
Scientific Committee on Consumer Safety

SCCS

# OPINION ON

# deoxyarbutin

# Tetrahydropyranyloxy Phenol

The SCCS adopted this opinion at its 10th plenary meeting

on 25 June 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.
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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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## 1. BACKGROUND

Deoxyarbutin CAS n. 53936-56-4 (4-[(tetrahydro-2H-pyran-2-yl)oxy]phenol) with INCI name Tetrahydropyranyloxy Phenol is a skin lightening agent synthesised through removal of hydroxyl groups from the glucose side-chain of ß-arbutin.
In the first opinion (SCCP/1158/08) on ß-arbutin adopted on 15th April 2008 the SCCP raised concerns with other substances resulting in the release and/or formation of hydroquinone.
However, Hydroquinone (CAS 123-31-9) is listed in Annex II/1339 of the Cosmetic Regulation No 1223/2009; therefore it is banned as cosmetic ingredient with the exception of entry 14 in Annex III. It is only permitted for professional use in artificial nail systems in a concentration in the final product up to 0.02%. Since Hydroquinone could not be used as a skin whitener after introduction of a ban, other substances have been used for that purpose, including Arbutin.
A dossier on the related substance, deoxyarbutin, was submitted to the European Commission by Girindius AG in 2008.

## 2. TERMS OF REFERENCE

*(1) Does the SCCS consider on the basis of the provided scientific data, the use of deoxyarbutin to be safe for consumers in cosmetic products in a concentration up to 3% in face creams?*

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

## 3. OPINION

### Introduction

Deoxyarbutin (4-[(tetrahydro-2H-pyran-2-yl)oxy]phenol) is a skin lightening agent synthesised through removal of hydroxyl groups from the glucose side-chain of β-arbutin.
In January 2012 the SCCS sent out a "*Request for additional data on arbutin and its derivatives*" to companies who had previously submitted a dossier on such agents. Whilst applicants for the other skin lightening agents (alpha- and beta-arbutin) responded, the mail to the applicant for deoxyarbutin, Girindius AG (Germany) was returned to sender.
A search revealed that the company has gone out of business (bought by Nicco Denco Avecia, which was then acquired by Merck & Co.).
This Opinion is based on the original submission of 2008 by Girindius AG.

*Note:* „Deoxyarbutin 100"=code for the company's compound name

### Overview on Studies conducted

Studies were generally carried out to the appropriate OECD/SCCP guidelines and to GLP. Where this was not the case, as in some very early studies, the rationale for their inclusion is stated.

| Study | Test material batch/lot code | Test Guidelines | GLP | Ref. |
|---|---|---|---|---|
| **Acute toxicity** | | | | |
| Acute Oral Toxicity Study with Rats (2005) | Deoxyarbutin 100/504007 | OECD 423 | Yes | 4 |
| Acute Oral Toxicity Study with Rats (1994) | Deoxyarbutin 100/HT0059.01 | Comparable to OECD 425 | Yes | 33 |
| Acute Oral Toxicity Study with Rats (1994) | Deoxyarbutin 100/HT0059.04 | Comparable to OECD 425 | Yes | 34 |
| Acute Dermal Toxicity Study with Rats (2005) | Deoxyarbutin 100/504007 | OECD 402 | Yes | 5 |
| Acute Dermal Toxicity Study with Rats (1994) | Deoxyarbutin 100/HT0059.01 | Comparable to OECD 402 | Yes | 35 |
| Acute Intraperitoneal Toxicity Study with Rats (1994) | Deoxyarbutin 100/HT0059.01 | - | Yes | 36 |
| **Skin irritation** In Vitro Skin Corrosion Study – Transcutaneous Electrical Resistance Test (2005) | Deoxyarbutin 100/501002 | OECD 430 | Yes | 6 |
| **Skin irritation** Acute Dermal Irritation/Corrosion Study with Rabbits (2005) | Deoxyarbutin 100/504007 | OECD 404 | Yes | 7 |
| **Eye irritation** Acute Eye Irritation/Corrosion Study with Rabbits (2005) | Deoxyarbutin 100/504007 | OECD 405 | Yes | 8 |
| **Eye irritation** Acute Eye Irritation Study with Rabbits (1994) | Deoxyarbutin 100/HT0061.01 | Comparable to OECD 405 | Yes | 37 |
| **Skin sensitization** Local Lymph Node Assay (2005) | Deoxyarbutin 100/504007 | OECD 429 | Yes | 9 |
| **Skin sensitization** Skin Sensitization Study (LLNA)(1994) **Dermal / Percutaneous Absorption** | Deoxyarbutin 100/HT0059.01 | Comparable to OECD 429 | Yes | 38 |
| **Repeated dose toxicity** 28-Day Oral Toxicity Study with Rats (2006) | Deoxyarbutin 100/504007 | OECD 407 | Yes | 18 |
| 28/91-day Percutaneous | Deoxyarbutin | - | Yes | 40 |

| | | | | |
|---|---|---|---|---|
| Toxicity (1994) | 100/HT0059.01 | | | |
| **Mutagenicity / Genotoxicity: *in vitro***<br>Ames test (1994)<br>*In Vitro* Mammalian Cytogenetic Study (2006) | Deoxyarbutin 100/HT0059.01<br>Deoxyarbutin 100/504007 | Comparable to OECD 471<br>OECD 473 | Yes<br>Yes | 41<br>19 |
| **Mutagenicity / Genotoxicity: *in vivo*** Micronucleus Test with Mice (oral application) (2006)<br>Micronucleus Test with Mice (oral application) (2006) | Deoxyarbutin 100/504007<br>Deoxyarbutin 100/509014 | OECD 474<br>OECD 474 | Yes<br>Yes | 21<br>20 |
| Mammalian Erythrocyte Micronucleus Test (intraperito-neal application) (2007) | Deoxyarbutin 100/509014 | OECD 474 | Yes | 21 |
| Spermatogonial chromosomal aberration test | Deoxyarbutin 100/509014 | OECD 483 | Yes | 25 |
| **Toxicokinetics**<br>Preliminary Penetration and Metabolism Study in Guinea Pigs (1994) | Deoxyarbutin 100/PBK 10082 | - | Yes | 1, 39 |
| **Photo-induced toxicity**<br>Photoirritation/Photoallergy in Guinea Pigs (1996) | Deoxyarbutin 100 / HT0059.05 | - | GLP | 43 |
| **Human Data**<br>Repeated Insult Patch test (2007)<br>Repeated Insult Patch test (1995) | SC23 (Deoxyarbutin 100) / 104544<br>Deoxyarbutin 100 / HT0072.01 | –<br>- | GLP<br>GLP | 24<br>42 |

## 3.1 Chemical and Physical Specifications

### 3.1.1 Chemical identity

#### 3.1.1.1 Primary name and/or INCI name

INCI Name: Tetrahydropyranyloxy Phenol

#### 3.1.1.2 Chemical names

(4-Tetrahydro-pyran-2-yloxy)-phenol

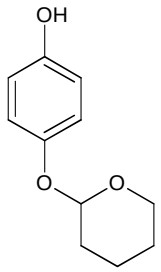
#### 3.1.1.3 Trade names and abbreviations

deoxyArbutin; "Deoxyarbutin" 100

#### 3.1.1.4 CAS / EC number

CAS No: 53936-56-4
EC: not available

### 3.1.1.5 Structural formula




Reference 17

### 3.1.1.6 Empirical formula

Empirical Formula: $C_{11}H_{14}O_3$

## 3.1.2 Physical form

White to off-white solid substance

## 3.1.3 Molecular weight

Molecular weight: 194.23 g/mol

## 3.1.4 Purity, composition and substance codes

Purity: ≥99.0 % (area)

Ref.: 17, 22, 28

## 3.1.5 Impurities / accompanying contaminants

| | |
|---|---|
| Hydroquinone (290 nm) | ≤0.05 % (w/w) |
| Benzoquinone (246 nm) | ≤0.05 % (w/w) |
| Sum Hydroquinone and Benzoquinone | ≤0.1 % (w/w) |
| NS 392 A* (290 nm) | ≤1.0 % (w/w) |
| 4-Benzyloxyophenol (290 nm) | ≤0.1 % (w/w) |
| Impurity RRT** = 1.48 (290 nm) | ≤0.2 % (area) |
| Impurity RRT** = 2.06 (290 nm) | ≤0.2 % (area) |
| Each unknown Impurity (290 nm) | ≤0.2 % (area) |

* NS 392A = 2(4-Benzyloxy-phenoxy)-tetrahydropyran

** RTT = relative retention time in HPLC

| | |
|---|---|
| Heavy Metals: | ≤20 ppm |
| Loss on Drying: | ≤0.5 % (w/w) |
| Residue on Ignition: | ≤0.5 % |
| Water Content: | ≤0.5 % |

Ref.: 17, 22, 28

## 3.1.6 Solubility

| | |
|---|---|
| Solubility in Methanol: | 667 g/L (24°C) |
| Solubility in Ethanol: | 500 g/L (24°C) |
| Solubility in Acetone: | 667 g/L (24°C) |

Solubility in water: 1.6 g/L (20°C), 2.0 g/L (30°C) (Directive 92/69/EEC)

Ref.: 14, 22, 28

### 3.1.7 Partition coefficient (Log $P_{ow}$)

Partition Coefficient (Log Po/w): 2.0 (at 35°C, pH 7.0 buffered)
(Directive 92/69/EEC)

Ref.: 15

### 3.1.8 Additional physical and chemical specifications

| | |
|---|---|
| Melting Point: | 88.6°C |
| Boiling Point: | 307.5 °C |
| Density: | 1.276 at 22°C (Directive 92/69/EEC) |
| pKa: | 10.1 |
| Ignition Point: | No self-ignition temperature was determined up to 10°C. Not classified as "self-igniting" (Directive 92/69/EEC) |
| Vapour pressure: | $4.3x10^{-4}$ Pa at 25 °C (experimental, Directive 92/69/EEC) 0.0137 Pa at 25°C (calculated) |

Ref.: 10, 11, 12, 13, 16, 22, 28

### 3.1.9 Homogeneity and Stability

**Stability during storage**:
Data in the table below indicate that deoxyarbutin, when protected from humidity, temperatures above 8°C and light, for is stable for at least 2 years (conditions: 2-8°C, argon atmosphere, desiccant).

| Test | Specification | 0 months | 26 months |
|---|---|---|---|
| | | 24.10.05 | 22.10.07 |
| | | Q-No. 41432 | Q-No. 107960 |
| Purity (HPLC) | | | |
| Deoxyarbutin 100 (290 nm) | ≥99.0 % (area) | 99.7 % (area) | 99.8 % (area) |
| Hydroquinone (290 nm) | ≤0.05 % (w/w) | 0.02 % (w/w) | 0.03 % (w/w) |
| Benzoquinone (246 nm) | ≤0.05 % (w/w) | <0.01 % (w/w) | 0.01 % (w/w) |
| Sum Hydroquinone and Benzoquinone | ≤1.0 % (w/w) | < 0.1 % (w/w) | 0.04 % (w/w) |
| NS 392 A* (290 nm) | ≤0.1 % (w/w) | n.d. | 0.1 % (w/w) |
| 4-Benzyloxy phenol (290 nm) | ≤0.2 % (area) | n.d. | n.d. |
| Impurity RRT** = 1.48 (290 nm) | ≤0.2 % (area) | 0.2 % (area) | n.d. |
| Impurity RRT** = 2.06 (290 nm) | ≤0.2 % (area) | 0.1 % (area) | 0.1 % (area)<br>RRT 2.17: <0,1 % |

* NS 392A = 2(4-Benzyloxy-phenoxy)-tetrahydropyran ** RTT = relative retention time in HPLC

Deoxyarbutin batch 509014 was manufactured on 12 September 2005 and was stored at ambient temperature then in cold storage from 30.11.2005.

After 3 months storage at 25°C and 60% relative humidity (Table below), deoxyarbutin showed partial decomposition with an increasing hydroquinone content above the specified limit.

| Batch 504007 | Specification | 0 months | 3 months |
|---|---|---|---|
| | | June 2005 | September 2005 |
| | | Q-No. 96830 | Q-No. 98223 |
| Purity (HPLC) | | | |
| Deoxyarbutin 100 (290 nm) | ≥99.0 % (area) | 99.5 % (area) | 99.1 % (area) |
| Hydroquinone (290 nm) | ≤0.05 % (w/w) | 0.02 % (w/w) | 0.20 % (w/w) |
| Benzoquinone (246 nm) | ≤0.05 % (w/w) | <0.01 % (w/w) | <0.01 % (w/w) |
| Sum Hydroquinone and Benzoquinone | ≤0.1 % (w/w) | 0.02 % (w/w) | 0.2 % (w/w) |
| NS 392 A* (290 nm) | ≤1.0 % (w/w) | 0.6 % (w/w) | 0.5 % (w/w) |
| 4-Benzyloxyphenol (290 nm) | ≤0.2 % (area) | n.d. | n.d. |
| Impurity RRT** = 1.48 (290 nm) | ≤0.2 % (area) | <0.1 % (area) | <0.1 % (area) |
| Impurity RRT** = 2.06 (290 nm) | ≤0.2 % (area) | n.d. | 0.1 % (area) |

* NS 392A = 2(4-Benzyloxy-phenoxy)-tetrahydropyran ** RTT = relative retention time in HPLC

Further tests on storage stability under "stress conditions" (40°C, 75% relative humidity) showed a decrease in deoxyarbutin compound (initially 99.6% to 90.3% after 3 months), and an increase in hydroquinone content from 1.67% (w/w), 2.93% (w/w) and 4.4% (w/w) after 1, 2 and 3 months, respectively.

(Reference 29)

**Stability in Solution**:
Aqueous solutions at pH 7 or at slightly basic conditions (pH 8) are stable. At pH 8 in solution (and with no protection against daylight), 0.03% parent compound degrades per hour, with no hydroquinone formation observed. Acidic aqueous solutions (pH <7) are not stable.
At pH 6, some decline in the content of deoxyarbutin with a corresponding increase in hydroquinone (HQ) is noted: Rate of HQ formation at pH 6 per hour is 0.02 % and at pH 5 0.2 % per hour. At pH 1 only HQ and no more deoxyarbutin is found in HPLC analysis.
Extensive exposure of deoxyarbutin to light, temperatures above >8°C and oxygen should be avoided. For analytical purposes, it is recommended to freshly prepare the test solutions at 8°C.

