.7 Carcinogenicity

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3.3.8 Reproductive toxicity

3.3.8.1 Two generation reproduction toxicity

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3.3.8.2 Other data on fertility and reproduction toxicity

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3.3.8.3 Developmental Toxicity

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3.3.9 Toxicokinetics and metabolism

***In vitro* metabolism**

Studies with pig and human skin homogenate indicate that metabolisation of alpha-arbutin is similar, with partial hydrolysis to hydroquinone which is pH-dependent and catalysed by microbial and/or skin glucosidases. See section 3.3.4 - subsection 3.2

***In vivo* toxicokinetics or metabolism**

One study of limited value (Mamabolo et al.; unpublished data) has measured hydroquinone (HQ) in tape strips of volunteers after topical application of alpha-arbutin, beta-arbutin and HQ (see section 3.3.11.3).

3.3.10 Photo-induced toxicity

3.3.10.1 Phototoxicity / photoirritation and photosensitisation

Guideline:	OECD 1997
Species/strain:	Dunkan Hartley guinea pigs
Group size:	5 animals per group in the main study phase with Group I for phototoxicity, Group II photoallergy; Group III positive photoallergy group
Test substance:	Hydroquinone-α-D-Glucoside
Batch:	H12-10,N-160
Purity:	99.7%
Vehicle:	water
Dose levels:	5 and 10% alpha-Arbutin
Dose volume:	0.025 ml / cm² skin
Positive control:	8-methoxypsoralen (0.005%) for phototoxicity 6-methylcoumarin (0.5%) for photosensitization
Route:	Topical application
UV source:	6 fluorescent tubes emitting UVA between 320-400 nm with peak intensity at 350 nm
UV intensity:	Source placed at 10 cm of the back of the guinea-pigs delivered irradiance of 10 joules/cm² skin
GLP:	In compliance
Study period:	9 Nov – 21 Dec 2000
Report:	20 Feb 2001

Phototoxicity determination was made on the day after the dorsal dose site was clipped and chemically depiliated; dosing was with a 10% test item solution applied to 2 anterior contra-

lateral sites and then 30-minutes later one side was occluded with aluminium foil to exclude irradiation while the right side was exposed to the UV light source set about 10 cm distant from skin for 105.9 minutes thereby delivering about 10 joules/cm^2 irradiance. Occlusive dressings were removed and all sites examined 3, 24, and 48 hours after the irradiation period and changes graded according to the Draize scale.

Photoallergenicity. On study day 4, animals in the remaining 2 groups had dorsal skin clipped and chemically depiliated; on day 5 the sites were tape-stripped by 4 consecutive applications of surgical tape followed by 4 localized intradermal injections of 0.1 ml each of Freund's Complete Adjuvant (1:1 in distilled water) at the corner of the dosing sites. Thereafter, test substance or 6-methylcoumarin was applied to the induction site of animals in the respective test groups and after 30 minutes exposed for 101 minutes to the UV irradiation source (10 joules/cm^2); thereafter, the dosed sites were rinsed with distilled water on a cotton wad. On days 6 through 9 each animal was again dosed, but without Adjuvant, with their respective treatment and irradiated for 103 to 104 minutes to deliver the 10 joules/cm^2 UVA dose.

Challenge dosing was on study day 34, the day after the dorsal sites were clipped of hair and depilated chemically. Test substance group animals each received alpha-Arbutin at 10% to 2 posterior contra-lateral sites and 5% on 2 anterior contra-lateral sites; application volume was 0.025 ml/cm^2 of skin. The positive control group substance was applied to 2 contra-lateral sites. The left side dose sites were occluded with aluminium foil and 30 min after application, the animals were exposed for 103 min to the UVA source (10 joules/cm^2). Thereafter, the aluminium foil was removed and 24 and 48 hours later the dose sites evaluated for relevant changes.

Bodyweights were recorded for each study phase.

Results: In all study groups, bodyweight and bodyweight change were not affected adversely during the study.

In the phototoxicity phase, 10% alpha-Arbutin did not induce skin reactions with or without UV irradiation; a phototoxic response did not occur. The positive control group showed the appropriate responses.

In the photoallergenicity phase, alpha-Arbutin at 5% and 10%, with and without UV irradiation did not show skin reactions to treatment; photosensitisation did not occur. The positive control group showed the appropriate responses.

Conclusion

Under the conditions of this test alpha-Arbutin up to 10% in distilled water was not phototoxic and was not photo-sensitising.

Ref.: (*29*)

3.3.10.2 Photomutagenicity / photoclastogenicity

/

SCCS Comment

The studies for phototoxicity and photoallergy are acceptable and neither phototoxicity nor photoallergy have been demonstrated for hydroquinone-alpha-D-glucoside in doses up to 10% w/v in distilled water.

