SCCS/1576/16
Final version of 6 October 2016
Corrigendum on 23 December 2016

Scientific Committee on Consumer Safety

SCCS

OPINION ON

Vitamin A

(Retinol, Retinyl Acetate, Retinyl Palmitate)

The SCCS adopted this Opinion at its 2nd plenary meeting

on 6 October 2016

- CORRIGENDUM adopted by written procedure on 23 December 2016 -

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SCCS

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

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For this Opinion, comments received resulted in the following changes that have been adopted on 23 December 2016 by written procedure: *chapter 3.2 Function and uses, SCCS general conclusion on the repeated dose toxicity of Vitamin A (page 51), chapter 3.3.7 Human data and the respectives conclusions number 2 and 3. A corrigendum on impurities on pages 11, 63 and 70 as well as acknowledgments.*

Keywords: SCCS, scientific opinion, Vitamin A (Retinol, Retinyl Acetate, Retinyl Palmitate), Regulation 1223/2009, CAS n. 11103-57-4 / 68-26-8 / 127-47-9 / 79-81-2, EC 234-328-2 / 200-683-7 / 204-844-2 / 201-228-5

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Table of Contents

1. BACKGROUND

Vitamin A (CAS n. 68-26-8 / 11103-57-4/ 116-31-4) constitutes a group of lipid-soluble compounds including retinol, retinyl palmitate, retinyl acetate, retinyl linoleate and retinal. Vitamin A is a lipophilic-soluble Vitamin and as such a micronutrient essential for most of mammalian species.

The risk characterisation for general use of Vitamin A for all age groups is based on the tolerable upper intake levels (UL) derived from earlier opinions from the Scientific Committee of Food (SCF)[1] and European Food Safety Authority (EFSA)[2]. In 2002, the SCF considered that the upper level of 3000 µg RE (retinol equivalents) /day is appropriate for all women of child-bearing age but also for men and for infants and children after correction for differences in metabolic rate. In 2008, EFSA considered that a maximum intake of 1500 µg RE/day would serve as a guidance level (GL) for individuals at greater risk of osteoporosis and bone fracture (particularly post-menopausal women).

In January 2012, the Commission received some documents from German authorities requesting a safety assessment of Vitamin A in cosmetics products (BfR, 2012). According to the Member State, the use of retinol and its esters in cosmetics should be restricted in view of increasing number of products containing Vitamin A, increasing concentrations and/or greater penetration (e.g. as a result of packaging in liposomes) and the fact that the UL is already exceeded by some parts of the population.

In February 2012, a safety dossier was submitted by Cosmetics Europe[3] to support the continuous use of Vitamin A in cosmetic product. It relates to the use of retinol, retinyl palmitate and retinyl acetate as cosmetic ingredients at maximum use concentrations of 0.05% RE in body lotions, 0.3% RE in hand and face creams as well as in other leave-on or rinse-off products.

In July 2013, The Commission received the Norwegian risk assessment of the use of Vitamin A (retinol and retinyl esters) in cosmetic products.

The Commission asked the opinion of the European Medicine Agency (EMA) to exclude the possibility that, at maximum use concentrations of 0.05% RE in body lotions, 0.3% RE in hand and face creams as well as in other leave-on or rinse-off products, Vitamin A could be considered a medicinal product instead of a cosmetic product. EMA replied that "*locally applied products containing Vitamin A at the maximum concentrations of 0.05% (retinol equivalents) in body lotions, 0.3% (retinol equivalents) in hand and face creams as well as in other leave-on or rinse-off products, are not considered to be medicinal products by virtue of their function*."

[1] http://ec.europa.eu/food/fs/sc/scf/out145_en.pdf
[2] http://www.efsa.europa.eu/en/ndatopics/docs/ndatolerableuil.pdf
[3] Cosmetics Europe- European Cosmetics Toiletry and Perfumery Association

2. TERMS OF REFERENCE

(1) On the basis of data provided does the Scientific Committee on Consumer Safety (SCCS) consider Vitamin A (retinol, retinyl palmitate, retinyl acetate, retinyl linoleate and retinal) safe when used as cosmetic ingredient:

a) in body lotions up to the maximum concentration of 0.05 % of retinol equivalent?

b) in hand/face cream, leave-on (other than body lotions) and rinse-off products up to the concentration of 0.3 % of retinol equivalent ?

If no, what concentration limits in the above mentioned categories of cosmetic products does the SCCS consider Vitamin A to be safe?

(2) The SCCS is asked, when making the assessment, to take into account the specific age and sex groups who might be particularly susceptible to the effects of Vitamin A, such as the use of lip products for fertile age and postmenopausal women.

(3) Does the SCCS have any further scientific concerns with regard to the use of Vitamin A (retinol, retinyl palmitate, retinyl acetate, retinyl linoleate and retinal) in cosmetic products?

3. OPINION

3.1 Chemical and Physical Specifications

As no data were specifically reported for retinal and retinol linoleate in the dossier submitted by the applicant, these two vitamin A derivatives are not included in this opinion.

3.1.1 Chemical identity

The term "Vitamin A" refers to a group of substances, the retinoids, including retinol (Vitamin A1) and substances with similar structures with the biological characteristics of retinol.

3.1.1.1 Primary name and/or INCI name

Retinol
Retinyl acetate
Retinyl palmitate

3.1.1.2 Chemical names

Retinol:
Chemical name: All-trans-3, 7-dimethyl-9-(2, 6, 6-trimethyl-1-cyclohexen-1-yl)-2, 4, 6, 8-nonatetraen-1-ol

Retinyl acetate:
Chemical name: All-trans-3, 7-dimethyl-9-(2, 6, 6-trimethyl-1-cyclohexen-1-yl)-2, 4, 6, 8-nonatetraene-1-yl acetate

Retinyl palmitate:
Chemical name: All-trans-3, 7-dimethyl-9-(2, 6, 6-trimethyl-1-cyclohexen-1-yl)-2, 4, 6, 8-nonatetraene-1-yl palmitate

(References: 18, 20, 37, 48, 49, 50, 51, 52, 76)

3.1.1.3 Trade names and abbreviations

Retinol: Acon, Afaxin, Agiolan, Alphsterol, Epiteliol, Testavol

Retinyl acetate: Vitamin Acetate

Retinyl palmitate: Arovit, Testavol S; Vitamin A Palmitate

(Reference: 71)

3.1.1.4 Synonyms

Retinol:

Synonyms: All-trans-retinol

All-trans-retinyl-alcohol
Vitamin A alcohol
15-apo-(3-caroten-15-ol)
Axerol
Axerophthol
Axerophtholum
Biosterol
(E)-3, 7-dimethyl-9-(2, 6, 6-trimethylcyclohex-enyl)-2, 4, 6, 8-nonatetraenol
(E)-3, 7-dimethyl-9-(2, 6, 6-trimethylcyclohexen- l -yl)-2, 4, 6, 8-nonatetraenol
(E)-9-hydroxy-3, 7-dimethyl-9-(2, 6, 6-trimethylcyclo-hexenyl)-1, 3, 5, 7-Nonatetraene
OleoVitamin A
Retinol
Trans-retinol
2-trans, 4-trans
Vitamin A
Vitamin A alcohol
Vitaminum A

Retinyl acetate:

Synonyms: All-trans-Vitamin A acetate
Vitamin A acetate
Acetic acid (E) -3, 7-dimethyl-9-(2, 6, 6-trimethyl-cyclohexenyl)-2, 4, 6, 8-nonatetraenylester
Acetic acid retinyl ester
All-trans-retinyl acetate
All-trans-retinol acetate
O-acetoxy-all-trans-retinol
O-acetyl-all-trans-retinol
Retinylacetate
2-trans, 4-trans, 6-trans, 8-trans-retinolacetate
2-trans, 4-trans, 6-trans, 8-trans-retinylacetate
Rac

Retinyl palmitate:

Synonyms: All-trans-Retinyl palmitate
Retinyl palmitate
Palmitic acid (E)-3, 7-dimethyl-9-(2, 6, 6-trimethyl-cyclohexenyl)-2, 4, 6, 8-nonatetraenyl ester
Palmitic acid retinyl ester
O-palmitoyl-all-trans-retinol
O-palmitoyl-retinol
Retinylpalmitate
2-trans, 4-trans, 6-trans, 8-trans-retinylpalmitate
2-trans, 4-trans, 6-trans, 8-trans-retinol palmitate
Retinol hexadecanoate
Trans-retinol palmitate
Trans-retinyl palmitate
RP

(References: 18, 20, 37, 48, 49, 50, 51, 52, 76)

3.1.1.5 CAS / EC number

Vitamin A:
CAS: 11103-57-4
EC: 234-328-2

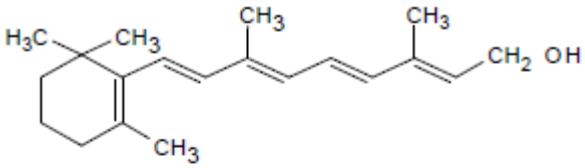
Retinol:
CAS: 68-26-8
EC: 200-683-7

Retinyl acetate:
CAS: 127-47-9
EC: 204-844-2

Retinyl palmitate:
CAS: 79-81-2
EC: 201-228-5

3.1.1.6 Structural formula

Retinol:



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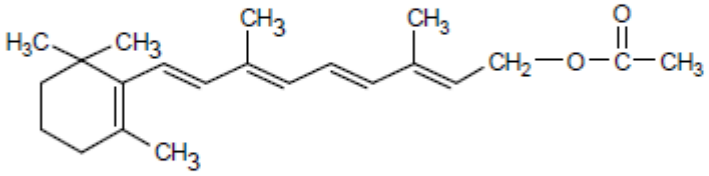
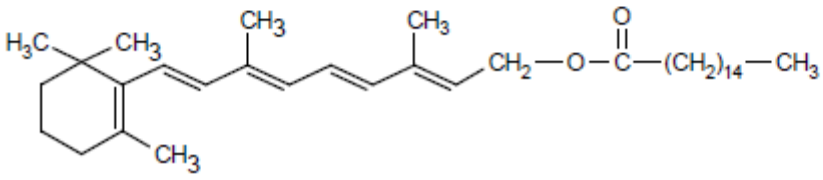


Retinyl Palmitate:



(References: 18, 20, 37, 48, 49, 50, 51, 52, 76)

3.1.1.7 Empirical formula

Retinol: $C_{20}H_{30}O$

Retinyl acetate: $C_{22}H_{32}O_2$

Retinyl palmitate: $C_{36}H_{60}O_2$

(References: 18, 20, 37, 48, 49, 50, 51, 52, 76)

3.1.2 Physical form

Retinol:
Pale yellow oil, which may crystallise at low temperatures

Retinyl acetate:
Pale yellow prisms or yellow supercooled melt, viscous liquid

Retinyl palmitate:
Yellow, crystalline or amorphous powder

3.1.3 Molecular weight

Retinol: 286.5 g/mol

Retinyl acetate: 328.5 g/mol

Retinyl palmitate: 524.9 g/mol

(References: 18, 20, 37, 48, 49, 50, 51, 52, 76)

3.1.4 Purity, composition and substance codes

Representative examples of marketed products are provided in the following paragraphs.

Retinol (e.g., Retinol 10 S, 15 D, 50 C):
Purity: ≥95% (all-trans retinol)
≤5% (cis-isomers)
International units (IU): 330000–370000 IU/g (Retinol 10 S)
500000–530000 IU/g (Retinol 15 D)
1425000–1650000 IU/g (Retinol 50 C)
Stabiliser: Butylhydroxytoluol (BHT) or Butylhydroxyanisol (BHA)

Retinyl acetate (e.g., Vitamin A acetate 1.5 mio IU/g):
Appearance: viscous-yellow oil, may crystallize on storage
Peroxide value: <10 meq/kg
Acid value: <2.0 mg/ KOH/g
International units (IU): 1500000 IU/g
Stabiliser: Tocopherol or BHT

Retinyl palmitate (e.g., retinyl palmitate 1.0 or 1.7 mio IU/g):
Appearance: viscous-yellow oil, may crystallize on storage
Peroxide value: <10 meq/kg
Acid value: <2.0 mg/ KOH/g
International units (IU): 1000000 or 1700000 IU/g
Stabiliser: Tocopherol, BHT, or BHA

All toxicological study data presented in section 6 (Toxicological evaluation) were checked for information on purity and composition. The respective information has been provided where available.

(References: 18, 20, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 57, 58)

Table 1 presents purity data for different batches of retinyl acetate, retinyl palmitate and retinol:

Compound	Batch No	Purity	Study (Ref.)
Retinyl acetate	6/17	1.53 mio IU/g USP XIX (526 mg RAc/g)	Acute toxicity (ref 7) Mucous membrane irritation (ref 6)
	805043	1.5 mio IU/g (515 mg RAc/g)	Skin irritation (Ref 129) Skin sensitisation (ref 132) Mucous membrane irritation / Eye irritation Batch (ref 130)
	/	1.0 mio IU/g (344 mg Rac/g)	Skin irritation (Ref 8)
Retinyl palmitate	710758	1.7 mio IU/g (935 mg RP/g)	Mucous membrane irritation / Eye irritation (ref 131) Skin sensitisation (ref 126)
	/	1.0 mio IU/g (550 mg Rac/g)	Skin irritation (Ref 10)
Retinol	50-2498	47.7 g/100 g	Mucous membrane irritation / Eye irritation (ref: 17)
	23-0136	47.1 g/100 g	Buehler test (a) (Ref.14), Open epicutaneous test (Ref. 16)
	Not provided	Not provided	Buehler test (c)
	82-0085-00	Not provided	Buehler test (b) (Ref.14)

3.1.5 Impurities / accompanying contaminants

Impurity data have not been provided.

SCCS comment
Data on purity determination was not submitted. According to the specification sheets, UV spectrophotometry was used to calculate the content of retinol, retinyl palmitate and retinyl acetate.

No information on the determination of impurities was provided for retinol, retinyl acetate and retinol palmitate as the applicant refers to the exception proposal (EU Monograph No 2034).

SCCS reminds the Applicant that retinoic acid is banned in cosmetic products in the EU (Annex 2, entry 375) and therefore it should not be present in the cosmetic products with the exception of occurring as an unavoidable trace impurity for which a justified limit is provided.

3.1.6 Solubility

Retinol: Soluble in most organic solvents (acetone, chloroform, dimethyl sulfoxide, diethyl ether, ethanol, hexane, isopropanol, methanol) and in fats and mineral oils (2.5 mol/L).
Practically insoluble in water (water solubility: 0.06 nmol/L) and glycerol.

Retinyl acetate: Soluble in most organic solvents (acetone, chloroform, ethanol, isopropanol) and in fats or oils (750 g/100 mL).
Insoluble in water and glycerol.

Retinyl palmitate: Soluble in most organic solvents (ethanol, iso-propanol, chloroform, acetone) and in fats and oils.
Insoluble in water and glycerol.

(References: 18, 37, 76)

3.1.7 Partition coefficient (Log P_{ow})

Retinol:
Log P_{ow}: 5.68 (measured)
7.6 (calculated KOWIN V 1.67, 2006)

Retinyl acetate:

Log P_{ow}: 9.4 (BASF SE, unpublished results, 1989)

Retinyl palmitate:
Log P_{ow}: 15.51 (calculated: KOWIN, V 1.67, 2006)

SCCS comment on calculated value
In case of a calculated value, the method should be specified. The P_{ow} strongly depends on the pH, especially for ionisable molecules, zwitterions etc. Therefore, a single calculated value of Log P_{ow}, usually without any reference to respective pH, cannot be correlated to physiological conditions and to the pH conditions of the dermal absorption studies.

3.1.8 Additional physical and chemical specifications

Retinol:

Melting point: 62–64° C
Boiling point: 137–138°C at $1x10^{-6}$ mm Hg / 421.2°C at 760 mm Hg
Flash point:
Vapour pressure: /
Density: 0.954 g/cm^3
Viscosity: /
pKa: /
Refractive index: /
pH: /
UV_Vis spectrum (….. nm): λmax = 325, $E^{1\%}_{1cm}$= 1820, ε=52140

Spectroscopy: Double-bond isomers of retinol do not show differences in their infrared spectra.

The ultraviolet (UV) absorption spectrum in ethanol: lambda max at 325 nm; E1%/1cm = 1835

The infrared (IR) and proton magnetic resonance (1H-NMR) spectra of retinol can be found in the relevant Aldrich Library volumes.

Fluorescence: Yellow-green at 510 nm after excitation at 327 nm and at 470 nm after excitation at 325 nm.

Retinyl acetate:

Melting point: 57-58° C
Boiling point: 440.5 °C at 760 mm Hg
Flash point:
Vapour pressure: /
Density: 0.968 g/cm^3
Viscosity: /
pKa: /
Refractive index: /
pH: /
UV_Vis spectrum (….. nm): lambda max 326 nm (in ethanol); $A^{1\%}$ 1cm 1550.
Fluorescence spectrum: Emission lambda max at 470nm for excitation at 325 nm.

Retinyl palmitate:

Melting point: 27-29° C
Boiling point: 607.5 °C at 760 mm Hg
Flash point:
Vapour pressure: /
Density: 0.92 g/cm^3
Viscosity: /
pKa: /
Refractive index: /
pH: /
UV_Vis spectrum (….. nm): λmax = 326, $A^{1\%}_{1cm}$= 960, ε=50.390

Spectroscopy: UV-visible: lambda max 325-328 nm (in ethanol); E1%/1cm 940-975.

Fluorescence: Emission lambda max at 470nm for excitation at 325 nm.

(References: 19, 37, 71, 76)

Analytics:

Recently, a novel sensitive analytical method was reported, including reversed-phase high performance liquid chromatography (HPLC) with ultraviolet (UV) detection for the quantification of retinol, retinyl palmitate, and retinoic acid in cosmetic preparations with respective recoveries from spiked cosmetic products of 95% or higher. The author emphasised that the method may be used to quantitatively determine several retinoids and their isomers in cosmetic products.

(Reference: 75)

SCCS comment
It is unclear if the HPLC method has been applied to the analysis of the batches used in toxicity testing. This method does not include retinyl acetate, retinyl linoleate or retinal.

3.1.9 Homogeneity and Stability

Retinol:

Photo-induced bond isomerisation from trans to cis gives the other known retinol isomers: 11-cis (neo b), 13-cis (neo a), 9, 13-di-cis (iso b), 9-cis (iso a), and 11, 13-di-cis (neo c).

Particularly in oil solution, retinol can be protected from isomerisation by preventing exposure to UV and sunlight.

Bond isomerisation can be caused by heat and iodine.

High levels of illumination can induce polymerisation.

Retinol is sensitive to oxygen, heat, light and heavy metals. It is optimally stored below 4°C under an inert gas (argon or nitrogen) or in the presence of an antioxidant (e.g. butylated hydroxytoluene, tocopherol).

Heat and trace metals accelerate retinol decomposition by oxygen and light.

Retinol is unstable to acids, which cause bond rearrangement to retro-Vitamin A, isomerisation, and dehydration to anhydro-Vitamin A, sometimes followed by solvent addition.

Retinol is also unstable to alkali in the presence of oxygen (unlike the palmitate ester).

Retinol and its acetate can bind strongly to polyvinyl chloride in plastics.

Stability of different retinol grades differ in respect to used antioxidant systems and ranged between 6–24 months, if stored below 15–20 °C.

Retinol in cosmetic formulations is stable for ≥6 months if manufactured under inert atmosphere and stored e.g., in aluminium tubes at ≤ 20 °C.

Retinyl acetate:
Slightly more stable than retinol but in general the same statements apply.

Retinyl palmitate:
Slightly more stable than retinol but in general the same statements apply.

(References: 18, 20, 37, 48, 49, 50, 51, 52, 76)

SCCS comment
No data are available on the stability.
Retinol in cosmetic products will need to be stabilised through final formulations.

General Comments to physicochemical characterisation
Calculations use international units (IUs) or retinol equivalents (REs). A conversion of the individual derivatives into IUs can be found in Table 2.

Retinol has a high estimated $LogK_{ow}$ indicating that the substance has a high potential to bioaccumulate and thus potentially fulfils the B/vB criteria of REACH Annex XIII. However, no experimental bioaccumulation data are available.

In terms of persistence, based on screening criteria, Retinol can be considered as not P/vP. According to experimental data on ready biodegradability test (OECD 301B), the substance exhibited 81% degradation in 28 days.

Table 2: Conversion of the various Vitamin A derivatives into international units (IUs)

Vitamin A derivative	1IU corresponds to
Retinol	0.300 µg
Retinyl acetate	0.345 µg
Retinyl propionate	0.359 µg
Retinyl palmitate	0.550 µg

Conversion factors for Vitamin A weight, international units or retinol equivalents (RE) (http://robert-forbes.com/resources/vitaminconverter)

	Vitamin A activity in International Units (IU)	Vitamin A activity in Retinol Equivalents (µg RE)
Retinol (1 mg)	3330	1000
Retinyl acetate (1 mg)	2900	870
Retinyl palmitat (1 mg)	1830	550

In this Opinion, SCCS has chosen to express Vitamin A amounts in RE.

3.2 Function and uses

Vitamin A is used as a cosmetic ingredient at maximum use concentrations of 0.05% (retinol equivalents) in body lotions, 0.3% (retinol equivalents) in hand and face creams as well as in other leave-on or rinse-off products. These products are usually presented as anti-wrinkle agents. In particular, retinol and its esters, mainly retinyl palmitates and acetates, are used in products such as face and eye creams, body lotions, sun lotions, lip products and baby creams, above all because of their anti-ageing effect. They induce biosynthesis of collagen in the skin and, at the same time, impede the UV-induced synthesis of collagen-reducing enzymes. These cosmetics promise to smooth wrinkles and fine lines in skin aged by both time and sun exposure. In toothpastes, Vitamin A serves to protect the gum epithelium against marginal parodontitis (Buddecke et al. 1981).