**Stability in o/w formulation**:
Cream (o/w) formulations of deoxyarbutin S/W (6%) are stable at a temperature of ≤8 °C, but somehow degrade under room temperature conditions turning from a white to a yellowish colour (see table below).

| Storage conditions / Test | 1 month | 2 months | 3 months |
|---|---|---|---|
| **Storage: 2 – 8°C** | | | |
| Description | white cream | white cream | white cream |
| Assay: Deoxyarbutin 100 | 5.8 % (w/w) | 5.7 % (w/w) | 5.7 % (w/w) |
| Assay: Hydroquinone | 0.001 % (w/w) | 0.001 % (w/w) | n.d. |
| **Storage: ambient temperature** | | | |
| Description | white cream | white cream | yellowish cream |
| Assay: Deoxyarbutin 100 | 5.7 % (w/w) | 5.8 % (w/w) | 5.7 % (w/w) |
| Assay: Hydroquinone | 0.002 % (w/w) | 0.004 % (w/w) | 0.006 % (w/w) |

(Reference: 29)

**SCCS comment**
The applicant reports that deoxyarbutin is stable for at least 2 years when protected from humidity, temperatures above 8°C and light. However, it has been shown that deoxyarbutin is thermolabile in aqueous solutions; this lack of stability may pose difficulties for its use in cosmetics (Yang et al., 2010). On the other hand, apparently stability can be achieved for oil in water formulations (creams) stored at low temperatures and protected from light, and in anhydrous emulsions also at higher temperatures (Lin et al., 2011).
The experiments determining the stability of deoxyarbutin look conclusive and were shown to be reproducible. It has been clearly stated that this compound and its formulations should be stored at low temperatures under exclusion of air and ambient light (storage recommendation for a lifetime of 24 months: 2-8°C, under argon atmosphere and addition of desiccant). Significant degradation occurs at pH-values lower than pH 7 (0.02% and 0.2% HQ per hour at pH 6 and 5, respectively) and above pH 7 (0.03% at pH 8) and thus under in-use conditions. It should be noted that the average pH-value of human skin is determined at approximately at pH 5.5.

### 3.1.10 Characterisation of deoxyarbutin used in current toxicity studies

Typical commercial batches of deoxyarbutin (batch 504007 and 509014) were used for the toxicological evaluation during the notification process. This material was characterised appropriately as summarised below.

| Testing Parameter | Requirements | Batch 504007 | Batch 509014 |
|---|---|---|---|
| Description | white to off-white solid substance | conforms | conforms |
| Identity (IR) | Conforms to reference spectrum | conforms | conforms |
| Loss on drying | ≤0.5% (w/w) | <0.1% (w/w) | 0.1% (w/w) |
| Water content | ≤0.5% (w/w) | 0.1% (w/w) | 0.1% (w/w) |
| Residue on ignition | ≤0.5% (w/w) | 0.1% (w/w) | 0.2% (w/w) |
| Heavy metals | ≤20 ppm | conforms | conforms |
| Purity (HPLC) | | | |
| Deoxyarbutin 100 (290 nm) | ≥99.0 % (area) | 99.5 % (area) | 99.7 % (area) |
| Hydroquinone (290 nm) | ≤0.05% (w/w) | 0.02 % (w/w) | 0.02 % (w/w) |
| Benzoquinone (246 nm) | ≤0.05% (w/w) | 0.01 % (w/w) | <0.01 % (w/w) |
| Sum of Hydroquinone and Benzoquinone | ≤0.1% (w/w) | 0.02 % (w/w) | <0.1% (w/w) |
| NS 329 A | ≤1.0% | 0.6 % | n.d. |
| 4-Benzyloxyphenol (290 nm) | ≤0.1% (w/w) | n.d. | n.d. |
| Imp. RRT 1.48 (290 nm) | ≤0.2% (area) | n.d. | n.d. |
| Imp. RRT 2.06 (290 nm) | ≤0.2% (area) | 0.1% (area) | 0.1% (area) |
| Each unknown impurity (290 nm) | ≤0.2% (area) | n.d. | RRT 1.1 at 0.1% (area) |

NS 392A = 2(4-Benzyloxy-phenoxy)-tetrahydropyran RTT = relative retention time

Analysis certificates for each batch can be found in the respective reports cited in section 3.5. (Toxicological evaluation)

## 3.2 Function and uses

Deoxyarbutin (4-[(tetrahydro-2H-pyran-2-yl)oxy]phenol) is synthesised through removal of hydroxyl groups from the glucose side-chain of β-arbutin, another skin lightening agent. It

is an inhibitor of the enzyme tyrosinase and therefore reduces formation of melanin. Deoxyarbutin is claimed to be more effective and less cytotoxic than hydroquinone (Yang et al 2010; Chawla et al 2008; Hu et al 2009). As it does not have the β-glucoside structure, it would not be expected to be a substrate for the β-glucosidase enzymes that are found in skin bacteria and in human tissues. Since deoxyarbutin lacks the hydroxyl groups on the glucose moiety, it is expected to be more lipophilic and more easily absorbed by the skin than arbutins (alpha- or beta-arbutin).
Deoxyarbutin is intended to be used as a skin lightening active ingredient in cosmetic preparations up to a maximum concentration of 3.0% in leave-on cosmetic products.

(References: 1, 28, 30)

## 3.3 Toxicological Evaluation

### 3.3.1 Acute toxicity

#### 3.3.1.1 Acute oral toxicity

| | |
|---|---|
| Date of report: | August 2005 |
| Guideline/method: | OECD 423 (2001) |
| Species/strain: | Rat/Sprague-Dawley (Crl:CD(SD)IGS BR) |
| Group size: | 12 females in total (3 per experimental group) |
| Test substance: | deoxyarbutin |
| Batch: | 504007 |
| Purity: | 99.5% (HPLC) |
| Dose levels: | 300, 2000 mg/kg bw |
| Dose volume: | 10 ml/kg bw |
| Vehicle: | Corn oil |
| Route: | oral (gavage) |
| Exposure: | single application |
| Observation period: | 14 days |
| GLP: | Yes |

The test substance was administered by oral gavage to each set of 12 female Sprague-Dawley rats in total. A stepwise approach was performed and each 3 animals received 300 mg/kg bw at step 1 and 2 as well as 2000 mg/kg bw at step 3 and 4 as an oily suspension in corn oil. The dosing volume was 10 ml/kg bw. The animals were observed for treatment-related effects within the periods 0-0.5, 0.5-1, 1-2, 2-4 and 4-6 hours after administration and then at least once a day for a total of 2 weeks. Observations included but were not limited to changes in skin, fur, eyes, the occurrence of secretions and excretions, autonomic activity, changes in gait, posture and the presence of convulsions. Bodyweights were determined prior to administration and on days 7 and 14 after application. Gross pathology was performed in rats that had died and in survivors that were sacrificed on day 14.

Results
At 300 mg/kg bw 2/6 animals showed clinical signs of reduced well-being, while at 2000 mg/kg bw all animals showed additional unspecific signs of nervous system impairment. The bodyweight gain of all animals was not affected. No mortality occurred at 300 mg/kg bw, but at 2000 mg/kg bw each 1/3 animals in step 3 and 4 died. Mortality was observed within 24 hours. No gross pathological findings were noted at necropsy in all surviving animals at 300 and 2000 mg/kg bw. In the 2 animals that died prematurely at 2000 mg/kg bw, gastrointestinal irritation was recorded beside unspecific signs of acute intoxication.

Conclusion
The acute oral toxicity (LD50) of deoxyarbutin was >2000 mg/kg bw for female Sprague-Dawley rats.

Ref.: 4

Other non-validated acute oral toxicity studies in Sprague-Dawley rats estimated LD50 values of 5039 mg/kg bw (CI: 3807–6670 mg/kg bw) in males and 1260 mg/kg bw (CI: 918–1729 mg/kg bw) in females with deoxyarbutin (batch: HT0059.01) dissolved in propylene glycol/absolute ethanol/physiological saline (60:20:20%, v/v/v), while the respective estimated LD50 values for deoxyarbutin batch HT0059.04 dissolved in propylene glycol were reported as 3068 mg/kg bw (CI: 2270–4146) for males and 1148 mg/kg bw (CI: 843-1564) for females.

Ref.: 33, 34

#### 3.3.1.2 Acute dermal toxicity

| | |
|---|---|
| Guideline/method: | OECD 402 (1987) |
| Species/strain: | Rat/Sprague-Dawley (Crl:CD(SD)IGS BR) |
| Group size: | 5 males and 5 females |
| Test substance: | deoxyarbutin |
| Batch: | 504007 |
| Purity: | 99.5% (HPLC) |
| Dose levels: | 2000 mg/kg bw |
| Route: | dermal (semi-occlusive) |
| Exposed area: | 10% of body surface (52 cm²) |
| Exposure: | single application for 24 hours |
| Observation period: | 14 days |
| GLP: | Yes |
| Date of report: | August 2005 |

Rats were administered the neat test substance soaked with distilled water on a clipped area of the back (52 cm²) by means of a cellulose patch fixed with a non-irritating tape and covered semi-occlusively by a dressing. After a 24-h exposure period, the dressing was removed and residues were removed with a cellulose tissue moistened with water. The animals were observed for treatment-related effects within the periods 0-0.5, 0.5-1, 1-2, 2-4 and 4-6 hours after administration and then at least once a day for a total of 2 weeks. Observations included but were not limited to changes in skin, fur, eyes, the occurrence of secretions and excretions, autonomic activity, changes in gait, posture and the presence of convulsions. Bodyweights were determined prior to administration and on days 7 and 14 after application. Gross pathology was performed on all animals, which were sacrificed on day 14.

Results
No deaths, no signs of irritation nor any signs of systemic toxicity were observed clinically or by gross pathology. All animals gained bodyweight during the observation period.

Conclusion
The acute dermal toxicity (LD50) of deoxyarbutin was >2000 mg/kg bw for male and female Sprague-Dawley rats.

Ref.: 5

An acute dermal toxicity study was previously performed in male and female New Zealand White rabbits but has limited validity. The study was performed as a limit test; each group of 3 male and female animals received a 30% (w/v) solution of deoxyarbutin dissolved in propylene glycol at a dose level of 2000 mg/kg bw on about 10% body surface area and held in place by an occlusive dressing for 24h. No mortality occurred during the course of the 14-day observation period. Thus the estimated LD50 value for New Zealand White rabbits was >2000 mg/kg bw for both male and female animals.

Ref.: 35

#### 3.3.1.3 Acute intraperitoneal toxicity

The acute intraperitoneal toxicity was investigated in male and female Sprague-Dawley rats. The rats received a single intraperitoneal injection of deoxyarbutin (batch: HT0059.01) dissolved in propylene glycol/absolute ethanol/physiological saline (60:20:20%, v/v/v) at dose levels of 240, 310, 400, 520, 680, and 1150 mg/kg bw. In addition, 3 male and 3 female rats received the vehicle at a dose volume of 5 ml/kg bw. Clinical signs of intoxication were recorded daily, the body weight was determined on day 7 prior to termination and all animals were subjected to gross pathology. All mortalities occurred by study day 5. This procedure resulted in an intraperitoneal LD50 value of 367 mg/kg bw in males (CI: 264–511 mg/kg bw) and 314 mg/kg bw in females (CI: 235–419 mg/kg bw).

Ref.: 36

***Applicants overall conclusion on acute toxicity***

The acute oral and dermal toxicity of deoxyarbutin can be regarded as low, with LD50 values for acute oral and dermal toxicity of >2000 mg/kg bw in rats.

### 3.3.2 Irritation and corrosivity

#### 3.3.2.1 Skin irritation and corrosivity

***In vitro***

| | |
|---|---|
| Guideline: | OECD 430 (2004) |
| Test system: | each 1 dorso-lateral skin disc of each 1 male Wistar rat |
| Group size: | 3 males |
| Test substance: | deoxyarbutin |
| Batch: | 501002 |
| Purity: | 99% (HPLC) |
| Dose level: | neat test substance |
| Route: | dermal (open) |
| Exposure: | single skin disc application for 24 hours at conditioned room temperature (22 ± 2 °C) |
| Negative control: | deionized water |
| Positive control: | 36% HCl |
| Measurement: | transcutaneous electrical resistance (TER) prior to application and after test substance removal after 24 hours |
| Equipment: | AIM electronic databridge 6401 LCR, 100 Hz |
| GLP: | Yes |
| Date of report: | May 2005 |

The test substance was investigated for its possible corrosive potential by the transcutaneous electrical resistance (TER) test. Skin discs were obtained from the dorso-lateral region of 3 male Wistar rats. Prior to sacrifice, the hair of the animals was clipped on the back and flanks and washed on two days with an antibiotic solution. The animals were sacrificed, the skin was removed, stripped of excessive subcutaneous fat and the skin was placed on the end of polytetrafluoroethylene (PTFE) tube. The integrity of the skin discs was determined prior to application of the test substance by means of transcutaneous electrical resistance measurements. Thereafter a sufficient amount of the neat test substance was applied with a spatula on the skin discs obtained from each of the 3 rats to cover the whole epidermis surface. Deionized water was added and the tubes were shaken. After 24 hours contact time, the test substance was removed by washing with tap water. Immediately thereafter, the TER was measured. Deionized water and 35% HCl solution were used as negative or positive controls, respectively.

Results
The mean TER of deoxyarbutin treated skins was 16 kΩ and for deionized water was 14 kΩ, while the positive control, 36% HCl revealed a mean TER 0.7 kΩ. The TER values of the negative and positive controls corresponded clearly to the respective guideline requirements and demonstrated the suitability and sensitivity of the test system.

Conclusion
Under the experimental conditions reported, deoxyarbutin did not exhibit any corrosive potential to the skin *in vitro*, when tested neat in the TER test.

Ref.: 6

**SCCS comment**
Based on this test, no conclusion on skin irritation potential can be drawn.

***In vivo***

| | |
|---|---|
| Guideline: | OECD 404 (2002) |
| Species/strain: | Rabbit/New Zealand White |
| Group size: | 3 females |
| Test substance: | deoxyarbutin |
| Batch: | 504007 |
| Purity: | 99.5% (HPLC) |
| Concentration: | 0.5 g of unchanged test substance moistened with 1.0 ml deionized water |
| Route: | intact skin (semi-occlusive) |
| Exposure: | 4 h |
| Observation period: | up to 72 h after patch removal |
| GLP: | Yes |
| Date of report: | August 2005 |

Deoxyarbutin was investigated for its acute dermal irritation potential in 3 female New Zealand White rabbits. The hair was clipped on the dorsal area of the trunk one day prior to application. An amount of 0.5 g of the test substance was moistened with 1.0 ml deionized water, applied to the test site (2.5 x 2.5 cm) and held in place using a non-irritating tape covered by a semi-occlusive dressing. The duration lasted 4 h and during that period, the animals were prevented from accessing the test area by being fitted with plastic collars. The animals were examined for erythema/eschar and oedema as well as for other local or systemic signs of toxicity after about 1, 24, 48 and 72 h after patch removal.

