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?*

   /

5. MINORITY OPINION

/

6. REFERENCES

References included in submission

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

1. Boissy RE, Visscher M, DeLong MA (2005) Deoxyarbutin: A novel reversible tyrosinase inhibitor with effective in vivo skin lightening potency, Experim. Dermatology, 14, 601-608
2. Bradley MO, Taylor VI, Armstrong MJ, Galloway SM (1987) Relationships among Cytotoxicity, Lysosomal Breakdown, Chromosome Aberrations, and DNA Double-strand Breaks, Mut. Res., 189, 69-79
3. ECB (European Chemicals Bureau, 2000) IUCLID Dataset, Hydroquinone (CAS 123-31-9),18 February 2000 (http://ecb.jrc.it/IUCLID-DataSheets/123319.pdf)
4. Girindus AG (2005a) „Deoxyarbutin 100": Acute oral toxicity study with rats (acute toxic class method), Report ARC-L-1632, Life Science, Toxicology, ARC Seibersdorf Research GmbH, Seibersdorf, Austria, unpublished data, August 2005
5. Girindus AG (2005b) „Deoxyarbutin 100": Acute dermal toxicity study with rats, Report ARCL-1625, Environmental and Life Science, Toxicology, ARC Seibersdorf Research GmbH, Seibersdorf, Austria, unpublished data, August 2005
6. Girindus AG (2005c) „Deoxyarbutin 100": In vitro skin corrosion study – Transcutaneous electrical resistance test, Report ARC-L-1489, Life Science, Toxicology, ARC Seibersdorf Research GmbH, Seibersdorf, Austria, unpublished data, May 2005

7. Girindus AG (2005d) „Deoxyarbutin 100": Acute dermal irritation/corrosion study with rabbits, Report ARC-L-1633, Life Science, Toxicology, ARC Seibersdorf Research GmbH, Seibersdorf, Austria, unpublished data, August 2005
8. Girindus AG (2005e) „Deoxyarbutin 100": Acute eye irritation/corrosion study with rabbits, Report ARC-L-1634, Life Science, Toxicology, ARC Seibersdorf Research GmbH, Seibersdorf, Austria, unpublished data, August 2005
9. Girindus AG (2005f) „Deoxyarbutin 100": Skin sensitization study (Local lymph node assay), Report ARC-L-1797, Life Science, Toxicology, ARC Seibersdorf Research GmbH, Seibersdorf, Austria, unpublished data, December 2005
10. Girindus AG (2005g) „Deoxyarbutin 100": Vapour pressure, Report ARC-L-1843, Life Science, Toxicology, ARC Seibersdorf Research GmbH, Seibersdorf, Austria, unpublished data, December 2005
11. Girindus AG (2005h) „Deoxyarbutin 100": Relative density, Report ARC-L-1842, Life Science, Toxicology, ARC Seibersdorf Research GmbH, Seibersdorf, Austria, unpublished data, December 2005
12. Girindus AG (2005i) „Deoxyarbutin 100": Boiling Temperature, Report ARC-L-1841, Life Science, Toxicology, ARC Seibersdorf Research GmbH, Seibersdorf, Austria, unpublished data, December 2005
13. Girindus AG (2005j) „Deoxyarbutin 100": Melting Temperature, Report ARC-L-1840, Life Science, Toxicology, ARC Seibersdorf Research GmbH, Seibersdorf, Austria, unpublished data, December 2005
14. Girindus AG (2005k) „Deoxyarbutin 100": Water solubility, Report ARC-L-1845, Life Science, Toxicology, ARC Seibersdorf Research GmbH, Seibersdorf, Austria, unpublished data, December 2005
15. Girindus AG (2005l) „Deoxyarbutin 100": Partition coefficient, Report ARC-L-1846, Life Science, Toxicology, ARC Seibersdorf Research GmbH, Seibersdorf, Austria, unpublished data, December 2005
16. Girindus AG (2005m) „ Deoxyarbutin 100":Relative Self-Ignition Temperature, Report ARC-L- 1851, Life Science, Toxicology, ARC Seibersdorf Research GmbH, Seibersdorf, Austria, unpublished data, December 2005
17. Girindus AG (2005n) Prüfanweisung (PA) Deoxyarbutin 100 [Test Instruction Deoxyarbutin 100, K WP PA 1965] Report No. F30123 K WP PA 1965/03, Girindus AG, Halle, Germany, unpublished data, 01 December 2005
18. Girindus AG (2006a) „ Deoxyarbutin 100": 28-day oral toxicity study with rats, Report ARC-L- 1876, Life Science, Toxicology, ARC Seibersdorf Research GmbH, Seibersdorf, Austria, unpublished data, January 2006
19. Girindus AG (2006b) „ Deoxyarbutin 100": In vitro mammalian cytogenetic study (chromosome analysis), Report ARC-L-1883, Life Science, Toxicology, ARC Seibersdorf Research GmbH, Seibersdorf, Austria, unpublished data, January 2006
20. Girindus AG (2006c) „Deoxyarbutin 100, Batch No. 509014": Micronucleus test with mice, Report ARC-L-1930, Life Science, Toxicology, ARC Seibersdorf Research GmbH, Seibersdorf, Austria, unpublished data, February 2006
21. Girindus AG (2006d) „ Deoxyarbutin 100, Batch No. 504007": Micronucleus test with mice, Report ARC-L-1929, Life Science, Toxicology, ARC Seibersdorf Research GmbH, Seibersdorf, Austria, unpublished data, February 2006
22. Girindus AG (2006e) Material Safety Data Sheet, Deoxyarbutin 100, Version 007/2006, Girindus AG, Bensberg, Germany, 21 February 2006
23. Girindus AG (2007a) Mammalian erythrocyte micronucleus test, Deoxyarbutin 100, BioReliance Study No. AB37AG.123.BTL, Sponsor Project No. 11430, BioReliance, Rockville, MD, USA, unpublished data, 25 January 2007
24. Girindus AG (2007b) Repeated insult patch test, O/W emulsion SC23 (6% dA 100), Project No. BLL-06-063, QC control No. Q-No 104544, CRL Study No. CRL17307, Clinical Research Laboratories, Piscataway, NJ, USA, unpublished data, 14 May 2007
25. Girindus AG (2007c) Mammalian spermatogonial chromosome aberration test, Deoxyarbutin 100, BioReliance Study No. AB37AG.109.BTL, Sponsor Project No. 12811, BioReliance, Rockville, MD, USA, unpublished data, 19 October 2007

