Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Risk assessment of vitamin A (retinol and retinyl esters) in cosmetics

Opinion of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics of the Norwegian Scientific Committee for Food Safety

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Ragna Bogen Hetland (Chair)

Berit Granum

Claus Lützow-Holm

Jan Ludvig Lyche

Jan Erik Paulsen

Vibeke Thrane

Contributors

Persons working for VKM, either as appointed members of the Committee or as *ad hoc* experts, do this by virtue of their scientific expertise, not as representatives for their employers. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has appointed a working group consisting of both VKM members and external experts to answer the request from the Norwegian Food Safety Authority. The members of the working group are acknowledged for their valuable work on this opinion.

The members of the working group are:

VKM members
Ragna Bogen Hetland, Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics (Chair)

Berit Granum, Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics

Jan Ludvig Lyche, Panel on Nutrition, Dietetic Products, Novel Food and Allergy

Jan Erik Paulsen, Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics

Vibeke Thrane, Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics

External experts
Claus Lützow-Holm, Huddoktoren, self-employed

Assessed by

The report from the working group has been evaluated and approved by the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics of VKM.

Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics:

Inger-Lise Steffensen (Chair), Jan Alexander, Mona-Lise Binderup, Knut Helkås Dahl, Berit Granum, Ragna Bogen Hetland, Trine Husøy, Jan Erik Paulsen, Tore Sanner, Vibeke Thrane.

Scientific coordinators from the secretariat

Tor Øystein Fotland, Inger Therese Laugsand Lillegaard.

Summary

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has at the request of the Norwegian Food Safety Authority (Mattilsynet) conducted an assessment of the risk related to the use of retinol and retinyl esters in cosmetic products. VKM was asked to assess both systemic and local effects of vitamin A for different age groups in the Norwegian population. The total exposure to vitamin A for different age groups should be estimated, taking into account both oral and dermal exposure routes, and include exposure scenarios that illustrate the influence of changing maximum authorised concentration levels of the retinol and retinyl esters used in cosmetics. The assessment has been performed by the VKM Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics.

Vitamin A constitutes a group of lipid-soluble compounds including retinyl esters, retinol and retinal. 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). Vitamin A is essential throughout life as it is required in numerous physiological functions. Retinol and retinyl esters are widely used ingredients in skin care products, such as anti-wrinkle products, moisturisers and sunscreens, due to their effects on various biological processes in the skin.

Both acute and chronic excessive intake of vitamin A may result in hypervitaminosis A which includes a number of systemic adverse effects. The teratogenic potential, effects on bone and local effects in the skin of vitamin A were considered as the most critical toxicological endpoints in this opinion and have therefore been dealt with in more detail than other possible adverse effects.

The risk characterisation for all age groups in the present opinion is based on the tolerable upper intake levels (UL) derived from earlier opinions from the Scientific Committee of Food (SCF) and European Food Safety Authority (EFSA). In 2002, the SCF derived an UL of 3000 µg retinol equivalents (RE)/day for all women of child-bearing age based on the teratogenic potential of vitamin A. Although teratogenicity is only relevant to women of child-bearing age, SCF considered that the upper level of 3000 µg RE/day is appropriate 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).

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) (Spedkost 2006-2007, Småbarnskost 2007, Ungkost 2000 and Norkost 3).

The Scientific Committee for Consumer Safety's (SCCS) Notes of Guidance for the Testing of Cosmetic Ingredients was used to estimate the systemic exposure dose (SED) from dermal absorption of retinol and retinyl esters. The study considered to best fulfil the SCCS's criteria for *in vitro* absorption studies was Yourick *et al*. (2008). Based on this study, the value of dermally absorbed retinol was estimated by VKM to be 5.7%.

The use of retinol and retinyl esters in cosmetic product is restricted in the Norwegian cosmetics regulations with maximum allowed concentrations of 0.3% retinol and 0.7% retinyl palmitate. According to the cosmetic industry, retinol and retinyl esters are used in the following concentrations: 0.01%-0.05% RE in body lotions and 0.01-0.3% retinol equivalents (RE) in face and hand creams. Thus the standard exposure scenarios in this opinion are based on a concentration of 0.05% RE in body lotions and 0.3% RE in face and hand creams. In the worst case scenarios, a concentration of 0.3% RE in body lotions and 1% RE in face and hand creams are used. These concentrations are based on maximum allowed concentration of retinol in Norway

and on information from the Norwegian Medicines Agency that products intended for cosmetic use may contain 1% retinol.

Application of vitamin A-containing baby skin care products such as body lotions and creams were considered relevant for 1- and 2-year old children. Similarly, application of body lotion for 4- and 9-year old children, body lotion and hand cream for adolescents and body lotion, face and hand creams for adults were included in the exposure estimates.

Based upon these premises, previous international and national risk assessments, and published literature, the following conclusions were drawn by VKM:

The critical adverse health effect of excess intake of vitamin A is teratogenicity. This effect is the basis for the tolerable upper intake level (UL) of 3000 µg RE/day.

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

The dietary intake of preformed vitamin A is high in parts of the Norwegian population. Consumption of food supplements contributes significantly to the total intake of preformed vitamin A in all age groups and will increase the proportion of the population exceeding the UL.

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) in all age groups. The estimated contribution of retinol and retinyl esters from cosmetics is most prominent for 13-year-old adolescents (23% of UL) and adults (29% of UL).

In the worst case scenarios based on the assumed increased concentrations in cosmetics (0.3% in body lotions and 1% in face and hand cream), the contribution from cosmetics would further increase the total exposure to vitamin A (retinol and retinyl esters). The estimated contribution of retinol and retinyl esters from cosmetics would reach 42-58% of the ULs for children, 98% of the UL for 13-year-old adolescents and exceed the UL for adults (115%).

The contribution from cosmetics is of special concern for women of fertile age, and a total exposure above the UL before and during pregnancy will increase the risk of birth defects.

For persons who are at higher risk for reduced bone mineral density, osteoporosis and fractures, especially post-menopausal women, a lower guidance level (GL) than the UL has been set, i.e. 1500 µg RE/day. About 10% of adult women in Norway exceed this GL by intake of vitamin A from food and food supplements alone. The additional contribution from cosmetics increases this proportion to approximately 75%. An increased exposure due to higher concentrations of vitamin A (retinol and retinyl esters) in cosmetic products would further augment the proportion of women at risk of osteoporosis.

Impaired skin may result in increased absorption of cosmetic products. This can occur in persons with diagnosed atopic dermatitis, in persons that suffer from dry skin and in small children with irritated skin in the nappy area.

Regarding local adverse effects in the skin, no information was found indicating that long-term use of topical retinoids may induce other effects than irritation and erythema. A NTP study indicates that retinol and retinyl palmitate may be photocarcinogenic in mice. However, these data do not provide sufficient information for a risk assessment of this effect of retinol and retinyl esters in cosmetics.

Norsk sammendrag

Vitenskapskomiteen for mattrygghet (VKM) har på oppdrag fra Mattilsynet gjennomført en risikovurdering forbundet med bruk av vitamin A (retinol og retinyl estere) i kosmetiske produkter. VKM ble bedt om å vurdere både lokale og systemiske effekter av vitamin A. Videre skulle den totale eksponeringen for vitamin A i ulike aldersgrupper i den norske befolkningen beregnes ved å ta hensyn til både oral og dermal eksponering, og gjennom å inkludere eksponeringsscenarioer som illustrerer betydningen av å endre den maksimalt tillatte konsentrasjonen av retinol og retinyl estere i kosmetiske produkter. Vurderingen er gjennomført av Faggruppen for tilsetningsstoffer, aroma, matemballasje og kosmetikk.

Vitamin A består av en gruppe fettløselige forbindelser inkludert retinyl estere, retinol og retinal. Hovedkildene til inntak av vitamin A fra kosten er retinol og retinyl estere fra animalske matvarer og kosttilskudd og karotenoider fra frukt og grønnsaker. Inntak av vitamin A er nødvendig siden det inngår i en rekke fysiologiske funksjoner i kroppen. På grunn av sine effekter i huden er retinol og retinylestere mye brukt som ingredienser i kosmetiske produkter, for eksempel antirynkekremer, fuktighetskremer og solkremer.

For høyt akutt eller kronisk inntak av vitamin A kan medføre hypervitaminose A som inkluderer flere negative systemiske effekter. De mest kritiske endepunktene for vitamin A-toksisitet synes å være risiko for teratogene effekter, effekter på beinbygningen og lokale effekter i huden. Disse effektene har derfor blitt omtalt mer detaljert enn andre mulige negative effekter av vitamin A i denne risikovurderingen.

Risikokarakteriseringen for de ulike aldersgruppene tar utgangspunkt i de øvre tolerable inntaksnivåene (UL) for vitamin A som er fastsatt av EUs tidligere vitenskapskomité på matområdet (SCF) og EUs mattrygghetsorgan (EFSA). Basert på risikoen for potensielle teratogene effekter av vitamin A, fastsatte SCF i 2002 en UL-verdi på 3000 µg retinolekvivalenter (RE)/dag. Selv om teratogenisitet kun er relevant for kvinner i fertil alder, vurderte SCF at det øvre tolerable inntaksnivået på 3000 µg RE/dag også er passende for menn og for spedbarn og barn etter at det er korrigert for forskjeller i metabolsk rate. I 2008 kom EFSA fram til at et maksimalt inntak på 1500 µg RE/dag kan fungere som et veiledende inntaksnivå (GL) for personer med høyere risiko for å utvikle osteoporose og beinskjørhet (spesielt kvinner etter menopausen).

Inntaksberegningene for vitamin A fra mat og kosttilskudd i denne risikovurderingen er basert på data fra de nasjonale kostholdsundersøkelsene for barn (1-, 2-, 4- og 9 åringer), ungdom (13-åringer) og voksne (18-70-åringer) (Spedkost 2006-2007, Småbarnskost 2007, Ungkost 2000 og Norkost 3).

EUs vitenskapelige komité for forbrukerprodukter (SCCS) sine retningslinjer for hvordan man skal beregne eksponering fra kosmetiske produkter har blitt benyttet til å estimere den systemiske eksponeringsdosen etter opptak av retinol og retinyl estere over huden. En studie publisert av Yourick og medarbeidere i 2008 ble vurdert å være den studien som best oppfylte SCCS's kriterier for *in vitro* absorpsjonsstudier. Basert på opplysningene i denne studien, estimerte VKM en verdi for dermal absorbsjon av retinol på 5.7 %.

I henhold til den norske kosmetikkforskriften er bruken av retinol og retinyl estere i kosmetiske produkter regulert med en maksimalt tillatt konsentrasjon på 0,3 % retinol og 0,7 % retinyl palmitat. Kosmetikkindustrien oppgir at det er vanlig å bruke følgende konsentrasjoner i aktuelle produkter: 0,01-0,05 % RE i body lotion og 0,01-0,3 % RE i ansikts- og håndkremer. Standard eksponerings-scenarioene for kosmetiske produkter i risikovurderingen tar derfor utgangspunkt i at konsentrasjonene er 0,05 % RE i body lotions og 0,3 % RE i ansikts- og håndkremer. I verste-fall-scenarioene er det benyttet konsentrasjoner på 0,3 % RE i body lotion og 1 % RE i ansikts- og håndkremer. Disse konsentrasjonene er basert på den maksimalt tillatte konsentrasjonen av retinol

i kosmetiske produkter i Norge og på informasjon fra Statens legemiddelverk som tilsier at produkter ment for kosmetisk bruk kan inneholde 1 % retinol.

Bruk av vitamin A-holdige kremer og bodylotion ment for babyer og små barn ble ansett som realistisk eksponering for 1- og 2 åringer. Tilsvarende ble bruk av body lotion for 4- og 9 åringer, en kombinasjon av body lotion og håndkrem for ungdom og en kombinasjon av body lotion, ansiktskrem og håndkrem for voksne ansett å være relevant.

Basert på forutsetningene nevnt ovenfor, tidligere internasjonale og nasjonale risikovurderinger samt publisert litteratur, har VKM trukket følgende konklusjoner:

Den avgjørende negative helseeffekten ved et for høyt inntak av vitamin A er teratogene effekter. Disse er basis for fastsettelse av det øvre tolerable inntaksnivået (UL) på 3000 µg RE/dag.

Den viktigste kilden til eksponering for vitamin A i befolkningen er kostholdet etterfulgt av kosttilskudd og deretter kosmetikk.