Results
There were no mortalities and no signs of systemic toxicity or irritation noted during the entire observation period. The mean scores for erythema/eschar and oedema were 0.0 for each animal at each observation time point.

Conclusion
Under the condition of this study, deoxyarbutin was shown to be non-irritating to the intact skin of New Zealand White rabbits.

Ref.: 7

Studies on the skin irritating potential of deoxyarbutin in humans are described in section 3.10.

### 3.3.2.2 Mucous membrane irritation / Eye irritation

| | |
|---|---|
| Guideline: | OECD 405 (2002) |
| Species/strain: | Rabbit/New Zealand White |
| Group size: | 3 females |
| Test substance: | deoxyarbutin |
| Batch: | 504007 |
| Purity: | 99.5% (HPLC) |
| Concentration: | approximate equivalent of 0.1 ml of neat test substance (57 and 2x64 mg) |
| Route: | instillation in the conjunctival sac of the right eye |
| Observation period: | up to 72 h after instillation |
| GLP: | Yes |
| Date of report: | August 2005 |

The potential irritant effect of deoxyarbutin to the mucous membrane was investigated by instillation of an approximate equivalent to 0.1 ml of the neat test substance (i.e. 57, 64 and 64 mg) into the right conjunctival sac of each of three animals. The eyes were shortly held close to prevent a loss of the test substance. The left eyes remained untreated and served as controls. Both eyes of the animals were examined within 24 h before application and about 1, 24, 48, 72 h after application. The entire eye, with focus on cornea, iris and conjunctivae, was examined using an otoscope lamp.

Results
The treatment had no effect on the cornea or iris of the rabbits at any time-point. Exposure resulted in conjunctival redness of grade 1 during 1 h – 48 h in all three animals, resulting in a mean score of 0.7 in all animals for the period between 24 – 72 hours. Conjunctival chemosis of grade 2 was observed in all three animals after 1 hour of instillation; grade 2 was observed in one animal and grade 1 in two animals after 24 h; grade 1 was observed in one animal after 48 hours, while no effect was observed at 72 hours. The respective mean scores for conjunctival chemosis were 0.3; 0.3 and 1.0 (24 – 72 hours), respectively.

Conclusion
Deoxyarbutin was slightly and transiently irritating to the eyes of 3 New Zealand White rabbits. However, according to current EU classification requirements, a test substance is considered an eye irritant if the mean score for conjunctival redness and chemosis is equal to or greater than 2.5 and 2, respectively, in at least two rabbits. Since these threshold scores were not reached under the conditions of this study, the substance should not be considered to be an eye irritant according to EU classification criteria.

Ref.: 8

**SCCS comment**
It is noted that according to the CLP criteria, the classification cut-off value for redness and chemosis now is ≥ 2. The SCCS agrees that classification of the test substance as an eye irritant is not warranted. However, as redness and particularly chemosis were observed during the observation period, the test substance is considered as a mild eye irritant.

There is another eye irritation study available using low volume and performed under GLP conditions. About 10 µl of 3% deoxyarbutin in a standard cream formulation was placed directly on the cornea of the right eye of each of 3 female New Zealand White rabbits. The other eye was left as untreated control. Animals were scored for signs of irritation at 24, 48, and 72 h post dosing using the Draize et al. (1959) scoring system. This low-volume eye irritation assay revealed no signs of eye irritation as the maximum average score was 0.0 at any time point.

Ref.: 37

**SCCS comment**
The low volume of test formulation (about 10 µl, i.e. 1/10 of the volume recommended in the OECD TG 405) used in this study precludes an evaluation of the eye irritating potential of the test formulation.

***SCCS overall conclusion on irritation***
Deoxyarbutin did not exhibit any corrosive potential to the skin in the *in vitro* TER test and was shown to be not irritating to the intact skin of New Zealand White rabbits.
In an eye irritation test with New Zealand White rabbits, the neat test substance was slightly and transiently irritating to the eyes. As redness and particularly chemosis were observed, the neat substance is considered as a mild eye irritant. Another test using standard cream formulation with 3% deoxyarbutin did not lead to signs of eye irritation in rabbits, but is not conclusive due to the very low volume of test formulation applied.

### 3.3.3 Skin sensitisation

**Local Lymph Node Assays (LLNA) in mice**

| | |
|---|---|
| Guideline: | OECD 429 (2002) |
| Species/strain: | Mouse/CBA/Ca |
| Group size: | 4 female animals per group |
| Test substance: | deoxyarbutin |
| Batch: | 504007 |
| Purity: | 99.5% (HPLC) |
| Concentrations: | 10%, 25% and 50% in DMF |
| Solvent: | Dimethylformamide (DMF) |
| Route: | Epidermal (topical) application on the dorsal ear lobe surface |
| Application volume: | 25 µl per ear of each animal |
| Application frequency: | single daily application for 3 consecutive days |
| Negative control: | DMF |
| Positive control: | 25% (v/v) solution of hexyl cinnamic aldehyde (HCA) in acetone:olive oil (AOO, 4:1, v/v) |
| GLP: | Yes |
| Date of report: | December 2005 |

The sensitising potential of deoxyarbutin was tested in the murine local lymph node assay. Each group of four female mice was treated by topical application to the dorsal surface of each ear lobe (left and right) with the test substance dissolved in DMF at concentrations of 10%, 25% and 50%, DMF alone as vehicle control and 25% α-Hexylcinnamaldehyde (HCA) in acetone:olive oil (AOO) 4:1 as positive control. The application volume was 25 µl per ear. The preparations were made freshly each day prior to application and were spread over the entire dorsal surface of the ear once daily for three consecutive days. Five days after the first application, all mice were injected with 20 µCi of $^{3}$H-thymidine in sterile saline. Five hours thereafter, all mice were sacrificed, the draining lymph nodes were excised, pooled and single cell suspensions of pooled lymph node cells were prepared. The cells were washed twice with phosphate buffered saline (PBS) and precipitated with 5% trichloroacetic acid (TCA) overnight at 4°C. The pellets were recovered by centrifugation and resuspended in 1 ml of TCA. The incorporation of $^{3}$H-thymidine was measured by β-scintillation counting. The calculated stimulation indices (SI, test substance/negative control ratio) were decisive for the grading of the potential of sensitisation: The mice were observed daily for mortalities and clinical signs of intoxication on regular intervals. The application sites were observed for signs of irritation daily.

Results
The test substance did not lead to any overt sign of systemic toxicity or local irritation and there was no mortality. All mice appeared normal and bodyweight gain was not affected. The exposure to deoxyarbutin at 10, 5%, 25% and 50% resulted in stimulation indices of 1.8, 4.2 and 1.3, respectively.
The results can be summarised as follows:

| Concentration | Substance | dpm | S.I. |
|---|---|---|---|
| 100% | DMF | 2057 | 1 |
| 10%(W/W) | Deoxyarbutin | 3674 | 1.8 |
| 25%(W/W) | Deoxyarbutin | 8714 | 4.2 |
| 50% (W/W) | Deoxyarbutin | 2608 | 1.3 |
| 25% (V/V) | HCA in AOO | 10770 | 19.8 |

DMF = negative (solvent) control, HCA = positive control, dpm = disintegration per minute, S.I = stimulation index

In the LLNA, a test material is considered to have skin sensitising activity if the stimulation index in any of the tested concentrations is equal to or greater than 3 to the concurrent vehicle control together with consideration of a dose-response. The positive control (25% HCA) led to a stimulation index (S.I.) of 19.8 confirming the sensitivity and suitability of the test system.

Conclusion
Based on these results, the authors of the study concluded that deoxyarbutin has to be regarded as a sensitiser. However, there was clearly no dose-response-relationship as the concentration of 10% resulted in a S.I. value of 1.8 and the highest tested concentration of 50% of a S.I. of 1.3, both values were clearly below the threshold value 3.0. Only the S.I. of the mid-concentration of 25% was above the threshold value. The applicant concluded that as deoxyarbutin did not reveal any clear sensitising potential at a concentration of 10%, it could be regarded as safe for the conditions of practical use. A possible low skin sensitising potential was noted at a concentration of 25% under the conditions of this murine local lymph node assay.

Ref.: 9

There is an additional local lymph node assay in mice with a slightly different study design, which was performed prior to the completion of the respective OECD 429 testing guideline.

| | |
|---|---|
| Guideline/method: | not mentioned but with minor deviations comparable to OECD 429 (2002) (4 instead of 3 daily applications, 5 instead of 4 mice, exposure to ventral and dorsal ear lobe instead of dorsal, no positive control) |
| Species/strain: | Mouse/CBA/J |
| Group size: | 5 female animals per group |
| Test substance: | deoxyarbutin |
| Batch: | PG21-69-1 |
| Purity: | 99.2% |
| Concentrations: | 3%, 10% and 20% in acetone |
| Solvent: | Acetone |
| Route: | Epidermal (topical) application on the dorsal and ventral ear lobe surface |
| Application volume: | 25 µl/animal (12.5 µl on ventral and dorsal ear lobe) |
| Application frequency: | single daily application for 4 consecutive days |
| Negative control: | a) untreated (naïve) mice<br>b) Acetone |
| GLP: | Yes |
| Date of report: | June 1994 |

In this murine local lymph node assay, each group of five female mice was treated by topical application to the ventral and dorsal surface of each ear lobe (left and right) with the test substance dissolved in acetone at concentrations of 3%, 10% and 20% and acetone alone as carrier control. One group of 5 female mice remained untreated as the naïve control group. The application volume was 25 µl in total (12.5 µl on each ear side). The preparations were prepared fresh each day prior to application and were spread over the entire ear surfaces once daily for four consecutive days. Six days after the first application, all mice were injected with 20 µCi of $^{3}$H-thymidine in sterile saline. Five hours thereafter, all mice were sacrificed, the draining lymph nodes were excised and single cell suspensions were prepared. The incorporation of $^{3}$H-thymidine was measured by β-scintillation counting. The calculated stimulation indices (SI, test substance/naive control ratio) were decisive for the grading of the potential of sensitisation: The mice were observed daily for mortalities, clinical signs of systemic intoxication or signs of irritation.

Results
The test substance did not lead to any overt sign of systemic toxicity or local irritation and there was no mortality. The exposure to deoxyarbutin at 3, 10% and 20% resulted in stimulation indices of 1.8, 1.5 and 2.0, respectively.
The results can be summarised as follows:

| Concentration | Substance | dpm (mean) | S.I. |
|---|---|---|---|
| | Untreated control | 100 | 1.0 |
| 100% | Acetone | 93 | 0.93 |
| 3% (W/W) | Deoxyarbutin | 177 | 1.77 |
| 10%(W/W) | Deoxyarbutin | 153 | 1.53 |
| 20% (W/W) | Deoxyarbutin | 201 | 2.01 |

dpm = disintegration per minute, S.I = stimulation index

Conclusion
It was demonstrated that under the conditions investigated, deoxyarbutin exhibited no potential to induce dermal sensitisation in this murine local lymph node assay when tested at concentrations up to 20% dissolved in acetone.

Ref.: 38

**SCCS comment / overall conclusion on skin sensitization**
The local lymph node assays performed indicate that deoxyarbutin is a moderate skin sensitiser according to the definitions provided in a SCCP memorandum (SCCP/0919/05).

### 3.3.4 Dermal / percutaneous absorption

**Percutaneous absorption *in vitro***

| | |
|---|---|
| Guideline: | OECD 428 (2004) |
| Test system: | Human skin |
| Test substance: | deoxyarbutin |
| Batch: | 509014 |
| Purity: | 99.8% (HPLC) |
| Concentrations: | 3% in a standard o/w emulsion |
| Skin preparation: | Dermatomed human skin from 3 female donors (approximately 400 – 500 µm thickness) |
| Replicates: | 6 samples/donor |
| Positive control substance: | Caffeine (batch 066K0085), 10 mg/l in Krebs-Ringer-buffer (KBR) at pH 7.4 |
| Skin temperature: | 32 ± 2 °C |
| Test chamber: | Static glass diffusion cell (Franz type, 3.0 cm$^2$) |
| Route: | topical application |

| | |
|---|---|
| Exposure time: | 24 h |
| Sampling time points: | 0.5, 2, 4, 14, 19 and 24 hours |
| Tape stripping of *stratum corneum*: | 20 tape strips (analysed individually or pooled per 6) |
| GLP: | Yes |
| Date of report: | December 2007 |

Deoxyarbutin was investigated for its permeation and penetration properties *in vitro* as a 3.0 % standard o/w emulsion on viable human skin. Initially, an analytical method for the determination of deoxyarbutin was implemented and validated. Within this process, the stability of the test item in the eluent and the test matrices (extraction + receptor medium) was tested for a period of 48 hours at 25°C ± 2°C and 4°C ± 2°C and additionally at 32°C ± 2°C for 48 hours for the permeation matrix. The content in the test formulation was determined by HPLC in triplicate. The study was performed under dynamic and non-occluded conditions using dermatomed human skin from 3 different female donors and 6 skin samples per donor. The skin thickness (approximately 400 - 500 µm) was measured immediately prior to each experiment and in total 6 samples were taken from the receptor medium at 0.5, 2, 4, 14, 19 and 24 hours. The test formulation was applied on each skin sample at an amount of 5 mg/cm², (~ 0.15 µg deoxyarbutin/cm²), left on the skin for 24 hours and then the residual amount was collected with cotton swabs. Thereafter, the concentration of deoxyarbutin in the skin was quantified. The *stratum corneum* was separated by tape stripping from the lower skin layers (dermis and epidermis), which were cryo-sectioned. The tape strips (20 strips total per sample) as sample for the *stratum corneum* were analysed as well as the cryosections as sample for the dermis/epidermis. Analysis was carried out by means of HPLC. A mass balance analysis was performed covering the initial and residual amounts in the formulation, in the *stratum corneum*, epidermis/dermis and acceptor compartment for the respective sampling time points.
The sensitivity and suitability of the test system was investigated in parallel with caffeine at a concentration of 10 mg/l in Krebs-Ringer-buffer at pH 7.4 and respective results were compared with the historical control permeability coefficients.