3.3.11 Human data

To analyse the efficacy of alpha-Arbutin the following two (3.3.11.1 and 3.3.11.2) studies with human volunteers were performed (sponsored by Pentapharm):

3.3.11.1 A cosmetic formulation, including either 1% alpha-Arbutin, kojic acid, beta-Arbutin or hydroquinone, were topically applied for 30 days, twice per day on the inner forearms of 20 healthy women each formulation. Skin lightening effects were evaluated by giving scores (from 0=light to 3=intense skin pigmentation) and chromameter measurements before and after application and compared to untreated control. The study was supervised by a dermatologist.

Result with regard to safety: All products were well tolerated during the study.

Ref.: (*30*)

3.3.11.2 Four cosmetic formulations containing either 1% alpha-Arbutin or 6% Tinosorb M or 1% alpha-Arbutin and 6% Tinosorb M and a placebo formulation were topically applied for 12 weeks, once per day on the inner forearm of 20 healthy volunteers. In addition formulations were applied 40 and 20 minutes before sunlight exposure (<1 MED three times a week). Skin colour, glossy effects, profilometry and biomechanical properties were assessed instrumentally. Volunteers were dermatologically examined by inspection and palpation at the beginning, after 4, 8 and 12 weeks.
Result with regard to safety: No peculiarities were observed. The products were well tolerated, also in combination with UV exposure. Skin changes, including allergic or pseudo-allergic reactions, did not occur during the trial period.

Ref.: (*31*)

SCCS comment
In the above studies, alpha-arbutin was applied as formulation with 1% active ingredient. But the applicant's request is for 2%.

3.3.11.3 A human study was initiated by the CTFA South Africa (Cosmetic, Toiletry, and Fragrance Association) to analyse whether Bearberry Extract and its related actives, alpha-Arbutin and beta-Arbutin are converted to hydroquinone in human skin. The study was performed by the Photobiology Laboratory of the University of Limpopo, Medunsa, South Africa (*32*).
For this purpose a simple gel without penetration enhancer, containing 3% of either alpha-Arbutin or beta-Arbutin was applied to the upper thigh area (2 mg/cm^2) of 30 Negroid (N) and 30 Caucasian (C) healthy female volunteers for 4 days, twice daily. The positive control was a gel with 1.9% hydroquinone and the negative control was the gel without active ingredient (placebo). One hour after the last application a series of 15 tape strips was taken from each of the four test sites as well as an untreated site to assess the hydroquinone levels in the stratum corneum. Each set of 15 strips was placed into 15 mL of 20% methanol and incubated for 45 min. A pilot study was conducted with 5 volunteers in October 2003 followed by the main study in May 2004. Samples were analysed by an HPLC assay method developed as part of a pilot study including 5 volunteers (C=Caucasian, N=Negroid).
As seen in the table below (taken from the final report), the hydroquinone levels measured in skin from the application of the 1.9% hydroquinone gel were taken as 100% and relative skin levels of hydroquinone were calculated to be 0.4% and 0.8% for alpha- and beta-Arbutin (based on the results from the main study only, respectively.

Table as in the Final report	Mean Hydroquinone levels detected (µg/mL)		
	Pilot study (Oct '03)	Main study (May '04)	
Number of volunteers	5 (3 C + 2 N)	30 C	30 N
Alpha-Arbutin	5.67	0.25	0.37
Beta-Arbutin	1.55	0.45	0.86
Placebo	0	0.39	0.41
Untreated site	0	0.13	0.06
Bearberry	0	Not used	Not used

Conclusion

Although the results of the pilot study and the main study were seemingly contradictory with regard the hydroquinone levels released from alpha- and beta-Arbutin, the authors concluded that the conversion of alpha-Arbutin and beta-Arbutin to hydroquinone is negligible when applied to the skin.

Ref.: (*32*)

Discussion (by Applicant, excerpt)**:**

"We found a separately prepared table of analytical results for the test samples and include it here for review. It is clear that analytical sensitivity or the method specificity was weak, or that sample processing steps were poorly executed. As a result, the very high standard deviations around the respective sample means do not allow differentiating the control from test groups or show distinct differences for any of the treatment groups. It may also be the case that no true differences exist. The study does not allow full conclusions to be made."

Table of Additional Analytical results (Mamabalo et al 2004)

Pilot Study (3C and 2N)	Hydroquinone (µg/ml)	
Treatment	**Mean**	**Std. Dev**
Hydroquinone	178.060	61.830
alpha-Arbutin	5.670	2.530
beta-Arbutin	1.550	1.620
Placebo	0.000	0.000
Untreated	0.000	0.000
Bearberry	0.000	0.000

Main study

30 Caucasian	Hydroquinone (µg/ml)	
Treatment	**Mean**	**Std. Dev**
Hydroquinone	144.807	99.546
alpha-Arbutin	0.252	0.442
beta-Arbutin	0.445	0.389
Placebo	0.385	0.535
Untreated	0.130	0.321