Retinoic acid is banned in cosmetic products in the EU, whatever the concentration (Annex 2, entry 375).

The maximum concentrations (in RE), of retinol, retinyl palmitate and retinyl acetate typically used in cosmetic preparations in the EU can be summarised as follows:

Product category	RE (%) [#]
Face and hand creams and other leave-on products	0.3
Body lotions	0.05
Rinse-off products	0.3
[#] RE = retinol equivalents, i.e. retinyl palmitate and retinyl acetate at corresponding retinol concentrations	

(References: 18, 22, 72, 92, 100, 150, 157)

External topical retinoids may reverse dermatological disorders most likely by interfering with local retinoid functions. Hence, topical retinoids have been used for clinical treatment of psoriasis, hyperkeratosis, acne, early aging and photodamage. The retinoids seem to play a role in the aging process of the skin, since many age-dependent changes may be reversed by topical application. In the dermis, topical retinoids may increase synthesis and inhibit degradation of collagen, changes that are associated with improvement of coarse wrinkling. In the epidermis, topical retinoids may cause hyperplasia, compaction of the *stratum corneum*, thickening of the granular layer and increased intercellular mucin deposition. These changes are associated with increased smoothness of the skin (VKM, 2012).

Apparently the anti-aging effect of topical retinoids is mainly linked to the receptor-mediated gene activation induced by the ligand retinoic acid modulating epidermal cell proliferation and differentiation, extracellular matrix production, angiogenesis, oxidative stress and melanocyte function (Sorg et al., 2006; Sorg and Saurat, 2014). According to the intracrine-proligand concept, the other topical retinoids have to be metabolised to retinoic acid by the skin to exert their genomic effects. This concept implies that topical application of any precursor retinoids may result in biological effects. However, the potency of the retinoid is strongly dependent on its metabolic distance to retinoic acid. Hence, the retinoid-like activity after topical application increases in the following order: retinyl esters << retinol < retinal < retinoic acid.

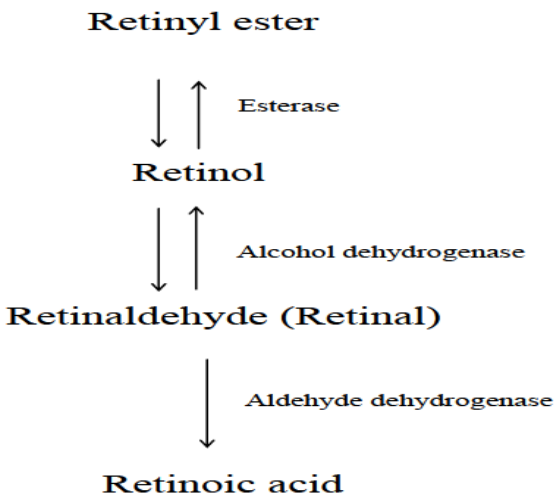


Figure 1: Metabolism of retinyl esters to retinoic acid

The retinoids retinyl esters, retinol and retinal are used in a large variety of cosmetic products such as anti-wrinkle creams, body lotions, hand creams and sunscreens. As active ingredients they are expected to provide the cosmetic product with a series of specific abilities to improve and counteract skin aging and photoaging, prevent oxidative stress, and control cutaneous bacterial flora.
Although retinyl esters did not show significant anti-aging activity, the retinyl ester - retinyl palmitate - is widely used in cosmetics because of its stability. With respect to sunscreen products, retinyl palmitate is extensively used because of its antioxidant, stabilising properties. However, in Europe and the USA, retinyl palmitate is not allowed to be added as UV-filter as such (VKM, 2012).

Several studies have demonstrated that topical retinol may induce the same cellular and molecular changes as retinoic acid although a 20 times higher dose is needed and the local irritation characteristics are less prominent. It has been shown that retinol could be effective in the treatment of skin aging and photoaging, but the effect was dependent on the vehicle used, as retinol is unstable and easily gets degraded to biologically inactive forms when exposed to light and air (VKM, 2012).

In several studies it has been demonstrated that retinal may be a useful topical agent in the treatment of aged and photoaged skin. Various cosmetic products containing retinal, primarily anti-aging preparations, are available on the European market.

Based on information provided by cosmetic industry, Vitamin A and esters are not used for children in the EU.

3.3 Toxicological Evaluation

In general, undesirable effects can arise from both a lack of Vitamin A and Vitamin A hypervitaminosis.

This Opinion focuses on those studies most relevant for the safety evaluation of Vitamin A (retinol, retinyl palmitate and retinyl acetate) used in topical cosmetic products.

The applicant noted that retinol, retinyl acetate and retinyl palmitate were also evaluated by the registrants according to REACH requirements for chemicals and respective dossiers with all relevant datasets are available publicly.

3.3.1 Acute toxicity

3.3.1.1 Acute oral toxicity

Retinyl acetate

Guideline:	Not mentioned but comparable to OECD 401 (1981)
Species/strain:	Rat/Sprague-Dawley
Group size:	5–10 males and 5–10 females per dose level
Test substance:	Retinyl acetate
Batch:	6/17
Purity:	1.53 mio IU/g USP XIX (526 mg RAc/g), not stabilised
Vehicle:	peanut oil
Dose levels:	3160, 3830, 4640, 5620, 6810 mg RAc/kg bw (10 animals/sex), 8250 mg RAc/kg bw (5 animals/sex)
Route:	oral gavage
Administration:	single administration
GLP:	No
Study period:	

Retinyl acetate was administered orally by gavage to a total of 55 male and 55 female Sprague-Dawley rats at dose levels between 3160–8250 mg/kg bw. Prior to application the animals were fasted for 15–20 h. Body weights were determined on regular intervals and the animals were observed for treatment-related effects including mortality for a 21-day observation period. Gross pathology was performed in rats that died and in survivors sacrificed at termination. Mortality ratios were calculated after 1, 24 and 48 hours and after 7 and 14 days according to the probit analysis of Finney (1971).

Results
Deaths occurred dose-dependently within 7 days after dosing and signs of intoxication were observed in the animals that died or recovered until termination. The surviving animals gained weight. Necropsy of the animals which died during the course of the study showed acute dilation of the heart including congestion, the liver was discoloured with broaded lobes and the gastro-intestinal tract revealed signs of irritation. Those rats which were sacrificed at the end of the 21-day observation period did not reveal any gross pathologic alterations in the tissues and organs examined.

Based on the mortality incidences, the acute oral LD50 for retinyl acetate in rats was calculated as 5290 mg RAc/kg bw in males, 4790 mg RAc/kg bw in females and 4980 mg RAc/kg bw combined for males and females (corresponding to 15.4 mio IU/kg bw in males, 13.9 mio IU/kg bw in females and 14.5 mio IU/kg bw combined, respectively).

(Reference: 7)

These LD50 values are in line with peer-reviewed literature data given for retinol and retinyl esters:

Retinol

LD50 oral, mouse:	2570 mg RE/kg bw	(8.5 mio IU/kg bw)
LD50 i.p., mouse:	1510 mg RE/kg bw	(5 mio IU/kg bw)

Retinyl acetate

LD50 oral, mouse:	4100 mg/kg bw	(11.9 mio IU/kg bw)
LD50 i.m., young monkey:	168 mg/kg bw (estimation)	(0.49 mio IU/kg bw)

Retinyl palmitate

LD50 oral, mouse:	6060 mg/kg bw	(11 mio IU/kg bw)
LD50 oral, rat:	7910 mg/kg bw	(14.4 mio IU/kg bw)

In general, in case of fatalities, the death was preceded by signs of acute toxicity in form of convulsions and paralysis, and those animals that survived had signs of malaise, decreased motor activity, stupor, muscular weakness, and occasionally changes in the gait.
The animals that survived recovered with no apparent residual toxic effects.

(References: 37, 68, 73, 76, 94, 121)

Overall conclusion on acute toxicity studies:

In acute oral toxicity studies, Vitamin A (retinol, retinyl palmitate, retinyl acetate) was found to be of low toxicity in laboratory animal species (LD50 values in rodents >2000 mg RE/kg bw).

The ranking of acute oral toxicity declined in the order of retinol > retinyl acetate > retinyl palmitate.

3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

Guideline:	OECD 404 (1987)
Species/strain:	Rabbit/New Zealand White
Group size:	3 animals (2 males, 1 female)
Test substance:	Retinyl acetate
Batch:	805043
Purity:	1.5 mio IU/g (515 mg RAc/g) stabilized with tocopherol
Vehicle:	pure
Dose level:	undiluted test substance
Dose volume:	0.5 mL
Route:	dermal application (semi-occlusive)

Exposure: 4 hours
Observation: up to 14 days after patch removal
GLP: Yes
Study period:

The irritation potential of retinyl acetate on the skin was investigated by application of 0.5 mL of the test substance to the intact and abraded skin of each of 3 New Zealand White rabbits on an area of 2.5 cm². After 4-hour exposure under semi-occlusive conditions, the patch was removed and the application site was washed with lukewarm water. The skin sites were scored at 1 h after removal of the patch, and at 24, 48 and 72 hours as well as after 7 and 14 days after the beginning of the exposure. The animals were observed daily for mortality and the body weights were determined on day of application and at termination.

Results:
There were no mortality or signs of systemic toxicity and the animals gained body weight. All animals revealed slight signs of irritation in form of very slight to well-defined erythema, but no edema was observed at any time. The scores for erythema for intact/abraded skin in the three animals were 0 or 1 after 1 hour and 24 hours, 1 or 2 after 48 hours, 2 after 72 hours, 1 after 7 days and 0 after 14 days.

Conclusion:
Retinyl acetate was shown to be slightly irritating to the skin of rabbits after 4 h semi-occlusive dermal exposure, when tested undiluted.

(Reference: 129)

SCCS comment
Under the conditions of this study, the test substance (Retinyl acetate) is a slight to moderate irritant to the rabbit skin. However, the identity of the test substance / purity in terms of retinyl acetate is not clear to the SCCS based on the information in the original study report.

Guideline: OECD 404 (1981)
Species/strain: Rabbit/Vienna White
Group size: 6 animals (3 males, 3 females)
Test substance: Retinyl acetate (substance number 82 / 202)
Batch: No data
Purity: 1.0 mio IU/g (344 mg RAc/g)
Vehicle: pure
Dose level: pre-formulated product in peanut oil DAB 8 (2.8 mio IU/g)
Dose volume: 0.5 mL
Route: dermal application (occlusive)
Exposure: 4 hours
Observation: up to 8 days after patch removal
GLP: No
Study period: July 1982

0.5 mL of the test substance was applied for a 4-hour exposure period onto the intact skin of each of 6 White Vienna rabbits to an area of 2.5 cm². After the occlusive exposure, the patch was removed and the application site was washed with water/Lutrol (1:1). The animals were observed for 8 days; the skin sites were scored at 30-60 minutes after

removal of the patch, and at 24, 48 and 72 hours as well as 8 days after the beginning of the exposure.

Results:
All animals revealed erythema after 4 hours (score 1), but no edema. Erythema and edema were observed after 24 hours (erythema: score 2 in 6/6; edema: score 1 in 6/6), 48 hours (erythema: score 2 in 4/6 and score 3 in 2/6; edema: score 1 in 6/6), and after 72 hours (erythema: score 2 in 3/6 and score 3 in 3/6; edema: score 1 in 6/6). Erythema (score 1) persisted up to day 8 in 1/6 rabbits and scaling was noted in all rabbits.

Conclusion:
Retinyl acetate was irritating to the skin of rabbits.

(Reference: 8)

SCCS comment
The scores of skin reactions were recorded at 30-60 minutes after removal of the occlusive dressing, but in the tabular summary the reactions were presented as scored at 4 hours after removal of the occlusive dressing.
Under the conditions of this study, the test substance (Retinyl acetate, substance number 82/202) is a moderate irritant to the rabbit skin. However, the identity of the test substance / purity in terms of retinyl acetate is not clear to the SCCS, based on the information in the original study report.

Guideline:	OECD 404 (1981)
Species/strain:	Rabbit/Vienna White
Group size:	6 animals (3 males, 3 females)
Test substance:	Retinyl acetate (substance number 82 / 203)
Batch:	No data
Purity:	1.0 mio IU/g (344 mg RAc/g)
Vehicle:	pure
Dose level:	pre-formulated product in peanut oil DAB 8 (2.8 mio IU/g)
Dose volume:	0.5 mL
Route:	dermal application (occlusive)
Exposure:	4 hours
Observation:	up to 8 days after patch removal
GLP:	No
Study period:	July 1982

0.5 mL of the test substance was applied for a 4-hour exposure period onto the intact skin of each of 6 White Vienna rabbits to an area of 2.5 cm². After the occlusive exposure, the patch was removed and the application site was washed with water/Lutrol (1:1). The animals were observed for 8 days; the skin sites were scored at 30-60 minutes after removal of the patch, and at 24, 48 and 72 hours as well as 8 days after the beginning of the exposure.

Results:
All animals revealed erythema after 4 hours (score 1), but no oedema. Erythema and oedema were observed after 24 hours (erythema: score 2 in 6/6; oedema: score 1 in 6/6), 48 hours (erythema: score 2 in 4/6 and score 3 in 2/6; oedema: score 1 in 6/6), and after 72 hours (erythema: score 2 in 3/6 and score 3 in 3/6; oedema: score 1 in 6/6). Erythema (score 1) persisted up to day 8 in 5/6 rabbits and scaling was noted in all rabbits.

Conclusion:
Retinyl acetate was irritating to the skin of rabbits.

(Reference: 9)

SCCS comment
The scores of skin reactions were recorded at 30-60 minutes after removal of the occlusive dressing, but in the tabular summary the reactions were presented as scored at 4 hours after removal of the occlusive dressing.
Under the conditions of this study, the test substance (Retinyl acetate, substance number 82/203) is a moderate irritant to the rabbit skin. However, the identity of the test substance / purity in terms of retinyl acetate is not clear to the SCCS based on the information in the original study report.

Guideline:	OECD 404 (1981)
Species/strain:	Rabbit/Vienna White
Group size:	6 animals (3 males, 3 females)
Test substance:	Retinyl palmitate (substance number 82 / 204)
Batch:	No data
Purity:	1.0 mio IU/g (550 mg RAc/g)
Vehicle:	pure
Dose level:	pre-formulated product in peanut oil DAB 8 (1.7 mio IU/g)
Dose volume:	0.5 mL
Route:	dermal application (occlusive)
Exposure:	4 hours
Observation:	up to 8 days after patch removal
GLP:	No
Study period:	July 1982

Six White Vienna rabbits received 0.5 mL of retinyl palmitate (1.0 mio IU/g) for 4 hours on intact skin. After 4-hour occlusive exposure to an area of 2.5 cm^2, the patch was removed and the application site was washed with water/Lutrol (1:1). The animals were observed for 8 days; the skin sites were scored at 30-60 minutes after removal of the patch, and at 24, 48 and 72 hours as well as after 8 days after the beginning of the exposure.

Results:
All animals revealed erythema after 4 hours (score 1), but no edema. Erythema and edema were observed after 24 hours (erythema: score 2 in 4/6 and score 1 in 2/6; edema: score 1 in 4/6), 48 hours (erythema: score 2 in 4/6 and score 1 in 2/6; edema: score 1 in 4/6), and after 72 hours (erythema: score 2 in 6/6; edema: score 1 in 4/6). Erythema (score 1) persisted up to day 8 in 1/6 rabbits and scaling was noted in all rabbits.

Conclusion:
Retinyl palmitate was irritating to the skin of rabbits.

(Reference: 11)

SCCS comment
The scores of skin reactions were performed at 30-60 minutes after removal of the occlusive dressing, but in the tabular summary the reactions were presented as scored at 4 hours after removal of the occlusive dressing.
Under the conditions of this study, the test substance (Retinyl palmitate, substance number 82/204) is a moderate irritant to the rabbit skin. However, the identity of the test substance / purity in terms of retinyl palmitate is not clear to the SCCS, based on the information in the original study report.

Peanut oil (DAB 8, substance number 82 / 205) as used formulation aid in the three studies was also separately tested for comparison under the same test conditions. Erythema (score 1) was noted in all 6 animals at 4 and 24 hours, in 5/6 animals at 48 hours, and in 3/6 animals at 72 hours. Oedema was not observed. At 8 days, scaling was noted in 3/6 animals.

(Reference: 10)

SCCS comment
The scores of skin reactions were recorded at 30-60 minutes after removal of the occlusive dressing, but in the tabular summary the reactions were presented as scored at 4 hours after removal of the occlusive dressing.
Under the conditions of this study, the test substance (peanut oil) is a slight irritant to the rabbit skin.

Overall SCCS conclusion on skin irritation
Under the conditions of the *in vivo* studies, retinyl acetate and retinyl palmitate are moderately irritating to the rabbit skin. The SCCS noted that the vehicle, peanut oil, is also slightly irritating to the rabbit skin under the conditions of the *in vivo* study. However, the identity of the test substance / purity in terms of retinyl acetate or retinyl palmitate is not clear to the SCCS based on the information in the original studies precluding a final conclusion on the skin irritation potential of retinyl acetate and retinyl palmitate.

3.3.2.2 Mucous membrane irritation / Eye irritation

Retinol

Guideline:	OECD 405 (1987)
Species/strain:	Rabbit/ White New Zealand
Group size:	3 animals (2 males, 1 female)
Test substance:	Retinol 50 C (main ingredients: retinol 47.1 g/100 g and Polysorbate 20)
Batch:	50-2498
Purity:	47.7 g/100 g
Vehicle:	undiluted test substance
Dose level:	undiluted test substance
Dose volume:	0.1 mL
Route:	instillation in the conjunctival sac of the right eye
Exposure:	24h
Observation:	7 days
GLP:	Yes
Study period:	November to December 2002

The potential of retinol to cause damage to the conjunctivae, iris or cornea was investigated by a single ocular application of 0.1 mL of the undiluted test material to one eye of three White New Zealand rabbits. About 24 hours after the application, the eye was rinsed with tap water. The readings were performed at 1, 24, 48 and 72 hours and on day 7; the untreated eye served as control. Examinations were made of the cornea, iris and the conjunctiva of each animal for signs of irritation that were graded according to Draize et al. (1959).

Results:
Slight to severe conjunctival redness (score 3 at 1 and 24 hours, score 2 or 3 at 48 hours, score 1or 2 at 72 hours), slight to marked conjunctival chemosis (score 2 at 1 hour, score 2

or 3 at 24 hours, score 1 at 48 and 72 hours) and moderate to severe discharge (score 2 at 1 hour and score 0, 2 or 3 at 24 hours) were observed during the course of the study. In addition, discharge of blood and injected sclera vessels in a circumscribed and circular area were noted. These ocular reactions were reversible in all animals within 7 days after application. No reactions were noted for cornea or iris.

Conclusion:
Retinol tested as a formulation (47.1 g/100 g) was shown to be slightly irritating to the eyes of 3 White New Zealand rabbits.

(Reference: 17)

SCCS comment
Under the conditions of this study, the test substance is a moderate irritant to the rabbit eye. However, the identity of the test substance / purity in terms of retinol is not clear to the SCCS based on the information in the original study report.

Retinyl acetate

Guideline:	/
Species/strain:	Rabbit/ Vienna White
Group size:	6 animals (2 males, 4 females)
Test substance:	Retinyl acetate (substance number 78 / 454)
Batch:	No data
Purity:	1.53 mio IU/g USP XIX (526 mg RAc/g) not stabilised, in peanut oil
Vehicle:	undiluted test substance
Dose level:	undiluted test substance
Dose volume:	0.1 mL
Route:	instillation in the conjunctival sac of the right eye
Exposure:	/
Observation:	3 days
GLP:	No
Study period:	/

0.1 mL of the undiluted test material was placed into the conjunctival sac of the right eye of each of 6 Vienna White rabbits (2 males, 4 females). The test substance was not washed out. The untreated eye served as control. The readings were performed at 24, 48 and 72 hours. Examinations were made of the cornea, iris and the conjunctiva of each animal for signs of irritation and the grades were scored according to Draize et al. (1959).

Results:
After 24 hours, slight conjunctival redness was observed in 3/6 rabbits (score 1) and moderate in 3/6 (score 2), and associated with slight secretion in 3/6 rabbits (score 1). At the 48-hour reading only, slight conjunctival redness was observed in 6/6 rabbits (score 1) and 3/6 showed slight secretion (score 1). After 72 hours, slight erythema of the conjunctiva was noted in 4/6 rabbits (score 1), but none of the animals showed secretion. Cornea or iris findings as well as conjunctival chemosis did not occur at any time in any animal.

Conclusion:
The test substance (Retinyl acetate, substance number 78/ 454) was shown to be slightly irritating to the eyes of 6 Vienna White rabbits. However, the identity of the test substance / purity in terms of retinyl acetate is not clear to the SCCS based on the information in the original study report.