Results
The HPLC method was successfully implemented and validated. The UV absorption at the wavelength of 223 nm was used for quantification of deoxyarbutin in the eluent mixture, the extraction medium and the acceptor medium. The LLOQ was 0.030 µg/ml in Krebs-Ringer bicarbonate buffer and eluent mixture. Deoxyarbutin was shown to be stable in the receptor medium during the course of the *in vitro* study. The recovery rate after incubation for 24 hours at 32°C was 103%.
The multiple endpoint analysis with caffeine revealed intact skin membranes demonstrating the suitability and sensitivity of the test system. After dermal application of a standard o/w emulsion containing 3% deoxyarbutin (about 160 µg deoxyarbutin/cm²) for 24 hours on the viable skin of three female donors, the mean amount of deoxyarbutin removed from the skin surface ranged from 43.28% to 62.58% of the dose applied in all experiments performed.
Deoxyarbutin was detected in all compartments relevant for assessment of dermal absorption and penetration, *e.g.* in the skin extracts and in the receptor solution samples; the chromatographic analyses revealed no indication for degradation. The mean recovery in the two first tape strips (upper *stratum corneum*) was 1.57% for all performed experiments. In the further 18 tape strips (lower *stratum corneum*), a mean recovery of 0.89 % was obtained.
The mean absorbed dose of deoxyarbutin representing the sum of the portions found in the viable epidermis, dermis and receptor medium, ranged from 42.11% to 70.85% in the three donor skin samples, with the greatest portion of deoxyarbutin in the receptor medium. The overall portion of the absorbed dose, dermally bioavailable, was 57.86%. The mean recovery of deoxyarbutin in the deeper skin layers of dermis and epidermis (cryocuts) represented only 0.26 % in total.

The total recovery rate for the three different skin donors ranged between 106.63% and 115.37% of the dose applied with an overall total recovery rate of 112.16% and was therefore in the acceptable range for a valid study. The details are provided in the following table:

| **Amount of deoxyarbutin** | **Expressed as µg/cm² of skin surface mean ± S.D. (n = 18)** | **Expressed as % of dose mean ± S.D. (n = 18)** |
|---|---|---|
| Formulation applied | 160.81 ± 1.58 | 100 |
| Receptor | 93.24 ± 20.05 | 57.86 ± 11.90 |
| Skin surface | 82.90 ± 12.18 | 51.58 ± 8.11 |
| 2 Tape strips | 2.52 ± 1.97 | 1.57 ± 1.23 |
| 18 Tape strips | 1.43 ± 0.12 | 0.89 ± 0.07 |
| Cryocuts (dermis/epidermis) | 0.42 ± 0.10 | 0.26 ± 0.06 |
| Dose absorbed (i.e. dermally bioavailable) | 93.66 ± 20.15 | 58.12 ± 11.96 |
| Total Recovery | 180.51 ± 34.42 | 112.16 ± 3.93 |

Conclusion
After dermal application of 3% deoxyarbutin in a standard o/w emulsion (160.81 µg deoxyarbutin/cm²) applied for 24 hours to the viable skin of three human donors *in vitro*, deoxyarbutin was detected in all relevant compartments; the recovery showed no indication of degradation of the test compound. Under the conditions of this study, a high dermal availability of 58.12%, corresponding to 93.66 µg deoxyarbutin/cm², was observed.

Reference: 27

In an older exploratory screening study, the penetration of deoxyarbutin (no information on concentration and application area) was investigated *in vitro* using dermatomed skin from hairless albino guinea pigs. The dermatomed skin had a thickness of about 450 µm and was placed on a flow-through system consisting of a Manostat cassette pump, ISCO fraction collectors, a circulating temperature controlled water bath, and two banks each of 7 Teflon® flow-through diffusion cells. Prior to use, the integrity of each skin sample was determined grossly. The receptor fluid (modified HBSS; Hanks Balanced Salt Solution) was perfused beneath and in contact with the skin sample by means of a peristaltic pump, and was collected automatically (1.5 mL/h) at 1-hour intervals. The skin penetration samples were analysed by means of HPLC. Deoxyarbutin was shown to penetrate rapidly through the guinea pig skin *in vitro.* The steady state permeability constant was calculated as Kp = 1.3 x $10^{-4}$ cm/h, indicating that 100% of a 0.5 mg dose of a 1% deoxyarbutin cream would penetrate through guinea pig skin in 24 hours.

Ref.: 39

**Percutaneous absorption *in vivo***

There is no validated scientific or regulatory *in vivo* skin penetration study in experimental animals available. However, an older exploratory screening study in pigmented Guinea pigs, which was primarily designed to investigate the metabolism of topically applied deoxyarbutin, indicated that deoxyarbutin may rapidly penetrate through the skin. The calculated effective permeability constant (Kpeff) was 1.9 x $10^{-4}$ cm/h. (39). In a pilot study with topical application of radiolabelled 3% deoxyarbutin to guinea pigs, 90% of the radioactivity was detected in urine after 48h, 52-57% as the glucuronide and ~30% as the sulphonated conjugate, so that the deoxyarbutin was nearly quantitatively absorbed.

***SCCS comment / conclusion on dermal/percutaneous absorption***

The human *in vitro* percutaneous absorption study showed that after the dermal application of 3% deoxyarbutin in a standard o/w emulsion (160.81 µg deoxyarbutin/cm²) applied for

24 hours to the viable skin of three female donors, deoxyarbutin was detected in all compartments relevant for assessment of dermal absorption and penetration. There was no evidence for degradation of the parent compound. A high dermal availability of 58.12 ± 11.96%, corresponding to 93.66 ± 20.15 µg deoxyarbutin/cm², was observed.
Because of some limitations in the *in vitro* study using human skin (low number of donors), the SCCS considers the mean+2 SD, *i.e.* 82.04% or 134 µg/cm$^2$ for dermal penetration.

### 3.3.5 Repeated dose toxicity

#### 3.3.5.1 Repeated Dose (28 days) oral / dermal toxicity

**28-day oral toxicity study in rats**

| | |
|---|---|
| Guideline: | OECD 407 (1995) |
| Species/strain: | Rat/F344 |
| Group size: | Main group: 5 males and 5 females per group |
| Satellite group: | 5 males and 5 females (control and high dose groups) |
| Test substance: | deoxyarbutin |
| Batch: | 504007 |
| Purity: | 99.5% (HPLC) |
| Dose levels: | 0, 100, 316, 1000 mg/kg bw |
| Dose volume: | 5 ml/kg bw |
| Vehicle: | Corn oil |
| Route: | Oral (gavage) |
| Exposure period: | 28 days |
| Exposure frequency: | daily |
| Recovery period: | 14 days for control and high dose groups |
| GLP: | Yes |
| Date of report: | January 2006 |

The subacute toxicity of deoxyarbutin was examined in a 28-day oral toxicity study in male and female F344 rats. Each of the 5 males and 5 females per group received the test substance as an oily solution (corn oil) at dose levels of 0, 100, 316 and 1000 mg/kg bw daily by gavage for 28 days (main group). The volume administered was 5 ml/kg bw. In addition, two groups of 5 males and 5 females each, i.e. one high dose satellite group and one control satellite group, were treated in the same manner as their corresponding groups, but were kept for a further 14 days without test substance administration to observe the reversibility or persistence of test substance-induced findings. The dose selection was based on a previous range-finding study with daily administration of oily solutions at dose levels of 0, 32, 100, 320 and 1000 mg/kg bw for 7 consecutive days. The test substance solutions were analysed for correct concentrations, homogeneity and stability over 4 hours.
The animals were observed for clinical signs and mortality at regular intervals including a detailed clinical observation once a week. A functional observation including measurements of motor activity and sensory reactivity was performed at the end of the administration period in all treated main group animals and towards the end of the recovery period. Bodyweights and food consumption were recorded weekly. Blood samples for haematological and clinical chemistry were taken at day 29 in all main group animals and at day 43 in the respective satellite animals. At termination of treatment and after recovery had elapsed, all animals were sacrificed and macroscopically examined, organs were weighed and histopathology was performed.

Results
The analysis confirmed the stability of the oily solutions for at least 4 hours at room temperature and the correctness of the concentrations. The homogeneity was also confirmed with the exception that the one high dose sample was slightly outside the

acceptable range. However, as the concentration was slightly higher, the validity of the study was not impaired.

Range-finding study: There was no mortality and only the animals of the 320 and 1000 mg/kg bw groups suffered transiently from unspecific signs of impaired health status. At 1000 mg/kg bw, the bodyweights and food consumption of the animals of both groups were slightly but not statistically significantly affected. At necropsy, no substance-related finding was noted in any animal. Therefore, 1000 mg/kg bw was selected as the highest dose level for the subsequent 28-day main study.

28-day main study:
There was no substance-related mortality but one high dose female of the main group died on day 2 due to a gavage error and was replaced with an animal from the recovery group. Substance-related findings occurred only in the animals of the 1000 mg/kg bw group and consisted of a transient occurrence of clinical findings, slightly reduced food consumption and retarded bodyweight gain during certain periods, minor haematological effects in females and slight impairment of single clinical chemistry parameters (increases of albumin and protein in males, reduced aspartate aminotransferase in females). At this dose level the relative liver weights of both sexes and the relative kidney weights in the males showed treatment-related increases. Slight deviations in the relative weights of the spleen, thymus and brain were observed and considered as secondary changes due to the impaired bodyweight gains at this dose level. There were no substance-related finding in histopathology and thus, no morphological correlates for the observed findings in clinical chemistry or the organ weight changes. In the low- and mid-dose animals, no biologically relevant findings were observed. The functional observation battery in the form of a detailed assessment of behaviour, motor activity, sensory reactivity, reflexes and grip strength revealed no indication of any neurological impairment at any dose level in any of the treated animals.

Conclusion
Deoxyarbutin was tolerated without mortality up to the high dose level of 1000 mg/kg bw. Slight signs of systemic toxicity occurred only at 1000 mg/kg bw in male and female rats but no specific mode of action or target organ was identified. There were no gross or histopathological findings and no impairment of any parameter in the comprehensive functional observation battery. Thus, the study authors stated that the no observed adverse effect level (NOAEL) was 316 mg/kg bw for male and female F344 rats under the conditions of this study.

Ref: 18

**28 day dermal toxicity study in the rabbit**

| | |
|---|---|
| Guideline/method: | / |
| Species/strain: | Rabbit/New Zealand White |
| Group size: | 5 males and 5 females per group |
| Test substance: | deoxyarbutin |
| Batch: | HT0059.01-04 |
| Purity: | 99% |
| Dose levels: | 3% deoxyarbutin in a cream formulation |
| Control: | Vehicle cream formulation |
| Dose volume: | 2 ml/kg bw |
| Route: | Dermal (open, intact skin) |
| Exposure period: | 28 days |
| Exposure frequency: | once daily for 6 h, 7days/week |
| GLP: | Yes |
| Date of report: | October 1994 |

The subacute dermal toxicity and cumulative irritation of deoxyarbutin were examined in a 28-day dermal toxicity screening study in male and female New Zealand White rabbits. Each animal, 5 males and 5 females per group, was administered a cream formulation with 3%

deoxyarbutin once a day for 6 hours, 7 days per week for 28 days. The application volume was 2 ml/kg bw and the covered area corresponded to about 15% of the body surface. The application area remained open but a collar was placed on each animal to prevent ingestion. The control animals of the same size received the cream formulation. After 6 hours of exposure, the skin was washed with propylene glycol, followed by a tepid water wash before drying. The animals were observed continuously for signs of toxicity. Bodyweight was recorded once a week. The skin at the application area was investigated before the daily exposure. Blood samples for haematology and clinical chemistry were taken in weeks 2 and 4 and urine samples in week 4. At termination, the animals were sacrificed and examined for gross pathological findings, organs were weighed and histopathology was performed.

Results
No animal died prematurely. The dermal application of 3% deoxyarbutin in a cream formulation led to a transient retardation of the bodyweight gain in males and dermal irritation in males and females characterised by moderate to marked erythema. Macroscopic findings in the form of thickening with red discoloration and microscopic lesions consisting of inflammation of the dermis and hyperkeratosis and acanthosis of the epidermis were limited to the treated skin. However, the incidences of all of these findings were higher with the vehicle cream formulation. With the exception of the skin findings, there were no further treatment-related clinical observations or findings in haematology, clinical chemistry, urinalysis or pathology.

Conclusion
Since the macroscopically and histopathologically observed dermal irritation was more pronounced in the vehicle control group, no final conclusion on the cumulative irritation potential of 3% deoxyarbutin can be drawn. In any case, the repeated application for 28 consecutive days led to no systemic toxicity in male and female rabbits under the condition of the study. However, based on the limitations, this study can only be considered as an exploratory screening study.

Reference: 40

### 3.3.5.2 Sub-chronic (90 days) dermal toxicity

| | |
|---|---|
| Guideline/method: | / |
| Species/strain: | Rabbit/New Zealand White |
| Group size: | 5 males and 5 females per group |
| Test substance: | deoxyarbutin |
| Batch: | HT0059.02 |
| Purity: | 99% |
| Dose levels: | 1, 5, 40% deoxyarbutin dissolved in propylene glycol/ethanol (75%/25%, V/V) |
| Control: | propylene glycol/ethanol (75%/25%, v/v) vehicle |
| Dose volume: | 2 ml/kg bw |
| Route: | Dermal (open, intact skin) |
| Exposure period: | 91 days |
| Exposure frequency: | once daily for 6 h, 7days/week |
| GLP: | Yes |
| Date of report: | October 1994 |

The subchronic toxicity of the test substance was examined in a 91-day dermal toxicity study in male and female New Zealand White rabbits. Each animal (5 males and 5 females per group), was administered 1, 5 and 40% deoxyarbutin dissolved in the vehicle once a day for 6 hours, 7 days per week for 91 days. The application volume was 2 ml/kg bw and the covered area corresponded to about 15% of the body surface. The application area remained open but a collar was placed on each animal to prevent ingestion. The control animals (same group size) received the vehicle (propylene glycol/ethanol (75%/25%, v/v)). After 6 hours of exposure, the skin was washed with propylene glycol, followed by tepid

water wash before drying. The animals were observed continuously for signs of toxicity. Bodyweight was recorded once a week. The skin at the application area was investigated before the daily exposure. Blood samples for haematology and clinical chemistry were taken in weeks 2 and 4 and at termination, and urine samples were taken at termination. After 91 days of treatment had elapsed, the animals were sacrificed and examined for gross pathological findings; organs were weighed and histopathology was performed.