Inntaket av vitamin A er høyt for deler av den norske befolkningen. Inntak av kosttilskudd bidrar signifikant til det totale inntaket av vitamin A for alle aldersgrupper og øker også andelen av befolkningen som overskrider det øvre tolerable inntaksnivået.

Ved standard-scenarioene (0,05 % i bodylotion og 0,3 % i ansikts- og håndkremer) vil påføring av kosmetiske produkter øke den totale eksponeringen for vitamin A (retinol og retinylestere) i alle aldersgrupper. Det estimerte bidraget fra retinol og retinylestere fra kosmetikk er mest fremtredende for 13 år gamle ungdommer og voksne (henholdsvis 23 og 29 % av UL).

I verste-fall-scenarioene basert på en økt brukskonsentrasjon av vitamin A i kosmetiske produkter (0,3 % i bodylotion og 1 % i ansikts- og håndkremer), vil bidraget fra kosmetikk kunne gi en ytterligere økning av den totale eksponeringen for vitamin A (retinol og retinylestere). Det estimerte bidraget av retinol og retinylestere fra kosmetikk kan komme opp mot 42-58 % av UL for barn, 98 % for 13 år gamle ungdommer og kan overskride UL for voksne(115 %).

Bidraget fra kosmetikk er av spesiell bekymring for kvinner i fertil alder hvor total eksponering over UL før og under graviditet vil kunne øke risikoen for medfødte skader hos barnet.

For personer som har en økt risiko for redusert beintetthet, osteoporose og beinbrudd (spesielt kvinner etter menopausen) er det satt et veiledende inntaksnivå på 1500 µg RE/dag (GL) som da er lavere enn det øvre tolerable inntaksnivået (UL). For rundt 10 % av voksne kvinner i Norge vil inntak av vitamin A gjennom kostholdet og kosttilskudd alene føre til en overskridelse av GL. Bidraget fra bruk av retinol og retinyl estere i kosmetikk (som estimert i standard-scenarioene) øker denne andelen til rundt 75 %. Verste-fall-scenarioene vil gi en ytterligere økning i andelen kvinner med en forhøyet risiko for å utvikle osteoporose.

Svekket/skadet hud kan føre til økt dermalt opptak av kosmetiske produkter. Dette gjelder spesielt for personer diagnostisert med atopisk dermatitt, for personer med tørr hud og for små barn med irritert/sår hud i bleieområdet.

Når det gjelder lokale effekter i huden, er det ikke funnet informasjon som tyder på at langsiktig bruk av produkter som inneholder retinoider vil kunne indusere andre effekter enn irritasjon og erytem. En studie fra National Toxicology Program (NTP) indikerer at retinol og retinyl palmitat kan være fotokarsinogent i mus. Disse dataene bidrar imidlertid ikke med tilstrekkelig informasjon for en risikovurdering av denne effekten av retinol og retinyl estere i kosmetiske produkter.

Abbreviations/Glossary

$1,25(OH)_2D$ – 1,25-dihydroxyvitamin D

25(OH)D – 25-hydroxyvitamin D

AFSSA – The French Food Safety Agency

AFSSAPS – The French Agency for the Safety of Health Products

BfR – Federal Institute for Risk Assessment

BMD – Bone mineral density

CNS – Central nervous system

CSF – Cerebro spinal fluid

EFSA – European Food Safety Authority

Erythema – Redness of the skin caused by any skin injury, infection or inflammation

FDA – Food and Drug Administration (US)

FSA – Food Standards Agency (UK)

GL – Guidance level

IU – International unit

LI – Lower level of intake

MMR – Matrix metalloproteinase

NNR – Nordic Nutrition Recommendations

NTP – National Toxicology Program

RAR – Retinoic acid receptor

RARE – Retinoid acid response element

RBP – Retinol-binding protein

RE – Retinol equivalent

Retinoids – all synthetic or natural compounds that have biological activity like that of vitamin A

RI – Recommended intake

RXR – Retinoid X receptor

RXRE – Retinoid X responsive element

SACN – Scientific Advisory Committee on Nutrition

SCCS – Scientific Committee on Consumer Safety

SCF – Scientific Committee for Food (1974 - 1997)

SCF – Scientific Committee on Food (1997 - 2003)

SED – Systemic exposure dose

SSL – Simulated solar light

UL – Tolerable upper intake level

VDR – Vitamin D receptor

VKM – Norwegian Scientific Committee for Food Safety

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Background

Vitamin A (retinol and retinyl esters, such as retinyl palmitate and retinyl acetate) are widely used ingredients in cosmetics products, such as anti-aging creams and other skin care products to generally improve the appearance of skin. Topical application of vitamin A in the form of retinol and retinyl esters has been shown to have beneficial effects on the skin when applied at low concentrations. Products which are marketed as especially suitable for making the skin of children soft and smooth could also contain vitamin A.

Currently, according to the national regulations for cosmetic products in Norway the maximum authorised concentrations of vitamin A are 0.3% in the form of retinol and 0.7% in the form of retinyl palmitate (Norwegian Cosmetic Regulations, 1995). There are no such specific restrictions for the use of vitamin A in cosmetic products laid down in the European Cosmetics Directive 76/768/EEC (EC, 1976).

Excessive intakes of vitamin A has been linked to increased risk for hypervitaminose A, retinol-induced teratogenicity and bone health problems. Another debated question related to vitamin A is whether frequent application of skin care products containing retinol or retinyl esters could result in long term effects in skin. Recently, some concerns were also raised regarding the phototoxic potential of retinol and its esters (NTP, 2011).

Amendments in the Norwegian Medicines Agency's procedures for classification of drugs have made it necessary to assess the use of pharmacological active substances in foods and cosmetics sold on the Norwegian market. At present, cosmetic products containing more vitamin A (retinol or retinyl esters) than the abovementioned maximum authorised concentrations would be illegal.

The current national regulations for retinol and retinyl palmitate in cosmetic products are based on an evaluation of retinol and retinol esters in cosmetic products, taking into account intake from dietary vitamin A and vitamin A supplements, performed by the Norwegian Institute of Public Health and the Ullevål University Hospital in 1997 (Paulsen *et al*., 1997). This previous evaluation is 15 years old and did not include relevant endpoints, such as osteoporosis. The Norwegian Food Safety Authority has therefore requested an updated risk assessment of vitamin A (retinol and retinyl esters) in cosmetic products from VKM.

The Norwegian Food Safety Authority will use VKM's risk assessment to consider if the national restrictions on the use of vitamin A (retinol and retinyl esters) in cosmetics should be maintained, and if so, aim for an amendment of the European Cosmetics Directive to have harmonised maximum authorised concentrations for vitamin A in cosmetic products within the European Union (EU).

The Norwegian Food Safety Authority has emphasized that the risk assessment from VKM should be based on the total exposure to vitamin A from cosmetic products and foods, including food supplements, in the Norwegian population.

This opinion has been performed and approved by the VKM Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics. As a basis for the final and approved opinion from VKM, a draft opinion was prepared by a working group consisting of members of the VKM Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics, the VKM Panel on Nutrition, Dietetic Products, Novel Food and Allergy, and one external expert.

Terms of reference

The Norwegian Food Safety Authority requests the Norwegian Scientific Committee for Food Safety to assess the risk related to use of retinol and retinyl esters in cosmetics. The following aspects should be considered in the assessment:

- The risk assessment should consider both systemic and local effects of vitamin A for children and adults in the Norwegian population.
- The SCCS's Notes of Guidance for the Testing of Cosmetic Ingredients and Their Safety Evaluation should be used to estimate the exposure from dermal absorption of retinol and retinyl esters from the use of cosmetics.
- The total exposure to vitamin A, taking into account the intake from foods and food supplements and exposure to cosmetic products should be estimated for different age groups in the Norwegian population.
- The risk assessment should include exposure scenarios that illustrate the influence of changing the maximum authorised concentration levels of the different forms of vitamin A used in cosmetics.

The risk assessment should be based on already existing national and international evaluations and opinions of vitamin A and on relevant data from the national food consumption surveys, including new results from Norkost 3 for adults.

Assessment

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 from VKM 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 (7^{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 have been considered for specific issues related to exposure to cosmetic products in this opinion.

VKM has focused on possible excessive intakes of preformed vitamin A in this opinion, as exposure to vitamin A (retinol and retinyl esters) from cosmetic products is considered an additional contribution to a potentially high intake of the substance in the population via food and food supplements. The teratogenic potential, effects on bone and local effects in the skin of vitamin A were considered as the most critical toxicological endpoints and have therefore been dealt with in more detail than other possible adverse effects in this opinion. Possible health effects of vitamin A deficiency are not relevant according to the terms of reference for this assessment and are therefore not discussed.

1 Introduction

1.1 Vitamin A – general background

Vitamin A is essential throughout life as it is required in numerous physiological functions such as vision, growth, proliferation and differentiation of epithelial tissues, immune functions, bone growth, reproduction and embryonic development. Vitamin A constitutes a group of lipid-soluble compounds including retinyl esters, retinol and retinal (retinyl aldehyde). The main sources of dietary vitamin A are preformed vitamin A in the form of retinol and retinyl esters from animal foods (dairy products, liver and fish liver oil) and supplements. The vitamin can also be derived from plants (dark green leefy vegetables, orange-yellow fruits and vegetables) in the form of provitamin A carotenoids which are enzymatically converted to vitamin A in the intestine and other tissues. Provitamin A carotenoids may also be a constituent of food supplements. Because of the well regulated bioconversion of carotenoids to preformed vitamin A, only intake of preformed vitamin A is considered relevant for vitamin A toxicity (Blomhoff *et al*., 2003; EFSA, 2008).

In industrialized countries there could be a potential health risk associated with excessive vitamin A intake from diet and food supplements (Blomhoff *et al*., 2003). Symptoms of hypervitaminosis A may occur in skin, nervous system, musculo-skeletal system, internal organs and embryo (EFSA, 2008). In contrast, in many developing countries, vitamin A deficiency is highly prevalent among children and pregnant woman, causing serious health effects such as reduced resistance against infections, reduced growth, severe anaemia, blindness and death (West *et al*., 2011). The foetal and postnatal life stages appear to be most sensitive to inadequate doses of vitamin A and both low and excess intake have the potential to induce birth defects as well as other serious developmental disorders.

1.1.1 Recommended intake and tolerable upper intake level

The recommended intake (RI) of a nutrient is the intake at which the risk of health effects due to inadequacy would be very small. It is assumed that adverse events occur above a threshold intake level, therefore the vitamin A intake which should not be exceeded because of safety concerns is defined as the tolerable upper intake level (UL). The UL states the maximum level of total chronic daily intake of a nutrient (from all sources) judged to be unlikely to pose a risk of adverse health effects to humans (SCF, 2000).

The risk of developing health problems along the intake scale of vitamin A can be illustrated by a U-shaped curve with a lower level of intake (LI) at 400 and 500 µg/day for women and men, respectively. RI is set to be 700 and 900 µg/day for women and men, respectively (NNR, 2004). Among women consuming vitamin A in the lower dose-ranges, no increase in the risk of vitamin A-associated birth defects has been observed at doses below 3000 µg/day and this dose is set as the UL for adults (SCF, 2002; EFSA, 2006a; 2008). However, long-term intakes of preformed vitamin A in excess of 1500 µg/day have been associated with increased risk of osteoporosis in older men and women. Therefore, this level serves as a guidance level (GL) for individuals at greater risk of osteoporosis and bone fracture (particularly post-menopausal women). Provitamin A carotenoids are not known to cause vitamin A toxicity; therefore ULs are expressed in terms of preformed vitamin A (EFSA, 2008). The UL for retinol applies to intakes from both foods and food supplement, whereas an additional contribution from cosmetic products has not been included.

1.1.2 Retinol equivalents

For nutritional purposes, the term "retinol equivalent" (RE) is used to convert all dietary sources of preformed vitamin A (retinol and retinyl esters) and provitamin A (carotenoids) into a single unit. Based on molecular weights, 1 RE is defined as 1 µg of all-*trans*-retinol. For conversion of preformed vitamin A activity expressed in International Units (IU) to RE, 1 RE is equivalent to 3.33 IU (Table 1). 1 µg of retinol is estimated to be biologically equivalent to 12 µg of carotenoids. In this opinion, VKM has chosen to express vitamin A activity in RE.

Table 1: Vitamin A activity[1].