(Reference: 6)

Guideline:	OECD 405 (1987)
Species/strain:	Rabbit/ White New Zealand
Group size:	3 animals (2 males, 1 female)
Test substance:	Retinyl acetate
Batch:	805043
Purity:	1.5 mio IU/g (515 mg RAc/g) stabilized with tocopherol)
Vehicle:	Neantine® (Diethylphthalate)
Dose level:	a) undiluted test substance b) 30% dilution
Dose volume:	0.1 mL
Route:	instillation in the conjunctival sac of the left (undiluted) and right eye (30% dilution)
Exposure:	/
Observation:	72 hours
GLP:	Yes
Study period:	October 1988

The potential of retinyl acetate (1.5 mio IU/g) to cause damage to the conjunctivae, iris or cornea was investigated by a single ocular application of 0.1 mL of the undiluted test material to the left eyes and of a 30% dilution in diethylphthalate to the right eyes of three White New Zealand rabbits. The eyes were not rinsed. The readings were performed at 1, 24, 48 and 72 hours. Examinations were made of the cornea, iris and the conjunctivae of each animal for signs of irritation and the grades were scored according to the OECD 405 guideline criteria (1987).

Results:
All animals treated either with the undiluted test material or the 30% dilution showed redness of the conjunctivae only at the 1-hour reading (grade 2); cornea or iris findings as well as conjunctival chemosis were not observed. At the 24, 48 and 72 readings, no findings were noted on the cornea, iris and conjunctivae irrespectively if tested undiluted or diluted. Slightly yellow to yellow staining of the eyelashes of the treated eyes due to pigmentation or colouring by the test material was observed in one rabbit treated with the undiluted test item after 1 hour, and in 2 rabbits from 1-48 hours after treatment. The same staining effect was observed in all rabbits receiving the 30% dilution, but at the one-hour reading only.

Conclusion:
Retinyl acetate tested undiluted and as a 30% dilution was shown to be well tolerated after instillation to the eyes of 3 White New Zealand rabbits.

(Reference: 130)

SCCS comment
Under the conditions of this study, the test substance (Retinyl acetate) is a slight irritant to the rabbit eye. However, the identity of the test substance / purity in terms of retinyl acetate is not clear to the SCCS based on the information in the original study report.

Retinyl palmitate

Guideline:	OECD 405 (1987)
Species/strain:	Rabbit/ White New Zealand
Group size:	3 animals (1 male, 2 females)
Test substance:	Retinyl palmitate

Batch:	710758
Purity:	1.7 mio IU/g (935 mg RP/g) stabilized with tocopherol)
Vehicle:	Neantine® (Diethylphthalate)
Dose level:	a) undiluted test substance b) 30% dilution
Dose volume:	0.1 mL
Route:	instillation in the conjunctival sac of the left (undiluted) and right eye (30% dilution)
Exposure:	/
Observation:	72 hours
GLP:	Yes
Study period:	October 1988

The potential of retinyl palmitate (1.7 mio IU/g) to cause damage to the conjunctivae, iris or cornea was investigated by a single ocular application of 0.1 mL of the undiluted test material to the left eyes and of a 30% dilution in diethylphthalate to the right eyes of three White New Zealand rabbits. The eyes were not rinsed. The readings were performed at 1, 24, 48 and 72 hours. Examinations were made of the cornea, iris and the conjunctivae of each animal for signs of irritation and the grades were scored according to the OECD 405 guideline criteria (1987).

Results:
All animals treated with the undiluted test material showed redness of the conjunctivae at the 1-hour reading (grade 2), which was noted as being less pronounced in one animal at the 24 hour (grade 1). The 30% dilution led to redness of the conjunctivae at the 1-hour reading (grade 2 in one animal and grade 1 in 2 other animals. Besides these initial and/or transient findings, no further findings were noted on the cornea, iris and conjunctivae with either the undiluted test material or 30% dilution. Slightly yellow to yellow staining of the eyelashes of the treated eyes due to pigmentation or colouring by the test article was observed in one rabbit treated with the undiluted test material from 1-24 hours and in 2 rabbits from 1-72 hours after treatment. The same staining effect was observed in all rabbits receiving the 30% dilution, but at the one-hour reading only.

Conclusion:
Retinyl palmitate tested undiluted and as a 30% dilution was shown to be well tolerated after instillation to the eyes of 3 White New Zealand rabbits.

(Reference: 131)

SCCS comment
Under the conditions of this study, the test substance (Retinyl palmitate) is a slight irritant to the rabbit eye. However, the identity of the test substance / purity in terms of retinyl palmitate is not clear to the SCCS based on the information in the original study report.

In 1987 CIR Expert Panel reported that the use of Retinyl palmitate at concentrations of 0.1% to 1% Retinyl palmitate in cosmetics were at most slightly irritating and did not result in skin sensitisation (CIR, 1987). The subsequent 2006 CIR Expert Panel's review of existing animal and human data concurred and a limit up to 5% for Retinyl palmitate was introduced (CIR, 2006).

Overall SCCS conclusion on eye irritation
Under the conditions of the *in vivo* study, retinol (tested as a formulation) is a moderate irritant to the rabbit eye, and retinyl acetate and retinyl palmitate (tested either undiluted or as a 30% dilution) are slightly irritating to the rabbit eye. However, the identity of the test substance / purity is not clear to the SCCS based on the information in the original

studies precluding a final conclusion on the eye irritation potential of retinol, retinyl acetate and retinyl palmitate.

3.3.3 Skin sensitisation

Guinea Pig Maximization test (GPMT)

Retinyl acetate

Guideline:	OECD 406 (1981)
Species/strain:	Albino Guinea pig/Himalayan
Group size:	20 female animals in the test group, 10 females per control group
Test substance:	Vitamin A acetate (Ro 01-5275)
Batch:	805043
Purity:	1.5 mIU/g (515 mg RAc/g)
Vehicles:	Olive oil
Route:	Intradermal induction, percutaneous booster and challenges
Dose levels:	Intradermal induction: 5% in olive oil Epicutaneous induction: 30% Epicutaneous challenge: 10% in olive oil
GLP:	Yes
Positive control:	DNCB in a separate group of animals
Study period:	Oct – Dec 1988

The skin sensitising property of retinyl acetate (1.5 mio IU/g) was investigated in a GPMT according to the protocol of Magnusson & Kligman, using female albino Himalayan guinea pigs. After a dose-range-finder experiment to find the minimal irritant concentration for the induction phase and a suitable non-irritant concentration for the challenge phase with intradermal as well as with topical application, 5% in olive oil was selected for intradermal induction, 30% for epidermal induction and 10% for epidermal challenge in the main study.

The dose was 0.1 mL and three pairs of intradermal injections were given simultaneously into an area of 4 x 6 cm (on 6 x 8 cm clipped dorsal skin on scapular region) according to the following scheme:

1st pair: Freund's complete adjuvant (FCA) emulsified 50:50 in distilled water.
2nd pair: 5% test article in olive oil
3rd pair: Test item concentration of 5% emulsified in Freund's complete adjuvant in the ratio 50:50 (w/w), and in the vehicle
The control group was treated accordingly without the test item.

For the epicutaneous induction, one week after the injections, the same area was clipped free and a 2 x 4 cm patch was saturated with the diluted test item (30% in olive oil) and placed on the skin and covered with aluminium foil. This was secured firmly by an elastic plaster wrapped around the trunk and additionally secured with impervious adhesive tape. The dressing was left in place for 48 hours.

The guinea pigs were challenged topically two weeks after the intradermal induction. A second challenge was performed two weeks after the first challenge according to the same procedure

Results
After 1st challenge 5/10 (50%) control animals showed slight erythema at 24 and 48 hours, but at 2nd challenge no skin reaction was observed in the control animals.

In the test group after 1st challenge, slight erythema was observed in 3/30 (15%) at 24 hours and 4/20 (20%) animals at 48 hours, but no skin reaction was noted at each time-point after the 2nd epidermal challenge.

Conclusion (according to the dossier):

Retinyl acetate (1.5 mio IU/g) showed slight skin sensitising reaction in female Guinea pigs after the 1st but not after the 2nd epidermal challenge. The transient and slight skin reaction observed in 50% of the control and 15-20% in the test group only after the 1st challenge were interpreted as signs of enhanced skin reactivity, a syndrome known to occur in animals treated with FCA and lipophilic test substances.

According to EEC (European Economic Community) classification criteria described in guidelines 83/467, September 16, 1983, this test article is not a sensitiser.

(Reference: 132).

Retinyl palmitate

Guideline:	OECD 406 (1981)
Species/strain:	Guinea pig/Himalayan
Group size:	20 female animals in the test group, 10 females as control group
Test substance:	Retinyl palmitate
Batch:	710758
Purity:	1.7 mIU/g (935 mg RP/g)
Vehicles:	Olive oil
Route:	Intradermal induction, percutaneous booster and challenges
Dose levels:	Intradermal induction: 5% in olive oil Epicutaneous induction: 100% Epicutaneous challenge: 30% in olive oil
GLP:	Yes
Positive control:	DNCB (separate group of 10 animals)
Study period:	Oct – Dec 1988

The skin sensitising property of retinyl palmitate (1.7 mio IU/g) was investigated in the GPMT according to OECD test guideline 406 using female albino Himalayan guinea pigs. After a dose-range-finder experiment to find the minimal irritant concentration for the induction phase and a suitable non-irritant concentration for the challenge phase with intradermal as well as with topical application, 5% in olive oil was selected for intradermal induction, 100% for epidermal induction and 30% for epidermal challenge in the main study.

The dose was 0.1 mL and three pairs of intradermal injections were given simultaneously into an area of 4 x 6 cm (on 6 x 8 cm scapular region clipped free of hair) according to the following scheme:

1st pair: Freund's complete adjuvant (FCA) emulsified 50:50 in distilled water.
2nd pair: 5% test article in olive oil
3rd pair: Test item concentration of 5% emulsified in Freund's complete adjuvant in the ratio 50:50 (w/w), and in the vehicle

The control group was treated accordingly without the test item.

For the epicutaneous induction, one week after the injections, the same area was clipped free and a 2 x 4 cm patch was saturated with the undiluted test item (100%) placed on the

skin covered with aluminium foil and secured firmly. The dressing was left in place for 48 hours. The guinea pigs were challenged topically two and four weeks after the intradermal induction.

Results

After the 1st and 2nd challenge, no skin reaction was observed in the control animals. Four out of twenty (20%) and 2/20 (10%) animals showed slight erythema at 24 and 48 hours, respectively, after the 1st challenge, but no skin reaction was noted at each time-point after the 2nd epidermal challenge.

Conclusion (according to dossier):
According to the results described above, the allergenic potency of the test article RO 01-5852 – Vitamin A Palmitate1.7 mIU/g is considered to be of a mild grade in this test when followed the rating of allergenicity described by Magnusson B and Kligman AM (1969) According to EEC (European Economic Community) classification criteria described in guidelines 83/467, September 16, 1983, this test article is not a sensitiser.

(Reference: 126).

Buehler test

Retinol

Guideline:	OECD 406 (1992),
Species/strain:	Guinea pig/ Dunkin Hartley (Hsd Poc: DH)
Group size:	20 female animals in the test group, 10 females per control group
Test substance:	a) Retinol 50 C (main ingredients: retinol 47.1 g/100 g and Polysorbate 20) b) Retinol 10 CM c) Polysorbate 20
Batch:	a) 23-0136 b) 82-0085-00 c) no information
Purity:	/
Vehicles:	Lutrol E 400 (polyethyleneglycol) Miglyol 812 N
Route:	Topical induction and challenge
Dose levels:	Epicutaneous induction: 25% in Lutrol E 400 Epicutaneous challenge: 1st challenge: 10% in Lutrol E 400 2nd challenge: 5% and 10% in Lutrol E 400 3rd challenge: 2.5% (Retinol 10 CM) in Miglyol 812 N 2.5% (Polysorbate 20) in Lutrol E 400
GLP:	Yes
Positive control:	alpha Hexylcinnamaldehyde (in a separate group)
Study period:	June – August 1999

The sensitising property of a retinol preparation was evaluated in a non-adjuvant skin sensitisation test according to the Buehler protocol (Buehler 1965) for delayed contact hypersensitivity using female albino Hartley guinea pigs.

After range-finding studies for the determination of the slightly irritating concentration to be used for induction and maximum non-irritating concentration for challenge, the animals were topically exposed (under occlusive dressing) with a 25% test substance preparation in Lutrol E 400.

Three challenges were carried out 14, 21 and 56 days after the 3rd induction by application of 0.5 mL test material formulation on gauze patches (2x2 cm) under an occlusive dressing for 6 hours according to the following scheme:

1st challenge: test and control group 1 treated with 10% test formulation in Lutrol E 400

2nd challenge: test and control groups 1 and 2 with the test substance formulations (5% and 10% in Lutrol E 400and Lutrol E 400 as vehicle)

3rd challenge: test and control groups 1, 2 and 3 were treated with 2.5% formulations of the main ingredients of the test substance (Retinol 10 CM in Miglyol 812 N and Polysorbate 20 in Lutrol E 400)

Approximately 24 and 48 h after removal of the occlusive dressing, the skin reaction was evaluated and scored using a four-point scale according to the grading of Magnusson and Kligman (1969).

Results:

The first induction caused no skin reaction, but the 2nd and 3rd induction led to discrete or patchy to intense erythema, swelling and scaling in the test group animals.

After the first challenge with the 10% formulation in Lutrol E 400, skin reactions similar to those seen in the induction phase were recorded in all test group animals and in addition, 3 control group animals revealed comparable erythema. The corresponding vehicle control group animals revealed no skin reaction. The observed skin reactions in the control group led to a second challenge with 5% and 10% test substance formulations in Lutrol E 400. After the 2nd challenge dose-related increased skin reactions were noted in test group and control group 1 receiving 5% and 10% formulation in Lutrol E 400 and also in animals of the control group 2, while the vehicle control animals showed no adverse reactions. Due to inconsistency of the observed skin findings in all groups, a 3rd challenge was performed.

Also within this challenge procedure, the differences in the composition of the respective test substance formulations were investigated. The skin readings after the 3rd challenge revealed different forms of erythema in the test group animals receiving 2.5% test substance preparation of retinol in Miglyol 812 N and, to a lesser incidence in some control groups, 1 and 2 animals were treated identical for challenge. A newly introduced control group 3 did not show any reactions after treatment with 2.5% retinol preparation.

The application of a 2.5% preparation of Polysorbate 20 or the respective vehicle controls evoked no skin reaction.

Conclusion (as stated in the dossier)

Based on the evaluation criteria cited, the results of this study show that the test substance has a sensitising effect on the skin of the guinea pig in the BUEHLER Test under the test conditions chosen. The ingredients of the test substance considered to be responsible for the sensitising effect is retinol.

(Reference: 14).

Open epicutaneous test

Retinol

Guideline: /
Species/strain: Guinea pig/ Dunkin Hartley (Hsd Poc: DH)

Group size: 8 female animals in the test group and control group
Test substance: Retinol 50 C (main ingredients: retinol 47.1 g/100 g and Polysorbate 20)
Batch: a) 23-0136
Purity: /
Vehicles: Lutrol E 400 (polyethyleneglycol)
Route: Topical induction and challenge
Dose levels: Epicutaneous induction: 0.2%, 0.4%, 0.75%, 2%, 5% in Lutrol E 400 (13 inductions) Epicutaneous challenge: 0.2%, 0.4%, 0.75%, 2%, 5% in Lutrol E 400 (1st and 2nd challenge)
GLP: Yes
Positive control: alpha-Hexylcinnamaldehyde (in separate group of animals)
Study period: Oct 1999 – March 2000

For the identification of a threshold concentration for the sensitising property of a retinol preparation an open epicutaneous test (OET) was performed using female albino Hartley guinea pigs, following the method of Klecak (1977).

For the determination of the minimum irritant and maximum non-irritant concentrations, range finding studies were performed. The animals were topically exposed to 0.2%, 0.4%, 0.75%, 2%, 5% test substance solution in Lutrol E 400 by applying 0.1 mL of each concentration to an area of 8 cm² on the clipped skin of the right flank. In total 13 inductions were performed with 1 application per workday during days 0 – 14 followed by 6 days rest; thereafter each dose was again applied once on days 21 and 22, followed by a rest of 3 days.
Two challenges were carried out, the 1st at 6 days and the 2nd 20 days after the induction phase, by application of 0.025 mL test material formulation on an area of 2 cm² with 0.2%, 0.4%, 0.75%, 2%, 5% test substance solution in Lutrol E 400. Approximately 24, 48 and 72h after application the skin reaction was evaluated and scored.

Results

During the induction period, the animals of all test groups exhibited skin inflammation indicated by erythema, swelling, severe scaling and eczematous skin reactions and residuals of the test substance formulation was noted in all groups at several times during this period. Due to these skin reactions, which were mainly observed in the animals receiving concentrations of 2% or 5%, the application areas were moved from cranial to caudal and were interrupted for all animals after the 11th induction for 6 days. After partial recovery of skin reactions, the animals received two further induction applications, but due to renewing severe skin reactions, further induction applications were omitted.

After the 1st and 2nd challenge, inflammatory skin reactions consisting of erythema, swelling, partially with open eczematous appearance were noted in a varying degree and incidence dependent on the induction pre-treatment and the respective applied challenge application.

Conclusion (according to the dossier)

Retinol was shown to exhibit a sensitising effect on the skin of guinea pigs in the Open Epicutaneous test under the conditions investigated. The induction threshold concentration was 0.4% preparation (corresponding to 0.2% retinol), while no skin sensitisation was induced with a test substance solution of 0.2% preparation (corresponding to 0.1% retinol).

(Reference: 16).

SCCS comment
The evaluation of both skin sensitisation tests (Buehler and OET) with retinol is hampered by a decreasing irritation threshold after repeated topical administration and in the Buehler

test by the usage of different vehicles. Skin reactions in the Buehler test suggest that skin sensitisation by retinol concentrations of 2.5% cannot be ruled out. The results of the open application test (according to the method of Klecak) are inconclusive and most likely due to irritation. The pre-study irritation dose finding was based on a single exposure of unknown duration. Retinyl acetate and palmitate exhibited no potential to induce dermal sensitisation in Guinea pigs in the Maximization test according to Magnusson and Kligman. Studies evaluating the sensitisation potential by LLNA could not be identified.

In view of the sparse case reports on sensitisation in humans despite widespread exposure in cosmetics (see 3.3.11 Human data), the SCCS considers the risk of sensitisation to retinol, retinyl acetate and retinyl palmitate as negligible.

3.3.3 Dermal / percutaneous absorption

In vitro

Guideline:	/
Species/strain:	Human
Test system:	Freshly biopsied human skin from abdominal surgery (split thickness skin layer: 200–320 µm)
Membrane integrity:	^{3}H water test
Group size:	2 donors – 3 replicates
Method:	Flow-through diffusion cells
Test substance:	Retinol
Batch:	No data
Purity:	>99%
Test item:	Hydroalcoholic gel or oil in water emulsion containing 0.3% [^{3}H]-retinol (specific activity: 47 Ci/mmol, radiochemical/chemical) corresponding to about 0.7 µCi/cell
Dose applied:	2 mg/cm²
Exposed area:	0.64 cm²
Exposure time:	24h
Sampling:	6-h fractions for a total of 24 or 72 h
Receptor fluid:	Hanks' balanced salt solution (HBSS) plus 4% bovine serum albumin plus 0.001 % butylhydroxytoluene (BHT)
Tape stipping:	Yes (10 times)
Method of Analysis:	Liquid scintillation counting
GLP:	Not in compliance
Study period:	/

Retinol was tested *in vitro* for dermal permeation by means of either a gel or oil-in-water emulsion with a content of 0.3% [^{3}H]-retinol. Freshly biopsied human skin from abdominal surgery of 2 volunteers was used. The subcutaneous fat was removed and the skin was cleaned with a 10% soap solution and thoroughly rinsed with distilled water. A split-thickness layer (200-320 µm) was prepared with a dermatome. Discs of dermatomed skin were obtained and mounted on the flow-through diffusion cell (exposed surface area, 0.64 cm²). The receptor fluid was HBSS + 4% bovine serum albumin + 0.001% BHT (pH 7.4). The flow rate of the receptor fluid was approximately 1.5 mL/h. The skin surface temperature was maintained at 32 °C by circulating 35 °C water through the diffusion cell holding block. The retinol dose (2 mg/cm² application amount) was applied to each diffusion cell for 24 h, and then washed off to remove any unabsorbed material. A fraction collector was used to collect receptor fluid as 6-h fractions for a total of 24 or 72 h. At the end of the study (24 or 72 h), the skin was removed from the diffusion cell and the amount of retinol remaining in the skin was determined. Skin discs were tape stripped ten times to remove the *stratum corneum*. Each tape strip was placed into a scintillation vial. Skin discs containing the viable epidermis/dermis were then frozen for later analysis. Skin discs were

thawed and homogenized on ice and dissolved. The viable skin content was determined from the amount of radioactivity in the skin homogenate by liquid scintillation counting.