Results
No animal died prematurely. The dermal application of 1, 5 and 40% deoxyarbutin in the propylene glycol/ethanol vehicle led to no adverse clinical signs and no effects on bodyweight gain. Slight erythema was noted in all groups. Moderate erythema was noted only in the high-dose group. The gross lesions were limited to the treated skin area of the high-dose animals and consisted of scaling and red discoloration. Histopathology revealed lesions in form of chronic inflammation of the dermis and hyperkeratosis and acanthosis of the epidermis in the vehicle control as well as in the test-substance treated animals. There were no treatment-related findings in haematology, clinical chemistry or urinalysis. Pathology revealed no macroscopic or microscopic treatment-related finding other than the skin effects and there were no substance-induced effects on any organ weight.

Conclusion
The results indicated that subchronic dermal treatment of deoxyarbutin at concentrations of 1, 5 or 40% in propylene glycol/ethanol vehicle was tolerated without mortality or adverse clinical findings or an effect on bodyweight gain. Dermal irritation scoring showed that both the vehicle and the test substance produced dermal irritation, slightly more pronounced at the high concentration. Histopathology revealed chronic skin lesions in all groups including the vehicle control; there were only slight differences in severity but not in the incidence, suggesting that the vehicle was also responsible for the chronic inflammatory skin effect. Therefore, no final conclusion on the chronic cumulative irritation potential of deoxyarbutin can be drawn. However, no signs of systemic toxicity were noted up to the highest concentration of 40% deoxyarbutin in propylene glycol/ethanol (corresponding to about 800 mg/kg bw) in male and female rabbits under the condition of the study.

Ref.: 40

#### 3.3.5.3 Chronic (> 12 months) toxicity

No chronic toxicity study with deoxyarbutin is available.

### *SCCS overall conclusion on repeated dose toxicity*

The systemic toxicity after repeated **oral** application for 28 days to male and female rats was low and effects could only be observed at the currently accepted limit dose level of 1000 mg/kg bw for repeated toxicity studies. They consisted of transient clinical findings, slightly reduced food consumption and retarded body weight gain, minor haematological effects in females and slight impairment of single clinical chemistry parameters. In addition, the relative liver weights of both sexes and the relative kidney weights of the males were increased, but without any histopathological correlates. The functional observation battery revealed no indication of any neurological impairment at any dose level. Clear or severe organ toxicity was not observed morphologically even at the limit dose of 1000 mg/kg bw. Oral dosing may be considered as worst-case scenario for deoxyarbutin since it is likely that the acidic stomach environment led to the formation of a (non-quantifiable) portion of hydroquinone, causing the observed effects.

The **No-Adverse-Effect-Level (NOAEL) for subacute toxicity after 28-day oral treatment was 316 mg/kg bw** in male and female rats. This value may be considered as the worst-case scenario for deoxyarbutin since the acidic stomach environment will lead to its degradation to hydroquinone which then causes the observed toxicity.

Repeated **dermal** application of deoxyarbutin to rabbits either as 3% cream formulation for 28 days or as 1, 5 and 40% in propylene glycol/ethanol solution for 91 days did not lead to any substance-related systemic findings up to the highest dose level investigated. The cumulative dermal irritation potential of deoxyarbutin cannot be assessed from these studies as the vehicle cream formulation led to more pronounced findings than the cream with 3% deoxyarbutin, and the propylene glycol/ethanol vehicle used in the 91-day study was *per se* irritating and led to chronic dermal inflammation. For subchronic dermal treatment; the highest dose of 40% deoxyarbutin in propylene glycol/ethanol vehicle, corresponding to about **800 mg/kg bw, was the No-Adverse-Effect-Level (NOAEL) for systemic toxicity in male and female rabbits**. This value will be used for risk assessment and the calculation of the margin of safety (MoS).

## 3.3.6 Mutagenicity / Genotoxicity

### 3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

**Bacterial reverse mutation assay**

| | |
|---|---|
| Guideline/method: | Comparable to OECD 471 |
| Test system: | *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538 and *Escherichia coli* strain WP2 *uvrA* and WP2 |
| Replicates: | triplicate plates |
| Test substance: | deoxyarbutin |
| Batch: | HT0059.01 |
| Purity: | 99.2% |
| Concentrations: | 0, 33, 100, 333, 1000, 3333, 5000 µg/plate without and with S9-mix |
| Solvent: | Acetone |
| Treatment: | direct plate incorporation method |
| Positive Controls: | without S9-mix: 2-nitrofluorene, sodium azide, 9-amino-acridine, methyl methanesulfonate<br>with S9-mix: 2-aminoanthracene, |
| GLP: | in compliance |
| Date of report: | March 1994 |

Deoxyarbutin was tested for mutagenicity in the reverse mutation assay on bacteria with and without metabolic activation. The S9 fraction was prepared from Aroclor 1254 induced male Sprague-Dawley rat liver. The plate incorporation method was used. The *S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538 and *E. coli* strain WP2 uvrA and WP2 were exposed to the test substance (dissolved in acetone) at concentrations ranging from 33 – 5000 µg/plate. For control purposes, the solvent and positive controls were also investigated.

Results
Precipitation was not observed with any *S. typhimurium* or *E. coli* strain up to the highest concentration tested. No bacteriotoxicity was noted in any strain with the exception of *E. coli* strain WP2 at high concentrations of ≥3333 µg/plate. The test substance did not induce a biologically relevant increase in revertant colony numbers in the bacterial strains at any concentration tested in the presence or absence of metabolic activation. The sensitivity and validity of the test system used was demonstrated by the expected induction of a significantly increased number of revertants with the positive controls.

Conclusion
Under the experimental conditions used, deoxyarbutin was not mutagenic in this gene mutation test in bacteria.

Ref.: 41

**Chromosome aberration test in cultured human lymphocytes**

| | |
|---|---|
| Guideline: | OECD 473 (1997) |
| Test system: | Human lymphocytes |
| Replicates: | duplicate culture in two experiments |
| Test substance: | deoxyarbutin |
| Batch: | 504007 |
| Purity: | 99.5% (HPLC) |
| Concentrations: | 72, 216, 647, 1940 µg/ml without S9-mix, 3 h treatment<br>24, 72, 217, 650 µg/ml with S9-mix, 3 h treatment<br>72, 217, 647, 650, 1940 without S9-mix, 20 h treatment |
| Treatment: | 3 h treatment without and with S9-mix; harvest time 20 h after the start of treatment<br>20 h treatment without S9-mix; harvest time immediately after the end of treatment |
| Solvent: | DMSO |
| Positive Controls: | without S9-mix: Methane sulfonic acid methylester; with S9-mix: cyclophosphamide |
| GLP: | in compliance |
| Date of report: | January 2006 |

Deoxyarbutin was tested for its potential to induce structural chromosome aberrations in human lymphocyte cultures prepared from the blood of three female and one male donor in two independent experiments. The test substance was tested in the presence and absence of metabolic activation. The S9 fraction was prepared from Aroclor 1254 induced Sprague-Dawley rat liver. The test article was dissolved in DMSO. After sampling of the primary lymphocytes, the cultures were incubated for 48 hours in the presence of phytohaemaglutinin. The cells were exposed to the test substance for 3 or 20 hours in the absence of metabolic activation and for 3 hours in the presence of S9-mix. Colcemid was added to the cultures 2 hours prior to harvest. The lymphocytes were processed, fixed and then treated with Giemsa stain. The mitotic indices were calculated from 2000 lymphocytes per culture to evaluate cytotoxicity. Methane sulfonic acid methylester for the non-activation set and cyclophosphamide requiring activation served as positive control substances. A solvent control (DMSO) was also included in the test.

Results
Cytotoxicity was observed in cultures in the presence or absence of metabolic activation. Without S9-mix and after 3 hours of incubation, no mitoses were found at 1940 µg/ml. The three remaining concentrations were evaluated and cytotoxicity complied with the requirements of the OECD TG.. After 20 hours without S9-mix, only the mitotic index of the lowest concentration complies with the OECD TG. Consequently, only this concentration gave reliable results. Treatment of culture for 3 hours in the presence of S9-mix resulted in no mitoses at 1940 µg/ml. Three other concentrations were evaluated and had cytotoxicity levels in agreement with the OECD TG.
A biologically relevant increase in the number of cells with chromosomal aberrations was not observed after the treatment for 3 hours without metabolic activation at any concentration. The same exposure period (3 h) with metabolic activation led to an increase in cells with structural aberrations at 650 µg/ml.
The prolonged treatment of 20 hours without S9-mix caused an increase in cells with structural aberrations at 24 µg/ml.
The sensitivity of the test system was shown since the vehicle control led to no findings but the positive control substances showed statistically expected increases in the number of cells with chromosomal aberrations.

Conclusion
Under the experimental conditions used, deoxyarbutin was genotoxic (clastogenic and/or aneugenic) in this *in vitro* chromosome aberration test with human lymphocytes.

Ref.: 19

### 3.3.6.2 Mutagenicity / Genotoxicity *in vivo*

**Mouse Micronucleus Assay with intraperitoneal application**

| | |
|---|---|
| Guideline: | OECD 474 (1997) |
| Species/strain: | Mouse/ICR |
| Group size: | 5 male and 5 female mice per dose group/sacrifice time |
| Test substance: | deoxyarbutin |
| Batch: | 509014 |
| Purity: | 99.8% (HPLC) |
| Dose levels: | 0, 62.5, 125 or 250 mg/kg bw |
| Exposure: | Single application |
| Route: | Intraperitoneal (i.p.) |
| Vehicle: | 1% Methylcellulose (MC) in phosphate buffered saline |
| Sacrifice Times: | 24 hours and 48 hours (vehicle and high dose only) after start of treatment |
| Negative control: | vehicle |
| Positive control: | Cyclophosphamide (CP) |
| GLP: | in compliance |
| Date of report: | January 2007 |

The ability of deoxyarbutin to cause chromosomal damage *in vivo* was investigated in the mouse bone marrow micronucleus test. In the range-finding study, 3 male and 3 female ICR mice were intraperitoneally (i.p.) injected with the test substance at 200, 250, 500, 1000 or 2000 mg/kg bw to determine the maximum tolerated dose to be used in the definitive micronucleus study. All mice were weighed immediately prior to dose administration and were observed immediately after test substance administration and daily thereafter for 3 days for clinical signs of toxicity. Bodyweights were recorded before dose administration on days 0 and 3.
In the micronucleus test, 5 male and 5 female mice per dose group received a single i.p. injection of the test substance at 62.5 and 125 mg/kg bw, while 15 animals of both sexes (including 5 replacement animals per sex) received 250 mg/kg bw. All animals were observed for clinical signs of intoxication at regular intervals throughout the study period and the bodyweights were determined prior to application. In addition, urine samples were collected at 2, 8 and 24 hours after treatment and blood samples were taken at 24 and 48 hours after treatment, but were not further analysed.
Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and total erythrocytes (PCE/NCE). The results obtained for the negative and positive controls were compared to historical control data for validation purposes.

Results
The test substance formed a cloudy white suspension at 100 mg/ml.
In the range-finding study, mortality was observed in all males and all females at 1000 and 2000 mg/kg bw. Lethargy was observed in all males and all females at 200 and 300 mg/kg bw and all males and 1 of 3 females at 500 mg/kg bw. In addition, irregular breathing and deep and prolonged prostration were recorded for all animals of both sexes at 500 mg/kg bw. Based on mortality and clinical signs, doses of 62.5, 125 and 250 were selected for the micronucleus test.
In the micronucleus test, no mortality was observed. Clinical findings were observed and consisted of piloerection in all males and all females at 125 and 250 mg/kg bw and lethargy

in all males and all females at 250 mg/kg bw. Prostration occurred immediately after dosing (for approximately 10-15 minutes post-dose) in 2 of 5 males and 1 of 5 females at 125 mg/kg bw and all males and all females at 250 mg/kg bw. All mice treated with the vehicle or the test article at 62.5 mg/kg bw appeared normal following dose administration and during the course of the study.
No biological relevant and statistically significant increase in the number of micronucleated polychromatic erythrocytes in any of the test substance treated groups relative to the respective vehicle control groups was observed at 24 or 48 hours after dose administration.
Exposure of the bone marrow was indicated as reductions in the PCE/NEC ratio of 24% and 18% were observed at 250 mg/kg in the 24 hour male and female groups relative to the respective vehicle controls. In addition, reductions of 11% and 17% in the 48-hour male and female groups at 250 mg/kg were also observed. In the vehicle group, the incidence of micronucleated PCEs did not exceed the historical control group ranges, while the positive control induced a significant increase in its incidence indicating the suitability and sensitivity of the test system.

Conclusion
Under the experimental conditions used, deoxyarbutin is not genotoxic (clastogenic and/or aneugenic) in this micronucleus test in mice.

Ref.: 23

**Mouse Micronucleus Assay with oral application**

| | |
|---|---|
| Guideline/Method: | OECD 474 (1997) |
| Species/strain: | Mouse/NMRI |
| Group size: | 5 male and 5 female mice per dose group/sacrifice time |
| Test substance: | deoxyarbutin |
| Batch: | 509014 |
| Purity: | 99.7% (HPLC) |
| Dose levels: | 0, 300, 600 and 900 mg/kg bw |
| Exposure: | Single application |
| Route: | Oral (gavage) |
| Vehicle: | Corn oil |
| Sacrifice Times: | 24 hours and 48 hours (vehicle and high dose only) after start of treatment |
| Negative control: | vehicle |
| Positive control: | Cyclophosphamide |
| GLP: | in compliance |
| Date of report: | February 2006 |

Deoxyarbutin was examined for the potential to induce chromosomal damage *in vivo* in the mouse bone marrow micronucleus test using the oral route of application. A range-finding study to determine the maximum tolerated dose level was performed in 1 male and 1 female NMRI mouse treated with dose levels of 500, 1000 or 1500 mg/kg bw. The mice were observed for mortality and clinical signs of toxicity until termination at 48 hours after application.
In the micronucleus test, 5 male and 5 female mice per dose group received a single oral administration of deoxyarbutin suspended in corn oil at 300 and 600 mg/kg bw, while 12 males and 15 females (including 2 male and 5 female replacement animals) received 900 mg/kg bw. All animals were observed for clinical signs of intoxication at regular intervals throughout the study period.
Mice were euthanised at 24 h and at 48 hours (high dose and vehicle control). Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and total erythrocytes (PCE/NCE). The results obtained for the negative and positive controls were compared to historical control data for validation purposes. A statistical analysis of the results was performed.