	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

[1]Modified from SCF 2002.

1.2 Vitamin A and the skin

The use of cosmetic products could be another important source of vitamin A exposure. Retinol and its esters are widely used ingredients in skin care products, such as anti-photoaging products and moisturizers. Consequently, dermal absorption of these ingredients could contribute to the total exposure of vitamin A in the population. Retinol toxicity has been shown to be a matter of concern for the Nordic countries where the dietary intake of retinol is relatively high (Blomhoff *et al*., 2003; NNR, 2004). It is therefore of special importance to monitor the additional contribution from the use of cosmetics in populations with an already high intake of vitamin A from food and food supplements.

The retinyl esters retinyl palmitate and retinyl acetate, and the retinol all-*trans*-retinol, are the main vitamin A forms used in cosmetics. Retinal is another ingredient which could be found in some cosmetic products, while retinoic acid, the biological active metabolite, is not allowed in cosmetics (Figure 1) (EC, 1976; Norwegian Cosmetic Regulations, 1995). Retinoic acid is, however, the active component in some drugs used in clinical dermatology (van de Kerkhof, 2006).

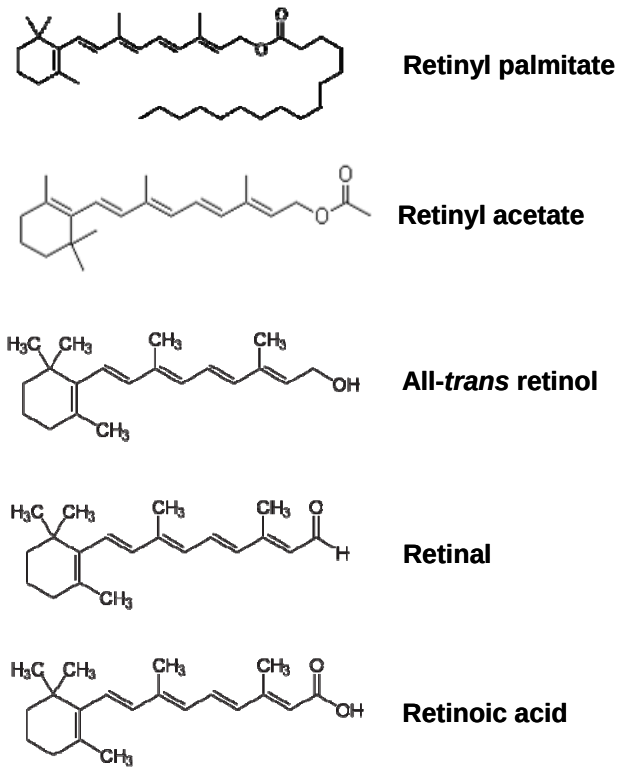

Figure 1: Structure formulas of retinyl palmitate, retinyl acetate, all-trans retinol, retinal and retinoic acid.

1.2.1 Structure and physiology of the skin

The skin is the largest organ of the human body, with a surface area of around 2 m^2 and a thickness between 0.5 and 4 mm. It is anatomically designed to exert a wide range of physiological functions as it is: a physical and chemical barrier between the external and internal environment; a sensory organ of touch, pressure, vibration, tissue injury and temperature; a termoregulating organ controlling heat loss from sweating and cutaneous blood flow. The specialized functions of the skin are made possible by its unique structure (Figure 2), which is primarily stratified into the epidermis, the dermis and the hypodermis.

The epidermis, the outermost layer, has a protective structure, which consists of distinct strata that reflect different stages of keratinocyte maturation: corneum, lucidum (only palms of hands and sole of feet), granulosum, spinosum and basale (Fu *et al.*, 2007; NTP 2010). In addition to keratinocytes, the epidermis contains melanocytes (produce the sunlight protecting pigment melanin), Langerhans cells (immune function) and Merkel (sensory) cells.

The dermis is a fibrous layer that support and strengthens the epidermis. It interfaces with the epidermis through a system of upward protrusions of dermal papillae, which provide a firm anchor to physically stabilize and support the epidermis. This papillary dermis contains a network of capillaries. Since the epidermis contains no blood vessels, the metabolic needs of the epidermis are met through diffusion exchange of nutrients and waste products between the epidermis and capillaries in the dermis. The deeper portion of dermis, the reticular region, is lesser vascularized. In aging, the synthesis of matrix proteins decelerates, while expression of matrix metalloproteinases (MMPs) which are essential for the turnover of extracellular matrix proteins accelerates. Chronic exposure to sunlight can further exacerbate these biochemical

changes (Darlenski *et al*., 2010). The dermis also contains hair follicles, sweat glands, sebaceous glands, apocrine glands and lymphatic vessels.

The hypodermis is a subcutaneous layer of fat beneath the dermis that supplies nutrients to the other two layers and cushions and insulates the body.

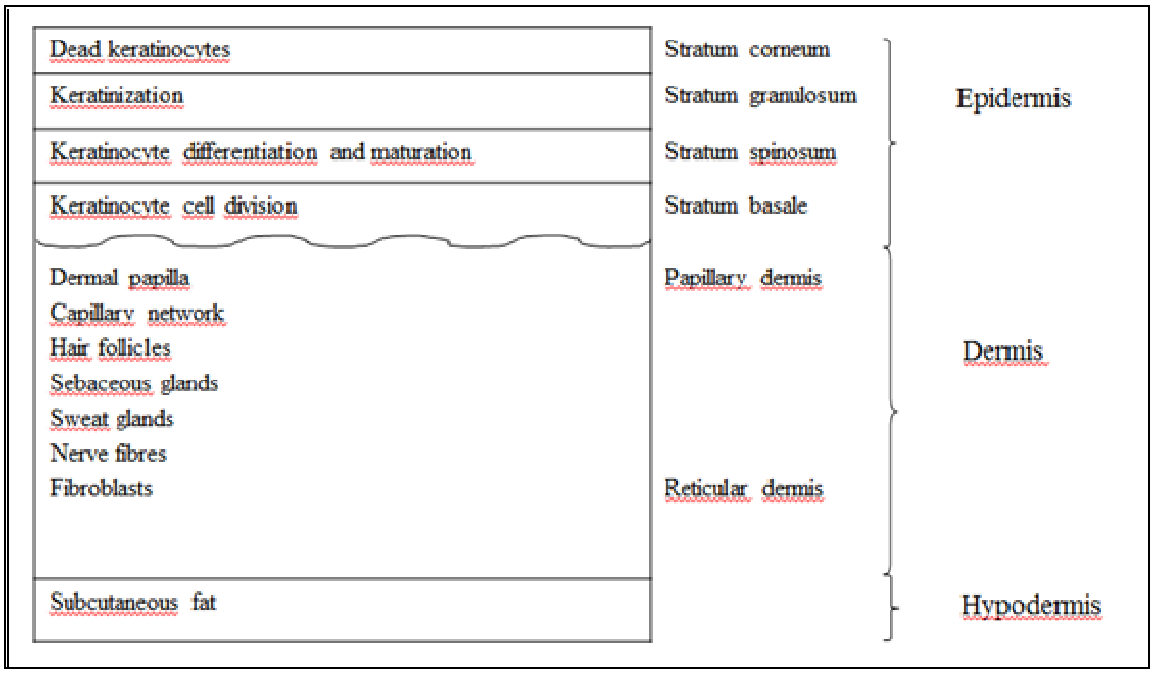

Figure 2: Principal components of the skin.

1.2.2 Retinoid function in the skin

Vitamin A has long been known to play a critical role in homeostasis of various epithelia including epidermis and is important for sustaining normal growth and differentiation. A diet deficient in vitamin A was early known to cause abnormal keratinization of epithelia (Bloch, 1921 cited in NTP, 2011). Even though epidermis is avascular it contains significant quantities of retinoids (Randolf and Siegenthaler, 1999 cited in NTP, 2011). This demonstrates that plasma retinol has access to and is taken up by the keratinocytes, but the mode of uptake is not clear (NTP, 2011). When absorbed, retinol may be esterified with fatty acids to form retinyl esters. Retinol may also be oxidized into retinal, which subsequently may be oxidized to retinoic acid (Figure 3).

Skin may also be supplied with vitamin A via topical application of cosmetics and drugs. 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 (Orfanos *et al*., 1997). The retinoids seem to play a role in the aging process of the skin, since many of the changes may be reversed by topical application (Darlenski *et al*., 2010). 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.

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). 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 (see Figure 3). 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 is increasing in the following order: retinyl esters << retinol < retinal < retinoic acid.

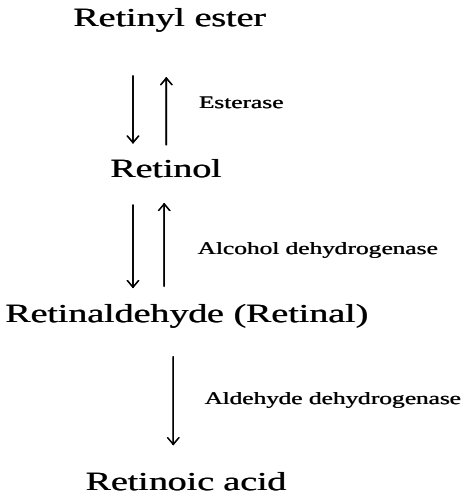

Figure 3: Metabolism of retinyl esters to retinoic acid.

1.2.3 Retinoids in cosmetics

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 (Serri and Iorizzo, 2008; Sorg *et al*., 2006).

Although retinyl esters did not show significant anti-aging activity, the retinyl ester retinyl palmitate is widely used in cosmetics because of its stability (Mukherjee *et al*., 2006). With respect to sunscreen products, retinyl palmitate is extensively used because of its antioxidant, stabilizing properties. However, in Europe and USA retinyl palmitate is not allowed to be added as UV-filter as such.

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 aging and photoaging, but the effect was dependent on the vehicle used, as retinol is unstable and easily gets degraded to biological inactive forms when exposed to light and air (reviewed in Mukherjee *et al*., 2006).

In several studies it has been demonstrated that retinal may be a useful topical agent in the treatment of aged and photoaged skin (reviewed in Mukherjee *et al*., 2006). Various cosmetic

products containing retinal, primarily anti-aging preparations are available on the European market, although these products are rarely traded in Norway.

1.2.4 Retinoids for medical use

Retinoids have been used mainly for treating skin diseases. The first use of vitamin A was published in 1943, but first by the work of Bollag *et al*. (1983), a wide range of retinoids were synthesized and tested for clinical use. Isotretinoin (13-*cis* retinoic acid) has been a very widespread and important drug for treating severe acne conditions, since the approval by the US Food and Drug Administration (FDA) in 1982. The monoaromatic retinoid etretinate, later replaced by acitretin, have also been widely used for treating severe psoriasis and other keratinisation conditions as ichtyosis. Recently also alitretinoin (9-*cis* retinoic acid) has been introduced for treating chronic hand eczema. Since these drugs are exhibiting similar effects and are highly teratogenic, they are under strict governmental and medical control for prescription. For local treatment the (polyaromatic) retinoids taxaroten and adapalen (differin) are widely used. Bexarotene is an oral and local retinoid with limited use in clinical dermatology, for the treatment of cutaneous T cell lymphoma. An imposing list of adverse effects are to be considered in clinical use, but clinical significant adverse effects are highly dose-related, and can be tolerated as the drugs are most often used for relatively short periods of months (isotretinoin), or for months and eventually lifelong (acitretion). Alternatives to these drugs do not exist for some dermatological conditions.

1.3 Previous assessments of vitamin A (retinol and retinyl esters)

1.3.1 Cosmetics

1.3.1.1 *Norwegian Institute of Public Health/Ullevål University Hospital (Paulsen et al., 1997)*

In 1997, the Norwegian Institute of Public Health/Ullevål University Hospital conducted an evaluation of retinol and retinyl esters in cosmetics. The objective of the evaluation, which was requested by the Norwegian Food Control Authority (Statens næringsmiddeltilsyn), was to assess if it was necessary to regulate the maximum permitted content of retinol and retinyl palmitate in cosmetics. The evaluation focused on both systemic and local effects. Since no relevant data on the systemic absorption of topical retinol and retinyl palmitate was available, the Norwegian assessment was then based on data for all-*trans*-retinoic acid and a systemic absorption rate of 7% (Nau, 1993).