Results

The vast majority of the applied [3H]-retinol, applied either as hydro-alcoholic gel or as oil-in water emulsion was washed off after 24 h of exposure. The labelled [3H]-retinol penetrated into and through the human skin. The recovery rates were in an acceptable range of 87 – 96%. The amount absorbed into the receptor fluid at 24-h was 0.3% of the applied dose for the gel vehicle and 1.3% for the emulsion. The major portion of the penetrated amount was related to the *stratum corneum* (SC) and amounted between 3.5–5.9% for the gel and emulsion at 24-h. At that time, the total amount in the SC and viable skin was 5.7% of the applied dose for the gel and 8.9% for the emulsion. There was an increase in retinol absorbed in the receptor fluid with the gel and emulsion vehicles, when data from 72 h was compared to those from 24 h. The details are provided in the following table:

Table 3 *In vitro* percutaneous absorption of [^{3}H]-retinol in human (means of 2 volunteers with each 3 replicates) skin using gel and oil-in-water emulsion vehicles after exposure of 24-h and determination after 24-h and 72-h

Recovery site	24h – gel (%)	72h – gel (%)	24h – emulsion (%)	72h – emulsion (%)
Receptor fluid	0.3 +/- 0.1	0.5 +/- 0.01	1.3 +/- 0.1	2.2 +/- 0.2
Stratum Corneum (SC)	3.5 +/- 0.4	2.8 +/- 0.8	5.9 +/- 1.4	4.8 +/- 0.8
Viable skin	2.1 +/- 1.2	1.0 +/- 0.1	3.0 +/- 0.6	2.9 +/- 0.6
Total amount in SC and viable skin	5.7 +/- 0.8	3.8 +/- 0.7	8.9 +/- 2.0	7.8 +/- 1.4
Bioavailable portion (viable skin, receptor fluid)	2.4	1.5	4.3	5.1
Recovery	87.3 +/- 6.3	95.9 +/- 0.2	94.8 +/- 2.6	96.3 +/- 5.3

Conclusion

The exposure of freshly biopsied human skin *in vitro* to cosmetic preparations in the form of either a hydro-alcoholic gel or oil-in water emulsions containing 0.3% [^{3}H]-retinol for 24 h showed that the majority of the test substance was washed off and the major portion was attached to the SC. Only small amounts remained in the viable skin (epidermis/dermis) or receptor fluid. The portion penetrated into the skin of human (SC, viable skin) amounted to 5.7% or 8.9% after 24 hours with values of 3.8% and 7.8% after 72 hours for the gel or emulsion, respectively.

The bioavailable portion amounted to 2.4% or 4.3% after 24 hours with values of 1.5% and 5.1% after 72 hours of the applied dose level for the gel or emulsion, respectively under the study conditions.

Reference: 168

SCCS comments
The number of donors used in this study is not in accordance with the SCCS Notes of Guidance. As this study concerns a scientific paper, not all raw data is available to SCCS. Only mean values are presented for the systemically-available amount. Standard errors are reported instead of standard deviations.

Guideline:	/
Species/strain:	Fuzzy rat (female)
Test system:	Freshly biopsied dorsal skin (split thickness skin layer: 200–320 µm)
Membrane integrity:	/
Group size:	3 (3-4 replicates)
Method:	Flow-through diffusion cells
Test substance:	Retinol
Batch:	No data
Purity:	>99%
Test item:	Hydroalcoholic gel or oil in water emulsion containing 0.3% [^{3}H]-retinol (specific activity: 47 Ci/mmol, radiochemical/chemical) corresponding to about 0.7 µCi/cell
Dose applied:	2 mg/cm²
Exposed area:	0.64 cm²
Exposure time:	24h
Sampling:	6-h fractions for a total of 24 or 72 h
Receptor fluid:	Hanks' balanced salt solution (HBSS) plus 4% bovine serum albumin plus 0.001 % butylhydroxytoluene (BHT)
Tape stipping:	Yes (10 times)
Method of Analysis:	Liquid scintillation counting
GLP:	Not in compliance
Study period:	/

Skin penetration of retinol was tested *in vitro* by using skin of female Fuzzy rats by means of either a gel or oil-in-water emulsion with a content of 0.3% [^{3}H]-retinol. Fuzzy rats were euthanised with carbon dioxide and the fine hair was cut with electric clippers. The dorsal skin and the subcutaneous fat were removed. The skin was cleaned with a 10% soap solution and thoroughly rinsed with distilled water. A split-thickness layer (200-320 µM) was prepared with a dermatome. Discs of dermatomed skin were obtained and mounted on the flow-through diffusion cell (exposed surface area, 0.64 cm²). The receptor fluid was HBSS + 4% bovine serum albumin + 0.001% BHT (pH 7.4). The flow rate of the receptor fluid was approximately 1.5 mL/h. The skin surface temperature was maintained at 32 °C by circulating 35 °C water through the diffusion cell holding block. The retinol dose (2 mg/cm² application amount) was applied to each diffusion cell for 24 h, and then washed off to remove any unabsorbed material. A fraction collector was used to collect receptor fluid as 6-h fractions for a total of 24 or 72 h. At the end of the study (24 or 72 h), the skin was removed from the diffusion cell and the amount of retinol remaining in the skin was determined. Skin discs were tape-stripped ten times to remove the *stratum corneum*. Each tape strip was placed into a scintillation vial. Skin discs containing the viable epidermis/dermis were then frozen for later analysis. Skin discs were thawed and homogenised on ice and dissolved. The viable skin content was determined from the amount of radioactivity in the skin homogenate by liquid scintillation counting.

Results

The vast majority of the applied [^{3}H]-retinol, applied either as hydro-alcoholic gel or as oil-in-water emulsion was washed off after 24 h of exposure. The labelled [^{3}H]-retinol

penetrated into and through the rat skin. The recovery rates were in an acceptable range of 91.0–99.9%. The amount absorbed into the receptor fluid at 24 h was 6.0% of the applied dose for the gel vehicle and 6.5% for the emulsion. The portion of the penetrated amount into the *stratum corneum* (SC) was 4.2% and 3.8% for the gel and emulsion at 24 h. At that time, the total amount in the SC and viable skin was 23.1% of the applied dose for the gel and 26.0% for the emulsion. There was an increase in retinol absorbed in the receptor fluid with the gel and emulsion vehicles, when data from 72 h was compared to those from 24 h. The details are provided in the following table:

Table 4 *In vitro* percutaneous absorption of [3H]-retinol in female Fuzzy rat skin samples (means of 3 rats with each 3-4 replicates) skin using gel and oil-in water emulsion vehicles after exposure of 24-h and determination after 24-h and 72-h

Recovery site	24h – gel (%)	72h – gel (%)	24h – emulsion (%)	72h – emulsion (%)
Receptor fluid	6.0 +/- 2.3	12.9 +/- 3.9	6.5 +/- 1.8	15.7 +/- 3.3
Stratum Corneum (SC)	4.2 +/- 1.0	3.3 +/- 0.5	3.8 +/- 0.5	2.8 +/- 0.4
Viable skin	18.9 +/- 1.9	14.6 +/- 2.4	22.2 +/- 1.4	16.2 +/- 3.1
Total amount in SC and viable skin	23 +/- 1.5	17.9 +/- 2.7	26.0 +/- 1.6	19.1 +/- 3.0
Bioavailable portion (viable skin, receptor fluid)	24.9	27.5	28.7	31.9
Recovery	93.6 +/- 5.2	98.5 +/- 2.0	91.0 +/- 3.2	99.5 +/- 5.2

Conclusion

The exposure of freshly biopsied female Fuzzy rat skin *in vitro* to cosmetic preparations in form of either a hydro-alcoholic gel or oil-in water emulsions containing 0.3% [^{3}H]-retinol for 24 h showed that the majority of the test substance was washed off. The portion penetrated into the skin of rats (SC, viable skin) amounted to 23.1% or 26.0% after 24 hours with values of 17.9% and 19.1% after 72 hours for the gel or emulsion, respectively. The bioavailable portion amounted to 24.9% or 28.7% after 24 hours with values of 27.5% and 31.9% after 72 hours of the applied dose level for the gel or emulsion, respectively, under the study conditions.

Reference: 168

SCCS comments

The number of donors used in this study is not in accordance with the SCCS Notes of Guidance. Membrane integrity of the rat skin has not been checked prior to the experiment. As this study concerns a scientific paper, not all raw data is available to SCCS. Only mean values are presented for the systemically available amount. Standard errors are reported instead of standard deviations.

A clear species' difference exists with a much higher *in vitro* dermal absorption of [^{3}H]-retinol observed in rat *versus* human skin. No vehicle effect is observed. These results are in contrast to dermal absorption in human skin, where a lower proportion of [^{3}H]-retinol was found in the viable skin compared to rats.

Retinyl Palmitate

Guideline:	/
Species/strain:	Human
Test system:	Freshly biopsied human skin from abdominal surgery (skin thickness: 1.37±0.007 mm)
Membrane integrity:	/
Group size:	3 female donors (4 replicates)
Method:	Flow-through diffusion cells
Test substance:	Retinyl palmitate (RP)
Batch:	No data
Purity:	>99%
Test item:	Cream containing 0.15% [^{14}C]-RP corresponding to about 3 µg [^{14}C]-RP/cm² with/without nanocapsules
Dose applied:	2 mg/cm²
Exposure time:	16h
Sampling:	After exposure time
Receptor fluid:	Isotonic aqueous solution (PBS and 0.25% Tween 80)
Tape stripping:	Yes (10-15 times)
Method of Analysis:	Liquid scintillation counting
GLP:	In compliance
Study period:	March - May 1997

Retinyl palmitate was tested *in vitro* for its dermal bioavailability on human skin by means of a cosmetic emulsion with a content of 0.15% [14C]-retinyl palmitate ([^{14}C]-RP) with/without nanocapsules. Freshly biopsied human skin from abdominal surgery of 3 female volunteers was placed on static diffusion cells. The receptor fluid consisting of an isotonic aqueous solution and was held at 37 °C. The cosmetic formulation was applied on the skin with the aid of a syringe to achieve an amount of about 2 mg/cm², corresponding to approximately 3 µg [^{14}C]-RP/cm². The application areas were not covered and were allowed exposure to natural day light. After exposure time of 16 hours, the skin surface was washed and the remaining test preparations were removed from the skin surface by wiping with Kleenex® paper. The receptor fluid was taken from the cell, the SC was removed by 10-15 applications of tape-stripping, the epidermis was separated from the dermis and cut into small pieces. Analysis of radioactivity was performed by liquid scintillation counting.

Results

The labelled [^{14}C]-retinyl palmitate penetrated into and through the human skin. Both emulsions (with/without nanocapsules) showed comparable results. The recovery rates were in an acceptable range of 85-90%±5%. The predominant portion of the penetrated amount was related to the SC and amounted to 8-9%, while less was absorbed in the epidermis and dermis and only minor amounts could be detected in the receptor fluid. The details are provided in the following table:

Table 5 [^{14}C]-retinyl palmitate quantification after exposure of 16 hours *in vitro* using freshly biopsied human skin from 3 female volunteers

Preparation	0.15% [^{14}C]-retinyl palmitate with nanocapsules	0.15% [^{14}C]-retinyl palmitate without nanocapsules
No. of measurements	11	12
Amount of cream applied	2.2±0.1 mg/cm²	1.9±0.1 mg/cm²
Amount in respect to active ingredient	3.52±0.18 µg/cm²	2.93±0.15 µg/cm²
Dislodgeable dose	2.63±0.14 µg/cm² 76.37±5.52%	2.30±0.15 µg/cm² 79.24±4.35%
Stratum Corneum (SC)	0.29±0.05 µg/cm² 8.14±1.11%	0.27±0.02 µg/cm² 9.28±0.66%
Epidermis	0.015±0.003 µg/cm² 0.44±0.11%	0.013±0.003 µg/cm² 0.47±0.13%
Dermis	0.020±0.004 µg/cm² 0.61±0.17%	0.020±0.007 µg/cm² 0.74±0.27%
Receptor Fluid	0.0012±0.0002 µg/cm² 0.0378±0.0075%	0.0010±0.0001 µg/cm² 0.0340±0.0045%
Recovery	85.59±5.29%	89.77±4.69%
Total amount in SC and viable skin	0.33±0.06 µg/cm² 9.23±1.12%	0.30±0.02 µg/cm² 10.53±0.81%
Bioavailable portion (viable skin, receptor fluid)	0.0362 µg/cm² 1.09%	0.0340 µg/cm² 1.24%

"surface recovery" as indicated in the above Table refers to the sum of tissue swabs + chamber wash-off (term changed in the table into "dislodgeable dose"); individual values for chamber wash-off and tissue swabs recalculated from the study results as:

Preparation	0.15% [14C]-retinyl palmitate with nanocapsules	0.15% [14C]-retinyl palmitate without nanocapsules
Chamber wash-off	0.16±0.04 µg/cm² 4.63±0.94%	0.16±0.03 µg/cm² 4.74±1.02%

Tissue swabs	2.47±0.15 µg/cm² 71.76±5.75%	2.14±0.15 µg/cm² 74.48±5.20%

Conclusion

The exposure of freshly biopsied human skin *in vitro* to cosmetic emulsions containing 0.15% [^{14}C]-retinyl palmitate for 16 h showed that the majority of the test substance was attached to the SC. Only small amounts could be detected in the remaining skin tissues and only negligible amounts in the receptor fluid. The portion penetrated into the skin (SC, epidermis, dermis) was in the range of 0.30-0.33 µg/cm² or 9.1–10.5% with respect to the applied dose. The bioavailable portion ranged between 0.033–0.036 µg/cm² corresponding to 1.1–1.24% of the applied dose level under the study conditions.

Reference: 98

SCCS comments

The number of donors and study duration were not in accordance to the SCCS Notes of Guidance. Membrane integrity of the human skin was not checked prior to the experiment. The composition of the nanocapsules was not provided to the SCCS. Only mean values were presented for the systemically available amount. Standard errors were reported instead of standard deviations. No effect of the nanocapsules was observed.

Guideline:	/
Species/strain:	Human (female)
Test system:	Freshly dermatomed human skin (400 µm)
Membrane integrity:	/
Group size:	4 donors (8 replicates)
Method:	Flow-through diffusion cells
Test substance:	Retinyl palmitate
Batch:	96129
Purity:	≥98%
Test item:	oil in water emulsion 0.3 % [^{14} C]-retinyl palmitate ([^{14} C]-RP) with or without 1% lipase SP644
Dose applied:	2 mg/cm²
Exposure time:	16h
Sampling:	After exposure time
Receptor fluid:	HHBSS with 4% bovine albumin
Tape stripping:	/
Method of Analysis:	Liquid scintillation counting
GLP:	In compliance
Study period:	July- September 1996

A further study investigated the influence of topically applied enzyme with retinyl palmitate related to the release of retinol into the skin by mixing ^{14}C- retinyl palmitate [^{14}C- RP] with the enzyme Lipase SP644 just prior to application and comparison to its further behaviour to that of the same cosmetic cream without enzyme. For this purpose formulations as O/W emulsions containing 0.3% ^{14}C- RP (batch: 96120; specific activity: 2169 MBq/mmol, radiochemical purity: ≥98%) with or without 1% Lipase SP644 were investigated *in vitro* under GLP conditions. The emulsions were applied for 16 h under yellow light conditions to freshly dermatomed human skin from abdominal surgery of 8 female volunteers (4 for absorption, 3 for skin metabolism, 1 for skin viability). The skin viability was maintained in the diffusion cells using oxygenated HBSS and the skin viability was confirmed by the MTT test. Dermal absorption and metabolism were studied *in vitro* using flow-through diffusion cells. The distribution in different skin layers was analysed by liquid scintillation counting.

The epidermis and dermis were homogenized and skin metabolites were analysed with HPLC. The amount of ^{14}C-RP and/or ^{14}C-metabolites remaining on the surface, dermal absorption, distribution, penetration, the influence of freezing the skin samples and the skin metabolism were each analysed in separate experiments.

The results of the cutaneous bioavailability/penetration part of the study are supplied in the following table.

Table 6 [14C]-retinyl palmitate quantification based on radioactivity measurement after exposure of 16 hours *in vitro* using freshly dermatomed human skin (mean value from 8 replicates)

Preparation	0.3% [^{14}C]-retinyl palmitate plus 1% lipase SP644	0.3% [^{14}C]-retinyl palmitate without 1% lipase SP644
Amount of cream applied	3.35±0.61 mg/cm²	3.62±0.66 mg/cm²
Amount in respect to active ingredient	5.34±1.13 µg/cm²	5.42±1.02 µg/cm²
Surface recovery	5.18±0.93 µg/cm² 99.05±5.68%	5.23±1.08 µg/cm² 97.92±7.44%
Epidermis + Stratum Corneum (SC)	0.19±0.09 µg/cm² 3.67±1.52%	0.15±0.05 µg/cm² 2.89±1.10%
Dermis	0.03±0.03 µg/cm² 0.48±0.64%	0.05±0.04 µg/cm² 0.69±0.82%
Receptor Fluid	0.03±0.02 µg/cm² 0.53±0.36%	0.02±0.01 µg/cm² 0.29±0.19%
Recovery	103.74±5.49%	101.79±8.13 %
Amount in epidermis plus SC, dermis and receptor fluid	0.28±0.14 µg/cm² 4.68±1.67%	0.20±0.08 µg/cm² 3.87±1.74%
Amount bioavailable portion (dermis, receptor fluid)	0.08±0.10 µg/cm² 1.02±0.91%	0.05±0.04 µg/cm² 0.98±0.86%

The Lipase enzyme quickly metabolises (within the first 5 minutes of application) retinyl palmitate into retinol at the skin surface. Within the epidermis, retinol was found in the samples treated with the Lipase enzyme, whereas only retinol palmitate was identified in non-lipase treated samples. Within the dermis, the ^{14}C radioactivity was below the limit of quantification. No endogenous metabolism of radiolabelled retinoids was observed.

No relevant influence of the dermal bioavailability was noted due to the supplementation of a Lipase enzyme. The amount penetrated into the dermis and the receptor fluid was generally low and represented only about 1% of the applied dose level. However, a scientifically reliable conclusion on the *in vitro* dermal bioavailability of retinyl palmitate

using human skin cannot be drawn from this study, since there was no separation of the SC from epidermis and it is generally accepted that the SC portion is not bioavailable. However, with respect to the results of other studies, it is considered very likely that the portion provided as the epidermal amount is primarily related to the SC.

References: 97, 102

SCCS comments

The number of samples and study duration are not in accordance with the SCCS Notes of Guidance.
Membrane integrity of the human skin was not checked.
No effect of lipase was observed.
The *stratum corneum* was not separated from the viable epidermis by tape stripping. The bioavailable amount reported is considered to be an underestimation as the portion present in the viable epidermis was not taken into account.

Guideline:	/ (modified method of Zesh/Schäfer)
Species/strain:	Naked rat and domestic pig
Test system:	Fresh naked rat and porcine skin
Membrane integrity:	/
Group size:	No data
Method:	Glass diffusion cells
Test substance:	Retinyl palmitate
Purity:	No data
Batch:	No data
Test item:	Unlabelled: Retinyl palmitate creams (A+C: 0.5%; B+D: 1.0% retinyl palmitate) Labelled: [^{3}H]-retinyl palmitate, 30 µCi/g retinyl palmitate (1 mio IU/g)
Dose applied:	6 mg/cm² corresponding to 30 µg retinyl palmitate /cm² for creams A and C, and 60 µg retinyl palmitate/cm² for creams B and D
Exposure time:	Naked rat skin: 6 and 16 h Porcine skin: 16 h
Sampling:	After exposure time
Receptor fluid:	Physiological salt solution
Tape stripping:	Yes 15 (+/- 6 times)
Method of Analysis:	Liquid scintillation counting
GLP:	No data
Study period:	April - May 1989

In a further skin penetration test, four creams containing retinyl palmitate were tested *in vitro* on the intact skin of naked rats and pigs. Labelled [3H]-retinyl palmitate was incorporated at concentrations of 0.5% (creams A+C) or 1.0% (creams B+D). Fresh dermatomed naked rat skin or porcine skin was put on the glass diffusion cells. The receptor fluid consisting of 0.9% aqueous sodium chloride solution and the glass cells were maintained at 32 °C. The creams containing the unlabelled retinyl palmitate concentrations were mixed with labelled material to achieve a radio-activity of about 1–5 µCi/5 cm² skin area. A total amount of 6 mg/cm² corresponding to 30 µg retinyl palmitate/cm² for creams A and C, and 60 µg retinyl palmitate/cm² for creams B and D was applied by smearing 30 mg tests creams on an area of 5 cm² for 30 sec with a glass spatula. After exposure times of 6 or 16 h for naked rat skin or 16 h for porcine skin, the test preparations were removed from the skin surface by means of cotton wool. The amount of labelled material in the cotton wool as measure for the remaining amount on the skin surface, in the SC, in the

stripped skin and in the chamber liquid was analysed by means of liquid scintillation counting.

Table 7 Results of skin penetration after exposure of creams containing retinyl palmitate to the skin of the naked rat for 6 and 16 h and of the pig for 16 h

	Skin surface		Stratum corneum		Remaining skin#		Receptor fluid		Bioavailable according to applicants##	
	µg/cm²	%	µg/cm²	%	µg/cm²	%	µg/cm²	%	µg/cm²	%
Naked rat, cream A (0.5% retinyl palmitate), 30 µg/cm²										
6 h	22.05	73.5	1.05	3.5	6.10	20.3	0.80	2.7	6.9	23.0
16 h	16.94	56.5	1.22	4.1	8.73	29.1	3.11	10.4	11.84	39.5
Pig, cream A (0.5% retinyl palmitate), 30 µg/cm²										
16 h	27.72	92.4	0.55	1.8	1.04	3.5	0.69	2.3	1.73	5.8
Naked rat, cream B (1.0% retinyl palmitate), 60 µg/cm²										
6 h	42.03	70.1	2.42	4.0	13.91	23.2	1.64	2.7	15.55	25.9
16 h	33.98	56.6	4.71	7.9	15.46	25.8	5.85	9.8	21.31	35.52
Pig, cream B (1.0% retinyl palmitate), 60 µg/cm²										
16 h	55.06	91.8	1.63	2.7	1.98	3.3	1.33	2.2	3.31	5.5
Naked rat, cream C (0.5% retinyl palmitate), 30 µg/cm²										
6 h	22.23	74.1	1.3	4.3	5.36	17.9	1.11	3.7	6.47	21.6
16 h	16.10	53.7	2.18	7.3	9.2	30.7	2.52	8.4	11.72	39.1
Pig, cream C (0.5% retinyl palmitate), 30 µg/cm²										
16 h	26.48	88.3	0.82	.27	1.95	6.5	0.75	2.5	2.70	9.0
Naked rat, cream D (1.0%retinyl palmitate), 60 µg/cm²										
6 h	44.2	73.7	2.4	4.0	11.13	18.6	2.27	3.8	13.40	22.3
16 h	31.14	51.9	4.01	6.7	19.72	32.9	5.13	8.6	24.85	41.4
Pig, cream D (1.0 % retinyl palmitate), 60 µg/cm²										
16 h	53.23	88.7	1.63	2.7	3.38	5.6	1.76	2.9	5.14	8.6

the remaining skin consisted of epidermis, dermis and connective tissue

the fraction penetrated into the remaining skin and through the skin into the liquid is considered as bioavailable by the applicants as the study author did not use the term "penetration" correctly.