Results
In the range-finding study, one male mouse died at 1000 and one female at 1500 mg/kg bw associated with severe clinical findings prior to death and in the concurrent surviving animal at 1500 mg/kg bw. At 500 mg/kg bw, the female showed decreased motor activity but no clinical findings were noted in the male animal. Both mice survived until termination. Based on mortality and clinical signs, doses of 300, 600 and 900 were selected for the micronucleus test.
In the micronucleus test, mortality was observed in 1 male and 5 females at 900 mg/kg bw. The death occurred between about 3 and 24 hours after administration of the test substance. The deceased animals were replaced by identically treated and dosed animals. All other animals survived until the scheduled sacrifice. Dose-dependently pronounced clinical findings were observed at 600 and 900 mg/kg bw consisting of decreased motor activities up to disturbed locomotion, tremours, unconsciousness and white-appearing eyeballs. No clinical signs of toxicity were noted in the low dose group animals.
Bone marrow cytotoxicity substantiated by markedly or statistically significantly lowered percentages of nucleated cells as well the percentages of polychromatic erythrocytes was noted at all tested dose levels 24 hours after the administration in the females and at the mid- and high-dose level in males as well as at the high-dose level at the 48-hour harvest time point in both sexes. The females appeared to be more susceptible than the males.
An increased number of polychromatic erythrocytes with micronuclei was observed in males and in females. While in females this effect was statistically significant and occurred at all dose levels and both time points, the effect in males achieved no statistical significance and was only observed at 600 and 900 mg/kg bw. In the vehicle group, the incidence of micronucleated PCEs did not exceed the historical control group ranges, while the positive control induced a significant increase in its incidence indicating the suitability and sensitivity of the test system.

Conclusion
Under the experimental conditions used, deoxyarbutin is genotoxic (clastogenic and/or aneugenic) in this micronucleus test with oral application in mice.

Ref.: 20

This result is in line with another oral micronucleus test in mice using a different batch of deoxyarbutin (batch 504007) and performed in parallel.

Ref.: 21

**SCCS comment**
A positive result in the micronucleus test with oral administration can be expected. The oral route is inappropriate when the substance is intended for dermal use since deoxyarbutin is hydrolysed at low pH values. In mice, the stomach pH is generally between 1 – 2, so that an immediate and complete breakdown of deoxyarbutin to hydroquinone and other compounds will occur. Thus, with oral application, not deoxyarbutin *per se* will be investigated but hydroquinone, a compound which is known to be clastogenic, both *in vitro* and *in vivo* (References: 3, 31). This has to be taken into consideration when discussing the value of the results of this mouse micronucleus test using the oral route of application. Consequently, SCCS considers the result of this oral micronucleus test not relevant to determine the genotoxicity of deoxyarbutin in the context of dermal application.

**Mammalian Spermatogonial Chromosome Aberration Test**

| | |
|---|---|
| Guideline/Method: | OECD 483 (1998) |
| Species/strain: | Mouse/ICR |
| Group size: | 5 male rats per dose group/sacrifice time |
| Test substance: | deoxyarbutin |
| Batch: | 509014 |

| | |
|---|---|
| Purity: | 99.8% (HPLC) |
| Dose levels: | 0, 62.5, 125 or 250 mg/kg bw |
| Exposure: | Single application |
| Route: | Intraperitoneal (i.p.) |
| Vehicle: | 1% Methylcellulose (MC) in phosphate buffered saline |
| Sacrifice Times: | 24 hours and 48 hours (vehicle and high dose only) after start of treatment |
| Negative control: | vehicle |
| Positive control: | Mitomycin C |
| GLP: | In compliance |
| Date of report: | October 2007 |

The chromosome-damaging potential of deoxyarbutin was investigated in a murine spermatogonial chromosome aberration test in ICR mice. Each of 5 male mice per dose group received a single i.p. injection of the test substance at 62.5 and 125 mg/kg bw, while 15 animals (including 5 replacement animals) received 250 mg/kg bw. Mice were euthanised 24 hours and 48 hours (high dose test substance group and vehicle control) after the single i.p. injection. The testes were removed from each mouse, and spermatogonial cells were isolated from the tubules, smeared onto the microscope slide and stained with Giemsa stain. One hundred metaphase cells (metaphase spreads) per mouse were examined by light microscopy and scored for chromatid-type and chromosome-type aberrations (structural aberrations). In addition, the mitotic index was recorded as the percentage of cells in mitosis based upon 1000 cells counted per mouse.

Results
No mortality occurred. Piloerection was observed in all mice at 125 and 250 mg/kg bw and lethargy and prostration were recorded in all mice at 250 mg/kg bw. No clinical findings were noted at 62.5 mg/kg bw or in any of the control groups.
There was no biologically relevant and statistically significant increase in the number of cells with structural chromosome aberrations relative to the respective vehicle control groups. There were no reductions in the mitotic indices at 24 or 48 hours after dose administration in the test substance treatment groups relative to the respective vehicle control groups, indicating that deoxyarbutin was not toxic to the spermatogonial cells. In the vehicle group the incidence of structural chromosome aberrations did not exceed the historical control group ranges, while the positive control induced a significant increase in its incidence indicating the suitability and sensitivity of the test system.

Conclusion
Under the experimental conditions used deoxyarbutin is not considered genotoxic (clastogenic and/or aneugenic) in this test.

Ref.: 25

**SCCS comment**
The lack of toxicity of deoxyarbutin may indicate that the spermatogonial cells were not exposed. SCCS considers this test of limited value.

***Overall SCCS conclusion on mutagenicity***
Overall, the genotoxicity of deoxyarbutin is sufficiently investigated in valid genotoxicity tests for the 3 endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy.
Deoxyarbutin did not induce gene mutations in bacteria. In human lymphocytes, deoxyarbutin showed a clastogenic potential with and without metabolic activation, However, as the clastogenic reactions were observed with and without metabolic activating enzymes, and deoxyarbutin is known to be unstable at lower pH values, chemical hydrolysis to hydroquinone can have occurred to a certain extent. Then, instead of deoxyarbutin, its chemical breakdown product hydroquinone, a known clastogenic and cytotoxic agent *in vitro* and *in vivo,* may have been the causative agent.

The positive *in vitro* results on clastogenicity were not confirmed in a micronucleus test with deoxyarbutin under the standard application procedure of intraperitoneal injection. In contrast, orally applied dose levels of deoxyarbutin induced micronuclei in the polychromatic erythrocytes of the bone marrow of male and female mice. This was expected due to the chemical breakdown of deoxyarbutin to hydroquinone in the acidic stomach environment. Thus, the oral application route to investigate the possible genotoxic or clastogenic potential of deoxyarbutin should be considered as inappropriate in the context of safety evaluation of the dermally applied substance. Deoxyarbutin did not induce an increase in the number of spermatogonial cells with structural or numerical chromosome aberrations. However, due to lacking indications of exposure of the spermatogionial cells, this test has limited value.
Based on the present reports, the parent compound deoxyarbutin can be considered to have no *in vivo* genotoxic potential and additional tests are unnecessary.
Arbutins are considered to be metabolised differently by different glycosidases or by spontaneous hydrolysis, yet they all form hydroquinone (HQ). The latter is considered as the genotoxic moiety; therefore a read-across based on HQ release is a possibility to assess the genotoxic potential of the arbutins as a group.

### 3.3.7 Carcinogenicity

No carcinogenicity study with deoxyarbutin is submitted.

### 3.3.8 Reproductive toxicity

No reproduction toxicity study with deoxyarbutin is submitted.

The repeated oral application of deoxyarbutin for 28 days up to the limit dose level of 1000 mg/kg bw in rats and the repeated dermal application for up to 3 months at the highest tested concentration of 40% (corresponding to about 800 mg/kg bw) led to no indication of any impairment of male or female reproductive organs.

Ref.: 18, 40

**SCCS comment**
Developmental and reproductive toxicity studies were not submitted.

### 3.3.9 Toxicokinetics and metabolism

***In vitro* metabolism**

| | |
|---|---|
| Guideline/method: | / |
| Test system: | Cryopreserved hepatocytes from female rabbits, rats, guinea pigs and human |
| Test substance: | deoxyarbutin |
| Batch: | 509014 |
| Purity: | 99.8% (HPLC) |
| Concentrations: | 5, 10 and 100 µM |
| Solvent: | Acetonitrile:5mM sodium hydroxide (1:1, V/V) |
| Positive Controls: | 7-Ethoxycoumarin (7-EC) |
| Analysis: | Reverse phase HPLC with MS/MS detection |
| GLP: | No |
| Date of report: | October 2007 |

Deoxyarbutin was investigated for its hepatic metabolism *in vitro* in female cryopreserved rabbit, rat, guinea pig and human hepatocytes to gain information on the relative quantities

of the metabolites and their rates of production. The test substance was dissolved and diluted with acetonitrile: 5 mM sodium hydroxide (1:1, v/v) and was used at concentrations of 5, 10 and 100 µM. Prior to use, the hepatocytes were thawed and the viability checked then incubated with test substance concentrations of 5, 10 and 100 µM. 7-Ethoxycoumarin was included as positive control. The hepatocytes were exposed for 0, 60, 120 and 240 min and the reaction was terminated by addition of acetonitrile. Thereafter, the exposed hepatocyte mixtures were centrifuged, frozen and stored at -70 °C until analysis (LC-MS/MS and LC-MS). All samples were analysed for the occurrence and amount of deoxyarbutin, deoxyarbutin-glucuronide, deoxyarbutin sulphate, 7-ethoxycoumarin, 7-hydroxycoumarin, 7-hydroxycoumarin-glucuronide, 7-hydroxycoumarin sulphate, hydroquinone and 5-hydroxypentanal.

Results
The metabolism of deoxyarbutin was very similar in the hepatocytes of all the species examined. The levels of deoxyarbutin declined quickly from the hepatocyte preparations. Glucuronide and sulphate conjugation were the major routes of metabolism in all species studied. The major metabolite was deoxyarbutin-glucuronide. Peak areas of this conjugate were larger than the peak areas for the sulphate conjugate in all of the species examined, except for the guinea-pig hepatocyte incubations, where the two conjugates were detected at comparable levels. The metabolic activity of the guinea pigs' hepatocytes was slightly higher and those of the human hepatocytes slightly lower compared to the other species. – In none of the hepatocyte preparation of any species were hydroquinone, 5-hydroxypentanal (breakdown product of hydroquinone) or the glucuronide or sulphate conjugates of hydroquinone were detected at any time point. The sensitivity and suitability of the test system was demonstrated as the positive control substance 7-ethoxycoumarin was metabolized to its major metabolites, 7-hydroxycoumarin glucuronide and 7-hydroxycoumarin sulphate.

Conclusion
The hepatic metabolism of deoxyarbutin *in vitro* was examined in hepatocytes from rabbits, rats, guinea pigs and human and shown to be fast and complete. Glucuronidation and sulphation were demonstrated as the major metabolic pathways. Virtually no metabolism to hydroquinone or its breakdown products was detectable.

Ref.: 26

**SCCS Comment**
A positive control with hydroquinone (HQ) was not done before the samples, exposed to deoxyarbutin, were analyzed for their content of metabolites also seen in samples exposed to HQ. It was mentioned that no HQ values above the background noise were present, but the LOD for this substance or its metabolites was not given.

### *In vivo* toxicokinetics or metabolism

There is no validated regulatory *in vivo* toxicokinetics or metabolism study in experimental animals available.
In an older exploratory screening study, the dermal penetration and metabolic fate of deoxyarbutin was investigated in pigmented guinea pigs. For comparison, hydroquinone (HQ) was investigated in parallel. Two 80% I-base creams were formulated with 5% deoxyarbutin in ethanol or 2.8% HQ in ethanol to yield final 0.05 M (1%, deoxyarbutin) or 0.05 M (0.56%) HQ in the creams. [$^{14}$C]-Deoxyarbutin or [$^{14}$C]-HQ was added to the creams (specific activity of 0.58 mCi/mL or 0.59 mCi/mL, respectively). During the 8-day study, the backs of the pigmented guinea pigs were depilated twice (days 1 and 3) and an area of 16 cm² was treated with 50 µl of each cream to each of 3 animals for four consecutive days. The urine was collected twice daily and stored at -20°C until analysis (scintillation counting, HLPC or thin layer chromatography (TLC). After the treatments were halted, the urines were collected until day 8 for a total of 167 hours. In addition, urine samples were collected from

a control guinea pig without any treatment. Prior to the analyses, the pH of the guinea pig urine was adjusted to 7 with HCl. The urine samples for TLC from deoxyarbutin or HQ treated animals prior to and after enzymatic digestion with ß-glucuronidase and sulfatase were run on silica G plates. The spots were visualized with iodine vapour and the plates were scanned on a radioscanner for radioactivity measurements.

Results
Deoxyarbutin was excreted more rapidly than HQ, with a half-life ($t_{1/2}$) of about 9 h. After 38 hours about 97% was recovered in the urine. The respective values for HQ were $t_{1/2}$ >167 hours and recovery of about 52%. Little radioactivity (4%) was detected in the faeces in each case. Two major metabolites were detected in the urine from deoxyarbutin treated animals and identified as glucuronide (≥57%) and sulfate (≥29%). The obtained data showed also that the metabolic fate of deoxyarbutin in guinea pigs was different from that of HQ. Further analysis of the metabolism data revealed an effective permeability constant (Kpeff) of 1.9 x $10^{-4}$ cm/h through guinea pig skin into the urine. The authors assumed that although this value represents an average flux through the entire animal, it is comparable to permeability constants determined from either steady state or finite dose skin penetration studies. A small amount (5%) of HQ/HQ-metabolites was also found in the urine of dA-treated animals. However, since the starting [$^{14}$C]-deoxyarbutin was contaminated, the presence of the HQ-metabolite was expected, and as the pH of the guinea pig urine had been adjusted to 7 for the enzymatic digestions, the presence of a 5±2% HQ/HQ-metabolite is consistent with the amount of HQ contamination in the dosing solution and from the expected breakdown in the stored urine samples.
Finally, an exploratory study in guinea pigs showed that deoxyarbutin penetrated rapidly through the skin; it was glucuronidated and sulphonated and then rapidly excreted.