26. Girindus AG (2007d) A study to determine the metabolism of deoxyarbutin in female cryopreserved rabbit, rat guinea pig and human hepatocytes, CXR Study No. CXR0548, CXR Bioscience Ltd., DUNDEE DD1 5JJ, Scotland, UK, unpublished data, 04 October 2007
27. Girindus AG (2007e) In vitro permeation and penetration properties of Deoxyarbutin 100 on human skin under GLP conditions, GLP Study Report STP 037/00, Report No. C-13120-152-0807, Across Barriers GmbH, Saarbruecken, Germany, unpublished data, 20 December 2007
28. Girindus AG (2007f) Product Profile Deoxyarbutin 100, Revision 07-26.2007, Girindus AG, Bensberg, Germany, unpublished data, 26 July 2007
29. Girindus AG (2007g) Stabilitätsbericht (SB) Deoxyarbutin 100 [Stability report of Deoxyarbutin 100], Report No. F30123 K WP SB 3290/01, Girindus AG, Halle, Germany, unpublished data, 17 December 2007
30. Hamed SH, Sriwiriyanont P, DeLong MA, Visscher MO, Wickett RR, Boissy RE (2006) Comparative efficacy and safety of a new tyrosinase inhibitory agent, deoxyarbutin, - J Cosmet Sci 57(4):291-308
31. OECD SIDS, Hydroquinone, UNEP Publications. (http://www.inchem.org/documents/sids/sids/123319.pdf)
32. Scott D, Galloway SM, Marshall RR, Ishidate M, Brusick D, Ashby J, Myhr BC (1991) Genotoxicity under Extreme Culture Conditions, A Report from ICPEMC Task Group 9, Mut. Res., 257, 147-205
33. The Proctor & Gamble Company (1994a) An acute oral toxicity study in rats with HT0059.01 and HT0060.01, SLS Study No. 3029.1886, Springborn Laboratories, Life Science Division, Spencerville, OH, USA, unpublished data, 20 July 1994
34. The Proctor & Gamble Company (1994b) An acute oral toxicity study in rats with HT0059.04, SLS Study No. 3029.1912, Springborn Laboratories, Life Science Division, Spencerville, OH, USA, unpublished data, 04 October 1994
35. The Proctor & Gamble Company (1994c) An acute dermal toxicity study in rats with HT0064.01, SLS Study No. 3029.1894, Springborn Laboratories, Life Science Division, Spencerville, OH, USA, unpublished data, 13 June 1994
36. The Proctor & Gamble Company (1994d) Up and down procedure for testing acute intraperitoneal toxicity in rats, SLS Study No. 3029.1887, Springborn Laboratories, Life Science Division, Spencerville, OH, USA, unpublished data, 28 June 1994
37. The Proctor & Gamble Company (1994e) A low volume eye irritation study in rabbits with HT0061.01, SLS Study No. 3029.1895, Springborn Laboratories, Life Science Division, Spencerville, OH, USA, unpublished data, 03 May 1994
38. The Proctor & Gamble Company (1994f) Murine local lymph node assay, IITRI Project No. L08321, Study No. 45, IIT Research Institute, Life Science Research, Chicago, IL, USA, unpublished data, June 1994
39. The Proctor & Gamble Company (1994g) The penetration and metabolic fate of the topically applied skin lightening active deoxyarbutin in Guinea pigs, Research and Development Department Report, Cosmetic and Fragrances Technology Division, The Procter & Gamble Company, Cincinnati, OH, USA, unpublished data, 31 August 1994
40. The Proctor & Gamble Company (1994h) 28-day and 91-day dermal toxicity study in rabbits, IRDC Study No. 191-1600, DRD No. HTSE-042, IRDC, Mattawan, MI, USA, unpublished data, 17 October 1994
41. The Proctor & Gamble Company (1994i) Salmonella/Mammalian microsome (Ames Test) and Escherichia coli WP2 mutagenesis assay, HT0059.01, Document No. HTSE-040, Laboratory Study No. TE257.508, Microbiological Associates, Rockville, MD, USA, unpublished data, 22 March 1994
42. The Proctor & Gamble Company (1995a) Human repeat insult patch test (HRIPT), HT0072.01, deoxyarbutin (3%) in a moisturizer vehicle with 0.3% Glydant, DRD No. HTSE-051, Report No. 03694, IS Consulting Limited, Haddington, Scotland, unpublished data, April 1995
43. The Proctor & Gamble Company (1996a) Photoirritation/Photoallergy study of HT0059.05 in Guinea pigs, DRD No. HTSE 054, Report No. CHW 51002116, Corning Hazleton Inc.