Conclusion

The dermal bioavailability was more species-, dose- and time-dependent than influenced by differences in the tested cream formulations. A large portion of the test substance was found in the SC. The percutaneous penetration of retinyl palmitate after topical application amounted to about 20 and 50% in rat skin, and approximately 10% in pig skin. In contrast to the procedure of the study author, who included the portion of the SC for its penetration consideration, only the bioavailable fractions were calculated and assessed. The applicants agree to the conclusion that the results obtained from pig skin are more reliable than the data from naked rats. Finally, it is concluded that the highest value of 5.14 µg/cm² corresponding to 8.6% obtained from pig skin and retinyl application of 60 µg/cm² reflects the highest dermal bioavailability obtained under these study conditions.

Reference: 127

SCCS comments
The number of donors and replicates used in this study is not known. The exposure time (6 and 16 hours) is not in accordance with the SCCS Notes of Guidance. Membrane integrity of the rat and porcine skin were not checked prior to the experiment. The composition of the tested cream formulations was not provided to the SCCS. Only mean values were presented, no standard deviations. Retinyl palmitate does not dissolve in the receptor fluid used and therefore the results of this study are not considered valid.

A further study which did not meet current testing and assessment requirements predominantly due to an insufficient recovery rate was carried out with retinyl palmitate *in vitro* on porcine skin, using an O/W preparation with content of 0.7% retinyl palmitate. The influence of different vehicles was additionally investigated. Freshly dermatomed porcine skin was put on the glass diffusion cells. A total amount of 20 mg/5 cm² (4 mg/cm²) cream was applied corresponding to a retinyl palmitate concentration of 140 µg/5 cm² or 35 µg/cm². The application areas were covered to avoid impairment from light. After an exposure time of 24 hours, the remaining test preparations were removed from the skin surface. The receptor fluid was taken of the cell and the SC was removed by 15–20 tape strippings. The epidermis was separated from the dermis after heating. Analysis was performed by HPLC.

The total recovery ranged between about 70 – 80% irrespectively of the vehicle used. However, the usage of aqueous vehicles led to about twice the rate of absorption than oily vehicles. The authors assumed that the observed loss may be due to oxidative metabolism or due to influence of light during further processing of the skin samples. The topical application for 24 hours showed that 96-100% of the retinyl palmitate that was absorbed (10-20%) was limited to the SC and in this skin compartment, it could only be detected in the upper 6–8 strips of the horny layer. Minor amounts <1% were found in the epidermis. Penetration into the receptor fluid was not observed.

Reference: 21

SCCS comment
SCCS agrees with the applicant that this study cannot be used in safety assessments.

In vivo

In animals

Retinol

Method:	Explorative percutaneous skin penetration *in vivo*
Species/Strain:	Female Fuzzy rat
Animal number:	3 - 9 per group
Test substance:	Retinol
Batch:	No data
Purity:	>99%
Test item:	Hydroalcoholic gel or oil in water emulsion containing 0.3% [^{3}H]-retinol (specific activity: 47 Ci/mmol, radiochemical/chemical) corresponding to about 0.7 µCi/cell
Dose applied:	2 mg/cm²
Exposure area:	3.0 cm²
Exposure time:	24 h
Samples:	Skin washes, dosed skin sites, carcass, urine, faeces
Sampling time:	24 or 72 h (urine, faeces)
Tape stripping:	Yes (10 times)
GLP:	Not in compliance
Study period:	/

The dermal absorption and distribution of Retinol was tested *in vivo* after exposure to groups of 3 – 9 female Fuzzy rats for 24 hours. An area of application of 3.0 cm² was delineated on the midscapular region of the rat. The dosing area was enclosed and protected with a Stomahesive® patch glued directly to the animal's skin. The rats were dosed with [^{3}H]-retinol at a dose level of 2 mg/cm² in hydroalcoholic gel or oil in water emulsion. The patch was covered with a screen and the animals were placed in metabolism cages for 24 or 72 h to collect urine and faeces. The rats were then euthanized. 24 h after application the treated skin site was washed with soap and water to remove unabsorbed material. The washes were collected to determine the amount of unabsorbed material. The skin dosing site and remaining carcass were each dissolved in concentrated potassium hydroxide. Aliquots of the urine, faeces, and dissolved carcass were analysed for radioactivity by liquid scintillation counting. The distribution of radioactivity was determined in the area of the dosing site. The skin with the patch still intact was cleaned of fat from the underlying tissue. The patch was then removed and the remaining glue on the skin was removed with hexane- soaked cotton swabs. A dermatome was used to create a split-thickness skin section (180-330 µm). The area containing the dosing site was punched into one or two skin discs. Each skin disc was tape stripped 10-times to determine the amount of [3H]-retinol remaining in the SC *versus* the epidermis/dermis.

Results

In vivo absorption studies were conducted for 24 h and 72 h in fuzzy rats for comparison with the *in vitro* results (see above). At 24 h, the total, systemic absorption (sum of the urine, faeces, and carcass levels) of retinol applied in the gel vehicle amounted to 4.1% and was similar to the corresponding *in vitro* value (i.e., receptor fluid level) at 24 h. The total amount in the skin and the total penetration of retinol *in vivo* at 24 h also agreed with values obtained in the corresponding *in vitro* studies. In the *in vivo* studies, there was no significant difference between the levels of absorbed material found in the receptor fluid at 24 h and 72 h. The recovery of radioactivity at the end of the 24-h study was greatly improved when charcoal filter paper was placed on top of the protective patch around the edges of the dosing area for 1 h after dosing the skin. Therefore, low recoveries in the *in vivo* studies appear to be due to loss of retinol from the dosing area.

Similar results were observed with the *in vitro–in vivo* comparison when retinol was applied to skin in the oil-in-water emulsion. The 24-h *in vivo* systemic absorption value closely agreed with the *in vitro* receptor fluid levels of retinol found at 24 h. In addition, the large skin reservoir (i.e., the levels measured as the total in the skin) observed in the *in vitro* studies was also seen in the 24-h and 72-h *in vivo* studies. The details are provided in the following table:

Table 8 *In vivo* percutaneous absorption of [^{3}H]-retinol in female Fuzzy rat (means of 2 – 9 rats) skin using gel and oil-in-water emulsion vehicles after exposure of 24- h and determination after 24-h and 72-h

Recovery site	24-h gel (%)a	72-h gel (%)b	24-h emulsion (%)c	72-h emulsion (%)d
Total systemic absorption (urine, feces, carcass)	4.1±0.4	4.4±0.03	6.5±0.2	5.6±0.1
Stratum corneum	5.5±1.3	2.6±0.7	3.4±0.3	7.5±1.4d
Total in skin	17.7±2.1	12.9±2.7	17.5±0.7	11.0±1.7d
Total penetration	21.8±2.2	17.3±2.7	24.0±0.6	16.6±1.7d
Recovery	83.1±4.5	70.7±2.3	77.9±1.5	81.9±1.0
Recovery (charcoal filter paper for 1 h)e	106.2±0.9			
a: mean ± SD for 9 rats, b, c, d: mean ± SD for 3 rats, e: mean ± SD for 2 rats				

Conclusion

The total systemic absorption of retinol was less than the total retinol found in the skin. Total systemic absorption of retinol *in vivo* ranged from 19–33% of total penetration no matter which vehicle was used or time point measured (24 h or 72 h). Unlike in the *in vitro* studies, there was no increase in total systemic absorption of retinol when 24-h values were compared to 72-h values in the *in vivo* studies. Thus, the author concluded that this is an indication for an *in vivo* skin reservoir. However, it has to be taken into consideration that in human studies there was no evidence that topically applied retinol, retinyl palmitate or retinoid metabolites induced detectable changes in the constitutive plasma levels.

Reference: 168

Retinyl palmitate

Method:	Explorative percutaneous skin penetration *in vivo*
Species:	SKH-1 hairless mice (female)
Animal number:	3 per group
Test substance:	Retinyl palmitate (RP, source: Sigma Chemical Co. St. Louis, MO, USA, analysed by HPLC)
Purity:	No data
Batch:	No data
Test item:	0.5 or 2% RP in an oil-in-water cream oil
Dose applied:	75 µL/mouse corresponding to approximately 2 mg cream/cm²
Exposure time:	Experiment 1: single dose 2% RP cream for 1, 2, 3, 6 days Experiment 2: single dose 0.5% RP cream for 1, 2, 3, 6 days Experiment 3: for 4 consecutive days single dose 2% RP cream for 1, 3, 6, 11, 11 days
Samples:	stratum corneum, epidermis, dermis
Sampling time:	1, 2, 3, 6, 11, 18 days post application
Tape stripping:	Yes (20 times)
Method of Analysis:	HPLC
GLP:	Not in compliance
Study period:	/

The dermal absorption and distribution of Retinyl palmitate (RP) as 0.5% or 2.0% oil in water cream was tested *in vivo* after exposure to three female SKH-1 mice per group after application of 75 µL to the dorsal skin corresponding to approximately 2 mg cream/cm². Three different experiments were performed. In both experiment 1 and 2, three mice were topically exposed to either 2% or 0.5% RP cream and euthanised 1, 2, 3, or 6 days later. Skin was collected, flash-frozen and stored at -80°C until use. In experiment 3, mice received daily topical applications of 2% RP cream on the dorsal skin area for 4 consecutive days and were euthanised on days 1, 3, 6, 11 or 18 and the skin was collected and processed as described above. The samples of skin of each mice were separated into SC (20 times tape stripping) and into epidermis and dermis by heat separation followed by cooling. RP and retinol from each sub-fraction and skin layer were extracted, identified and quantified by HPLC analysis.

Results

Table 9 Levels of RP and retinol in the SC, epidermis, and dermis of mice treated once with 2%-RP cream.

Retinoid	Skin layer	Day after last treatment			
		One	Two	Three	Six
RP (ng/mg)	Stratum corneum	32.34±2.36	24.68±2.36	7.02±2.36	0.34±2.36
	Epidermis	120.49±5.09	70.40±5.09	12.02±5.09	3.68±5.09
	Dermis	10.28±0.48	5.66±0.48	1.85±0.48	1. 16±0.48
	Intact skin	18.8±1.6 (237.3)#	12.9±1.6 (327.6)	3.3±0.2 (350.9)	1.1±0.3 (283.4)
Retinol (ng/mg)	Stratum corneum	2.21±0.39	1.76±0.39	0.50±0.39	ND
	Epidermis	21.78±1.98	17.19±1.98	3.78±1.98	2.33±1.98
	Dermis	2.20±0.18	2.2±0.18	1.10±0.18	0.47±0.18
	Intact skin	2.9±0.4	2.8±0.4	1.2±0.4	0.45±0.09
Values are Least Square mean±SD; n = 3, ND = not determined, #= intact skin weight (mg)					

Table 10 Levels of RP and retinol in the SC, epidermis, and dermis of mice treated with 0.5%-RP cream for one day

Retinoid	Skin layer	Day after last treatment			
		One	Two	Three	Six
RP (ng/mg)	Stratum corneum	2.63±0.26	1.67±0.26	0.88±0.26	0.50±0.26
	Epidermis	24.57±0.47	12.07±0.47	5.52±0.47	2.21±0.47
	Dermis	2.15±0.12	1.33±0.12	0.95±0.12	0.86±0.12
	Intact skin	2.7±0.3 (275.6)#	1.6±0.3 (308.6)	1.1±0.2 (300.0)	0.85±0.2 (243.0)

Retinoid	Skin layer	Day after last treatment			
		One	Two	Three	Six
Retinol (ng/mg)	Stratum corneum	0.35±0.72	0.48±0.72	0.24±0.72	ND
	Epidermis	5.35±0.35	6.73±0.35	3.57±0.3 5	1.23±0.35
	Dermis	0.74±0.09	0.82±0.09	0.65±0.09	0.50±0.09
	Intact skin	0.77±0.2	0.89±0.08	0.69±0.2	0.45±0.09
Values are Least Square mean±SD; n = 3, ND = not determined, #= skin weight (mg)					

Table 11 Levels of RP and retinol in the SC, epidermis, and dermis of mice treated with 2%-RP cream for four consecutive days.

Retinoid	Skin layer	Day after last treatment				
		One	Three	Six	11	18
RP (ng/mg)	Stratum corneum	14.20±0.84	3.84±0.84	0.79±0.84	0.66±0.84	0.69±0.84
	Epidermis	53.28±0.70	13.61±0.70	7.29±0.70	2.67±0.70d	1.74±0.70
	Dermis	6.67±0.38	2.56±0.38	2.34±0.38	0.97±0.46b,c	0.71±0.38
	Intact skin	11.1±2.4 (131.3)#	3.4±0.6 (312.0)	2.4±0.4 (245.9)	1.0±0.2 (239.7)	0.76±0.04 (234.5)
Retinol (ng/mg)	Stratum corneum	0.49±0.07	0.36±0.07	ND	0.12±0.07	ND
	Epidermis	8.87±0.46	2.93±0.46	1.65±0.46	0.74±0.46	0. 55±0.46
	Dermis	1.40±0.08	1.33±0.08	0.52±0.08	0.30±0.10	0.23±0.08
	Intact skin	1.8±0.4	1.3±0.2	0.5±0.2	0.28±0.04	0.21±0.05
Values are Least Square mean±SD; n = 6, ND = not determined, #= skin weight (mg)						

Conclusion

It was shown that RP rapidly diffuses into the SC and epidermal skin layers within 24 h following the application of RP-containing creams. Of the three skin layers, the highest level of RP and retinol per weight unit (ng/mg) at all-time points was found in the epidermis. Levels of RP and retinol were lowest in the dermal layer and intermediate in the SC. The levels of RP and retinol in the separated skin layers and in the intact skin decreased with time, but levels of RP remained higher than control values for a period of up to 18 days.

These results indicate that the application of RP to mouse skin can alter the normal physiological levels of RP and retinol in the skin of mice.

Reference: 166

In human

Topical application of a 0.05% retinal cream on 14 consecutive days (7 mg/day) covering around 40% of the body surface (back, chest, abdomen, external aspects of the arms) on male volunteers did not induce an alteration of the plasma levels of retinoids (retinol, all-*trans* retinoic acid, retinyl palmitate/oleate, 13-*cis*-retinoic acid and 4-oxo-13-*cis*-retinoic acid) during the treatment period. However, there was a slight, but not statistically significant, increase in the levels of all-*trans*-retinoic acid, retinol and retinyl palmitate/oleate until one week after the end of the treatment (Reference: 146).

In a study by Nohynek *et al.* (2006) (Reference: 117), two groups of female volunteers were treated topically for 21 days with creams containing **0.3% retinol or 0.55% retinyl palmitate** on about 3000 cm^2 of their body surface (back, upper legs). Daily, 3.5 g of cream comprising 9 mg of retinol or 16 mg of retinyl palmitate was applied. Plasma levels of retinol, retinyl palmitate, retinyl oleate, retinyl stearate, 9-*cis*-, 13-*cis*-, all-*trans*-, 13-*cis*-4-oxo- or all-*trans*-oxo-retinoic acids were measured 0, 1, 2, 4, 6, 8, 12, 14-16 and 24 hours after each application. On day 21, no changes in plasma retinoid levels were observed.

Franz and Lehman (1990) examined the absorption **of [^{14}C] retinoic acid** in 8 male subjects with mild to moderate facial acne. Four of the subjects had dermatitic skin (signs of mild irritation induced by treatment with non-radioactive 0.05% Retin-A cream during the

prior two weeks). A commercially available 0.05% ATRA (all-*trans*-retinoic acid) cream containing radioactive ATRA was applied on the forehead and neck (100 mg/50 cm^2). The application sites were washed after 10 hours. There were no significant differences in the absorption of ATRA through normal acne skin and dermatitic acne skin (urinary excretion of radioactivity: 1.1% (±0.2) and 1.5% (±0.4) through normal and dermatitic skin, respectively). When corrected for excretion by non-urinary routes (factor 0.21 obtained from experiments on rhesus monkeys) total absorption was 5.3 and 7.2%, respectively, for the normal and the dermatitic skin. The rate of absorption continually increased until the time the normal skin was washed, but peaked and started to decline prior to time the dermatitic skin was washed (10 vs 5 hours).

A further study in female volunteers investigated whether and to what extent retinyl-palmitate, present in a cosmetic cream, penetrates the skin and if changes in plasma concentrations of retinyl- palmitate, retinol and/or the metabolites 13-cis retinoic acid and all trans retinoic acid after a single dermal application could be noted. Young healthy female volunteers (age 21.3 +/- 1.3) were examined according to an approved protocol. The individual plasma kinetics of all females was investigated after intravenous injection of 5.5 mg retinyl-palmitate. This was compared with the effect of a single dermal application of cream containing retinyl palmitate (1% or 10% w/w) onto the whole forearm (about 500 com^2) for 12 hours.

The intravenous administration resulted in significantly increased plasma level of retinyl palmitate (up to 0.8 mg/l) but had no effect on the endogenous retinol level. The semi-occlusive topical application of the cream containing 1% or 10% retinyl palmitate on the skin surface of 500 cm2 resulted in no increase of plasma levels of retinol, retinyl palmitate, 13-cis retinoic acid or all-trans retinoic acid. (Reference: 108).

The bioavailability after repeated topical application of retinol and retinyl palmitate containing creams and its impact on metabolism and endogenous metabolite profile was investigated in a total of 36 women of child-bearing age after approval of protocol under GLP conditions. The three groups of female volunteers received 10g of O/W creams containing either 0.236% retinol palmitate (about 41 000 IU), 0.147 retinol (about 25 000 IU as retinol) or no retinoid for comparison on a large body surface on the trunk and thighs daily for a period of 56 days. Each volunteer was issued with food diaries for monitoring of exogenous Vitamin A intake. Blood samples were collected each week and detailed profiles were determined on days 0, 28, 56 and as well as 7 days after termination of application. All females were subjected to a complete dermatological assessment for skin findings. The plasma samples were analysed for retinol, retinyl palmitate, and various retinoic acids, including all-trans-retinoic acid. Retinol and retinyl palmitate were extracted and analysed by HPLC without acidification of the plasma, while retinoic acids were analysed by HPLC after acidification.

In general, the consecutive topical application of the creams was well tolerated. Only occasionally did a few participants note transient skin effects consisting of slight itching or the development of a rash.

The treatment for 56 days did not produce an overall trend for an increase in plasma concentrations of retinyl palmitate, retinol, all-trans retinoic acid, 13-cis retinoic acids, 13-cis-4-oxo retinoic acid, all-trans-4-oxo retinoic acid or 9-cis retinoic acid. (Reference: 156).

Skin metabolism

The metabolic pathways of retinoids in the skin were comprehensively reviewed by several authors with regard to the enzymes and binding proteins that are mainly involved in the activation, modulation, and cleavage of retinoids in human skin. The involvement of these enzymes/binding proteins in the pathogenesis of skin disorders, especially malignancies and

disorders of keratinisation were also emphasised. In addition, the xenobiotics that are capable of modulating the steady-state of tissue retinoid concentrations and their impact on the enzyme systems that regulate the metabolic pathways were considered. In addition, in rats it was shown that metabolites of retinol formed in the skin are less teratogenic when compared with the activity of retinol (References: 103, 143, 160).

Retinol (0.4%) applied to the skin of human volunteers under occlusion for 6h to 4d was mainly metabolised to metabolites such as 14-hydroxy-4,14-retro-retinol (principal metabolite), anhydro-retinol and retinyl esters (minor metabolites). The level of 14-hydroxy-4, 14-retro-retinol was increased from undetectable at time 0 to 326 ng/g wet weight of tissue at 6h (6% of the retinol level) and maintained approximately the same concentration at 24h to 409 ng/g wet weight (1.9% of the retinol level); it decreased to 48 ng/g wet weight of tissue (12% of its maximum level) by 4d. In contrast to all-trans-retinol, 14-hydroxy-4, 14-retro-retinol had no effect on epidermal thickness and mRNA expression of cellular retinoic acid binding protein II. Retinoic acids were not detected in this study. These results suggest that the metabolism of retinol *in vivo* in human skin predominantly yields metabolites that are biologically less active than retinol itself (Reference: 53).

Similar results were obtained in mice, where no detectable levels of retinoic acids were found in the skin after topical administration of retinol or retinaldehyde (Reference: 145).