Reference: 39, 1

**SCCS Comments**
The data for the animals exposed to hydroquinone (HQ) are not given.

***Applicants conclusion on toxicokinetics and metabolism***
The hepatic metabolism of deoxyarbutin *in vitro* examined in hepatocytes from rabbits, rats, guinea pigs and human was shown to be fast and complete. Glucuronidation and sulphonation were demonstrated as the major metabolic pathways. Virtually no metabolism to hydroquinone or its breakdown products was detectable *in vitro*. The available study on skin penetration and metabolic fate of deoxyarbutin in guinea pigs is not in agreement with current guideline requirements and was not performed under GLP condition but can be considered as a scientifically valid screening study. This study indicated that deoxyarbutin penetrated rapidly through the skin, was glucuronidated and sulphonated, and then rapidly excreted.
Deoxyarbutin was found to have a different metabolic profile than hydroquinone (HQ) suggesting that HQ is not generated in the skin or systemically after topical application of deoxyarbutin.

**SCCS comment**
Data are available on the metabolism of deoxyarbutin in hepatocytes from several species; however data on its metabolism in (human) skin is lacking. Information from an *in vivo* study with topical application to guinea pigs indicates high dermal absorption and suggests extensive phase II metabolism of deoxyarbutin and about 5% breakdown to HQ in the dosing material.

### 3.3.10 Photo-induced toxicity

**Combined phototoxicity and photoallergy study in guinea pigs**

| | |
|---|---|
| Guideline/method: | / |
| Species/strain: | Guinea pig/albino Crl: (HA)BR |
| Group size: | Photoirritation phase: 6 animals/group (9 males and 9 females in total) |
| Photoallergy phase: | 5 – 10 animals/group (14 males and 11 females in total) |
| Test substance: | deoxyarbutin |
| Batch: | HT0059.05 |
| Purity: | 98.7% |
| Route: | Occlusive epicutaneous induction and challenge (Hill Top Chambers®) |
| Vehicle: | deoxyarbutin: Propylene glycol |
| Positive controls: | Acetone (photoirritation/-allergy phase) |
| Dose level: | Photoirritation phase: 1, 5, 20 and 50% in propylene glycol |
| Photoallergy phase: | 50% in propylene glycol |
| Light source: | Bank of 8 fluorescent black light lamps (Sylvania F20BLB) |
| Irradiation: | 10 J/cm² UV-A (320 – 400 nm) |
| Negative control: | Propylene glycol |
| Positive controls: | Photoirritation phase: 0.01% 8-methoxypsoralen (8-MOP) |
| Photoallergy phase: | Musk ambrette (0.1% for challenge and 10% for induction) |
| GLP: | Yes |
| Date of report: | March 1996 |

The phototoxicity and photosensitising properties of deoxyarbutin were evaluated according to a modified protocol of Ichikawa et al., 1981, Gerberick and Ryan, 1989 and Nilsson et al., 1993 using male and female albino Crl:(HA)BR guinea pigs.

Photoirritation phase

Prior to application, the dorsal surfaces of the albino guinea pigs were depilated. Duplicate 0.3 ml applications of the test or control substances were applied to four sites per animal for 2 hours under occlusive conditions (Hill Top Chamber®, 25-mm diameter). The tested deoxyarbutin concentrations were 0, 1, 5, 20 and 50% in propylene glycol. The positive control was 0.01% 8-methoxypsoralen (8-MOP) in acetone. Thereafter, the patches were removed and the application sites wiped and covered with aluminium foil. The animals were exposed to 10 J/cm$^2$ of Ultraviolet-A (UV-A) light (320–400 nm). Following exposure, the foil was removed and the skin was examined 1, 4, 24, and 48 hours for dermal irritation and graded on a scale of 0 – 3 (0, no erythema; 1, slight but confluent or moderate patchy; 2, moderate; 3, severe with or without edema).

Photoallergy phase

Prior to the induction application, the nuchal region of the albino guinea pigs was shaved and depilated. The induction area was pre-treated with 4 intradermal injections of 0.1 ml of Freund's Complete Adjuvant (FCA) at each corner. Afterwards, the animals were tape stripped repeatedly to glisten the skin. The prepared skin areas were treated for 2 h with 0.3 ml of 50% deoxyarbutin in propylene glycol or 10% w/v musk ambrette in acetone as positive control via an adhesive patch (Hill Top Chamber®). The animals were kept in the restrainers and the skin was occluded. Thereafter, the patches were removed and the sites were wiped. The untreated lumbar regions were shielded and animals were exposed to 10 J/cm$^2$ of UVA light (320–400 nm). Induction was repeated on days 2, 4, 7, 9, and 11 with depilation performed on days 4, 7, and 11. Challenge was performed on day 22 on a naïve, depilated lumbar site by application of 0.3 ml/patch of the test material (50% deoxyarbutin) and positive control substance. The sites were occluded for 2 h followed by patch removal and wiping of the skin sites. The left challenge site and the induction areas were shielded with aluminium foil under tape (right challenge site uncovered) followed by exposure to 10 J/cm$^2$ of UVA light. Afterwards, the foil was removed and at 24 and 48 hours after challenge the skin was graded. The re-challenge control group remained untreated.

**Results**

*Phototoxicity*

There was no sign of dermal irritation to any of the test substance concentration on either the UVA irradiated or non-irradiated sites except for a very slight patchy erythema (grade 1) at two sites exposed to 1% deoxyarbutin (1 UV-A irradiated, 1 not irradiated) and one exposed to 50% deoxyarbutin and UV-A. However, compared to the reactions of the positive control, these very slight dermal findings were considered as not indicative for photoirritation. The positive control resulted in moderate to severe erythema at the UVA irradiated sites in all animals indicating the sensitivity and suitability of the test system.

*Photoallergy*

Grade 1 erythema at the UV-A irradiated challenge site was noted in 3/10 animals treated with 50% deoxyarbutin. These reactions were observed at 24 hour in 2/10 and at both 24 and 48 hour observations in 1/10 animals. One of the 10 challenge sites not receiving UV-A radiation also exhibited a grade 1 erythema at 24 and 48 h scoring observation. Two of five animals in the primary challenge control group showed a grade 1 erythema at the UV-A treated site. No signs of irritation were recorded at the sites without UV-A exposure. There were no relevant differences in the incidence or the severity of challenge reactions when comparing the test group sites receiving UV-A irradiation and those not receiving UV-A exposure. In addition, there were also no relevant differences observed between challenge reactions in the UV-A exposed sites in the test substance-treated groups and the primary challenge irradiation control groups. Musk ambrette as positive control substance was proven to be a photoallergen under the conditions of the study as an increased incidence and severity of skin reactions at the UV-A exposed sites occurred, demonstrating the suitability and sensitivity of the test procedure.

Conclusion

Deoxyarbutin was shown to have no phototoxic and no photoallergic potential when tested in concentrations up to 50% in propylene glycol under the conditions of this combined study in male and female guinea pigs.

Ref.: 43

**SCCS comment**

The test results indicate that deoxyarbutin is not phototoxic or photosensitising.

### 3.3.11 Human data

**Human repeated insult patch tests (HRIPTs)**

| | |
|---|---|
| Guideline/Method: | Approved study protocol and standard operating procedures |
| Species: | Human |
| Group size: | 229 induced volunteers and 208 completed (males and females) |
| Test substance: | deoxyarbutin |
| Batch: | 509014 |
| Purity: | 99.8% (HPLC) |
| Route: | Dermal occlusive application by patch |
| Induction: | Applications on Monday, Wednesday and Friday for 24 hours for a total of 9 applications |
| Rest period: | about 2 weeks |
| Challenge: | 1 application for 24 hours |
| Concentration: | 6.0% deoxyarbutin in SC23 emulsion (O/W) |
| GLP: | Yes |
| Date of report: | May 2007 |

Deoxyarbutin was tested for potential irritation and sensitisation on human skin in a repeated insult patch test (HRIPT) as a 6% emulsion (O/W, SC23). Prior to the application, the test area was wiped with 70% isopropyl alcohol and allowed to dry. The test material emulsion was occlusively applied to the upper back (between the scapulae) of each of the

229 male and female healthy volunteers and was allowed to remain in direct skin contact for a period of 24 hours. Patches were applied to the same site on Monday, Wednesday, and Friday for a total of 9 applications during the induction period. The sites were graded for dermal irritation 24 hours after removal of the patches on Tuesday and Thursday and 48 hours after removal on Saturday.
Following a rest period of about 2 weeks, the challenge patches were applied to previously untreated skin sites for 24 hours. After removal of the patches, the sites were evaluated for dermal findings and 48 and 72 hours thereafter.

Results
208 male and female volunteers completed the study and 21 discontinued, but not due to test material reaction. During the induction phase and after challenge, no visible skin reaction was noted in any of the volunteers at any time point.

Conclusion
Under the conditions of the repeated insult patch test, no skin reactions were noted in any of the 208 male and female volunteers. Thus, deoxyarbutin investigated as a 6.0% O/W emulsion did not demonstrate any potential for skin irritation or sensitisation in humans.

Ref.: 24

This study result is in line with a previously performed HRIPT in 99 male and female volunteers, of which 94 volunteers completed the study. Deoxyarbutin was tested as a 3% moisturiser cream formulation containing 0.3% Glydant under comparable occlusive conditions as described above. The moisturizer cream without deoxyarbutin was tested in parallel as vehicle control. Among the 94 volunteers who completed the study, there was 1 female showing skin reactions indicative for sensitisation during late induction and after challenge. This female had a history of stress asthma and indicated by re-questionnaire that she was allergic to a penicillin type and has to avoid certain perfumes. Two re-challenges indicated that deoxyarbutin was not the causative agent for the dermal findings. Finally, it can be concluded that deoxyarbutin investigated as a 3.0% moisturizer cream showed no clear indication for skin irritation or skin sensitisation under the conditions of this human repeated insult patch test.

Ref.: 42

**SCCS comment**
The SCCS does not consider HRIPT studies for determining sensitisation potential to be ethical.

**Other Human tests**

The efficacy of deoxyarbutin as a skin lightening substance in humans was investigated in two studies which are reported here in brief for the sake of completeness and with regard to notable undesirable effects.
50 female volunteers (34 Caucasians and 16 mixed ethnic persons) received application of a 3% deoxyarbutin containing moistening cream including a placebo control, each on a 100 $cm^2$ area on the dorsal surface of the forearm. The daily treatment was for 12 weeks from mid-November to mid-February at a clinical site in the United Kingdom. No signs of skin irritation were reported during the course of this study.

Ref. 1

A further human clinical trial was performed with 25 male and female volunteers with Fitzpatrick skin types of III or IV. Three skin sites on the back of each volunteer were exposed for 10-20 min daily for 7 consecutive days to UV light from a tanning bed. At the end of the tanning regime, one of each of the coded test sites was left untreated, treated with 3% deoxyarbutin or treated with 4% hydroquinone. The test substance preparations were topically applied 3 times per week for 5 weeks, at 12.5 µL / 2 $cm^2$ in moisturizer (oil-

in-water emulsion type), using an occlusive patch system and modified to a semi-occlusive system when necessary to manage irritation. After the 5-week treatment period, the percent of tan remaining in the untreated and treated sites was compared. Other endpoints or side effects were not reported.

Ref. 30

### 3.3.12 Special investigations

***In vitro* studies**

The effects of deoxyarbutin (dA) and hydroquinone (HQ) on cultured human cells (primary cultures of human melanocytes, keratinocytes and fibroblasts established from neonatal foreskin of dark and light skin individuals) were compared in assays for viability (cell counts), tyrosine hydroxylase activity and melanin content: The maximum concentration of dA that allowed 95% viability was 4-fold higher than HQ in human keratinocytes, indicating that dA is less cytotoxic than HQ. At the maximum concentration allowing normal cellular viability, dA effectively inhibited tyrosinase activity and melanin content in human melanocytes, where HQ was marginally inhibitory. Upon removal of dA, the tyrosinase activity and melanin content was normalised within 5 days (ref. 30). Apparently, dA inhibits human tyrosine hydroxylase and DOPA oxidase in a reversible manner and is a more robust competitive inhibitor than HQ (Chawla et al. 2008). Further studies in cultured melanocytes showed that deoxyarbutin not only inhibited tyrosinase, but also decreased the protein expression (Hu et al. 2009).

**Tests in experimental animals**

Two studies, conducted to assess efficacy of deoxyarbutin as a skin lightening substance, are briefly reported for completeness, but only with respect to undesirable effects.

Hairless pigmented guinea pigs were treated 9 weeks with 3% deoxyarbutin containing cream and for comparison with hydroquinone, kojic acid and arbutin containing creams. The deoxyarbutin-treated sites showed no signs of skin irritation.

Ref. 1

Efficacy was also examined in human xenografts on female ICD-SCID mice: the grafts were left untreated for 2 months until hyperpigmentation was reached, then topical treatment with 5% deoxyarbutin or HQ or tert-butylphenol containing emulsions was started. Also the dose-response (0.1, 0.3, 1, 3%) and reversibility (8 weeks after termination of treatment) were studied. No signs of inflammation or abnormal morphology were noted.

Ref. 30

### 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 in 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]

The Cosmetic Ingredient Review (CIR) Expert Panel conducted a safety assessment of hydroquinone as used in cosmetics and concluded that it is safe at concentrations ≤ 1% in hair dyes and is safe for use in nail adhesives. Hydroquinone should not be used in other leave-on cosmetics. With regard to the use of HQ in topical formulations as skin bleaching and depigmenting agent, the CIR report of 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=HQ Ref. F]: Total permeation of HQ after 24h was 43.3% of the

[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)

dose *in vitro*; flux was 2.93 µg/h-cm$^2$. An average of 45.3±11.2% of the dose was recovered in the urine of volunteers after application of HQ to forehead skin.
For the safety evaluation of HQ exposure resulting from the use of skin bleaching products in comparison to internal HQ doses which may induce ochronosis, the SCCS will use 50% dermal absorption for HQ.

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

***Safety evaluation for deoxyarbutin*** (3% in face cream, applied once per day)

Application of face cream (1.54 g/day, according to the SCCS NoG, Table 3) with 3% a.i. equals 46.2 mg deoxyarbutin/person/day; adjusted to 60 kg b.w. = 0.77 mg/kg x day.
For calculation of a margin of safety, the NOAEL of a subchronic dermal study (ref. 40) is divided by the human external dose.