The main conclusions in the evaluation was that the teratogenic risk of topical retinol and retinyl palmitate was low, and that a cream containing 0.3-0.6% retinol or 0.6-1.2% retinyl palmitate can be considered as safe. The risk of local adverse effects of short-term topical treatment of retinol or retinyl palmitate was also found to be low and a cream with the abovementioned concentrations of retinol or retinyl palmitate was considered as safe. Information on possible adverse effects of long-term topical treatment of retinol or retinyl palmitate was not available.

It was further concluded that cellular and molecular changes after topical treatment of 0.3% retinol was equivalent to changes observed with 0.03% retinoic acid, a dose used for clinical treatment. This indicates that the topical use of retinol and retinyl palmitate for treatment of skin disorders should be assessed by the drug authorities.

1.3.1.2 *Federal Institute for Risk Assessment (BfR) (2006, 2009 and 2010)*

The Committee for Cosmetics of the Federal Institute for Risk Assessment in Germany (BfR) has in recent years discussed and assessed the use of vitamin A (retinol and retinyl esters) in cosmetic products (BfR, 2004; 2008; 2009; 2010).

Relevant toxicological data from both animal and human studies, including pharmacological studies with focus on the pharmacokinetics and skin penetration of retinol and retinyl esters and their influence on plasma levels, were considered in the German assessment.

In its most recent statement (protocol from the meeting on 6th May 2010), the BfR Committee for Cosmetics has looked into how high an additional exposure from vitamin A (retinol and retinyl esters) in cosmetics could be, on top of the dietary intake of vitamin A, without causing any health risks for the consumer. Taking into account data on the dietary intakes of retinol in the German population, the BfR Committee for Cosmetics has concluded that the additional contribution from cosmetics should not exceed 10% of the UL (3000 µg RE/day).

The BfR Committee for Cosmetics estimated an additional contribution of retinol from cosmetic products of approximately 7.5% of the UL taking into account assumptions of twice daily application of a 0.3% vitamin A-containing oil/water emulsion to the face as well as a once daily administered 0.05% vitamin A-containing oil/water body lotion. This estimate was based on *in vitro* penetration rate data for retinol and its esters between 1.24 and 4%. With such conditions, the BfR Committee for Cosmetics is of the opinion that there are no concerns over the use of vitamin A-containing cosmetics for persons with a healthy skin. However, it should be noted that these considerations presume that no additional vitamin A-containing products are used and that the penetration of retinol or its esters is not reinforced (e.g. through penetration enhancers). For persons with atopic eczema, higher penetration rates could be expected (BfR, 2009). According to the BfR Committee for Cosmetics, a new risk assessment should be performed in situations were additional vitamin A-containing cosmetic products are used.

Based on its work on vitamin A, the BfR Committee for Cosmetics has proposed that the use of retinol and retinyl esters in cosmetic products should be restricted in the legislation for cosmetic products.

1.3.1.3 *The French Agency for the Safety of Health Products (AFSSAPS) (2011)*

The Norwegian Scientific Committee for Food Safety (VKM) is aware of a coming, but so far not published, risk assessment on vitamin A in cosmetic products from the French Agency for the Safety of Health Products. The Norwegian Food Safety Authority has, however, forwarded to VKM information received from AFSSAPS referring that skin irritation with face cream and body lotion at concentrations of 0.1% and 0.048% retinol, respectively, have been reported to the French cosmetovigilance system. Based on studies on potential phototoxicity of retinol and retinyl palmitate, the Committee on Cosmetology within AFSSAPS has suggested a maximum concentration corresponding to 0.15% retinol equivalents in cosmetic products (AFSSAPS, 2011).

1.3.2 Food and food supplements

1.3.2.1 *Scientific Committee on Food (2002)*

The Scientific Committee on Food (SCF) expressed an opinion on the UL of preformed vitamin A (retinol and retinyl esters) in 2002. SCF noted that determining an UL for preformed vitamin A is difficult, as the margin between the population reference intake and the intakes associated with adverse effects is narrow. The teratogenic risk, hepatotoxicity and a possible increase in risk of bone fracture were especially addressed in the derivation of an upper level for the intake of vitamin A. SCF established an UL at 3000 µg RE/day of preformed vitamin A for adults, based on the risk to women of child-bearing age. This value was about 2.5-fold lower than the lowest daily intake associated with hepatotoxicity during chronic intake. The tolerable intake levels for infants and children were based on the adult value with correction for differences in basal metabolic rate compared to adults (body weight$^{0.75}$).

The SCF further considered that the tolerable upper intake level may not provide an adequate margin of safety in relation to the possible decrease in bone density and the risk of bone fracture, and that it would be advisable that postmenopausal women, who are at greater risk of osteoporosis and bone fracture, should restrict their intake of preformed vitamin A to 1500 µg RE/day.

1.3.2.2 *European Food Safety Authority (2008)*

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) published a scientific opinion on the consequences for the consumer of the use of vitamin A in animal nutrition in 2008. The EFSA opinion reviewed two recent reports on the safety of vitamin A performed by the UK Scientific Advisory Committee on Nutrition (SACN, 2005) and the Agence Francaise de Securite Sanitaire des Aliments (AFSSA, 2005). The review of Ribaya-Mercado and Blumberg (2007) on the potential risk of vitamin A on osteoporosis and bone fracture was also considered in this EFSA opinion.

The FEEDAP Panel was of the opinion that new available data published later than the SCF opinion from 2002 would not substantially alter the risk assessment for preformed vitamin A. Consequently, EFSA still considered the UL of 3000 µg RE/day from preformed vitamin A as being appropriate. The FEEDAP Panel further referred to the advice from SCF that a maximum intake of 1500 µg RE/day from preformed vitamin A would serve as a guidance level (GL) for individuals at a greater risk of osteoporosis and bone fracture, until new data indicates the necessity of a re-evaluation.

Both relevant national studies on the intake of vitamin A and data from the food consumption survey within the European Prospective Investigation into Cancer and Nutrition (EPIC) project were considered in the intake calculations presented in the EFSA opinion. The mean intake of preformed vitamin A in the adult population in Europe was estimated between 400 and 1200 µg RE/day in men and between 350 and 1000 µg RE/day in women. A small proportion of the European population had an intake of preformed vitamin A above the UL. Consumption of liver was found to be the predominant source of preformed vitamin A (about 60-80%) in France, Greece, Italy and Spain. Milk, including butter and other dairy products represented the highest proportion (45-60%) of preformed vitamin A in Germany, the Netherlands, Norway and Sweden. The FEEDAP Panel concluded that the risk of exceeding the UL (and GL) for preformed vitamin A was predominantly related to the consumption of liver, but also to the consumption of supplements containing vitamin A.

It was further concluded that preformed vitamin A may raise safety concerns because of its high levels in some foods of animal origin and of individual consumption patterns. Therefore, feeding practice should seek to avoid any unnecessary high concentrations in those foods. As a consumer protection measure, the FEEDAP Panel recommended a general introduction of maximum contents of vitamin A in feed for food-producing animals.

1.3.2.3 *Nordic Council of Ministers (TemaNord 2003:502) (Blomhoff et al., 2003)*

A working group appointed by the Nordic Council of Ministers published in 2003 a scientific report on the health risks related to high intake of preformed retinol (vitamin A) in the Nordic countries (Blomhoff *et al*., 2003). As vitamin A (i.e. preformed retinol) intake in Denmark, Sweden, Norway, Finland and Iceland is known to be higher than in most other countries, the objective of this work was to evaluate whether vitamin A toxicity could be a real threat to the health of people in the Nordic countries. The potential health risk related to dietary intake of vitamin A and use of supplements containing retinol (i.e. retinol and retinyl esters) was evaluated in the report.

The intake calculations presented in the Nordic report showed that men have a higher intake of retinol than women in all countries. The mean intake varied from 990 to 3180 µg RE/day in men, and from 560 to 2050 µg RE/day in women, with the highest intakes found among Icelanders, followed by Norwegians. The daily dietary intake among high consumers (90 percentile) in the Nordic countries was between 1800 and 6500 µg RE in men and between 1100 and 5000 µg RE in women.

There have been no reports in the Nordic countries describing neither chronic nor acute hypervitaminosis A due to consumption of foods containing large amounts of retinol (except Arctic explorers eating Polar bear liver). Furthermore, 17 suspected cases of chronic hypervitaminosis A have been reported due to consumption of supplements containing retinol.

The Nordic report emphasized that it is important to note that intake of retinol in various physical forms may have different thresholds for toxicity. Intake of retinol from non-fortified foods is generally below the dosages where hypervitaminosis A may be induced, with a possible exception being consumption of liver where the retinol content in some cases may be very high.

In vitamin A-fortified foods, water miscible/emulsified preparations of retinol are mainly used. Such preparations of retinol are presumed to have higher toxicity than oily preparations, indicating that intake of vitamin A-fortified foods may increase the likelihood that the safe upper intake could be exceeded. The Nordic experts therefore recommended that fortification with water miscible/emulsified and solid preparations of retinol should be kept to a minimum and that oil-based preparations of retinol, with a lower toxicity threshold, should be preferred if fortification is needed. The same arguments were found relevant in connection with retinol-induced toxicity from supplements.

Several studies demonstrate teratogenicity of high doses of preformed retinol in various experimental animals. However, due to the limited occurrence of scientific data in humans, it was not possible to establish a threshold level of intake of retinol during pregnancy below which there is no increased risk of giving birth to a malformed child. The Nordic working group was of the opinion that available epidemiological data indicate that intakes up to 3000 µg RE (10000 IU) per day during pregnancy were not associated with increased risk for malformations. They found it prudent to continue to apply precautionary measures and to recommend that daily intake of retinol during pregnancy should not exceed 3000 µg RE.

The Nordic report further stated that the evidence for lower bone mineral density and increased risk of hip fracture after retinol intakes marginally above the recommended dietary intake is limited and not consistent. Since there was evidence that links a high intake of preformed retinol with negative effects on the skeleton, further studies addressing the issue were warranted.

1.3.2.4 *Nordic Nutrition Recommendations (2004)*

The Nordic countries have for several decades collaborated in setting guidelines for dietary composition and recommended intakes of nutrients. The recent recommended intake of vitamin A in the Nordic countries is described in the 4th edition of the Nordic Nutrition Recommendations published in 2004 (NNR, 2004).

The Nordic recommendations have been based on estimated requirements that assure adequate body stores of retinol where no clinical signs of deficiency are observed, adequate plasma retinol levels are maintained and there is protection against vitamin A deficiency for approximately 4 months on a vitamin A-deficient diet, as described by the U.S. Food and Nutrition Board of the Institute of Medicine in 2001 (FNB, 2001). Using a factorial method for Nordic reference subjects, the estimated average requirement for vitamin A was found to be very similar as for U.S. reference subjects, i.e. close to 600 and 500 µg/day for men and women, respectively. Based on these considerations, the present Nordic recommended intakes for adults were set to 900 µg RE/day for men and 700 µg RE/day for women. The recommended intake for pregnancy has been set to 800 µg RE/day to cover individual variation, while an additional intake of 400 µg RE/day is recommended during lactation to counteract the loss of vitamin A through breast milk.

The recommended intakes for children and adolescents have been extrapolated from the adult values by using a scaling factor (body weight$^{0.75}$). The following recommended intakes are valid for Nordic children and adolescents: 2- to 5-year-olds (350 µg RE/day), 6- to 9-year-olds (400 µg RE/day) and 10- to 13-year-olds (600 µg RE/day).

The Nordic Nutrition Recommendations are currently under revision and the 5th edition is due to be published in 2013.

2 Hazard identification and characterisation

2.1 Retinoid uptake and metabolism after oral intake

In food derived from animal products, the major forms of preformed vitamin A are long-chained fatty acid esters of retinol (reviewed in Harrison, 2005). Prior to uptake essentially all of the esters are hydrolysed in the intestinal lumen into retinol. Free retinol is then taken up by intestinal cells where it is re-esterified with long-chained fatty acids before they are incorporated into chylomicrons. Studies using the human intestinal cell line, Caco-2, show that retinol uptake is a rapid process with a half-life of minutes (Nayak, 2001). The absorption efficiency of retinol is generally high, in the range of 70-90% (NNR, 2004). However, it may be reduced if diets are low in fat or if individuals suffer from fat malabsorption syndrome (EVM, 2003).