30 Negroid	Hydroquinone (µg/ml)	
Treatment	**Mean**	**Std. Dev**
Hydroquinone	120.189	80.426
alpha-Arbutin	0.366	0.367
beta-Arbutin	0.861	0.813
Placebo	0.408	0.561
Untreated	0.063	0.201

"Despite the many shortcomings of the study and its reporting we include this information because, to our knowledge and from our own literature searches, we believe such information is not readily available on studies of the release of hydroquinone from alpha-Arbutin in vivo. However, the study results suggest that any hydroquinone released from alpha-Arbutin on the skin is far below the hydroquinone levels obtained from application of the positive control gel containing 1.9% hydroquinone. Considering that hydroquinone is about 40% of the molecular (formula) weight of the alpha-Arbutin molecule, a complete release of hydroquinone from alpha-Arbutin (which was applied as a 3% formulation) would have resulted in a similar HQ exposure as from the positive control 1.9% hydroquinone formulation."

SCCS comment

The SCCS shares the applicant's view that this study is only of limited value with regard to quantifying hydroquinone (HQ) release from arbutins *in vivo*. It may be taken as indication that the extent of HQ release in skin treated with 3% alpha- or beta-Arbutin formulation is far below amounts resulting from exposure to gels with 1.9% HQ.
On the other hand, HQ release in skin samples from volunteers with repeated application of beta-Arbutin has been investigated and revealed that HQ can represent up to 12% of the absorbed fraction of beta-Arbutin (section 3.3.4.2 in ß-Arbutin opinion, SCCS/1550/2015). However, an *in vivo* study of similar quality (which covers also the possible influence of skin microbes on the stability of the applied product) is not available for alpha-Arbutin.
In the absence of better data, the SCCS will assume that HQ can represent 20% of the dermally absorbed fraction of alpha-Arbutin (submission II, page 27 and ref. 6).

3.3.12 Special investigations

Metabolism of alpha-Arbutin and beta-Arbutin by mouse skin enzymes

To assess the metabolism of alpha-Arbutin compared to beta-Arbutin by skin enzymes, the stability of both substances was measured 1) in the presence of mouse skin and 2) in the presence of mouse skin homogenate.
For the first experiment 2 mL of alpha-Arbutin and beta-Arbutin (0.037 M in Na citrate-phosphate buffer, pH 7) were incubated with 0.5 and 2.0 g freshly prepared mouse skin in phosphate buffer at 37°C. After 19 and 40 hours samples were centrifuged and alpha- and beta-Arbutin was determined in the supernatant by HPLC. Each sample was tested in triplicate in all 4 groups and mean residual rates were calculated.
For the second experiment a skin homogenate was prepared from mice (2.6 g skin in 5.2 mL 100 mM Na citrate-phosphate buffer, pH 7.0) and mixed 1:1 (v/v) with the 0.037 M alpha- or beta-Arbutin solutions. Samples were taken after 4 and 20 hours of incubation at 37°C, centrifuged and supernatants were analysed as described above.

Result: Neither alpha-Arbutin nor beta-Arbutin was metabolised after 19 hours of incubation with 0.5 g of skin (~95% recovery), while in the samples containing 2.0 g of skin the initial amounts were reduced to 85% and 87%, respectively.
About 87.5% of alpha-Arbutin could be recovered after 40 hours of incubation with 0.5 g of skin, while beta-Arbutin could not be detected anymore. In the samples containing 2.0 g skin the residual amount was 34.4% and 3.7% for alpha-Arbutin and beta-Arbutin, respectively. With the skin homogenate the residual alpha-Arbutin and beta-Arbutin was 98.9% and 95.6%, respectively, after 4 hours. After 20 hours the residual was 85.6% and 31.5%, respectively.

Table 1: Residual alpha-and beta Arbutin amounts after incubation with skin for 19 and 40 hours			
Time	Amount of skin/sample	Residual alpha-Arbutin (%)	Residual beta-Arbutin (%)
19 h	0.5 g	~95% (no exact numbers were given in the report)	~95% (no exact numbers were given in the report)
	2.0 g	~85% (no exact numbers were given in the report)	~87% (no exact numbers were given in the report)
40 h	0.5 g	87.5%	0%
	2.0 g	34.4%	3.7%

Table 2: Residual alpha-and beta Arbutin amounts after incubation with skin homogenate for 4 and 20 hours.		
Time	Residual alpha-Arbutin (%)	Residual beta-Arbutin (%)
4 h	98.9%	95.6%
20 h	85.6%	31.5%

Conclusion (of applicant): The results indicate that alpha-Arbutin is more stable against degradation by skin enzymes compared to beta-Arbutin.

Ref.: (*10*)

SCCS comment

This study used skin of mice; the results on the recovery of alpha- and beta-arbutin are of limited value because of known differences in skin enzyme activities including hydrolases between various species (Mier & van den Hurk 1976; Chang et al. 1991; Oesch et al. 2014).