Additional References – added by the SCCS

- Chawla S, deLong MA, Visscher MO, Wickett RR, Manga P, Boissy RE (2008) Mechanism of tyrosinase inhibition by deoxyArbutin and its second-generation derivatives. Brit J Dermatol 159 (6) 1267-1274
- Deisinger PJ, Hill TS, English JC (1996) Human Exposure to Naturally Occurring Hydroquinone, J Toxicol Environ Health. 47, 31-46.
- Hu ZM, Zhou Q, Lei TC, Ding SF, Xu SZ (2009) Effects of hydroquinone and its glucoside derivatives on melanogenesis and antioxidation: Biosafety as skin whitening agents. J Dermatol Sci 55(3) 179-84
- Lin CC, Yang CH, Chang NF, Wu PS, Chen YS, Lee SM, Chen CW (**2011)** Study on the stability of Deoxyarbutin in an anhydrous emulsion system. Int J Mol Sci 12, 5946-5954
- Yang CH Chen YS, Lai JS, Hong WW, Lin CC (2010) Determination of the thermodegradation of deoxyArbutin in aqueous solution by high performance liquid chromatography. Int J Mol Sci 11(10):3977-3987. doi: 10.3390/ijms11103977
- Yang CH, Chang NF, Chen YS, Lee SM, Lin PJ, Lin CC (2013) Comparative study on the photostability of arbutin and deoxy-arbutin: sensitivity to ultraviolet radiation and enhanced photostability by the water-soluble sunscreen, benzophenone-4. Biosci Biotechnol Biochem 77(5):1127-1130

References in section 3.3.13 on Hydroquinone [HQ Ref. A-F]

A. OECD SIDS. Hydroquinone, CAS No. 123-31-9 (above Ref. 31)

B. International Programme on Chemical Safety, IPCS (1994). Environmental Health Criteria 157, Hydroquinone, WHO, Geneva

C. McGregor D (2007) Hydroquinone: an evaluation of the human risks from its carcinogenic and mutagenic properties. Crit Rev Toxicol 37(10):887-914.

D. Levitt J (2007) The safety of hydroquinone: a dermatologist's response to the 2006 Federal Register. Journal of the American Academy of Dermatology 57(5):854-872.

E. Andersen FA, Bergfeld WF, Belsito DV, Hill RA, Klaassen CD, Liebler DC, Marks JG, Shank RC, Slaga TJ, Snyder PW [Cosmetic Ingredient Review (CIR) Expert Panel] (2010) Final amended safety assessment of hydroquinone as used in cosmetics. Intl J Toxicol. 29 (suppl 4): 274S-287S

F. Wester RC et al. (1998) Human *in vivo* and *in vitro* hydroquinone topical bioavailability, metabolism, and disposition. J Toxicol Environ Health, Part A, 54: 301-317.