The metabolism of radio-labelled retinol, retinaldehyde, and retinoic acid by fresh human skin as well as by cultured human dermal fibroblasts were investigated *in vitro* and demonstrated that topical application of retinoids resulted in gradient concentrations within the skin. For each fraction, metabolites and unchanged product portions were determined by HPLC. After treatment with retinol and retinal, low but significant amounts of retinoic acid were detected in the epidermis, as well as in the dermis (30-90 pM). In comparison, treatments with retinoic acid itself, led to higher level of retinoic acid in the epidermis and in the dermis (respectively 2050 and 420 pM). Cultured human dermal fibroblasts, treated with retinol and retinal formed retinoic acid as well as several other metabolites. Thus, the obtained results can be interpreted as an indication of conversion of retinol or retinal in the skin to retinoic acid due to the metabolic function of the dermis (Reference: 4)

In summary, studies on the metabolic fate of topically applied retinol or retinyl esters concluded that topically applied retinol is minimally absorbed by the viable epidermis and is extensively metabolised by keratinocytes. There is certain evidence that the skin metabolizes Vitamin A preparations to metabolites that are biologically less active than retinol itself.

SCCS general comments on dermal/ percutaneous absorption

Several *in vitro* and *in vivo* investigations on dermal absorption in experimental systems are available. The majority of these studies was performed with retinyl palmitate at cosmetic use concentrations, and also investigated the effect of different vehicles or formulation procedures. In addition, recent *in vitro* skin penetration data investigating retinol are also available. The studies utilised animal (rat, mice, pig) or human skin as test systems. Most of the studies were not performed under GLP conditions, and suffered from deviations from the current testing requirements/guidelines with regard to methodology and assessment. All available studies showed that, irrespective of the origin of the investigated skin, the major portion of the applied dose could be removed from the application site after the studied exposure periods. A few studies observed some skin penetration, although most found that the vast majority of the test material was adsorbed by the *stratum corneum* and was, thereby, considered non-bioavailable. Penetration into deeper skin layers (epidermis, dermis), or penetration through the skin (receptor fluid) was also observed but consisted of very small amounts when compared with the total applied dose in most of the studies.

In addition, the known differences in the skin permeability of the investigated species were in the order of rat/mouse>pig≥human. Differences due to the selected vehicles for the respective formulation were noted as e.g., aqueous vehicles led to about twice the rate of absorption than oily vehicles.

In the *in vivo* studies cited above, no significant increase in plasma levels of retinoids could be detected after repeated applications of retinal, retinol or retinyl palmitate (References: 117, 146). This may be due to several factors such as dose and area of application. In the study by Sass, the application area was about 40% of the total body surface area and the dose corresponded to 7 mg of retinal daily for 14 days. In the study by Nohynek (Reference: 117), around 19% (3000 cm^2) of the total body surface area was covered daily by an amount of 3.5 g of cream for 21 days. This corresponds to a daily dose of 9 mg of retinol or 16 mg of retinyl palmitate. According to the SCCS Notes of Guidance (SCCS, 2015), 7.82g of body lotion is the estimated daily exposure level. Furthermore, the mean exposed skin surface area for body lotion is 15 670 cm^2. Thus, both the doses applied and the application area in the studies by Sass and Nohynek are much lower than values used by SCCS. In addition, serum retinol concentrations are not considered as an efficient biomarker of exposure in individual patients because of the homeostatic regulation of Vitamin A (VKM, 2012). An increase in plasma levels of retinoids after topical application may not be expected due to the storage capacity and the tightly controlled low-level conversion of retinol to retinoic acid in the skin. However, both *in vivo* and *in vitro* studies demonstrate that topical application is effective with respect to loading the skin with substantial levels of retinoids. Furthermore, the topically applied retinol and retinol palmitate have been shown to trigger biochemical (e.g. increased expression of retinol and retinoic acid binding proteins, increased levels of enzymes that metabolise retinoic acid) and histological (e.g. epidermal hyperplasia, dermal collagen synthesis and degradation) changes in the skin that might be expected from perturbation of previously established retinoid homeostasis (VKM, 2012).

For the calculation of the MoS, the SCCS will use the study from Yourick et al. 2008 (Reference: 168) described above for dermal penetration. As in this study, standard errors (SEM) were provided instead of Standard Deviation (SD). SD were calculated by SCCS as followed:

2 donors are used with 3 replicates per donor which result in a total number of samples (n) of 6

- viable skin penetration: 3.0 ± 0.6 %
- receptor fluid penetration: 1.3 ± 0.1 %

knowing that SEM = SD/$\sqrt{n}$, SD = SEM x $\sqrt{6}$

Thus values ± SD instead of SEM become:
- viable skin: 3.0 ± 1.47 %
- receptor fluid: 1.3 ± 0.245 %

-> dermal absorption: 4.30 ± 1.72 %

For the MoS calculation, because the number of donors was not according to the SCCS requirements, mean + 2SD will be used which means 7.74 %

3.3.4 Repeated dose toxicity

Numerous safety studies on different species including mice, rats, rabbits, dogs, guinea pigs, cattle, chicken, pigs, ducks, hamsters, monkeys, sheep and cats have been performed to investigate the toxicity of Vitamin A. In these studies Vitamin A and its metabolites were tested *via* oral, intravenous, intramuscular, subcutaneous, intraperitoneal and dermal administration routes. The toxicity of Vitamin A was also assessed by several regulatory or expert bodies: e.g. EFSA (2006, 2015), EMEA (2015), CIR (2013), COT (2013), BfR (2012), VKM (2012), ANSM (2010), SCF 2002. Toxicity appears to occur when the amount of vitamin A in plasma exceeds the capacity of Retinol Binding Proteins, leading to a change in the ratio of free retinol to retinol-RBP complexes. Only a summary of the main points related to the toxicity of Vitamin A that can be used to assess the risk for cosmetic exposure is included in this section.

The teratogenic potential of Vitamin A and effects on bone were considered as the most critical toxicological endpoints and have therefore been dealt with in more detail than other possible adverse effects.

In 2002, the SCF reviewed possible adverse effects of long-term intake of retinol and retinyl esters (SCF, 2002). The following adverse effects were identified: hepatotoxicity, changes in lipid metabolism and in bone density, and teratogenicity. The lowest continuous daily consumption in patients with cirrhosis was 7500 mg RE/day taken over 6 years. A case of cirrhosis from 7500 mg RE/day for 6 years has been reported (Kowalski et al., 1994), in which progressive liver failure led to the death of the patient. Cases of hepatotoxicity have not been reported below 7500 mg RE/day, and it can be hypothesised that this value might be the upper threshold of the storage capabilities of the liver. The SCF also identified the daily dose of 7500 µg RE/day for four years as the LOAEL for changes in lipid metabolism, resulting in a 2-3% increase in blood cholesterol concentration in a placebo-controlled trial involving 2,297 63 years old subjects, which could lead to an increased risk of cardiovascular disease. The SCF reported reduced bone density and increased rates of fracture in women aged 40-76 years with daily intake of Vitamin A greater than 1500 µg RE/day in comparison with intakes of less than 500 µg RE/day and commented that middle aged and elderly women were the group most sensitive to such effects. It was not known however, whether the same dose-response relationship would apply in men or in children.

Based on epidemiological data, no association has been found in the majority of case-control studies between daily doses of Vitamin A of 3000 mg RE or less and foetal malformation. However, in each of these studies, the number of women consuming high amounts of Vitamin A was too limited to give a reliable estimate of a safe intake value.

A prospective study involving 22,748 pregnant women was large enough to stratify the population according to the Vitamin A intake. Moreover, the origin of the Vitamin A intake (supplement or food) was available for all subjects. Women taking more than 4500 mg RE of total Vitamin A (from food and supplement) daily had a 3.5 times higher risk of giving birth to a child with cranial-neural-crest defects, than mothers ingesting less than 1500 mg RE/day. When the analysis was restricted to the supplemental intake of Vitamin A only, the relative risk for mothers ingesting more than 3000 mg RE/day was 4.8 higher than those ingesting 1500 mg RE/day. The authors fitted a regression curve to their data, which indicated a rise in the ratio of prevalence of birth defects associated to the cranial-neural crest at doses greater than 3000 mg RE/day of Vitamin A (food and supplement). The conclusions of the study remained the same when several potential confounding factors were considered. The quantitative conclusion from this study was that 3000 mg RE/day of supplemental Vitamin A can be considered as a threshold for teratogenicity, which would be associated with a low or negligible risk of teratogenicity (SCF, 2002).

The SCF set a Tolerable Upper Intake Level (UL) for preformed Vitamin A[4] (retinol, retinal, retinyl esters and retinoic acid) of 3000 µg RE/day (or 10 000 IU) for women of childbearing age and men, based on the risk of teratogenicity and hepatotoxicity. This UL also applies during pregnancy and lactation. ULs for children were extrapolated from the UL for adults, based on allometric scaling (body weight to the power of 0.75). ULs were set at 800 µg RE/day for children aged 1–3 years, 1 100 µg RE/day for children aged 4–6 years, 1 500 µg RE/day for children aged 7–10 years, 2 000 µg RE/day for children aged 11–14 years and 2 600 µg RE/day for children aged 15–17 years.

In a subsequent assessment which considered studies published until 2004, the Scientific Advisory Committee on Nutrition (SACN, 2005) concluded that the evidence of an association between high intake of retinol and poor bone health was inconsistent. The Committee noted that some epidemiological data suggest that a retinol intake of 1500 µg/day and above is associated with an increased risk of bone fracture; the evidence was considered not robust enough to set a Safe Upper Level, and a Guidance Level for retinol intake of 1 500 µg/day was set for adults for individuals at greater risk of osteoporosis and bone fracture (particularly post-menopausal women) (EFSA, 2008, 2013).

The EFSA NDA Panel[5] (EFSA, 2015) was aware that additional observational studies on possible associations between retinol and Vitamin A intake and bone health have been published since the SCF and SACN assessments. An overview of prospective cohort and nested case–control studies which investigated an association of retinol or "Vitamin A" intake with the risk of bone fracture has been performed. Based on this review, the Panel considered that evaluation of the data published since the SCF assessment does not change the conclusion from that of the SCF with respect to the association between retinol or Vitamin A intake and risk of bone fracture in postmenopausal women.

In summary, the critical adverse effects of high intakes of Vitamin A are different at different stages of life, such as bulging fontanelles in infants, decreased bone density and increased bone fracture in middle aged and elderly women, and teratogenicity in women of child-bearing age. Hepatotoxicity and altered lipid metabolism are also relevant for adults. The LOAEL of preformed Vitamin A identified by SCF as causing these adverse effects are shown in table 12.

Table 12: Lowest doses identified as associated with adverse effects (SCF, 2002)

Effect	Lowest dose (per person)
Bulging fontanelles	7 500 µg RE, single dose
Hepatotoxicity	7 500 µg RE/day
Altered lipid metabolism	7 500 µg RE/day
Decreased bone density/increased bone fracture	1 500 µg RE/day
Teratogenicity	>3 000 µg RE/day

SCCS general conclusion on the repeated dose toxicity of Vitamin A

The SCCS considers that the teratogenic potential of Vitamin A and effects on liver and local effects in the skin are the most critical toxicological endpoints. For assessing the systemic toxicity of Vitamin A after cosmetic exposure, the SCCS has relied on the Tolerable Upper Intake Level (UL) for preformed Vitamin A of:

[4] Preformed Vitamin A consist predominantly of retinol and retinyl esters, which are supplied in the diet by animal-derived products (EFSA, 2015)

- 3 000 µg RE/day (or 10 000 IU) for women of childbearing age and men. The UL also apply during pregnancy and lactation.
- 800 µg RE/day (2700 IU) for children aged 1–3 years,
- 1 100 µg RE/day (3700 IU) for children aged 4–6 years,
- 1 500 µg RE/day (5000 IU) for children aged 7–10 years,
- 2 000 µg RE/day (6700 IU) for children aged 11–14 years and
- 2 600 µg RE/day (8700 IU) for children aged 15–17 years.

To take into account more susceptible population groups such as women suffering from osteoporosis or children above 6 years old who may also be exposed to Vitamin A *via* cosmetic products, SCCS will use the value of 1500 µg RE/day (5000 IU) for the safety assessment of Vitamin A in cosmetic products. This value will be appropriate for women of childbearing age and also for middle age and elderly women who may suffer decreasing bone density, as well as for men and children.

Based on information provided by the applicants, Vitamin A and esters are not currently used for children in the EU. However, application of Vitamin A-containing baby skin care products, such as body lotions and creams, are considered by the SCCS relevant for 1- and 3-years old children. Therefore, based on a theoretical scenario, exposure to Vitamin A *via* these products has been assessed in this opinion. For children aged 1-3 years SCCS will use the value of 800 µg RE/day (2700 IU) for the safety assessment of Vitamin A in cosmetic products.

For children aged 4-6 years SCCS will use the value of 1100 µg RE/day (3700 IU) for the safety assessment of Vitamin A in cosmetic products.

Moreover, the SCCS is aware that undesirable effects can also arise from a lack of Vitamin A. Therefore population reference intake values are recommended which is the level of intake that is adequate for virtually all people in a population group. For Vitamin A, the recommended values by EFSA are as shown in the following Table:

EFSA, 2015

Table 7: Summary of Population Reference Intakes for vitamin A

Age	Population Reference Intake (µg/day)
7–11 months	250
1–3 years	250
4–6 years	300
7–10 years	400
11–14 years	600
15–17 years (M)	750
15–17 years (F)	650
≥ 18 years (M)	750
≥ 18 years (F)	650
Pregnancy	700
Lactation	1 300

F, females; M, males.

3.3.5 Toxicokinetics

3.3.5.1 Toxicokinetics in laboratory animals

The pharmacokinetics of orally administered Vitamin A compounds were assessed in several prenatal developmental toxicity studies in *Cynomolgus* monkeys.

Retinyl palmitate orally administered to *Cynomolgus* monkeys at doses of 7500 and 20000 IU/kg bw/day (4.1 and 11 mg RP/kg bw/day) produced mean AUC values of retinyl esters approximately 30 times higher than those of the control group. However, there was no statistically significant difference between the AUC values of retinyl esters at these dose levels.

Compared to controls, the AUC values for all-trans-retinoic acid were 4.5 times higher in animals treated with 7500 IU/kg bw/day and 30 times higher at 20000 IU/kg bw/day. The AUC for 13-cis-retinoic acid in plasma was found to be 5 times higher at 7500 IU/kg bw/day and 19 times higher at 20000 IU/kg bw/day. In contrast, the AUC values for retinol in the groups receiving 7500 and 20000 IU/kg bw/day were only slightly increased when compared with those in the control group.

The comparison of the plasma levels after oral administration of a retinyl palmitate formulation and a diet containing Vitamin A-enriched liver at dose of 80000 IU/kg bw/day (44 mg RP/kg bw/day) resulted in large inter-individual differences in AUC values of four retinoic acids without statistically significant differences between the groups. The plasma concentrations of retinyl esters were higher in the group fed Vitamin A-enriched liver when compared with those in the group receiving the water-soluble formulation.

3.3.5.2 Toxicokinetics in humans

Retinoid metabolism is complex and involves many different retinoid forms, including retinyl esters, retinal, retinoic acid and oxidized and conjugated metabolites of both retinol and retinoic acid. In addition, retinoid metabolism involves carrier proteins and enzymes that are specific to retinoid metabolism, as well as other proteins, which may be related to triglyceride and/or cholesterol metabolism (Reference: 40).

In human studies, there is no evidence that topically applied retinol or retinoid metabolites induce detectable changes in their constitutive plasma levels. In a PK investigation by Nohynek et al. (2006) (Reference: 117), the systemic availability of oral Vitamin A and its metabolites after repeated and prolonged topical application of 30000 IU Vitamin A/subject/day was studied. The systemic availability after topical application was compared to plasma levels of retinol, retinyl palmitate, oleate and stearate, 9-cis-, 13-cis, all-trans, 13-cis-4-oxo- or AT-4-oxo-retinoic acids after administration of a single oral dose of 10000 IU or 30000 IU retinyl palmitate. On days 1 or 21 of topical treatment, no changes were measured in individual group mean plasma Cmax, AUC 0-24 h or other pharmacokinetic parameter of REL, retinyl esters or RAs relative to pre-study data.

Although serum/plasma retinol concentration has been used as a biomarker of intake, serum/plasma retinol concentration is under homeostatic control and, in the usual range, is not related to observed levels of habitual Vitamin A intake. Therefore, it is not considered a reliable marker of Vitamin A or retinol intake (Section 2.4.2).

Vitamin A status is best expressed in terms of total body store of retinol (i.e. as free retinol and retinyl esters) or, alternatively, as liver concentration of the vitamin. A concentration of 20µg retinol/g liver (0.07 µmol/g) in adults represents a level assumed to maintain adequate plasma retinol concentration, to prevent clinical signs of deficiency and to provide adequate stores. The EFSA Panel (2015) considered that this can be used as a target value for establishing the Average Requirement (AR) for Vitamin A for all age groups. The relationship between dietary intake of Vitamin A and retinol liver stores has been explored with stable isotope dilution methods but available data are considered insufficient to derive an AR. A factorial approach was applied. This approach considered a total body/liver retinol

store ratio of 1.25 (i.e. 80 % of retinol body stores are in the liver), a liver/body weight ratio of 2.4 %, a fractional catabolic rate of retinol of 0.7 % per day of total body stores, an efficiency of storage in the whole body of ingested retinol of 50 % and the reference body weights for women and men in the EU of 58.5 and 68.1 kg, respectively. On the basis of this approach, ARs of 570 µg RE/day for men and 490 µg RE/day for women were derived after rounding. Assuming a coefficient of variation (CV) of 15 % because of the variability in requirement and the large uncertainties in the dataset, **Population Reference Intakes (PRIs) of 750 µg RE/day for men and 650 µg RE/day for women were set after rounding.**

For infants aged 7–11 months and children, the same target concentration of retinol in the liver and the same equation as for adults was used to calculate ARs. Specific values for reference body weight and for liver/body weight ratio were used. There are some indications that retinol catabolic rate may be higher in children than in adults, but data are limited. The EFSA Panel decided to apply the value for catabolic rate in adults and correct it on the basis of a growth factor. Estimated ARs range from 190 µg RE/day in infants aged 7–11 months to 580 µg RE/day in boys aged 15–17 years. PRIs for infants and children were estimated based on a CV of 15 % and range from 250 to 750 µg RE/day.

For pregnant women, the Panel assumed that a total amount of 3600 µg retinol is accumulated in the foetus over the course of pregnancy. Considering that the accretion mostly occurs in the last months of pregnancy, and assuming an efficiency of storage of 50 % for the foetus, an additional daily requirement of 51 µg RE was calculated for the second half of pregnancy. In order to allow for the extra need related to the growth of maternal tissues, the Panel applied this additional requirement to the whole period of pregnancy. Consequently, an AR of 540 µg RE/day was estimated for pregnant women. Considering a CV of 15 % and rounding, a PRI of 700 µg RE/day was derived for pregnant women.

For lactating women, an increase in the AR was based on the Vitamin A intake required to compensate for the loss of retinol in breast milk. Based on an average amount of retinol secreted in breast milk of 424 µg/day and an absorption efficiency of retinol of 80 %, an additional Vitamin A intake of 530 µg RE/day was considered sufficient to replace these losses. An AR of 1 020 µg RE/day was estimated and, considering a CV of 15 % and rounding down, a PRI of 1 300 µg RE/day was proposed for lactating women.

Vitamin A metabolism is highly regulated, involving many different forms of retinoid, enzymes and carrier or storage proteins. A finely tuned transport mechanism allows a sustained and specific delivery of retinol to cells and organs that need vitamin A for their function. In plasma, retinol circulates bound to retinol-binding protein (RBP or RBP4; molecular weight - MW -, 24 kDa), the specific Vitamin A carrier protein synthetised mainly by the liver, which in turn forms a high MW complex with transthyretin (TTR, 55 kDa). In some tissues, retinol-bound RBP (holo-RBP) is specifically recognised by a receptor stimulated by retinoic acid 6 (STRA6), which transports retinol into cells. Upon delivery of retinol, RBP dissociates from TTR (becoming apo-RBP) and is rapidly eliminated by glomerular filtration followed by reabsorption and catabolism in renal tubules. The highly specific interaction between RBP and STRA6 ensures that retinol be delivered only to cells that have a means to store it, which prevents excessive uptake and random diffusion of retinol. Under fasting conditions, more than 95% of retinol in the circulation is bound to RBP (holo-RBP). Under non-fasting conditions, by contrast, approximately one third of the dietary retinoid is delivered as bound chylomicrons (i.e. as retinyl esters) and their remnants to tissues other than the liver. It has been established that about 25% of postprandial retinyl is taken up by extra-hepatic tissues, including white adipose tissue, skeletal muscle, heart, lungs, and kidneys. In the postprandial state, chylomicron retinyl ester is elevated in plasma and can quantitatively predominate over retinol-RBP.

While many advances have been made in understanding the metabolism of retinol absorbed by the oral route, little is known about the metabolic fate of retinol or retinoids after skin absorption. What has been established is that these lipophilic compounds are absorbed across the skin with estimated absorption rates in the range of 5-8%. It is has also been established that the skin can metabolise retinoids and synthetise cellular RBP, meaning that the skin has some storage capacity for retinol. By contrast, there is a complete lack of data regarding the distribution of absorbed retinol and importantly, regarding the proportion of absorbed retinol that is not taken by the RBP-dependent transport and storage pathways. Because of their lipophilic character, retinol or retinyl esters do not need carrier proteins to be transported by diffusion and to cause adverse effects on cells and organs with no capacity to store retinol. In the literature, this phenomenon is referred to as the random diffusion toxicity of retinol or the RBP-independent toxicity pathways. According to some reports, humans might be about 100 times more sensitive than rodents to the toxicity associated with random retinoid diffusion. More specifically, these considerations raise the question on whether there is a risk that through random diffusion, retinol absorbed across the skin accumulates in the subcutaneous white fat contributing thereby to increase plasma RBP. Another possibility is that retinol, which is frequently applied on the face and the neck, might be transported by diffusion or bound to lipoproteins across the blood/brain barrier in a similar way as it can cross the placental barrier.