Since arbutins are hydrolysed by different alpha- and beta-glucosidases (enzymes in skin and skin resident microbes), the extent of metabolism to HQ can differ for both substances. Thus, internal HQ exposure should be based on data (rather than a simple read-across).

Inhibition of tyrosinase

Alpha-Arbutin was shown to exert inhibitory effects on tyrosinases from B16 mouse melanoma and HMV II human melanoma cells, as well as in a three-dimensional human skin model, but, in contrast to beta-Arbutin, did not inhibit tyrosinase from mushroom (*13-16*). Alpha-Arbutin showed stronger inhibitory activity on tyrosinase isolated from human melanoma cells than beta-Arbutin (4-hydroxyphenyl β-glucopyranoside), with inhibitory IC_{50} values of 2.1 mM and >30 mM, respectively. Further experiments with additional α-glycosides suggest that the α-glycosidic linkage plays an important role in the inhibitory effect (*13, 17*). The effect of alpha-Arbutin on reduced skin pigmentation seems not to be due to inhibition of cell growth or decreased tyrosinase gene transcription, but rather to the inhibition of the enzyme at protein level (*16*).

3.3.13 Information on the toxicity of hydroquinone

Taken in part from the SCCP/1158/08 Opinion and updated in SCCS/1550/2015

LD_{50}-oral-rat = 298 mg/kg
Slightly irritating to the eye
Sensitising to the skin
NOEL (28d/90d-oral-rat) = 20 mg/kg/day

NOAEL (28d/90d-dermal-rat) = 74 mg/kg/day
NOEL (developmental toxicity-rabbit) = 25 mg/kg/day (dams)
NOEL (developmental toxicity-rabbit) = 75 mg/kg/day (teratogenic effects).
NOEL (1-generation reproduction toxicity-rat) = 15 mg/kg/day (general toxicity).
NOEL (1-generation reproduction toxicity-rat) = 150 mg/kg/day (reproductive toxicity).
Negative in the Ames test, the dominant lethal assay and the mouse spot test.
Positive in the *in vitro* chromosome aberration test (+S9)
Positive (i.p.) and weakly positive (oral) in the *in vivo* micronucleus test.
Equivocal conclusions on potential carcinogenic effects at dosage levels ≥ 25 mg/kg/day.
[HQ Refs. A, B]

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

[HQ Ref. A]

According to IARC, hydroquinone is not classifiable as to its carcinogenicity to humans. This conclusion was based upon limited evidence in experimental animals and inadequate evidence in humans (IARC 1999). In the EU, hydroquinone is classified as Carc Cat 2 H351 (suspected of causing cancer) based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI[1].
A more recent literature review on the carcinogenicity of hydroquinone concludes that indeed renal tumours were observed in male rats, but that the mode of action (exacerbated chronic progressive nephropathy) appears to be a rat-specific disease that appears to lack a human counterpart. The available cohort studies (all involving occupational exposure) failed to show a clear causal relationship between exposure to hydroquinone and the development of several types of malignancies.

[HQ Ref. C]

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

[HQ Ref.D]

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

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

Studies on the kinetics (ADME) of hydroquinone (HQ) in humans and rodents (reviewed by McGregor 2007=HQ Ref.C and Anderson et al. 2010=HQ Ref.E) indicate rather high bioavailability and rapid clearance after oral or dermal administration. The dermal penetration in humans was examined for 2% HQ in cream *in vitro* and *in vivo* with similar results [Wester et al. 1998= ref.9 in subm II]: Total permeation of HQ after 24h was 43.3% of the dose *in vitro*; flux was 2.93 µg/h-cm^2. An average of 45.3±11.2% of the dose was recovered in urines of volunteers after application of HQ to forehead skin. It is worth noting that dermal penetration of HQ is considerably higher than that of α– or ß-Arbutin.
For the safety evaluation of HQ exposure resulting from the use of skin bleaching products in comparison to internal HQ doses which may induce ochronosis, the SCCS will use 50% dermal absorption for HQ.

3.3.14 Safety evaluation

Safety evaluation for alpha-arbutin

Alpha-Arbutin exposure calculation

Scenario A: Face, hand and neck

Alpha-Arbutin at 2% in cream formulation is applied at 2 mg product/cm^2 skin on face, neck and hands (ca. 700 cm^2); with two daily applications about 2.8 g (max. 4 g) product is applied with 56 mg (max. 80 mg) active ingredient per day (or A=0.933 mg/kg bw/day or max. 1.334 mg/kg bw/day).

Using the value of 0.53% skin absorption (0.27%+2SD) yields about 0.3 mg (or max 0.424 mg) alpha-Arbutin in the relevant skin layers.

Exposure to active ingredient	A	= 0.933 mg/kg bw/day or max 1.334
Skin Area Surface	SAS	= 700 cm^2
Application Frequency per day	F	= 2
Concentration in finished product	C	= 2 %
Typical body weight of human		= 60 kg
Dermal absorption	DA_p	= 0.53 %
Systemic exposure dose (SED)	A x C /100 x DAp	= 0.005 mg/kg bw (or max. 0.007 ….)