The chylomicrons are released into the blood circulation via the lymph where they undergo a remodelling process involving primarily the hydrolysis of triglyceride and acquisition of

apolipoprotein E from the circulation, resulting in the formation of chylomicron remnants. The majority of the chylomicron remnants are taken up by the liver (66-75%). However extrahepatitic uptake of remnants in tissues such as bone marrow, peripheral blood cells, spleen, adipose tissue, skeletal muscle and kidney, do also occur (D'Ambrosio, 2011; Blomhoff *et al*., 2003).

Although much of the absorbed retinol is secreted into lymph in esterified form, there is evidence that a significant amount is also secreted into portal circulation, probably as free retinol (Harrison, 2005). This transport is expected to be physiologically significant in pathologic conditions that affect the secretion of chylomicrons, and thus may be an essential back-up mechanism for the homeostasis of vitamin A under some conditions.

In liver parenchymal cells, the retinyl esters are hydrolysed and retinol may be transferred to retinol-binding proteins (RBP). The retinol-RBP complex can be secreted directly into the circulation or they are transferred to the hepatic stellate cells and stored in the form of long-chained fatty esters (Debier and Laronedelle, 2005; Harrison, 2005).

Before the release of retinyl esters from the stellate cells, the retinyl esters are hydrolysed and free retinol binds to RBP and are secreted into the circulation (Harrison, 2005). The secreted retinol-RBP forms a ternary complex with plasma transthyretin to prevent renal elimination (Tzimas and Nau, 2001). Circulating retinol-RBP can then enter the target cells, and depending on the tissue type, retinol is either transformed into retinyl ester, retinal or retinoic acid (Debier and Larondelle, 2005; Evira 2007; 2008) (Figure 3). The conversion of retinol into active metabolites occurs usually in the target cells by complex metabolic pathways: 1) esterifying within the tissue, 2) activation by oxidation of the side chain into retinal and retinoic acid, which can in turn be further oxidised into 4-hydroxy metabolites, or 3) conjugation with glucuronic acid which leads to retinoyl- and retinyl glucuronides, which results in their elimination in faeces and urine (reviewed in Blomhoff *et al*., 2003; EFSA, 2006a).

The release of retinol from the liver is tightly controlled, and in vitamin A-sufficient conditions, circulation levels of retinol-RBP are maintained at a constant level (1-3 μmol/l) The serum level of retinol is indicating the vitamin A storage in the liver only if there is an extreme depletion or over-consumption of vitamin A (EFSA, 2008; Evira 2007; 2008). If hepatic storage capacity is exceeded, plasma levels of retinyl esters increase, but not retinol itself (EVM, 2003).

Serum retinol concentrations have been extensively used to identify populations at risk of vitamin A toxicity, but are not considered as an efficient biomarker of exposure in individual patients because of the homeostatic regulation of vitamin A. Other factors may also affect the serum concentration without any changes in the total body stores. For example, retinol-binding protein (RBP) is a negative acute phase protein. Therefore, serum retinol and RBP concentrations will fall during times of infection. The status of other nutrients, particularly iron and zinc deficiency, may also negatively affect serum retinol concentrations without any depletion of the liver store. However, studies in humans suggest that retinoic acid and its metabolites instead of plasma retinol may be the biomarker of choice when evaluating the risk for vitamin A toxicity (reviewed in Penniston and Tanumihardjo, 2006).

2.1.1 Retinoid-dependent signalling

The biological functions of vitamin A are mediated by the active metabolite retinoic acid which acts as the natural ligand for two distinct nuclear receptors, the retinoic acid receptor (RAR) and the retinoid X receptor (RXR). While RARs bind the two physiological forms of

retinoic acid, all-*trans* retinoic acid and 9-*cis* retinoic acid, RXRs have been reported to only bind 9-*cis* retinoic acid. The RAR-RXR heterodimer recognizes retinoic acid response elements (RAREs) on the DNA whereas the RXR-RXR homodimer recognizes retinoid "X" response elements (RXREs) in the promoter regions of target genes. This interaction with the transcriptional machinery and regulation of gene expression indicates that the biological effects of vitamin A are regulated at the gene level (reviewed in Blomhoff and Blomhoff, 2006; Theodosiou *et al*., 2010; Gutierrez-Mazariegos *et al*., 2011). Furthermore, there are indications that retinoic acid can serve as ligand for other nuclear receptors. Examples are the peroxisome proliferators-activated receptor (PPAR)β/δ, which may be involved in regulation of skin integrity, and the retinoic acid receptor related orphan receptors (RORβ). Effects of retinoic acid that are independent of gene transcription have also been reported, both with and without the involvement of retinoid receptors. However, the relevance of these observations is still unclear (Theodosiou *et al*., 2010).

2.2 Systemic adverse effects of retinoids (hypervitaminosis A)

It is well documented that both acute and chronic excessive intake of vitamin A may result in a number of adverse effects. Acute hypervitaminosis A, following the ingestion of a single very high dose or several repetitive very high doses over a few days, is characterised by various symptoms such as nausea and vomiting, fatigue, abdominal pain and skin disorders. In neonates and infants, one single or series of large doses of vitamin A may lead to the development of reversible bulging fontanelle. In adolescents and adults, headache and blurred vision is frequently reported during hypervitaminosis A. Both these symptoms, as well as bulging fontanelle may possibly arise from increased cerebrospinal fluid (CSF) pressure.

Chronic hypervitaminosis A results from continued ingestion of high doses for months or years and the clinical picture of symptoms are varied and non-specific. The onset and severity of the various toxic effects are also dependent on dose and duration of the exposure, as has been clearly demonstrated for vitamin A-induced damage to the liver. The teratogenic potential of retinoids is well documented and excessive exposure to retinol during embryonal development may lead to congenital malformations in many different target organs. Other symptoms of chronic vitamin A toxicity include increased CSF pressure and chronic headache, various skin disorders and erythema, bone pain and reduced bone density (Blomhoff *et al*., 2003; FNB, 2001; SCF, 2002; EFSA, 2006a).

Based on the weight of evidence neither retinol nor retinyl esters are considered genotoxic (EVM, 2003). Retinoids seem not themselves to induce tumours in animals. However, studies on the effects of retinoids on carcinogenesis in animals show contradictory results as some studies show a protective effect and other an enhancing effect on tumour formation. *In vitro* studies suggest that retinoids may inhibit certain steps of carcinogenesis and enhance other steps (Rivedal and Sanner, 1992; Miller *et al*., 2000).

Among the adverse toxic reactions of vitamin A, the teratogenic effects of chronic excess intake of vitamin A, as well as the effects on bone mineral density and osteoporosis are referred more closely in the following paragraphs. Furthermore, local effects in the skin and a potential contribution to systemic effects of vitamin A from cosmetic products are discussed in more details (see section 2.3 and 2.4).

2.2.1 Teratogenic effects

Extensive research carried out over the last 100 years has established that vitamin A plays crucial roles in embryonic development by regulating organogenesis, cell proliferation, differentiation and apoptosis. Because of the importance of vitamin A during development, the transport via the placenta is tightly regulated and it is documented that both low and excess dietary levels of vitamin A may induce malformations in experimental animals and humans indicating that the concentration must be within a narrow range to avoid birth defects (SCF, 2002; Blomhoff *et al*., 2003; Gutierrez-Mazariegos *et al*., 2011).

Because vitamin A bioaccumulate in the human liver, intake of large doses in the months before conception may lead to increased teratogenic risk. In a prospective study performed by Rothman *et al*. (1995) which included 22748 pregnant women, it was concluded that intake of more than 10000 IU (3000 µg RE) of supplemental vitamin A per day was associated with increased risk of birth defects. In this study, 1.3% of babies born to women who took 5000 IU (1500 µg RE) or less of vitamin A supplement had cleft lip, cleft palate, hydrocephalus or major heart defects, while 3.2% of infants born to women who took over 10000 IU/day had such defects (Rothman *et al*., 1995). One prospective study including exposures of more than 50000 (>15000 µg RE) and 100000 IU (>30000 µg RE) per day and available epidemiological data on intake in the lower-dose ranges support the conclusion that daily intake of 10000 IU (3000 µg RE) per day would be associated with a low or negligible risk of teratogenicity (Mastroiacovo *et al*., 1999; SCF, 2002; Blomhoff *et al*., 2003).

Teratogenic effects after oral exposure to vitamin A in humans are best documented in children born to mothers treated with the pharmaceutical Accutane, 13-*cis*-retinoic acid (isotretionin) during pregnancy. Accutane entered the U.S. market in 1982 for the treatment of severe acne, and already 3 years later Lammer *et al*. (1985) reported high risk for spontaneous abortion, premature birth, perinatal mortality and major malformations associated with exposure to the therapeutic dose. The pattern of malformations reflected the birth defects observed in animal studies including craniofacial, cardiac, thymic, and CNS deformities. The most frequently occurring category was CNS malformations (Lammer *et al*., 1985; 1988). Furthermore, longitudinal follow-up of the children, both with and without major malformations, indicated that 47% of the children had mental ability scores in the borderline to the mentally retarded range (Adams and Lammer, 1993).

As opposed to oral intake, more than 25 years of topical use of RA for acne are without evidence for increased teratogenicity. In a case control study of 215 mothers exposed to tretinoin during pregnancy, the prevalence of major foetal malformations was 1.9 in the treatment group versus 2.6 in the control group (Jick *et al*., 1993 cited in Mukherjee *et al*., 2006). However, it is still being warned against using the drug retinoic acid during pregnancy.

2.2.2 Osteoporosis

2.2.2.1 *Skeletal effects of vitamin A*

Osteoporosis is an age-related condition characterised by low bone mass and increased risk of fracture. This is a major health problem among older adults, and especially among postmenopausal white women (discussed in Ribaya-Mercado and Blumberg, 2007). It is well described that large doses of retinol interfere with calcium and bone metabolism, as demonstrated by abnormalities such as hypercalcemia, ligamentous calcification, bone pain, demineralisation of bones or osteopososis (reviewed in NNR, 2004). However, association between slightly greater than recommended intake level of retinol and poor bone mineral density or increased risk of hip fracture have also been demonstrated. The risk of both

reduced bone mineral density (BMD) and fracture was increased for retinol intake > 1500 μg RE/day (Melhus *et al*., 1998; Michaëlsson *et al*., 2003) and self-reported fractures were increased for total vitamin A intake ≥ 3000 μg RE/day and retinol intake (food + supplements) ≥ 2000 μg/day, respectively (Fescanich *et al*., 2002). Additional studies since the SCF opinion from 2002 support the apparent increased risk of bone fracture over an intake range similar to that normally consumed from food and supplements (SCF, 2002; SACN, 2005; AFSSA, 2005; EFSA, 2008). Several cases of skeletal problems have also been reported in children with severe hypervitaminosis A or after treatment with synthetic retionoids for several years. The symptoms involve abnormalities in ossification and calcification, decreased cortical thickening and decreased bone density (SCF 2002; Ahmadieh and Arabi, 2011).

Among studies examining relationships of vitamin A intake and BMD or fractures in humans, both low and high intakes of retinol were associated with low BMD in a study by Promislow *et al*. (2002). In contrast, there are others that suggest a certain protective effect of vitamin A against bone loss (reviewed in Ribaya-Mercado and Blumberg, 2007; Crandall, 2004; NNR, 2004) and also a few observational studies, and one small short-term clinical trial, where little or no associations between vitamin A intake from food and supplements and bone health were found. In studies where serum or plasma retinol or retinyl esters were measured as markers of vitamin A status, increased risk of hip fracture was demonstrated for high serum retinol (Melhus *et al*., 1998; Michaëlsson *et al*., 2003) and for high as well as low serum retinol + retinyl esters combined (Opotowsky *et al*., 2004). Certain positive relations have also been shown for plasma retinol and BMD, whereas no correlations were found for carotenoids (Maggio *et al*., 2003; Ribaya-Mercado and Blumberg, 2007; Crandall, 2004).

Previous studies with retinoids used as drugs have identified musculoskeletal symptoms that mimic those of hypervitaminosis A, including cortical hyperostosis and changes in tendons and ligaments. With respect to bone demineralisation, a number of human studies from the 1980s reported contradictory evidence. Retinoids have been shown to alter bone turnover and decrease BMD in some smaller studies, whereas other studies have demonstrated no or minor decrease in BMD (DiGiovanna, 2001; Blomhoff *et al*., 2003; DiGiovanna, 2010). In a recent case-control study obtained from two nationwide registers in Denmark, it was concluded that risk of fractures is not associated with dose or duration of systemic retinoids (isotretinoin and acitretin) (Vestergaard *et al*., 2010).