90-day dermal rabbit toxicity study for deoxyarbutin:
NOAEL for systemic toxicity (mg/kg bw) about 800
Human external dose (mg/kg bw) 0.77
**MOS:** 800 / 0.77 = **1039**

*NOTE: An additional MOS calculation - based on the systemic exposure dose (SED) for deoxyarbutin using the mean+2 SD, i.e. 80.04% or 134 µg/cm$^2$ from the in vitro dermal penetration study (ref. 27) and the NOAEL of 316 mg/kg bw from the 28-day oral toxicity study in rats (ref. 18) - arrives also at a sufficient margin of safety (of 250). The oral NOAEL is considered as worst-case scenario for deoxyarbutin since the acidic stomach environment will lead to its degradation to hydroquinone which then causes the observed toxicity.*

***Safety evaluation for the Hydroquinone (HQ) formed***

Other than alpha- or beta-arbutin (SCCS/1550/2015 and SCCS 1552/2015) where partial hydrolysis of the absorbed fraction can occur within the skin, the dermally absorbed deoxyarbutin undergoes apparently extensive phase-II metabolism with no measurable release of hydroquinone in skin. However, hydroquinone can be present as impurity in the cosmetic product itself as a consequence of partial degradation of deoxyarbutin.

**Considerations on the stability of deoxyarbutin with regard to formation of HQ as impurity in the cosmetic preparations:**

Deoxyarbutin seems to be stable in anhydrous emulsions or in O/W formulations at pH 7 when stored at low temperatures (8°C) and protected from light (Lin et al. 2011). However, under real use conditions (elevated temperatures on skin and pH of about 5.5), some degradation to hydroquinone (HQ) can occur. The extent of HQ formation under real use conditions on human skin has not been studied.
Approximations are derived from *i)* stability tests in solution which show at slightly acidic pH some decline in the content of deoxyarbutin with a corresponding increase in HQ: already at 1 hour HQ content was 0.03% at pH 6 and 0.25% at pH 5; after 12 hours the HQ content was 0.2% at pH 6 and 2.42% at pH 5 (ref. 29, page 19). *ii)* The kinetic study in guinea pigs with dermal application of deoxyarbutin in I-base cream reported "contamination" of the starting material with about 5±2% HQ, and this was also reflected by the presence of a similar fraction of HQ/HQ metabolites in urine samples (ref. 39).
In the absence of other data, the SCCS assumes 10% HQ as impurity in deoxyarbutin under in use conditions.

**Estimates:**
Application of 1.54 g/day cream with 3% deoxyarbutin (dA) equals 46.2 mg dA/person/day: If 10% of the initial ingredient (dA) degraded to HQ, the external exposure amounts to 4.62 mg (dA) and corrected for molecular weights of dA (194.23) and HQ (110.11),
(4.62 x 110.11/194.23) = **2.62 mg HQ per person/day**.

As HQ is known to readily penetrate the skin (dermal absorption about 50%), the presence of this impurity in the product would result in **internal HQ dose of 1.31 mg/day**
and (: 60 kg) **SED for HQ of 0.0281 mg/kg bw/day.**

The estimated exposure to hydroquinone (HQ) from application of deoxyarbutin containing cosmetic products is assessed below and compared to risks related to a) repeated dose toxicity, b) induction of ochronosis, and c) carcinogenicity.

**a)** *HQ repeated dose toxicity*

In this part of the safety assessment, MOS values are calculated from literature data on **sub/-chronic toxicity** tests with hydroquinone (see section 3.3.13)

| | |
|---|---|
| NOAEL (90 day oral - rat): | 20 mg/kg bw/day |
| NOAEL (1-generation reprotox - rat) | 15 mg/kg bw/day |
| SED for HQ: | 0.0218 mg/kg bw/day |

→ **MOS (NOAEL/SED)** values would be
**917** (90 day) or **688** (reprotox)

**b)** *HQ induction of ochronosis*

HQ is also suspected to cause exogenous **ochronosis** (EO). As a NOAEL has not been established for exogenous ochronosis, 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 HQ-induced ochronosis: | 1 % |
| Maximum quantity of application: | 0.8 g formulation |
| Frequency of application per day: | 2 |
| Absorption of HQ though skin | 50 % |

**total estimated HQ amount resulting from deoyarbutin skin application:**
of **1.31 mg HQ** per day 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 6.1**

The minimum exposure level of HQ which may cause ochronosis is calculated as being only 6 times higher than the estimated exposure resulting from application of 3% desoxyarbutin formulations with 10% HQ as impurity.

**c)** *Carcinogenicity*

In the EU, hydroquinone is classified as Carc Cat 2 H351 (suspected human carcinogen) Muta Cat 2 H341 (suspected of causing genetic defects) based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI.
The SCCS has carried a calculation to determine lifetime cancer risk (as in section 3-7.4 of the 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 by using the following formula:

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

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

Assuming a systemic exposure dose of **0.0218** mg/kg bw/d, the risk is calculated to be about **3 x $10^{-4}$**.

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

Whilst it can be taken into account that this tumour type is considered rodent specific and may have little relevance for humans, the lifetime risk calculated by linear extrapolation (in this case a very conservative approach), would exceed the usually accepted risk limits.

Moreover, the estimated systemic HQ exposure from the use of deoxyarbutin-containing cosmetic products (0.0281 mg/kg bw/d) would be higher than oral intake of HQ with food (0.017 mg/kg bw/d; Deisinger et al. 1996).

## 3.4 Discussion

**Physico-chemical properties**
The applicant reports that deoxyarbutin is stable for at least 2 years when protected from humidity, temperatures above 8°C and light. However, deoxarbutin is not stable in slightly acidic medium and hydrolyses to hydroquinone at room temperature [29]. This is of relevance when it is used in cosmetic products on skin (which has a slightly acidic pH), and when there is exposure to higher temperatures and light. Therefore, under in use conditions, it has to be considered that deoxyarbutin can undergo hydrolysis into hydroquinone.
An approximation of the possible HQ content in deoxyarbutin formulations was derived from stability tests in solution and the reported "contamination" of the starting material with about 5±2% HQ in I-base cream applied in a kinetic study with guinea pigs. In the absence of other data, the SCCS assumes 10% HQ as impurity in deoxyarbutin under in use conditions.

**Acute toxicity**
The acute oral and dermal toxicity of deoxyarbutin can be regarded as low. Studies performed according to current testing guidelines under GLP conditions with characterised test material resulted in $LD_{50}$ values for acute oral and dermal toxicity of > 2000 mg/kg bw in rats. Former acute oral, dermal and intraperitoneal studies with limited validity generally confirmed the low acute toxicity for all application routes in the species tested.

**Skin and mucous membrane irritation**
Deoxyarbutin did not exhibit any corrosive potential to the skin in the *in vitro* TER test and was shown to be non-irritating to the intact skin of New Zealand White rabbits. In an eye irritation test using New Zealand White rabbits, it was slightly and transiently irritating to the eyes, but threshold scores for classification as an eye irritant were not reached. However, as redness and particularly chemosis were observed during the observation

period, the test substance is considered as a mild eye irritant. A standard cream formulation containing 3% deoxyarbutin led to no signs of eye irritation in rabbits, but the low volume of test formulation (about 10 µl, i.e. 1/10 of the volume recommended in the OECD TG 405) used in this study precludes an evaluation of the eye-irritating potential of the test formulation. Human repeated insult patch tests did not reveal a skin-irritating potential for deoxyarbutin at a concentration of up to 6.0%.

**Skin sensitisation**
The sensitising potential of deoxyarbutin was investigated in two murine local lymph node assays. The more recent study investigated concentrations of 10% - 50% and was carried out in accordance of the actual OECD and EC guidelines. The former study investigated concentrations of 3% - 20%, but a slightly higher number of animals and exposure days as well as individual lymph node measurements were used. The local lymph node assays performed indicate that deoxyarbutin is a moderate skin sensitiser.

**Dermal/percutaneous absorption**
The human *in vitro* percutaneous absorption study performed under current guideline requirements and under GLP conditions showed that after dermal application of 3% deoxyarbutin in a standard o/w emulsion (160.81 µg deoxyarbutin/cm²) applied for 24 hours to the viable skin of three female donors, deoxyarbutin was detected in all compartments relevant to assess dermal absorption and penetration. The recovery revealed no indication for degradation of the test material. A high dermal availability of 58.12±11.96 %, corresponding to 93.66±20.15 µg deoxyarbutin/cm² was observed. Because of some limitations in the *in vitro* study (low number of donors), the SCCS considers to use the mean+2 SD, i.e. 80.04% or 134 µg/cm$^2$ for dermal penetration in the safety assessment.

**Repeated dose toxicity**
The systemic toxicity after repeated oral application to male and female rats for 28 days was low and effects could only be observed at the current internationally accepted limit dose level of 1000 mg/kg bw consisting of transient clinical findings, slightly reduced food consumption and retarded bodyweight gain, minor haematological effects in females and slight impairment of single clinical chemistry parameters. The relative liver weights of both sexes and the relative kidney weights of the males were increased but without any histopathological correlation. The functional observation battery revealed no indication of any neurological impairment at any dose level. The No-Adverse-Effect-Level (NOAEL) for subacute toxicity in rats after 28-day oral treatment was 316 mg/kg bw. This value may be considered as the worst-case scenario for deoxyarbutin since the acidic stomach environment will lead to its degradation to hydroquinone which then causes the observed toxicity. Also in light of the pronounced route effect observed in the *in vivo* genotoxicity tests (see 3.3.6.), the SCCS concluded that the oral application route should be considered as inappropriate for investigating safety of desoxyarbutin in the context of its dermal application, and therefore chose the subchronic dermal toxicity study to derive a NOAEL for the calculation of a margin of safety. **Repeated dermal** application of deoxyarbutin in **rabbits** either as 3% cream formulation for 28 days or as 1, 5 and 40% propylene glycol/ethanol solution for 91 days did not lead to any substance-related systemic findings up to the highest dose level investigated. The respective **NOAEL** for systemic toxicity in rabbits was 40% deoxyarbutin corresponding to about **800 mg/kg bw**. This value will be used for risk assessment and the calculation of the margin of safety (MoS).

**Mutagenicity/Genotoxicity**
Overall, the genotoxicity of deoxyarbutin is sufficiently investigated in valid genotoxicity tests for the 3 endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy.
Deoxyarbutin did not induce gene mutations in bacteria. In human lymphocytes deoxyarbutin showed a clastogenic potential with and without metabolic activation, However, as the clastogenic reactions were observed with and without metabolic activating enzymes, and deoxyarbutin is known to be unstable at lower pH values, chemical hydrolysis

to hydroquinone can have occurred to a certain extent. Then, instead of deoxyarbutin, its chemical breakdown product hydroquinone, a known clastogenic and cytotoxic agent *in vitro* and *in vivo,* may have been the causative agent. The positive *in vitro* results on clastogenicity were not confirmed in two separate micronucleus tests with deoxyarbutin under the standard application procedure of intraperitoneal injection. In contrast, orally applied dose levels of deoxyarbutin induced micronuclei in the polychromatic erythrocytes of the bone marrow of male and female mice. This was expected due to the chemical breakdown of deoxyarbutin to hydroquinone in the acidic stomach environment. Thus, the oral application route to investigate the possible genotoxic or clastogenic potential of deoxyarbutin should be considered as inappropriate in the context of safety evaluation of the dermally applied substance. Deoxyarbutin did not induce an increase in the number of spermatogonial cells with structural or numerical chromosome aberrations. However, due to lacking indications of exposure of the spermatogonial cells, this test has limited value.
Based on the present reports, the parent compound deoxyarbutin can be considered to have no *in vivo* genotoxic potential and additional tests are unnecessary.
Arbutins are considered to be metabolised differently by different glycosidases or by spontaneous hydrolysis, yet they all form hydroquinone (HQ). The latter is considered as the genotoxic moiety; therefore a read-across based on HQ release is a possibility to assess the genotoxic potential of the arbutins as a group.

**Reproduction toxicity**
Developmental and reproductive toxicity studies were not submitted.
The repeated oral application of deoxyarbutin for 28-days in rats up to the limit dose level of 1000 mg/kg bw as well as the repeated dermal application in rabbits for up to 3 months and the highest tested concentration of 40% (corresponding to about 800 mg/kg bw) led to no indication of any impairment of male or female reproductive organs.

**Toxicokinetics and metabolism**
The hepatic metabolism of deoxyarbutin *in vitro,* examined in hepatocytes from rabbits, rats, guinea pigs and humans, was shown to be fast and complete. Glucuronidation and sulphonation were demonstrated as the major metabolic pathways. No metabolism to hydroquinone or its breakdown products was detectable *in vitro*. Whilst information on the hepatic metabolism of deoxyarbutin is available, data on its metabolism in human skin is lacking.
The available screening study on skin penetration and metabolic fate of deoxyarbutin in guinea pigs indicated high absorption of deoxyarbutin that penetrated rapidly through the skin; it was glucuronidated and sulphonated, and then rapidly excreted.
Deoxyarbutin was found to have a different metabolic profile than hydroquinone (HQ), suggesting that HQ may not be generated in the skin or systemically after topical application of deoxyarbutin. This was supported by the fact that in a dermal penetration study *in vitro* using human skin, the recovery revealed no indication for degradation of the test material. Information from an *in vivo* study with topical application to guinea pigs suggests extensive phase II metabolism of deoxyarbutin. However, in the same study about 5±2% breakdown to hydroquinone (HQ) in the dosing material was reported. Therefore, (nonenzymatic) degradation to HQ under in use conditions has to be considered as well (see also above section 'Physico-chemical properties').

**Photo-induced toxicity**
Deoxyarbutin was shown to have no photoirritation and no photoallergic potential when tested in concentrations up to 50% in propylene glycol under the conditions of a combined study in male and female guinea pigs.

**Human data**
Two independent human repeated insult patch tests showed that deoxyarbutin up to a concentration of 6.0% did not demonstrate any potential for skin irritation or sensitisation in humans.

## 4. CONCLUSION

1. *Does the SCCS consider, on the basis of the provided scientific data, the use of deoxyarbutin to be safe for consumers in cosmetic products in a concentration up to 3% in face creams?*

   Although on the basis of the provided scientific data the use of deoxyarbutin as such can be considered safe for consumers in cosmetic products in a concentration up to 3% in face creams, hydroquinone will be formed at levels which raise concerns with regard to the safety of such products during life-cycle of the product (e.g. storage conditions and stability under in-use conditions). Therefore, the overall conclusion of the SCCS is that the use of deoxyarbutin up to 3% in face creams is not safe.

2. *And/or does the SCCS have any scientific concerns with regard to the use of deoxyarbutin or related substances known to release hydroquinone in cosmetic products?*

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## 5. MINORITY OPINION

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## 6. REFERENCES

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**Note:** „Deoxyarbutin 100"=code for the company's compound name

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