Scenario B: Body Lotion

Alpha-Arbutin at 0.5% in formulation applied at 2 mg product/cm^2 skin on the body; for two daily applications then about 7.82 g (SCCS Notes of Guidance, Tab.3) product is applied with 39.1 mg active ingredient per day (or A=0.65 mg/kg bw/day).

Using the value of 0.53% skin absorption (0.27%+2SD) yields about 0.21 mg alpha-Arbutin in the relevant skin layers.

Exposure to active ingredient	A	= 0.65 mg/kg bw/day
Skin Area Surface*	SAS	= 17000 cm^2
Application Frequency per day	F	= 2
Concentration in finished product	C	= 0.5 %
Typical body weight of human		= 60 kg

Dermal absorption	DA_p	= 0.53 %
Systemic exposure dose (SED)	A x C /100 x DAp	= 0.0034 mg/kg bw

Total exposure to alpha-Arbutin from both exposure scenarios:		
External amount:	56 (max 80) + 39.1 = (range) 96 - 129	mg/person/day
External dose:	0.933 (max 1.334) + 0.65 = 1.583 (max 1.984)	mg/kg bw/day
Internal dose (SED):	0.005 (max 0.007) + 0.0034 = 0.0084 (max 0.0104)	mg/kg bw/day
SED expressed **in µg/person/day**: 300 (or max 420) + 204 = **(range) 504 - 624**		

* *(area body – area face and hand)*

Estimation of Margin of Safety

Without a repeated dose toxicity study with alpha-Arbutin (only a 14-day dermal irritation study) there is no basis to derive a NOAEL for a classical MOS calculation for systemic safety by topical exposures.

The applicant has suggested using the TTC approach[2]. Without a justification the applicant assigned Cramer class I (safe dose: 1800 µg/person/day) for alpha-arbutin applied topically under the assumption that the molecule remains intact as applied. However, the SCCS considers that hydroquinone can be released when alpha-arbutin is applied to the skin and can also be present in the formulation as an impurity. Furthermore, alpha-arbutin is predicted as a Cramer Class III substance (90 µg/person/day) albeit with genotoxicity alerts by the ToxTree software programme. The TTC for substances with a genotoxicity alert is 0.15 µg/person/day. The total SED for alpha-arbutin is in the range of 504 - 624 µg/person/day leading to the conclusion "*Risk assessment requires compound-specific toxicity data*" when applying the TTC decision tree.

Alternatively, it can be hypothesized that the systemic toxicity of alpha-arbutin is mainly due to hydroquinone formation in the body. Based on the worst-case assumption of complete cleavage, based on the SED of max. 624 µg/person/day corrected for molecular weight (624 µg alpha-arbutin x 110.11 / 272.25 = 252 µg hydroquinone) and a body weight of 60 kg a dose of 4.2 µg/kg bw/day hydroquinone can be derived. Compared to the NOEL of hydroquinone of 20 mg/kg bw/day a margin of safety of 4800 is calculated.

Further safety considerations will focus on ingredient-related topical exposure to hydroquinone, taking into account information on the stability and metabolic fate of alpha-arbutin in and on skin, to estimate hydroquinone release.

Safety evaluation for hydroquinone

Background information on the toxicity of hydroquinone is provided in section 3.3.13 of this opinion.

Exposure to hydroquinone (HQ) from application of alpha-arbutin containing products (face cream plus body lotion) is derived as follows:
a) Using 0.53% skin absorption (0.27%+2SD) for the glycoside yields about 504 µg (or max 624 µg) alpha-Arbutin into the relevant skin layers (see above 3.3.14.1) of which 20% (ref. 6, subm. II) could be converted to HQ and related reaction products, *i.e.* 100 µg or

[2] For a TTC approach, the SCCS (and other SCs) accept in principle the division into Cramer Classes I and III (SCCP/1171/08). When assigning a chemical to the lowest toxicity class (Class I, 1800 µg/person/d for substances with no genotoxicity alert), classification should be carefully considered and justified. The applicant proposed to compare an SED for alpha-arbutin to Cramer class I compounds, but provided no justification for this classification, aside from the structural similarity with beta-arbutin.

125 µg per person and day. It is not known whether this fraction (~0.1 mg HQ+reaction products) will remain within the skin bound to protein, be metabolized, or pass to systemic circulation.
b) Additional HQ exposure may occur from the possible presence of HQ as impurity and/or hydrolysis of alpha-Arbutin by skin resident microbes. With regard to the latter, about 6% degradation of alpha-Arbutin to HQ was noted for *pig skin* in the absence of antibiotics (section 3.3.4.2). Information on the effect of human skin resident microbes on the stability of alpha-Arbutin is not available, as all incubations with *human skin* contained antibiotics.
For the presence of HQ as impurity in the applied products, the SCCS has based an estimate on results from stability tests (section 3.1.9.2) which indicated about 13 ppm HQ in 'stabilized formulations' after several weeks. For the applied products (crème 2.6 g and body lotion 7.82 g), one can add 135 µg HQ (external amount) and use 50% dermal absorption for HQ, resulting in an extra 68 µg HQ per day.