Animal studies support the clinical findings that modest excesses of retinol is associated with increased fracture risk and effects on bone growth and bone remodelling (reviewed in Ribeya-Mercado, 2007). In rats, it has been demonstrated that subclinical hypervitaminosis led to increased bone fragility (Johansson *et al*., 2002), however, BMD measurements did not reveal adverse skeletal changes in the same group (Lind *et al*., 2006). Excess retinoid treatment induced bone thinning in both young and old rodents, and the measured BMD was decreased in cortical but increased in trabecular bones (Kneissel *et al*., 2005). In a recent study in rats, high doses of retinol for one week led to weakening and higher degree of mineralisation of bones with little apparent effect on BMD. Furthermore, the findings indicated a reduced endosteal/marrow blood flow leading to hypoxia and pathological mineralisation (Lind *et al*., 2011). In a long-term study, vitamin A supplementation induced a transient increase in medullary and decrease in cortical thickness in young rats. Furthermore, no effects on resistance to fracture or on bone mineral content were observed in old rats (Wray *et al*., 2011).

One of the major roles of retinol is regulation of differentiation and proliferation of various cell types and both osteoblasts (bone formation) and osteoclasts (bone resorpsion) express retinoid receptors. Retinoic acid has been shown to suppress osteblast activity and stimulate

osteoclast formation and activity, resulting in decreased bone formation and increased bone resorption (Binkley and Kreuger, 2000; NNR, 2004; SCF, 2002).

In summary, the data on bone density and risk of fracture provides basis for concern since effects are reported at lower daily intakes of vitamin A than for other adverse effects. The data is, however, not considered sufficient to establish causality and can therefore not be used to derive a tolerable upper intake level.

2.2.2.2 *Combined effects of vitamins A and D*

Vitamin D regulates normal serum calcium concentrations and an adequate serum level is of great importance for bone health. Deficiency of vitamin D results in secondary hyperparathyroidism, increased bone turn over and bone loss, and is therefore a main risk factor in osteoporosis-related fractures (Holick *et al*., 2007; Lips P, 2001).

An antagonistic effect of vitamin D and retinol was previously found on serum calcium after combined intake in humans (Johansson *et al*., 2001). The past high retinol concentration in cod liver oil was also suggested as a possible explanation for the observed negative association between childhood cod liver consumption and bone mineral density in a population-based cohort of peri- and postmenopausal Norwegian women (Forsmo *et al*., 2008). In a recent study on postmenopausal Spanish women, it was concluded that high retinol levels together with vitamin D deficiency increase the risk of osteoporosis (Mata-Granados *et al*., 2010). Also among participants of the Women Health Initiative Observational Study, a modest increase in total fracture risk with high vitamin A and retinol intakes was observed in the low vitamin D-intake group (Caire-Juvera *et al*., 2009). In a meta-analysis of case-reports on toxicity induced by intakes of excessive amounts of dietary retinol, it was observed that the mean dose retinol causing chronic hypervitaminosis A was higher when retinol was taken together with vitamin D (Myhre *et al*., 2003). This may indicate that the antagonistic effects of vitamin A and vitamin D occur mainly at low dietary levels of vitamin D. Furthermore, a possible effect of high intakes of retinol on serum levels of 25-hydroxyvitamin D (25(OH)D) has also been demonstrated (Birgisdottir *et al*., 2011; Mata-Granados *et al*., 2008).

Several animal studies support an antagonism between retinol and vitamin D at high dietary retinol intake (reviewed in Blomhoff *et al*., 2003). Also at physiological levels antagonism of retinol on effects of vitamin D has been demonstrated in rats (Lind *et al*., 2006; Rohde *et al*., 1999). A potential antagonistic effect of retinol on vitamin D may also be illustrated by the observation that high intake of retinol lowers vitamin D status in mice and rats (Hetland *et al*., 2009, Lind *et al*., 2006).

Various mechanisms may be involved in an antagonistic effect between retinol and vitamin D. Competition between the active metabolites of retinol and vitamin D, retinoic acid and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), for the receptor RXR which forms heterodimers both with RAR and the vitamin D receptor (VDR), has been suggested by several (NNR, 2004, Rohde and DeLuca, 2005). However, retinol could also interfere with the absorption, transport and conversion to the active form or degradation of vitamin D. In male rats, however, results suggests that retinol does not antagonise the action of calciferol (vitamin D_3) by altering the metabolism of calciferol or its active metabolite $1,25(OH)_2D$ (Rohde and De Luca, 2005).

In summary, both human and animal studies indicate an antagonistic effect of retinol on the effects of vitamin D on serum calcium and bone metabolism. Furthermore, data indicate that high intake of retinol may affect the serum levels of vitamin D.

2.3 Retinoid uptake and metabolism after topical application

One of the skin functions is to limit the chemical exchange between the body and the environment. The stratum corneum is a barrier to the absorption of most topically applied chemicals (NTP, 2010). This layer, which is composed of approximately 40% lipids, 40% proteins and 20% water, is generally permeable only to small lipophilic molecules. Although the thickness of the stratum corneum may modulate absorption, the major barrier to topical absorption of chemicals is the intercellular lipid channels. The barrier function may be affected by lipid extraction, inhibition of lipid synthesis, and penetration enhancers that biochemically alter lipid composition. The skin of mouse, rat and guinea pig is more permeable with respect to vitamin A than human skin. Thus absorption studies using these animals have not been included in the present opinion.

2.3.1 *In vivo* absorption

Topical application of a 0.05% retinal 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 (Sass *et al*., 1996).

In a study by Nohynek *et al.* (2006), 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 was applied comprising 9 mg of retinol or 16 mg of retinyl palmitate. 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 commercial 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 of the skin wash of normal skin, but peaked and started to decline prior to the wash in dermatitic skin (10 vs 5 hours).

2.3.2 *In vitro* absorption

In *in vitro* experiments, Boehnlein *et al.* (1994) applied 20 μg/cm^2 of radioactive retinyl palmitate in an aceton vehicle on viable human skin obtained from two female donors (skin obtained from abdominoplasty and breast reduction). After incubation in a flow-through

diffusion cell for 24 hours under dark conditions, the skin surface was washed. Then the skin was homogenised and radioactivity measured. In addition, aliquots of the receptor fluid (Hepes buffered Hanks' balanced salt solution (HHBSS) w/4% bovine serum) fraction were analysed. Of the applied dose, 18% (±1) was absorbed in the skin (about 3.6 μg/cm^2). Only 0.2% (±0.01) of the absorbed material partitioned into the receptor fluid from the skin. The authors suggest that this low amount may be caused by retinyl palmitate being insoluble in water and thus not readily partition from skin into the diffusion cell receptor fluid.

An oil-in-water formulation containing 0.3% of retinyl palmitate was applied for 16 hours on human abdominal tissue (2 mg/cm^2, yellow light conditions, flow-trough diffusion cell). Cutaneous absorption (epidermis + dermis + receptor fluid (HHBSS w/4% bovine serum albumin)) was found to be about 4% (Leclerc, 1997).

Percutaneous absorption of retinol was studied by Yourick *et al*. (2008) using an *in vitro* model with human skin (from abdominoplasty) in a flow-through diffusion cells. Two vehicles were used: gel (hydroxypropylcellulose, butylhydroxytoluene, ethanol) and oil-in-water emulsion (polyglyceryl-3-distearate, cetyl stearyl alcohol, propylene glycol, light mineral oil, methyl- and propyl-*p*-hydroxybenzoate, butylhydroxytoluene, distilled water). Retinol (0.3%) formulations (gel or oil-in-water emulsion) containing ^{3}H-retinol were applied and absorption was measured at 24 and 72 hours. After 24 hours, unabsorbed retinol on the top of the skin constituted 87.3% (±6.3) for gel and 94.8% (±2.6) for oil-in-water emulsion, whereas 3.5% (±0.4) and 5.9% (±1.4) remained in the stratum corneum for gel and oil-in-water emulsion, respectively. In the viable skin (dermis/epidermis), 2.1% (±1.2) of the applied doses for gel and 3.0% (±0.6) for oil-in-water emulsion were absorbed. Retinol was found in the receptor fluid (HHBSS w/4% bovine serum albumine) at a concentration of 0.3% (±0.01) and 1.3% (±0.1) after application of the gel and oil-in-water emulsion vehicles, respectively. The levels of retinol increased over 72 hours in the receptor fluid (0.5% (±0.01) and 2.2% (±0.2) for gel and oil-in-water emulsion vehicles, respectively), but decreased or was unchanged in the viable skin (1.0% (±0.1) for gel vehicles and 2.9% (±0.6) for oil-in-water emulsion vehicles).

2.3.3 Factors that may influence dermal absorption

2.3.3.1 *Vehicles*

By using different vehicles, skin penetration enhancers and carrier systems the penetration or deposition rate may be altered. An increase in the water content in stratum corneum may result in increased transdermal delivery of both hydrophilic and lipophilic substances. Skin hydration can be achieved by using both occlusion (ointments, water-in-oil emulsion) and by providing water from the vehicle to stratum corneum (oil-in-water emulsions) (Otto *et al*., 2009). However, to further increase the dermal absorption, penetrations enhancers are often used in cosmetic products. Chemical penetration enhancers act by lipid fluidisation and lipid extraction. Surfactants may have different effects on the skin (inflammation, direct cytotoxic effects, lipid extraction) that can impair the skin barrier function and thus increase dermal penetration. In an *in vitro* study by Föster *et al.* (2011), three surfactants (esters of polyethyleneglycol) were used in preparing three oil-in-water emulsions and three surfactant solutions all containing retinol (0.5 wt%). All the surfactant solutions showed higher penetration rate compared with the corresponding emulsion, and the penetration behaviour appeared to be dependent on the surfactant used in the formulation. In an *in vivo* study, two skin penetration enhancers, Triton X 100 (octoxynol) and oleic acid were added to a cream (Myritol: caprylic/capric acid triglyceride), containing 0.3% all-*trans*-retinol (Mélot *et al*.,

2009). Myritol is a non skin penetration oil in which retinol is highly soluble. Solutions were applied once on the volar forearm on male voluntaries and dermal absorption of retinol was measured up to 6 hours after treatment using Raman spectroscopy. After treatment with retinol and Myritol, retinol hardly penetrated the skin. When oleic acid or Triton X 100 was added, retinol penetrated much more efficiently within the skin. Oleic acid, one of the most commonly used penetration enhancers in cosmetic products, resulted in a better penetration of retinol compared to Triton X 100.

Carrier systems such as nanoparticles can be used to alter the dermal absorption. Nanoparticles can be divided into soft and rigid particles where soft particles are made of organic materials (e.g. lipids, proteins, polymers) and rigid particles are made of inorganic materials (e.g. metals, metal oxides, ceramics). Particles penetrate preferentially into the hair follicle canal, thus enabling high concentrations within the reservoir of the follicular infundibulum (reviewed in Papakostas *et al*., 2011). In *in vitro* studies, skin permeability of encapsulated retinoic acid was shown to be lower than for free retinoic acid. However, the encapsulated retinoic acid yielded a continuous increase in drug concentrations in the stratum corneum and in the viable skin tissues (dermis/epidermis), whereas when applied as free retinoic acid the concentration of retinoic acid decreased gradually in all skin layers. Thus, the higher, sustained levels of retinoic acid may be due to retarded diffusion and not to enhanced dermal absorption (Masini *et al*., 1993; Fresno Contreras *et al*., 2005). The same dermal absorption pattern has been shown for retinol. Although the time needed to cross 20 µm of epidermis was found to be 7 minutes for encapsulated retinol and 11 minutes for free retinol (in oil-in-water emulsion), the release was highest for free retinol. However, the encapsulated retinol remained for a longer time in the skin than free retinol (Failloux *et al*., 2004). The use of nanoparticles may thus change both the penetration pathway and kinetics of topically applied substances.

2.3.3.2 *Impaired skin*

Cosmetic products are meant to be used on normal skin, however, they may also be applied on non-healthy skin. In these individuals, the barrier properties of the skin may be impaired. The prevalence of dry and sensitive skin is very high in the population at all age groups. Halvorsen *et al*. (2008) found that 34% of 2670 healthy adolescents in Norway had dry skin, and 13% dermatitis. The extent of dry skin varies, but dry skin is clearly related to reduced barrier properties and increased penetration of cutaneously applied cosmetics. Similar high percentages of dry and sensitive skin are reported from other studies in the USA (Misery *et al*., 2011).