The studies by Sass et al. (1996) and Nohynek et al. (2006) (described above – see section 3.3.3.) with retinol-based cosmetic products provide little insight into the fate of retinol after regular skin application. These studies, focused on measurements in plasma, found no significant increase of circulating retinol or other retinoids, which is not surprising. Because of the tight homeostatic regulation, plasma retinol is indeed known to be an insensitive biomarker of the hepatic store of retinol and *a fortiori* of the extra-hepatic stores. In addition, these studies did not measure plasma RBP, which as explained above is a key factor in the metabolism of retinol. Last, there is no indication in these studies that blood samples were taken under fasting conditions. Even though volunteers were under dietary restriction, the possibility cannot be excluded that the results were confounded by the postprandial increase of retinol bound chylomicrons, which may transiently predominate over RBP-bound retinol in plasma.

3.3.6 Photo-induced toxicity

3.3.6.1 Phototoxicity / photo-irritation and photosensitisation

Phototoxicity *in vitro*

The phototoxic potential of retinyl palmitate was studied *in vitro* using the 3T3 Neutral Red Uptake (NRU) assay. Results show that retinyl palmitate (n=2) was not phototoxic *in vitro* (Gomes Benevenuto et al., 2015).

Phototoxicity/photoirritation *in vivo*

Guideline:	/
Species/strain:	Guinea pig/Himalayan white spotted (outbred)
Group size:	5 male and 5 females
Test substance:	Retinyl Palmitate (1.7 mIU/g (935 mg/g)) with 2% DMSO
Batch:	710758
Purity:	approximately 95%
Vehicle:	/
Dose levels:	Undiluted
Dose volume:	0.025 ml per 2 cm^2
Route:	Open epicutaneous induction

Negative control:	Exposed to retinyl palmitate, but not to UV
Positive control:	/
Light source:	UV Lamp Westinghouse FS 40 "Black Lamp" (1x10 exp. 4 Ergs/cm²/sec, 320 – 400 nm)
Irradiation:	20 J/cm² UV-A
Duration of irradiation:	30 minutes
GLP statement:	In compliance
Study period:	October – December 1988

The phototoxic property of retinyl palmitate was evaluated in guinea pigs according the Cosmetic Toiletry and Fragrance Association (CTFA) safety testing guidelines and methodology published by Harber and Shalita (1975). Animals were exposed to the test item (0.025 mL per 2 cm²) by an open epicutaneous application. To enhance skin penetration, 2% DMSO was incorporated in the test substances. About 30 min after application of the test item to the left flank, the animals were exposed to UV-A irradiation (20 J/cm²). After irradiation, the right flanks were treated with the test item but remained unexposed to light, serving as control sites. Skin reactions were examined 24, 48 and 72 after application for signs of erythema and edema and were graded according a scale from 0 to 4. In addition, the animals were observed at least once daily for mortality and clinical signs and were weighed five days prior to treatment and at day one and at termination.

Results
A transient discoloration of the treated skin was observed during day 2 – 4 of the study due to pigment or colouring from the test item. No skin reactions indicative for a phototoxic reaction could be noted in any of the treated animals either with or without UV-A irradiation.

Conclusion
Under the conditions of the study undiluted retinyl palmitate (1.7 mio IU/g (935 mg RP/g)) was shown to have no phototoxic or photo-irritant potential in male and female guinea pigs.

Reference: 125

Photosensitisation *in vivo*

Guideline:	/
Species/strain:	Guinea pig/Himalayan white spotted (outbred)
Group size:	Control group: 5 male and 5 females Test group: 10 males and 10 females
Test substance:	Retinyl Palmitate (1.7 m.i.U/g (935 mg/g)) stabilized with Tocopherol
Batch:	710758
Purity:	approximately 95%
Vehicle:	Olive oil
Dose levels:	Induction 10%; Challenge 3, 10, 30 and 100%
Dose volume:	0.1 ml applied on an area of 8 cm^2
Route:	Open epicutaneous induction and challenge
Negative control:	No topical treatment with vehicle
Positive control:	/
Light source:	UV Lamp Westinghouse FS 40 "Black Lamp" (1x10 exp. 4 Ergs/cm²/sec, 320 – 400 nm); UV-B: UV-B Sunlamp TL/12
Irradiation:	UV-A: 10 J/cm² UV-A; UV-B: 1.8 J/cm²
Duration of irradiation:	30 minutes
GLP statement:	In compliance
Study period:	October – December 1988

The photosensitising potential of retinyl palmitate was evaluated according CTFA safety testing guidelines and methodology published by Harber and Shalita (1975). For the induction phase, animals first received 4 intradermal injections of Freund's complete adjuvant and physiological saline 50:50 (0.1 mL) into the corners of shaved skin of 8 cm² in

the neck. This was followed by application of 0.1 mL of 10% retinyl palmitate. After 30 minutes the test site was exposed to 1.8 J/cm² UV-B and 10 J/cm² UV-A irradiation. The topical application and irradiation procedure was repeated 4 times in 2 weeks without injection of the adjuvant. Control animals received 4 intradermal adjuvant injections in the neck area as well, without any further topical treatment.

Three weeks after the induction animals were challenged by open application of 0.025 mL/2 cm² of 3, 10, 30 and 100% of the test item on left flanks. After 30 minutes animals were exposed to 10 J/cm² UV-A irradiation. The right flanks were treated in a similar way, but were not irradiated. Skin reactions were examined 24, 48 and 72 after application for signs of erythema and edema and were graded according a scale from 0 – 4. In addition, mortality, clinical signs and body weight gains was monitored during the experiment.

Results
With the exception of one spontaneous death of a control female, no mortality or clinical sign of toxicity occurred and the body weight gain was not affected. Control animals and test animals showed a transient edema and erythema in the neck area of grade 1–4 lasting from day 2 to 7. Skin necrosis was observed from day 8–12 and exfoliation from day 13–26. These findings were attributed to the intradermal adjuvant injections. No erythema or edema was observed at any of the challenge readings, either in the irradiated or non-irradiated animals.

Conclusion
Under the conditions of this study retinyl palmitate (1.7 mio IU/g (935 mg RP/g)) was shown to possess no photosensitisation potential.

Reference: 128

SCCS overall conclusion on phototoxicity/photo-irritation and photosensitisation
In view of the low skin permeability of the test compound, the duration of exposure in the photosensitisation study may have been too short. The results from the *in vitro* and *in vivo* studies do not indicate that retinyl palmitate has a phototoxic/ photo-irritant or a photosensitising potential.

3.3.6.2 Photocarcinogenicity

In 2000, the Center for Food Safety and Applied Nutrition (CFSAN) within the US-FDA referred retinyl palmitate to National Toxicology Program (NTP) for phototoxicity and photocarcinogenicity testing. In 2003, NTP begun a one-year photococarcinogenesis study to determine whether the topical application of creams containing retinoic acid or retinyl palmitate would alter the process of photocarcinogenesis in more than 70 hairless (SKH-1) mice exposed to simulated solar light (SSL), UVA, or UVB (NTP, 2012).

The NTP report concluded that under the conditions of these studies, the topical treatment of SKH-1 mice with the control cream (vehicle without retinyl palmitate) resulted in higher incidences and multiplicities of squamous cell neoplasms of the skin when compared to untreated controls in the absence and presence of SSL. The control cream consisted of 85% base cream (EDTA, glycerin, carbopol981, mineral oil, BRIJ 721, stearic acid, cetearyl alcohol, octyl palmitate, germaben, water) and 15% diisopropyl adipate. Compared to the control cream, retinyl palmitate further enhanced the photocarcinogenic activity of SSL in SKH-1 mice based upon increased incidences and multiplicities of squamous cell neoplasms of the skin.

The findings were reviewed by the Norwegian Scientific Committee on Food Safety (VKM 2012) and by Wang et al (2010). The VKM report states that although the causative mechanisms of photococarcinogenesis demonstrated in the NTP study are not clear, one

plausible mechanism may be related to the formation of free radicals and photomutagenic effects observed in cells exposed to retinoids and UV light. It is reported that retinyl palmitate in combination with UVA light produces genotoxic effects in mouse lymphoma or human Jurkat T-cells via a photoclastogenic mode of action, most likely based on oxidative DNA damage from enhanced free radicals production (Mei et al., 2005; 2006; 2010, Fu et al., 2007). Vitamin A in the skin resides in a complex environment that in many ways is very different from the chemical environment in solution and in *in vitro* test systems. Relevant clinical studies or studies in animal models are therefore needed to establish whether the pro-oxidant activity of photoexcited vitamin A is observed *in vivo*, and to assess the related risks (Fu et al., 2007).

Hairless mice are also particularly sensitive to the development of UVR-induced skin cancer (Benavides, 2009). It should be noted that in the NTP study the irradiated sites treated with the vehicle (without retinyl palmitate) developed more skin tumours than the blank irradiated sites. According to the VKM opinion (VKM, 2012), extrapolation of the NTP results from highly susceptible hairless mouse skin to human skin cannot be done directly. The mouse is a typical nocturnal animal with a skin normally covered with fur, and is not adapted to intense UV light. The functional profile of the natural skin retinoids may be different in man and mouse. In humans, the local retinoids may in fact be involved in the protection system of various antioxidants in the skin.

The commentary by Wang et al. (2010) (Reference: 160) calls for extreme caution when extrapolating the NTP animal study to humans. Their abstracted data show that the increased number of malignant skin neoplasms over the vehicle exposed skin was only apparent from the lower (6.75 mJ/cm^2) UV dose, while there was no difference between vehicle and retinyl palmitate cream in the higher (13.7 mJ/cm^2) UV dose. They point out that after more than 40 years use of topical retinoids, and the medical follow up, there is no published evidence of increased risk of photocarcinogenesis, and that oral and topical retinoids are in fact used for chemoprevention of skin cancers in individuals at high risk.

A CIR Expert Panel also suggested that the NTP photococarcinogenesis study was confounded and compromised by the effects of the control cream, containing diisopropyl adipate in the vehicle. The Panel reported findings of a photopatch study in volunteers, demonstrating that formulations containing up to 17% diisopropyl adipate in the vehicle were not phototoxic, primary irritant or sensitiser, thus indicating that SKH-1 hairless mice used in the NTP photococarcinogenesis study are much more sensitive to the potential phototoxicity of diisopropyl adipate than human subjects. It was argued that the use of 13% to 15% diisopropyl adipate in the vehicle in the NTP study limits the use of the study results for the safety and risks assessment of dermal exposure to RP in cosmetic products (CIR, 2013).
The SCCS is aware that a new study on photocarcinogenicity is currently ongoing at the US National Center for Toxicological Research (study E0218501).

SCCS conclusion
The SCCS agrees with the VKM Opinion that the NTP data may indicate that retinyl palmitate could be photocarcinogenic in hairless mice, but that these results do not provide conclusive information for a risk assessment based on this effect of retinol and retinyl esters in cosmetics.

3.3.7 Human data

There are only sporadic case reports on contact allergy to retinol palmitate, retinyl palmitate and retinyl acetate, indicating that the risk of sensitisation, despite widespread use, is negligible (Blondeel, 1984; Clemmensen, 2007; Heidenheim 1995).

The VKM report has an overview of studies on the effects of different retinoids applied on the skin which were not primarily intended to evaluate skin irritation, but which allowed an extraction of skin irritancy data (VKM, 2012). Safe upper concentrations could not be defined.

While the retinoic acids are known to enhance non-specific UV sensitivity, there are no case reports that show phototoxic or photosensitising properties of retinol, retinyl acetate, retinyl palmitate, retinyl linoleate or Retinal. A study in humans on UV filter formulations containing retinyl palmitate showed no phototoxicity. However, the design of this study, in which retinyl palmitate was not tested as an individual ingredient but in formulations that contained also UV-filters, does not permit a conclusion on the phototoxicity nor photosensitisation of retinyl palmitate in humans (Gomes Benevenuto et al., 2015).

No case reports or other human studies with retinyl linoleate and retinal could be identified.

The CIR has reviewed a study on the phototoxicity of a face cream containing 0.4% retinyl propionate in 10 healthy adult subjects. It was concluded that the face cream does not possess a detectable phototoxic potential in human skin (CIR 2013).

3.3.8 Special investigations

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3.4 Exposure Evaluation

3.4.1 Exposure from cosmetic products

For the calculation of a Margin of Safety, the SCCS recommendations presented in the Notes of Guidance (2015) were followed. The exposure estimation is based on the maximum concentrations (in RE or IU) of retinol, retinyl palmitate and retinyl acetate typically used in cosmetic preparations in the EU and are summarised in the following table.

The applicants have pointed out that to the best of their knowledge these ingredients are not used in sunscreen cosmetic products sold in the EU.

The maximum concentrations (in RE), of retinol retinyl palmitate and retinyl acetate typically used in cosmetic preparations in the EU can be summarised as follows:

Product category	RE (%) [#]
Face and hand creams and other leave-on products	0.3
Body lotions	0.05
Rinse-off products	0.3
[#] RE = retinol equivalents, i.e. retinyl palmitate and retinyl acetate at corresponding retinol concentrations	

According to the known usage concentrations and, based on standard exposure parameters cited in the SCCS Notes of Guidance, the following exposure scenarios have been taken into consideration for the calculation of the systemic exposure dose (SED) and the respective margin of safety (MoS). A cumulative exposure includes the recognised uses of these ingredients in cosmetic products and is used to estimate an overall MoS for cosmetic products.

For adults:

Product type	Estimated daily amount applied	Retention factor [6]	Calculated daily exposure (g/day)	Calculated relative daily exposure (mg/kg bw/day) *
Face cream	1.54 g	1.0	1.54	25.67
Hand cream	2.16 g	1.0	2.16	36.00
Body Lotion	7.82 g	1.0	7.82	130.33
Rinse-off products	53.05g	0.01	0.53	8.83
Lipstick, lip salve	0.057 g	1.0	0.057 g	1
Total Aggregated			12.11	201.83
Aggregated (without Lip products)			12.05	200.83

* For an adult with a body weight of 60 kg

In principle, the SED and the MoS can be calculated using dermal absorption data expressed as µg/cm² or as percentage (%) using the respective exposure parameter and the appropriate calculation procedure.

[6] The retention factor was introduced by the SCCNFP to take into account rinsing off and dilution of finished products by application on wet skin or hair (e.g. shower gels, shampoos, …) [SCCNFP/0321/00]

Calculation of the systemic exposure dose (SED in mg/kg bw/day)

Product type	Calculated relative daily exposure (mg/kg bw/day)	Maximum Vitamin A concentration in the product (RE)	Amount of Vitamin A applied (RE mg/kg bw/day)	Dermal Absorption [7]	Vitamin A SED exposure (RE µg/kg bw/day)	Vitamin A SED exposure (RE IU/kg bw/day
Face cream	25.67	0.3%	0.08	7.7%	5.93	19.74
Hand cream	36.00	0.3%	0.11	7.7%	8.32	27.69
Body Lotion	130.33	0.05%	0.07	7.7%	5.02	16.71
Rinse-off products	8.83	0.3%	0.03	7.7%	2.04	6.79
Lip stick	1	0.3%	0.003	100%	3.00	9.99
Total aggregated			0.28		24.3	80.93
Aggregated (without Lip products)			0.28		21.3	70.94

3.4.2 Exposure from other sources

The most important source of Vitamin A in the population is the diet, followed by food supplements and then cosmetics.

Certain foods, such as breakfast cereals, juices or margarines, are often enriched with Vitamin A, and the absorption *via* food supplements also contributes to overall exposure.

The main sources of dietary Vitamin A are preformed Vitamin A in the form of retinol and retinyl esters (from animal foods and supplements) and proVitamin A carotenoids (from plants).

On the basis of data from 12 dietary surveys in nine EU countries, Vitamin A intake was assessed using food consumption data from the EFSA Comprehensive Food Consumption Database and Vitamin A composition data from the EFSA nutrient composition database (EFSA, 2015). Average Vitamin A intake ranged between 409 and 651 µg RE/day in children aged 1 to < 3 years, between 607 and 889 µg RE/day in children aged 3 to < 10 years, between 597 and 1 078 µg RE/day in children aged 10 to < 18 years and between 816 and 1 498 µg RE/day in adults.

In Norway, estimations of the intake of Vitamin A from the diet and supplements were based on national food consumption surveys for children (1-, 2-, 4- and 9-year-olds), adolescents (13-year-olds) and adults (18-70-year-olds) (VKM, 2012).

Consumption studies show that the UL of 10000 IU is already exceeded at the 97.5th percentile of adults. There is clearly no upwards flexibility for additional retinol. The BfR's committee for cosmetic products takes the view that the additional contribution of a substance from cosmetic products should not exceed 10% of the UL (BfR, 2012).

[7] The retention factor was introduced by the SCCNFP to take into account rinsing off and dilution of finished products by application on wet skin or hair (e.g. shower gels, shampoos, …) [SCCNFP/0321/00]

3.4.3 Safety evaluation (including calculation of the MoS)

The BfR in 2002 concluded that the additional uptake of retinol, retinyl palmitate, retinyl linoleate, and retinyl acetate *via* cosmetic products could constitute up to 20% of the Upper Limit (UL) of 10,000 IU/day of Vitamin A. In the case of postmenopausal women and the recommendation by the SCF of an UL of 5000 IE/day, the relative uptake of Vitamin A *via* cosmetic products could be as high as 40%. It should be noticed that these calculations were based on a maximum concentration of 0.1% for body products whereas the maximum concentration level requested by the applicant was 0.05%.

In the VKM opinion (2012), the Panel calculated that in the worst-case scenario based on 0.3% in body lotions and 1% in face and hand cream, the contribution of retinol and retinyl esters from cosmetics could reach 42-58% of the ULs for children. The estimates are based on the assumption that absorption of retinol from topical products is 5.7%.

Based on the calculations above (see section 3.4.2), exposure to Vitamin A (retinol, retinyl palmitate, and retinyl acetate) may lead to daily systemic dose of:

- *via* face cream 5.93 RE µg/kg bw/day or 19.7 IU /kg bw/day which is equivalent to 1185 IU for an adult of 60 kg. This exposure could constitute up to **24%** of the UL of 5000 IU/day of Vitamin A. The estimates are based on the assumption that absorption of retinol from topical products is 7.7 %.
- *via* hand cream 8.32 RE µg/kg bw/day or 27.7 IU /kg bw/day which is equivalent to 1661 IU for an adult of 60 kg. This exposure could constitute up to **33%** of the UL of 5000 IU/day of Vitamin A. The estimates are based on the assumption that absorption of retinol from topical products is 7.7 %.
- *via* body lotion 5.02 RE µg/kg bw/day or 16.7 IU /kg bw/day which is equivalent to 1003 IU for an adult of 60 kg. This exposure could constitute up to **20%** of the UL of 5000 IU/day of Vitamin A. The estimates are based on the assumption that absorption of retinol from topical products is 7.7 %.
- *via* rinse-off products 2.04 RE µg/kg bw/day or 6.8 IU /kg bw/day which is equivalent to 408 IU for an adult of 60 kg. This exposure could constitute up to **8.1%** of the UL of 5000 IU/day of Vitamin A. The estimates are based on the assumption that absorption of retinol from topical products is 7.7 %.
- *via* lip products 3 RE µg/kg bw/day or 10 IU /kg bw/day which is equivalent to 600 IU for an adult of 60 kg. This exposure could constitute up to **12%** of the UL of 5000 IU/day of Vitamin A.

Based on the calculations above, exposure to Vitamin A (retinol, retinyl palmitate, and retinyl acetate) *via* all cosmetic products (including lip products) may lead to a daily systemic dose of 24.3 RE µg/kg bw/day or 80.9 IU /kg bw/day, which is equivalent to 4855 IU for an adult of 60 kg. This exposure could constitute up to **97%** of the UL of 5000 IU/day of Vitamin A. This calculation is based on a worst-case scenario assuming that all the cosmetic products used (hand and face cream, body lotion, rinse-off products, products for the lips) contain Vitamin A at the maximum concentrations.

Based on the calculations above exposure to Vitamin A (retinol, retinyl palmitate, and retinyl acetate) *via* all cosmetic products (without lip products) may lead to daily systemic dose of 24.3 RE µg/kg bw/day or 70.94 IU /kg bw/day which is equivalent to 4256 IU for an adult of 60 kg. This exposure could constitute up to **85%** of the UL of 5000 IU/day of Vitamin A. This calculation is based on a worst case scenario assuming that all the cosmetic products used (hand and face cream, body lotion, rinse-off products) contain Vitamin A at the maximum concentrations.

For children between 1 and 3 years old, based on the same calculation as above but with a body weight of 15 kg, exposure to Vitamin A (retinol, retinyl palmitate, and retinyl acetate) may lead to daily systemic dose of:

- *via* face cream to 296 IU. This exposure could constitute up to **11%** of the UL of 2700 IU/day of Vitamin A.
- *via* hand cream to 415 IU. This exposure could constitute up to **15.3%** of the UL of 2700 IU/day of Vitamin A.
- *via* body lotion to 251 IU. This exposure could constitute up to **9%** of the UL of 2700 IU/day of Vitamin A.
- *via* rinse-off products to 102 IU. This exposure could constitute up to **3.8%** of the UL of 2700 IU/day of Vitamin A.