Below the exposure to hydroquinone from application of alpha-arbutin containing products (sum of a) and b) above) is assessed and compared to risks related to 1.) repeated toxicity, 2.) induction of ochronosis and 3.) carcinogenicity.

Internal exposure of **190 µg HQ-equivalents** per person/day divided by 60 kg results in an

SED of 3.166 µg/kg bw/day or **0.00317 mg/kg bodyweight**

1.) *HQ repeated toxicity*

NOEL from repeated oral toxicity test:	20 mg/kg bw	→ **MOS 6309**
NOEL from 2-generation reprotoxicity test:	15 mg/kg bw	→ **MOS 4731**

2.) *HQ induction of ochronosis*

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

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

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

total estimated HQ amount resulting from alpha-arbutin skin application:
of 190 µg HQ per day = **0.19 mg in skin** (internal amount)

compared to the HQ internal (skin) amount resulting from 1% HQ crème:
≈ 8 mg (for 50% dermal HQ absorption)
→ SED Ratio of 42

The minimum exposure level of HQ causing exogenous ochronosis is calculated as being 42 times higher than the maximum HQ exposure level formed from alpha-arbutin. Therefore it can be concluded that alpha-arbutin does not pose a risk with regard to induction of exogenous ochronosis in humans (under the conditions of use specified above).

3.) *HQ carcinogenicity*

According to IARC (1999) HQ is not classifiable as to its carcinogenicity for humans (group 3). In more recent reviews on the carcinogenicity of HQ (McGregor 2007; CIR 2010) a nongenotoxic mode of action that involves exacerbation of a spontaneously occurring rodent renal disease, chronic progressive nephropathy (CPN), has been proposed. CPN is

particularly prominent in male rats and the evidence is consistent with an absence of a human counterpart.
In the EU, hydroquinone is classified as Carc Cat 2 H351 (suspected human carcinogen) Muta Cat 2 H341 (suspected of causing genetic defects) based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI.
The SCCS has carried out a calculation to determine lifetime cancer risk (as in section 3-7.4 of the Notes of Guidance SCCS). First an animal dose descriptor (T25) for carcinogenic potency is determined and then converted to a human dose descriptor (HT25) based on comparative metabolic rates.

For the critical effect (renal tubular cell adenomas in male F344 rats; NTP study 1989), the **T25 dose descriptor** for hydroquinone is **61.4 mg/kg bw/day**. From this a **HT25 value of 18.2 mg/kg bw/d** was obtained. Assuming a systemic exposure dose of 0.00317 mg/kg bw/d, the **risk** is calculated to be **4.35 x 10^{-5}**.

The calculated lifetime cancer risk for hydroquinone (HQ) exposure, resulting from use of cosmetic products with alpha-Arbutin, is higher than the commonly accepted level of concern with regard to carcinogenicity.

However, it can be taken into account that the tumour type induced by HQ is considered rodent specific and may have little relevance for humans and risk has been calculated by linear extrapolation (in this case a highly conservative approach).

3.4 Discussion

Physico-chemical properties *and stability*
Alpha-arbutin is the synthetic α-glucopyranoside of hydroquinone, and thus the α-isomer of arbutin (ß-arbutin) and readily soluble in water. 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. Data are also presented on the stability of alpha-arbutin in different (stabilised, unstabilised) cosmetic product formulations: In the stabilised formulations the concentrations of hydroquinone are initially between 1 - 3 ppm and increase to about 13 ppm under accelerated storage conditions, but remain much lower than in the nonstabilised formulations.
Yet, it is to be considered that alpha-arbutin might also undergo cutaneous hydrolysis into hydroquinone due to enzymatic activity (through α-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). This is discussed in the context of data from *in vitro* studies on dermal penetration.

Toxicity
The single dose oral toxicity of alpha-Arbutin in water administered to rats revealed an LD50 between 300 and 500 mg/kg body weight. Two acute oral toxicity tests in rats with either a 5% or 10% alpha-Arbutin cream formulation did not reveal lethality after single gavage doses at 2000 mg formulation/kg bw (or >125 and >250 mg/kg bw active ingredient). No toxicity studies with repeated dosing has been carried out, except for a 14-day study in guinea pigs with dermal application. However, due to its focus on irritation and the low number of animals, this study is not suitable for conclusions on systemic effects or derivation of a NOAEL.
Without a repeated dose toxicity study with alpha-Arbutin there is no basis to derive a NOAEL for a classical MOS calculation for systemic safety by topical exposures. Thus, the applicant has suggested using the TTC approach, and assigned (without any justification) Cramer class I for alpha-arbutin applied topically under the assumption that the molecule remains intact as applied. However, the SCCS considers that hydroquinone can be released when alpha-arbutin is applied to the skin and can also be present in the formulation as an impurity. Furthermore, alpha-arbutin is predicted as a Cramer Class III substance with genotoxicity alerts by the ToxTree software. On the other hand, it can be hypothesised that

the systemic toxicity of alpha-arbutin is mainly due to hydroquinone (HQ) formation in the body. Based on the worst-case assumption of complete cleavage to HQ, the SCCS has calculated a margin of safety (see section 3.3.14).