In a study by García Ortiz *et al.* (2009), six adults with atopic dermatitis received one topical application (4 mg/cm^2; non-occluded) of metronidazole (1%) in a cream formulation on areas on the forearm with active atopic dermatitis and on areas with uninvolved (normal) skin. The cutaneous penetration of the metronidazole cream was 2.4-fold higher when applied on areas with active atopic dermatitis compared to cream applied on uninvolved skin. In the study by Franz and Lehman (1990), the absorption of retinoic acid after topical application on dermatitic skin was found to be 7.2% as compared to 5.3% after application on normal skin.

2.3.4 Skin metabolism of retinol and retinyl esters

In the *in vitro* experiments by Boehnlein *et al.* (1994) where radioactive retinyl palmitate in an aceton vehicle was applied on viable human skin, about 44% of the absorbed dose was

metabolised to retinol. No other additional metabolites were observed indicating that topically applied retinyl palmitate may deliver significant amounts of retinol into the skin.

Antille *et al.* (2004) applied oil-in-water creams containing 0.05% retinoids (retinoid acid, retinal, retinol or retinyl palmitate) onto fresh surgically excised human abdominal skin (25 skin explants from 5 females aged 26-66 years). After 24 hours incubation at 37 °C (mounted on Franz perfusion cells), all retinoids penetrated well into the epidermis. Retinal induced a slight increase in endogenous retinol and retinyl esters, and a small part was transformed into retinoic acid. Retinol and its palmitic esters increased endogenous retinol and retinyl esters, but no retinal or retinoic acid was found. Retinoic acid did not undergo metabolism.

In human volunteers, Kang *et al.* (1995) applied varying concentrations of retinol (up to 1.6%), 0.025% retinoic acid and a vehicle (70% ethanol/30% propylene glycol) to buttock skin (100 µl/18 cm^2). The sites were occluded under plastic wrap and covered with light-proof dressing for four days. Biopsies were obtained on day 4. After removal of stratum corneum, the epidermal content of retinyl ester and retinoic acid were measured (reverse-phase HPLC). The four day occlusion with 0.4% retinol increased the skin content of retinol, 13-*cis*-retinol and retinyl esters in comparison with vehicle. In another group of volunteers, biopsies were performed after retinol (0.4%) occlusive patches had been in place for 0, 6, 24 and 96 hours. At 24 hours, the levels of retinol had increased 70-fold, 13-*cis*-retinol 280-fold and retinyl esters 260-fold. Retinoic acid was undetectable or found at trace levels only. In untreated skin, retinyl ester was found in relatively small amounts compared to retinol (41±27 vs 305±28 ng/g wet weight). Of the esters, retinyl linoleate was the predominant ester at all time points examined. Other identified retinyl esters were oleate, palmitate, laurate and stearate (each made up less than 10% of the total).

Metabolisms of retinol into retinyl esters seem to be the primary route for disposition of retinol in the skin, suggesting that retinol may be metabolised into retinyl ester to prevent further conversion of retinol to retinoic acid (Figure 3). Retinol and retinyl esters account for more than 99% of total epidermal retinoids (reviewed in Sorg *et al*., 2006). The lack of detectable increase in retinoic acid in the skin following application of retinol may be explained by a tightly controlled, low-level conversion of retinol to retinoic acid at specific physiologically relevant sites (Kang *et al*., 1995). Adequate retinol levels in the skin are obtained by retrieval of retinyl esters from their storage sites in the skin followed by hydrolysis of the esters into retinol. The intracellular storage site is, however, not known. Retinol is then oxidised to retinoic acid by a two-step pathway: i) Oxidation of retinol to retinal by a cytoplasmic alcohol/retinol dehydrogenase and a microsomal, short-chain retinol dehydrogenase. Both unbound retinol and retinol-RBP can be oxidised. ii) Oxidation of retinal to retinoic acid where aldehyde dehydrogenase may play an important role. The formation of retinal is considered to be the rate-limiting step (Fu *et al*., 2007).

2.3.5 Dermal absorption and systemic availability of topical retinoids

In the *in vivo* studies cited in section 2.3.1, 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), 7.82 g

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 recommended 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 (Penniston and Tanumihardjo, 2006). 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 has 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 pertubation of previously established retinoid homeostasis (Kang *et al*., 1995; Duell *et al*., 1997; Fu *et al*., 2007).

SCCS has in the Notes of Guidance for the Testing of Cosmetic ingredients (SCCS, 2010) provided general guidelines to estimate the systemic availability (SED: systemic exposure dose) of a cosmetic ingredient by taking into account the daily amount of finished cosmetic product applied, the concentration of the ingredient, the dermal absorption of that particular ingredient and a mean human body weight value. Preferably, SED should be calculated based on the absolute amount bioavailable (µg/cm^2) after a certain time period, but calculations based on the percentage dermally absorbed may also be used.

According to the SCCS' Notes of Guidance for the Testing of Cosmetic ingredients, dermal absorption is defined as the amount measured in the dermis, epidermis (without stratum corneum) and the receptor fluid (SCCS, 2010). When studies fulfil the SCCS basic requirements for *in vitro* dermal absorption studies (Appendix I), the mean + 1SD should be used when calculating the margin of safety (MoS). However, in case of significant deviations from the protocol and/or very high variability, the mean + 2SD should be used. None of the *in vitro* studies assessed in the present opinion (Boehnlein *et al*., 1994; Leclerc, 1997; Yourick *et al*., 2008) fulfil the SCCS requirements. When it comes to the *in vitro* experimental conditions (e.g. diffusion cell designs, receptor fluid, skin preparation, dose/volume/contact time), they are comparable. The main differences between the studies are the choice of vehicles and measurements of the test substance in the relevant compartments (excess on the skin (dislodgeable dose), stratum corneum, living epidermis (without stratum corneum), dermis, receptor fluid). In the study by Boehnlein *et al*. (1994), information on the absorption in all relevant compartments is not available. The amount of absorbed retinyl palmitate is defined as the sum of absorbed in skin and receptor fluid. However, there is no information on whether the stratum corneum was removed before the homogenisation of the skin samples. In addition, there is no information on the dislodgeable dose. Lerclerc *et al.* (1997) define the absorbed amount as the sum of absorbed in epidermis, dermis and receptor fluid. The amount in the different compartments is shown, but only in three-dimensional figures making it difficult to define the exact absorption rates. There is no information on the dislodgeable dose.

The study considered to best fulfil the SCCS's criteria was Yourick *et al*. (2008) where the amount absorbed in all the different compartments were measured. However, the standard error has been used when calculating dermal absorption due to lack of information on the standard deviation. Data derived from the oil-in-water emulsion was chosen because it best mimics the vehicle used in cosmetic products as moisturisers. The absorptions after 24 hours were in viable skin 3.0%±0.6 and receptor fluid 1.3%±0.1. The value of dermally absorbed retinol in agreement with the SCCS's guideline (mean absorption in viable skin + receptor

fluid + 2 SD) was estimated by VKM to be 5.7%. This value correspond well with the absorption value of 5.3% calculated from the human *in vivo* study by Franz and Lehman (1990) after topical application of retinoic acid.

2.4 Local adverse effects of retinoids

2.4.1 Skin irritation

The main effect of topical retinoids is believed to be the receptor-mediated gene activation induced by the ligand retinoic acid. Accordingly, retinoic acid is the most potent topical retinoid and not allowed in cosmetics. Apparently, the other topical retinoids have to be metabolised to retinoic acid by the skin to exert their genomic effects and their potency are strongly dependent on their metabolic distance to retinoic acid. Hence, the retinoid-like activity after topical application is increasing in the following order: retinyl esters << retinol < retinal < retinoic acid. Notably, the similar ranking is seen for their capacity to induce skin irritation, the most pronounced local adverse effects. Retinoic acid is the most toxic retinoid, and the ability of a topical retinoid to enhance the local content of retinoic acid seems to predict its toxicity profile. For all topical retinoids the skin irritation effect is dose-dependent, and the adverse effect may be avoided with a topical dose below 0.3% (retinol) or 0.55% (retinyl esters) (Nohynek *et al*., 2006).

Irritated skin is characterized by redness, dryness and flaking of the skin at the treated site (Varani *et al*., 2008). Epidermal hyperplasia and features of abnormal differentiation are seen at the histological level. The molecular events that underlie retinoid-induced irritation are not clear.

2.4.2 Photocarcinogenesis

Experimental studies have indicated that topically applied retinoids can, under some conditions of testing, enhance photocarcinogenesis. In 2000, the Center for Food Safety and Applied Nutrition (CFSAN) within the FDA in USA nominated retinyl palmitate to National Toxicology Program (NTP) for phototoxicity and photocarcinogenicity testing. The nomination was based on the increasingly widespread use of this compound in cosmetic retail products for use on sun-exposed skin, the biochemical and histological cutaneous alterations elicited by retinyl palmitate, and the association between topical application of retinoids and enhancement of photocarcinogenesis (FSA CFSAN, 2000). 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 SKH-1 mice exposed to simulated solar light (SSL), UVA, or UVB. A draft report of this study was available in 2010 (NTP, 2010).

In the Technical Reports Peer Review Meeting January 26, 2011 (NTP, 2011), the panel agreed that under the conditions of these studies, the topical treatment of SKH-1 mice with the control cream (vehicle without retinoids) resulted in earlier onsets of in-life skin lesions and higher incidences and multiplicities of in-life skin lesions, 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. The topical treatment of SKH-1 mice with control cream 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. Compared to the control cream, retinoic acid and retinyl palmitate further enhanced the

effects of SSL in SKH-1 mice based upon earlier onsets and increased multiplicities of in-life skin lesions. 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.

In the same meeting, it was expressed some concern about the methodology used, the significant effect of the control cream, possible effects of animals scratching themselves as a result of irritation from high doses of retinoids and exclusion of animals because of skin condition. It was also commented that the experimental protocol for the study was appropriately chosen, the lines of evidence all point to the photocarcinogenic effect of retinyl palmitate in combination with sunlight, and the findings of the NTP study are in agreement with the research database on the phototoxicity and photococarcinogenicity of retinoid compounds.

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 produce genotoxic effects in mouse lymphoma or human Jurkat T-cells via a photoclastogenic mechanism (Mei *et al*., 2005; 2006, Yan *et al*., 2005). Other promotional mechanisms could also be involved, such as stimulation of cell proliferation, hyperplasia, irritation and erythema. These effects of topical retinoids are well known in human skin (Sorg *et al*., 2006).

The NTP photocarcinogenesis study of retinyl palmitate and retinoic acid in hairless mouse skin has introduced safety concern about the use of topical retinoids in combination with sun exposure. However, 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 it is not adapted to intense UV light. In contrast, the human skin is practically hairless and in general relatively well adapted to sunlight, but with some variation because of different pigmentation. The function 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. Wang *et al*. (2010) has critically analysed the safety of retinyl palmitate in sunscreens with particular focus on the NTP study. They point out two observations from clinical medicine that provide the strongest challenges to the notion that topical retinoids may be photocarcinogenic in humans: after more than 40 years use of topical retinoids and medical follow up there is no published evidence of increased risk of photocarcinogenesis, and oral and topical retinoids are in fact used for chemoprevention of skin cancers in individuals at high risk.

In conclusion, VKM notes that the NTP data may indicate that retinol and retinyl palmitate could be photocarcinogenic in mice. However, these results do not provide sufficient information for a risk assessment of this effect of retinol and retinyl esters in cosmetics. More studies are needed to clarify mechanisms of possible retinoid photocarcinogenesis as well as characteristics needed for the extrapolation from mouse skin to human skin.

2.4.3 Other potential local biological adverse effects

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. Topical retinoids from cosmetics are absorbed and loaded in the skin, and they interfere with the normal vitamin A function in a long-term perspective, for many users a life-long perspective.

No published materials have indicated that long-term use of topical retinoids may induce local biological adverse effects other than skin irritation, even though these retinoids have been used in cosmetics and medical treatment for more than 30 years.