Based on the calculations above exposure to Vitamin A (retinol, retinyl palmitate, and retinyl acetate) *via* all cosmetic products (without lip products) may lead to daily systemic dose of 1064 IU for a child of 15 kg. This exposure could constitute up to **39%** of the UL of 2700 IU/day of Vitamin A. This calculation is based on a worst-case scenario assuming that all the cosmetic products used (hand and face cream, body lotion, rinse-off products) contain Vitamin A at the maximum concentrations.

For children between 4 and 6 years old, the same calculation will lead to lower exposure estimation to vitamin A compared to the UL of 3700 IU/day for this sub population.

Topical application of cosmetic products as estimated in the standard scenarios (0.05% in body lotions and 0.3% in face and hand cream), increases the total exposure to Vitamin A (retinol and retinyl esters). Based on the maximum concentration of Vitamin A in the cosmetic products and a skin penetration of 7.7 %, the systemic exposure to Vitamin A calculated is below the Upper Limit for the dedicated subpopulation (adult including women suffering osteoporosis and children between 1 and 6 years old).

3.4.4 Discussion

The risk assessment of Vitamin A (retinol and retinyl esters) in cosmetics and the considerations of the total exposure to Vitamin A in this opinion are based on earlier opinions from the Scientific Committee on Food (SCF) and the European Food Safety Authority (EFSA) on the Tolerable Upper Intake Level (UL) of preformed Vitamin A (retinol and retinyl esters). The Scientific Committee on Consumer Product's (SCCS's) Notes of Guidance for the Testing of Cosmetic Ingredients and Their Safety Evaluation (8^{th} revision) and recent evaluations on the use of retinol and its esters in cosmetics from the Federal Institute for Risk Assessment (BfR) in Germany, from the Norwegian Scientific Committee for Food Safety (VKM) have been considered for specific issues related to exposure to cosmetic products in this Opinion.

Physicochemical properties

The purity, impurities and stability tests have not been performed according to the SCCS Notes of Guidance:

-No raw analytical data has been provided regarding purity for the batches used in these studies. According to the specification sheets UV spectrophotometry was used to calculate the content of retinol, retinyl palmitate and retinyl acetate. It is unclear if the HPLC method mentioned as an article publication in Ref 75 was applied to the analysis of the batches used

in toxicity testing. In addition, this article (ref 75) does not include retinyl acetate, retinyl linoleate or retinal.

-No information for the determination of impurities was provided for retinol, retinyl acetate or retinol palmitate.
- SCCS reminds the Applicant that retinoic acid is banned in cosmetic products in the EU (Annex 2, entry 375) and therefore it should not be present in the cosmetic products with the exception of occurring as an unavoidable trace impurity for which a justified limit is provided.
-No data are available on the stability. The use of retinol in cosmetic products needs to be stabilized through final formulations.

Function and uses

Vitamin A is used as a cosmetic ingredient at maximum use concentrations of 0.05% (retinol equivalents) in body lotions, 0.3% (retinol equivalents) in hand and face creams as well as in other leave-on or rinse-off products. These products are usually presented as anti-wrinkle products.
Retinoic acid is banned in cosmetic products in the EU whatever the concentration (Annex 2, entry 375).
The maximum concentrations (in RE), of retinol retinyl palmitate and retinyl acetate typically used in cosmetic preparations in the EU can be summarised as follows:

Product category	RE (%) [#]
Face and hand creams and other leave-on products	0.3
Body lotions	0.05
Rinse-off products	0.3
[#] RE = retinol equivalents, i.e. retinyl palmitate and retinyl acetate at corresponding retinol concentrations	

Toxicological Evaluation

Acute toxicity

Acute Vitamin A toxicity is rare and associated with hypervitaminosis A, which in children is more likely to occur due to overdosage with Vitamin A supplements than due to following high Vitamin A intake from food, when the plasma Vitamin A concentration exceeds the capacity of Retinol Binding Protein (RBPs) leading to a change in the ratio of free retinol to retinol-RBP complexes and binding of free retinol to lipoproteins. High food intakes of preformed Vitamin A but not beta-carotene and other proVitamin A carotenoids can be acutely toxic and weight, food fat content and general health are the crucial factors in determining the Vitamin A acute toxicity dose. Commonly observed clinical symptoms of Vitamin A acute toxicity in children include anorexia, bulging fontanelles, drowsiness, lethargy, irritability and vomiting.

In experimental acute oral toxicity studies, Vitamin A (retinol, retinyl palmitate, retinyl acetate) was found to be of low toxicity in laboratory animal species (LD50 values in rodents >2000 mg RE/kg bw).

The ranking of acute oral toxicity declines in the order of retinol > retinyl acetate > retinyl palmitate.

Local toxicity

Under the conditions of the *in vivo* studies, retinyl acetate and retinyl palmitate are moderately irritating to the rabbit skin. The SCCS noted that the vehicle, peanut oil, is also slightly irritating to the rabbit skin under the conditions of the *in vivo* study. However, the identity of the test substance / purity in terms of retinyl acetate or retinyl palmitate is not clear to the SCCS based on the information in the original studies precluding a final conclusion on the skin irritation potential of retinyl acetate and retinyl palmitate.

Under the conditions of the *in vivo* study, retinol (tested as a formulation) is a moderate irritant to the rabbit eye, and retinyl acetate and retinyl palmitate (tested either undiluted or as a 30% dilution) are slightly irritating to the rabbit eye. However, the identity of the test substance / purity is not clear to the SCCS, based on the information in the original studies precluding a final conclusion on the eye irritation potential of retinol, retinyl acetate and retinyl palmitate.

Considering that Vitamin A in cosmetics is used in a variety of products at concentrations not exceeding 0.3% RE in hand/face creams and in other leave-on or rinse-off products, and up to 0.05% RE in body lotions, it can be concluded that there is no risk of skin or eye irritation for the consumer from these exposures to cosmetic products.

Sensitisation

The evaluation of both skin sensitisation tests (Buehler and OET) with retinol is hampered by a decreasing irritation threshold after repeated topical administration and in the Buehler test by the usage of different vehicles. Skin reactions in the Buehler test suggest that skin sensitisation by retinol concentrations of 2.5% cannot be ruled out. The results of the open application test (according to the method of Klecak) are inconclusive and most likely due to irritation. The pre-study irritation dose finding was based on a single exposure of unknown duration. Retinyl acetate and palmitate exhibited no potential to induce dermal sensitisation in Guinea pigs in the Maximization test according to Magnusson and Kligman. Studies evaluating the sensitisation potential by LLNA could not be identified.

In view of the sparse case reports on sensitisation in humans despite widespread exposure in cosmetics (see 3.3.11 Human data), the SCCS considers the risk of sensitisation to retinol, retinyl acetate and retinyl palmitate as negligible.

Dermal absorption

Several *in vitro* and *in vivo* investigations on dermal bioavailability in experimental systems are available. The majority of these studies was performed with retinyl palmitate at cosmetic use concentrations and also studied the effect of different vehicles or formulation procedures. In addition, recent *in vitro* penetration data investigating retinol are also available. The studies utilized animal (rat, mice, pig) or human skin as test systems. Most of the studies were not performed under GLP conditions, and suffered from deviations from the current testing requirements/guidelines with regard to methodology and assessment. All available studies showed that, irrespective of the origin of the investigated skin, the major portion of the applied dose could be removed from the application site after the respective exposure periods. A few studies observed some skin penetration, although most of these studies found that the vast majority was adsorbed by the *stratum corneum* and was, thereby, non-bioavailable. Penetration into deeper skin layers (epidermis, dermis) or penetration through the skin (receptor fluid) was also observed but consisted of very small amounts when compared with the total applied dose in most of the studies.

In addition, there were known differences in the skin permeability of the investigated species - in the order of rat/mouse>pig≥human. Differences due to the selected vehicles for the respective formulation were noted as e.g., aqueous vehicles led to about twice the rate of absorption than oily vehicles.

In the *in vivo* studies cited above, no significant increase in plasma levels of retinoids could be detected after repeated applications of retinal, retinol or retinyl palmitate (Sass et al., 1996; Nohynek et al., 2006). This may be due to several factors such as dose and area of application. In the study by Sass, the application area was about 40% of the total body surface area and the dose corresponded to 7 mg of retinal daily for 14 days. In the study by Nohynek, around 19% (3000 cm^2) of the total body surface area was covered daily by an amount of 3.5 g of cream for 21 days. This corresponds to a daily dose of 9 mg of retinol or 16 mg of retinyl palmitate. According to the SCCS's Notes of Guidance (SCCS, 2010), 7.82g of body lotion is the estimated daily exposure level. Furthermore, the mean exposed skin surface area for body lotion is 15 670 cm^2. Thus, both the doses applied and the application area in the studies by Sass and Nohynek are much lower than those used by SCCS for risk assessment. In addition, serum retinol concentrations are not considered as an efficient biomarker of exposure in individual patients because of the homeostatic regulation of Vitamin A. An increase in plasma levels of retinoids after topical application may not be expected due to the storage capacity and the tightly controlled low-level conversion of retinol to retinoic acid in the skin. However, both *in vivo* and *in vitro* studies demonstrate that topical application is effective with respect to loading the skin with substantial levels of retinoids.

For the calculation of the MoS, the SCCs has used for dermal penetration the study from Yourick et al. 2008 (Reference: 168) described above. As in this study, standard errors (SEM) were provided instead of Standard Deviation (SD), SD were calculated by SCCS. Because the number of donors was not according to the SCCS requirements, mean + 2SD were used which resulted in an estimated value of 7.7 % skin penetration to be used for the calculation of the margin of safety.

Repeated dose toxicity

Both acute and chronic excessive intake of Vitamin A may result in hypervitaminosis A, which includes a number of systemic adverse effects. The teratogenic effect of excessive intake of Vitamin A or specific retinoids is well documented in both animals and humans.

The SCCS considers that the teratogenic potential, effects on liver and local effects in the skin of Vitamin A are the most critical toxicological endpoints. For assessing the systemic toxicity of Vitamin A after cosmetic exposure, the SCCS has relied on the Tolerable Upper Intake Level (UL) for preformed Vitamin A of:

- 3 000 µg RE/day (or 10 000 IU) for women of childbearing age and men. This UL also applies during pregnancy and lactation.
- 800 µg RE/day (2700 IU) for children aged 1–3 years,
- 1 100 µg RE/day (3700 IU) for children aged 4–6 years,
- 1 500 µg RE/day (5000 IU) for children aged 7–10 years,
- 2 000 µg RE/day (6700 IU) for children aged 11–14 years and
- 2 600 µg RE/day (8700 IU) for children aged 15–17 years.

To take into account more susceptible population, such as women suffering from osteoporosis or children above 6 years old who may also be exposed to Vitamin A *via* cosmetic products, the SCCS has used the value of 1500 µg RE/day (5000 IU) for the safety assessment of Vitamin A in cosmetic products. This value is appropriate for women of

childbearing age but also for middle age and elderly women who may suffer decreasing bone density, as well as for men and children.
For children aged 1-3 years the SCCS has used the value of 800 µg RE/day (2700 IU) for the safety assessment of Vitamin A in cosmetic products. Application of Vitamin A-containing baby skin care products such as body lotions and creams were considered by SCCS relevant for 1- and 3-year old children.

For children aged 4-6 years, the SCCS has used the value of 1100 µg RE/day (3700 IU) for the safety assessment of Vitamin A in cosmetic products.

Toxicokinetics

While many advances have been made in understanding the metabolism of retinol absorbed by the oral route, little is known about the metabolic fate of retinol or retinoids after skin absorption. What has been established is that these lipophilic compounds are absorbed across the skin with estimated absorption rates in the range of 5-8%. It has also been established that the skin can metabolise retinoids and synthesise cellular RBP, meaning that the skin has some storage capacity for retinol. By contrast, there is a complete lack of data regarding the distribution of absorbed retinol and importantly, regarding the proportion of absorbed retinol that is not taken by the RBP-dependent transport and storage pathways.

The studies by Sass et al. (1996) and Nohynek et al. (2006) with retinol-based cosmetic products provide little insight into the fate of retinol after regular skin application.

Phototoxicity/photo-irritation and photosensitisation

Phototoxicity and photo sensitisation have been studied for retinyl pamitate. The results from the *in vitro* and *in vivo* studies do not indicate that retinyl palmitate has a phototoxic/ photo-irritant or a photosensitising potential. There are no clinical reports and other human data that indicate a phototoxic or photosensitising capacity of Retinol, Retinyl acetate, Retinyl palmitate, Retinyl linoleate or Retinal.
Although there are indications that topical retinyl palmitate could be photocarcinogenic in hairless mice, the ongoing scientific debate on this issue (which includes a challenge of the appropriateness of the test-vehicle used) precludes an extrapolation of these findings from the very susceptible mouse skin to human skin.

Exposure via cosmetic products

For adults, based on the calculations above, exposure to vitamin A (retinol, retinyl palmitate, and retinyl acetate) *via* all cosmetic products (including lip products) may lead to daily systemic dose of 24.3 RE µg/kg bw/day or 80.9 IU /kg bw/day which is equivalent to 4855 IU for an adult of 60 kg. This exposure could constitute up to **97%** of the UL of 5000 IU/day of Vitamin A. This calculation is based on a worst-case scenario assuming that all the cosmetic products used (hand and face cream, body lotion, rinse-off products, products for the lips) contain Vitamin A at the maximum concentrations.

Based on the calculations above, exposure to Vitamin A (retinol, retinyl palmitate, and retinyl acetate) *via* all cosmetic products (without lip products) may lead to daily systemic dose of 24.3 RE µg/kg bw/day or 70.94 IU /kg bw/day which is equivalent to 4256 IU for an adult of 60 kg. This exposure could constitute up to **85%** of the UL of 5000 IU/day of Vitamin A. This calculation is based on a worst-case scenario assuming that all the cosmetic products used (hand and face cream, body lotion, rinse-off products) contain Vitamin A at the maximum concentrations.

For children between 1 and 3 years old, based on the calculations above, exposure to Vitamin A (retinol, retinyl palmitate, and retinyl acetate) *via* all cosmetic products (without lip products) may lead to daily systemic dose of 1064 IU for a child of 15 kg. This exposure could constitute up to 39% of the UL of 2700 IU/day of Vitamin A. This calculation is based on a worst-case scenario assuming that all the cosmetic products used (hand and face cream, body lotion, rinse-off products) contain Vitamin A at the maximum concentrations.

For children between 4 and 6 years old, the same calculation will lead to lower exposure estimation to Vitamin A comparing to the UL of 3700 IU/day for this sub population.

4. CONCLUSION

1. *On the basis of data provided does the Scientific Committee on Consumer Safety (SCCS) consider Vitamin A (retinol, retinyl palmitate, and retinyl acetate,) safe when used as cosmetic ingredient:*

(a) in body lotions up to the maximum concentration of 0.05 % of retinol equivalent?

The SCCS has estimated that exposure to Vitamin A (retinol, retinyl palmitate, and retinyl acetate) *via* **body lotion** at the maximum concentration of 0.05% may lead to a daily systemic dose of 1003 IU for an adult. This exposure would constitute up to 20% of the Upper Limit (UL) of 5000 IU/day of Vitamin A. Therefore, the SCCS considers that the use of Vitamin A **in body lotions** *per se* is safe.

(b) in hand/face cream, leave-on (other than body lotions) and rinse-off products up to the concentration of 0.3 % of retinol equivalent?

The SCCS has estimated that exposure to Vitamin A (retinol, retinyl palmitate, and retinyl acetate):

- *via* **hand cream** at the maximum concentration of 0.3% may lead to daily systemic dose of 1661 IU for an adult. This exposure could constitute up to 33% of the UL of 5000 IU/day of Vitamin A. Therefore, the SCCS considers that the use of Vitamin A in **hand cream products** *per se* is safe.

- *via* **face cream** at the maximum concentration of 0.3% may lead to daily systemic dose of 1185 IU for an adult. This exposure could constitute up to 24% of the UL of 5000 IU/day of Vitamin A. Therefore, the SCCS considers that the use of Vitamin A **in face cream products** *per se* is safe.

- *via* **rinse-off products** at the maximum concentration of 0.3% may lead to a daily systemic dose of 408 IU for an adult. This exposure could constitute up to 8.8% of the UL of 5000 IU/day of Vitamin A. Therefore, the SCCS considers that the use of Vitamin A **in rinse-off products** *per se* is safe.

The SCCS has also estimated that exposure to Vitamin A (retinol, retinyl palmitate, and retinyl acetate) from **all cosmetic products** (including lip products) may lead to a daily systemic dose of 4855 IU for an adult. This exposure could constitute up to **97%** of the UL of 5000 IU/day of Vitamin A. Excluding lip products, the daily systemic dose is estimated at 4256 IU for an adult, which constitutes up to **85%** of the UL of 5000 IU/day of Vitamin A.

It is of note that these estimates are based on a worst-case scenario assuming that all the cosmetic products used (hand and face cream, body lotion, rinse-off products, products for the lips) contain Vitamin A at the maximum concentrations.

If no, what concentration limits in the above mentioned categories of cosmetic products does the SCCS consider Vitamin A to be safe?

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2. *The SCCS is asked, when making the assessment, to take into account the specific age and sex groups who might be particularly susceptible to the effects of Vitamin A, such as the use of lip products for fertile age and postmenopausal women.*

The SCCS has considered that the teratogenic potential of Vitamin A, and effects on liver and local effects in the skin are the most critical toxicological endpoints. For assessing the systemic toxicity of Vitamin A after cosmetic exposure, the SCCS has relied on the Tolerable Upper Intake Level (UL) for preformed Vitamin A (section 3.4.4).

- To take into account more susceptible population groups such as women suffering osteoporosis or children above 6 years old who may also be exposed to Vitamin A *via* cosmetic products, the SCCS has used the value of 1500 µg RE/day (5000 IU) for the safety assessment of Vitamin A in cosmetic products. This value is appropriate for women of childbearing age and also for middle age and elderly women who may suffer decreasing bone density as well as men and children above 6 years. Based on information provided by the cosmetic industry, Vitamin A and esters are not used for children in the EU. However, based on a theoretical scenario, exposure to Vitamin A *via* these products has been assessed in this opinion for children above 1 year.

- The SCCS has used the value of 800 µg RE/day (2700 IU) for the safety assessment of Vitamin A in cosmetic products for children aged 1-3 years. Application of Vitamin A-containing baby skin care products such as body lotions and creams were also considered by SCCS relevant for 1- and 3-years old children. The SCCS has estimated that exposure to Vitamin A (retinol, retinyl palmitate, and retinyl acetate) *via* all cosmetic products may lead to a daily systemic dose of 1064 IU for a child of 15 kg. This exposure could constitute up to **39%** of the UL of 2700 IU/day of Vitamin A.

It is of note that these estimates are based on a worst-case scenario assuming that all the cosmetic products used (hand and face cream, body lotion, rinse-off products) contain Vitamin A at the maximum concentrations. Based on these estimates, the SCCS considers that the use of Vitamin A in the respective cosmetic products at the maximum notified concentration *per se* is safe for children above 1 year old.

3. *Does the SCCS have any further scientific concerns with regard to the use of Vitamin A (retinol, retinyl palmitate, and retinyl acetate,) in cosmetic products?*

- Based on information provided by the applicants, Vitamin A and esters are not used **in sunscreen products** in the EU. Therefore exposure to Vitamin A *via* these products has not been assessed in this Opinion.

- Based on information provided by the cosmetic industry, Vitamin A and esters are not used for children in the EU. However, application of Vitamin A-containing baby skin care products, such as body lotions and creams, were considered by the SCCS safe for 1- and 3-year old children. Exposure to Vitamin A *via* these products for children below 1 year has not been assessed in this Opinion.

- Retinyl linoleate and retinal may also be used in cosmetic products. However, since no specific data were provided by the applicant, these two Vitamin A derivatives have not been assessed in this Opinion.

- Exposure to Vitamin A may also occur from sources other than cosmetic products. The most important source of Vitamin A in the population is diet, followed by food supplements and cosmetics. This assessment has not taken into account people taking dietary supplement containing Vitamin A.

- On the basis of data from 12 dietary surveys in nine EU countries, Vitamin A intake was assessed and average intake ranged between 409 and 651 µg RE/day in children aged 1 to <3 years; between 607 and 889 µg RE/day in children aged 3 to <10 years; between 597 and 1 078 µg RE/day in children aged 10 to <18 years; and between 816 and 1498 µg RE/day in adults. Therefore exposure to Vitamin A *via* food may already be very close to the UL and any additional source of exposure, including cosmetic products, may exceed this UL. It is however not up to the SCCS to advise which portion of the UL should be dedicated to the different sources of exposure. For example, when assessing exposure to chemicals *via* toys or drinking water, usually 10% or 20% of the reference value is considered. In the case of Vitamin A, these portions would be equivalent to 150 or 300 µg RE/day, which means that at the maximum-notified concentrations, the use of hand and face cream products, rinse-off products, body lotion and cosmetic products for lips may lead to exceeding this value.

- No information for the determination of impurities was provided for retinol, retinyl acetate and retinol palmitate. SCCS reminds the Applicant that retinoic acid is banned in cosmetic products in the EU (Annex 2, entry 375) and therefore it should not be present in the cosmetic products with the exception of occurring as an unavoidable trace impurity for which a justified limit is provided.

- No data are available on the stability of Vitamin A in different product formulations. The use of retinol in cosmetic products will need to be stabilised through final formulations.

5. MINORITY OPINION

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6. REFERENCES

1. References from dossier on Vitamin A (Retinol, Retinyl Acetate and Retinyl Palmitate) in cosmetic products – Cosmetics Europe - October 2011

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