Local toxicity
Alpha-arbutin when tested as 10% solution was non-irritant to rabbit skin and only minimally irritant to rabbit eye. In a repeated once-daily dermal dosing of guinea pigs with alpha-Arbutin (0.05 g plus 0.1 ml water) consecutively for 14-days it was considered "practically non-irritant" in this study design.
In two studies with human volunteers and topical application of cosmetic formulations with 1% alpha-arbutin for 30 days or 12 weeks no adverse reactions were observed and the products were well tolerated during the studies. The SCCS notes that the applicant's request is for 2% active ingredient.

Skin sensitisation
In a guinea pig maximisation test with up to 50% alpha-arbutin, the test agent was found to be a mild skin contact sensitiser. No skin contact sensitisation occurred in another study with alpha-arbutin up to 20% which tested also cross-reactivity to hydroquinone (HQ) induction. Since HQ challenge (0.2% or more) caused strong sensitisation reactions, the negative results for alpha-arbutin imply that HQ formation in skin was below the level that elicits sensitisation in the guinea pig.

Dermal absorption *and formation of hydroquinone*
An early study with frozen/thawed skin from just one pig is of limited value with regard to hydrolysis and degradation of alpha-arbutin in skin. The total absorption of about 1.3% is similar to or lower than that found in follow-up studies, which was about 2% in pig skin.
In response to the 2011-2012 SCCS call for data on the stability of alpha-Arbutin on skin and the concern for release of hydroquinone (HQ) to skin from the use of skin lightening products, the applicant has completed three *in vitro* studies to assess alpha-Arbutin skin penetration and distribution as well as stability and metabolism when associated with skin.
Overall, the results indicate that human skin *in vitro* is about 8-fold less permeable to alpha-Arbutin than pig skin, with a total dermal absorption of 0.27 (±0.13) % of the applied dose for human skin, and 2.13 (±0.41) % for pig skin.
About 80% of recoverable ^{14}C-labeled material remains as alpha-Arbutin with some increase in HQ in the lower skin layers, and intact skin incubated *in vitro* with alpha-Arbutin shows only limited release of HQ in (pig and human) skin samples treated with antibiotics. 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 HQ and related degradation products.
Incubation with *homogenate* from pig skin and human skin showed that alpha-Arbutin was hydrolysed enzymatically to hydroquinone, but with somewhat different kinetics related to surrounding pH: At pH 5.4 the reaction kinetics were similar between human and pig skin homogenates whereas at pH 7.4 the reaction kinetics with human skin homogenate was much slower than with pig homogenate; about 85% alpha-Arbutin and 15% hydroquinone was found after 24 hours. It is worth noting that metabolism in homogenates (broken cell preparations) is usually more rapid than in intact skin.
In the absence of an *in vivo* studies with repeated topical application of a skin-lightening agent and analysis of parent compound and hydroquinone in skin biopsies (as conducted for beta-arbutin), the SCCS considers that 20% of the absorbed alpha-arbutin fraction in skin is present as hydroquinone.

Systemic toxicity
No toxicity studies with repeated dosing has been carried out, except for a 14-day study in guinea pigs with dermal application. However, due to its focus on irritation and the low number of animals, this study is not suitable for conclusions on systemic effects or derivation of a NOAEL.

Mutagenicity/genotoxicity
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 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.

Carcinogenicity
No data available. Discussion on hydroquinone: see section 3.3.14.

Reproductive toxicity
No data available.

Toxicokinetics
Studies with pig and human skin homogenate indicate that metabolisation of alpha-arbutin is similar, with partial hydrolysis to hydroquinone which is pH-dependent and catalysed by microbial and/or skin glucosidases (see above dermal absorption).
One study in human volunteers with topical application of a gel containing either 3% alpha-arbutin, 3% beta-arbutin or 1.9% hydroquinone (HQ) is of limited value with regard to quantifying hydroquinone (HQ) release from arbutins *in vivo*, but of interest with regard to demonstrating that the extent is far below amounts resulting from exposure to gels with 1.9% HQ. *[Considering that hydroquinone is about 40% of the molecular (formula) weight of the alpha-Arbutin molecule, a complete release of hydroquinone from alpha-Arbutin (applied as a 3% formulation) would have resulted in a similar HQ exposure as from the control 1.9% hydroquinone formulation.]*

Photo-induced adverse effects

In studies conducted in guinea-pigs neither phototoxicity nor photoallergy occurred with alpha-arbutin (hydroquinone-alpha-D-glucoside) in doses up to 10% w/v in distilled water.

Issues related to hydroquinone (HQ) in skin lightening products
If HQ is released in relevant amounts either in the product or during the use of arbutins (alpha-Arbutin, β-Arbutin or similar ingredients), the product could not be considered safe, since HQ has been assessed as being unsafe for use in skin-lightening applications due to the danger of exogenous **ochronosis** and leukomelanoderma [41] and consequently is currently banned for this use in the EU. The HQ product concentrations at which ochronosis has been described are of 1% and higher [44]. A 2% formulation of ^{14}C–hydroquinone was shown to have 45.3% ± 11.2% availability with a 24-hour application in human volunteers (6). The bioavailability was calculated as 43.3% of the dose with a flux of 2.85 µg/cm^2/h.
As no data is available on HQ concentration levels below 1%, a lower threshold for the occurrence of ochronosis is difficult to establish. Although the risk for ochronosis may be relatively low and largely confined to individuals with very dark skin, the occurring cases can be severe and irreversible.
The use of arbutins in skin-bleaching products is a complex situation for which the application level and the local availability of hydroquinone cannot be generalised. The factors causing HQ release at the skin surface will depend on the activity of skin bacteria and enzymes (glycosidases) in any one individual and there is insufficient information on their quantitative variation. Moreover, dermal penetration of the active ingredient and its stability in formulations can differ. Therefore, a **case-by-case evaluation** is needed.

Dermal absorption of alpha-arbutin is rather low with human skin (0.53% of the applied dose) and higher with pig skin (2.1% of the applied dose). The metabolism of alpha-arbutin

in skin *homogenates* from both species is similar with release of hydroquinone (HQ) catalysed by skin enzymes (glycosidases), but with somewhat different kinetics related to surrounding pH. In percutaneous penetration studies with (split or intact) pig skin the use of antibiotics reduced the degradation/hydrolysis of alpha-arbutin by skin resident microbes on pig skin. For human skin, information on the effect of skin resident microbes on the stability of alpha-arbutin is lacking. In dermal penetration studies with pig skin, about 80% of the recoverable material remains as alpha-arbutin, with some increase in HQ in lower skin layers. On this basis the SCCS considers that 20% of the absorbed alpha-arbutin in human skin is converted to hydroquinone (HQ and related products). When using this value of 20% conversion, the use of 2% of alpha-arbutin in creams applied twice per day to face, neck and hands as well as use of 0.5% alpha-arbutin in body lotions, could give rise to a systemically available dose of about 100 to 125 µg of hydroquinone per person and day. Considering also the presence of HQ as impurity in cosmetic formulations with alpha-arbutin (and the high dermal penetration of HQ itself), an extra 68 µg HQ were taken into account.
Thus, a total internal exposure value for HQ of 190 µg was used in safety assessments related to ochronosis and other endpoints.
In comparison, the internal exposure resulting from use of a product with 1% hydroquinone (taking a dermal penetration of about 50%) would amount to about 8 mg hydroquinone. This dose (which may elicit ochronosis) is 42 times higher than that resulting from the use of products with alpha-arbutin. Therefore it can be concluded that alpha-arbutin does not pose a risk with regard to induction of exogenous ochronosis in humans (under the conditions of use specified in the terms of reference).
The general potential toxicity of HQ has been reported as being highly sex-, species- and strain- specific, with nephrotoxicity and carcinogenicity primarily limited to male F-344 rats. Epidemiology and occupational studies of workers with extensive exposure to HQ have not reported any evidence of adverse *systemic* health effects.
Since hydroquinone is classified in the EU as Carc Cat 2 H351 (suspected of causing cancer) based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, the SCCS has carried a calculation to determine a lifetime cancer risk. Risk was calculated to be 4.35×10^{-5}, based on a systemic exposure dose of 0.00317 mg/kg bw/day and a HT25 dose derived from the T25 dose descriptor for renal tubular cell adenomas in male F344 rats (NTP study 1989).
Taking into account that risk has been calculated by linear extrapolation (in this case a highly conservative approach), and that the tumour type considered may have little relevance for humans, hydroquinone (HQ) exposure resulting from alpha-arbutin containing cosmetic products (under the conditions of use specified in the terms of reference) can be considered to be of low concern with regard to carcinogenicity.

4. CONCLUSION

(1) *Does the SCCS consider on the basis of the provided scientific data, the use of α-Arbutin to be safe for consumers in cosmetic products in a concentration up to 2% in face creams and up to 0.5 % in body lotions?*

The SCCS considers the use of *α-Arbutin* safe for consumers in cosmetic products in a concentration up to 2% in face creams and up to 0.5 % in body lotions.

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

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

5. MINORITY OPINION

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