2.5 Critical effect and upper intake levels

It is considered difficult to determine a tolerable upper intake level for preformed vitamin A due to the narrow margin between the RDI and intakes that are associated with adverse health effects. The teratogenic potential of vitamin A has received the most attention in setting an UL for intake, and dose-response relationship has been demonstrated in several human studies. In 2002, the Scientific Committee on Food (SCF) derived an UL of 3000 µg RE/day for all women of child-bearing age based on the teratogenic potential of vitamin A (SCF, 2002). The UL value is also lower than the daily intake that has been demonstrated to cause hepatotoxicity (7500 µg RE/day). The lowest reported effect doses for selected adverse effects are presented in Table 2 (adapted from SCF 2002; EFSA, 2006a).

Table 2: Reported lowest effect doses for a selection of adverse effects[1].

Effect	Lowest reported effect dose
Teratogenesis	>3000 µg RE/day (from Rothman *et al*., 1995)
Bone density/fracture	1500 µg RE/day (trend analysis do not show a threshold)
Hepatotoxicity	7500 µg RE/day for 6 years
Bulging fontanelle	7500 µg RE/day (as a single dose in infants)
Lipid metabolism	7500 µg RE/day for 4 years (only a minor change)

[1]Data from SCF, 2002.

The UL set by SCF is derived by fitting a curve to the dose response data from the large number of subjects in the at risk group included in the study cohort (Rothman *et al*., 1995). SCF acknowledged that a clear threshold for teratogenicity was difficult to establish, but it was found appropriate to use the value of 3000 µg RE/day. Since data from other studies indicated that the true threshold for an effect could be higher, SCF considered it not necessary to use any uncertainty factor.

Although teratogenicity is only relevant to women of child-bearing age, SCF considered that the upper level of 3000 µg RE/day is appropriate for men and for infants and children after correction for differences in metabolic rate, because it is 2.5-fold lower than the lowest daily intake associated with other toxic effects such as hepatotoxicity during chronic intake. The tolerable upper intake levels for different age groups presented in Table 3 is based on the value of 3000 µg RE/day for adults, with correction for differences in basal metabolic rate compared to adults using a scaling factor (body weight$^{0.75}$) (SCF, 2002; EFSA, 2008).

Table 3: Tolerable Upper Intake Levels (UL) for preformed vitamin A (retinol and retinyl esters) for different age groups (SCF, 2002; EFSA, 2008).

Age (years)	Tolerable Upper Intake Level (UL) for preformed vitamin A (retinol and retinyl esters) (µg RE/day)
1–3	800
4–6	1100
7–10	1500
11–14	2000
15–17	2600
Adults*	3000

* Women of child-bearing age and men

The UL derived by SCF may, however, not adequately address the possible risk of bone fracture in particularly vulnerable groups, such as postmenopausal women. SCF therefore recommended that it would be advisable for postmenopausal women, who are at greater risk of osteoporosis and fracture, to restrict their intake to 1500 µg RE/day (SCF, 2002).

In a more recent scientific opinion published by the EFSA FEEDAP Panel in 2008, the UL set by SCF was considered as still being appropriate, taking into account new available data published later than 2002 (SACN, 2005; AFSSA, 2005; Ribaya-Mercado and Blumberg, 2007). EFSA further concluded that quantitative correlations between retinol intake and bone health risk could not justify the establishment of a lower UL for a specific population subgroup (elderly people). Until new data indicates the necessity of a re-evaluation, EFSA therefore 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) (EFSA, 2008).

The main contribution to vitamin A intake comes from food and food supplements. However, an additional source to the total vitamin A exposure is the use of cosmetic products containing retinol, retinyl esters and retinal. Consequently, the assessments for the different age groups of the population are based on the total estimated exposures from food, food supplements and cosmetics. For the risk characterisation in this opinion, VKM has chosen to use the age-differentiated UL levels (Table 3) and the GL level for individuals at risk of osteoporosis and bone fracture established by SCF/EFSA (SCF, 2002; EFSA, 2008).

3 Exposure characterisation

3.1 Exposure to retinol and retinyl esters from the use of cosmetics

Vitamin A, in the form of retinol and retinyl esters, is widely used in cosmetic products. Retinol and its esters, such as retinyl palmitate or retinyl acetate are used to improve the appearance of skin by reducing fine lines and wrinkles and to generally improve the appearance of skin (Yourick *et al*., 2008). These ingredients are listed as skin conditioning agents in the EU inventory list for cosmetic products and are mainly used in various face creams with anti-photo aging purposes and in moisturizers.

According to Nohynek *et al*., (2006), retinol and retinyl esters are used in skin care products and cosmetic preparations at concentrations of up to 0.3% (retinol) or 0.55% (retinyl palmitate). Given that higher concentrations tend to be irritating to the skin, they are considered to be unsuitable for cosmetic use (Ries and Hess, 1999; Fluhr *et al*., 1999). Recent information from the cosmetic industry and the BfR refers to the following concentrations of retinol and retinyl esters in different cosmetic products (personal communication G. Nohynek, L'Oreal, October 2011; BfR, 2010):

- Face and hand creams: 0.01-0.3% RE*
- Body lotions: 0.01-0.05% RE*
- Rinse-off products: 0.01-0.3% RE*

*RE = retinol equivalents, i.e. retinyl palmitate and retinyl acetate at corresponding retinol concentrations (see section 1.1.2).

The use of retinol and retinyl esters in cosmetic products is restricted in the Norwegian cosmetics regulations with maximum allowed concentrations of 0.3% (retinol) and 0.7% (retinyl palmitate).

3.1.1 Estimated exposure to retinol and retinyl esters from the use of cosmetic products in different age groups in Norway

In this opinion, VKM has estimated the systemic exposure dose (SED) to vitamin A (retinol and retinyl esters) from topical application of cosmetic products in different age groups of the Norwegian population.

The different exposure scenarios presented in Table 4-6 are based on default values for exposure to the relevant cosmetic products described in the SCCS's Notes of Guidance for the Testing of Cosmetic Ingredients and Their Safety Evaluation (SCCS, 2010). The SED has been calculated as follows:

SED = Daily exposure to a cosmetic product (mg/kg bw/day) x concentration of the ingredient (%)/100 x dermal absorption (%)/100

Various concentrations of vitamin A (retinol and its esters) in the products have been used to illustrate how the estimated SED could be altered in the different scenarios.

3.1.1.1 *Daily exposure to skin care products used in the exposure scenarios*

In the new version of the Notes of Guidance (7th revision), more recent and robust cosmetic exposure data, provided by the European Cosmetics Association (Colipa), have been incorporated for 6 product types (body lotion, deodorant, facial moisturiser, shampoo, lipstick and toothpaste). The following default values for daily exposure to different skin care products (Table 3 in Notes of Guidance) are used in this opinion:

- Body lotion: 7.82 g/day, equivalent to 123.20 mg/kg bw/day
- Face cream: 1.54 g/day, equivalent to 24.14 mg/kg bw/day
- Hand cream: 2.16 g/day, equivalent to 32.70 mg/kg bw/day

The daily exposure values presented in Table 3 in the Notes of Guidance are valid for adults. For the younger age groups, the daily amount applied has been adjusted to the difference in skin surface area over body weight ratio (SSA/BW) between adults and children. The SSA/BW ratios for children from 0 to 10 years described in Notes of Guidance have been used in the different exposure scenarios:

- 1.6 fold at 12 months (used for 1- and 2-year-olds)
- 1.5 fold at 5 years (used for 4-year-olds)
- 1.3 fold at 10 years (used for 9-year-olds)

The daily exposure to skin care products for 13-year-old adolescents has been assumed to be similar to adults, as there is no correction factor for SSA/BW ratio above 10 years in the Notes of Guidance (SCCS, 2010).

3.1.1.2 *Concentrations of retinol and retinyl esters in skin care products used in the exposure scenarios*

The exposure scenarios are based on different concentrations of retinol and retinyl esters in the relevant cosmetic products. The maximum allowed concentrations for retinol and retinyl esters in Norway, and the concentration levels referred to by the cosmetic industry and BfR have been used in the estimates (Norwegian Cosmetic Regulations, 1995; BfR, 2010). In addition, a scenario for cosmetic products containing as much as 1% retinol has been included (information in a letter from the Norwegian Medicines Agency (2009) regarding an application for classification of medicinal products).

The concentrations of retinol and retinyl esters in cosmetic products are apparently limited at concentrations up to 0.3% retinol or 0.55% retinyl palmitate because higher concentrations will irritate the skin (Nohynek *et al.*, 2006). Since the effects of retinyl esters are related to their retinol activity (i.e. 0.55% retinyl palmitate comprises 0.3% retinol), all concentrations have been given in % or μg RE (see also 1.1.2).

The following concentrations have been used for different cosmetic product types in the exposure scenarios:

- Body lotion:
  - Standard scenario: 0.05% (BfR, 2010)
  - Worst case scenario: 0.3% (maximum allowed concentration in Norway)
- Face cream:
  - Standard scenario: 0.3% (BfR, 2010; maximum allowed concentration in Norway)
  - Worst case scenario: 1% (based on information from the Norwegian Medicines Agency (2009) that products for cosmetic use contain such concentrations).

- Hand cream:
  - Standard scenario: 0.3% (BfR, 2010; maximum allowed concentration in Norway)
  - Worst case scenario: 1% (based on information from the Norwegian Medicines Agency (2009) that products for cosmetic use could contain such concentrations).

It should also be noted that the German database "Codecheck.info" (http://www.codecheck.info/kosmetische_mittel.kat), which contains around 40000 cosmetic products offered for sale in German retailer outlets, gives information of cosmetic products with even higher concentrations of retinol than 1%. These products are especially intended for the niche market of anti-aging products/beauty saloons. However, products with retinol concentrations above 1% have not been considered in this risk assessment.

3.1.1.3 *Dermal absorption data used in the exposure scenarios*

The dermal absorption rate for retinol and retinyl esters has been based on the data for oil-in-water emulsions in the study of Yourick *et al*. (2008). Taking into account the basic criteria for dermal/percutaneous absorption studies described in SCCS's Notes of Guidance (SCCS, 2010), a dermal absorption rate of 5.7% has been calculated and used in the exposure scenarios in this opinion from VKM. For more details, see section 2.3.

3.1.2 Estimated exposure to retinol and retinyl esters from the use of cosmetic products for infants and children

The estimated exposure to retinol and retinyl esters from topical application of cosmetic products for 1-year old infants, 2-, 4- and 9-year-old children is shown in Table 4. Application of baby skin care products containing vitamin A, such as e.g. body lotions or creams is considered relevant for 1 year-old infants and 2-year-old children. A similar exposure situation from application of body lotions has been assumed for 4- and 9-year-old children.

Two different exposure scenarios for topical application of body lotions containing retinol and retinyl esters for different age groups of children are presented in Table 4; first a scenario based on a concentration of 0.05% in body lotions referred to by the cosmetic industry and BfR (standard scenario), and secondly a worst case scenario based on the maximum allowed concentration of retinol and retinyl esters in cosmetic products in Norway (0.3%).

Table 4: Exposure scenarios for the application of skin care products containing retinol and retinyl esters for infants and children.

Cosmetic product type	Estimated daily amount applied (g)[1]	Concentration of retinol and retinyl esters (%[2])[3]	Dermal absorption (%)[4]	SSA/BW[5]	Systemic exposure dose (SED) (µg RE/day)	UL
1-year-old infants						
Body lotion	1.22	0.05	5.7	1.6	56	800
Body lotion	1.22	0.3	5.7	1.6	334	800
2-year-old children						
Body lotion	1.58	0.05	5.7	1.6	72	800

Body lotion	1.58	0.3	5.7	1.6	432	800
4-year-old children						
Body lotion	2.22	0.05	5.7	1.5	95	1100
Body lotion	2.22	0.3	5.7	1.5	569	1100
9-year-old children						
Body lotion	3.94	0.05	5.7	1.3	146	1500
Body lotion	3.94	0.3	5.7	1.3	876	1500

[1]Based on default exposure levels from Table 3 in SCCS Notes of guidance and adjusted for the mean body weight of 1-year-old infants (9.9 kg), 2-year-old children (12.8 kg), 4-year-old children (18 kg) and 9-year-old children (32 kg).
[2]Retinyl palmitate and retinyl acetate at corresponding retinol concentrations.
[3]Standard scenario: 0.05% in body lotion, Worst case scenario: 0.3% in body lotion.
[4]The different scenarios are based on a dermal absorption rate of 5.7% (Yourick *et al*., 1998; SCCS, 2010).
[5]Factor for the difference in skin surface area (SSA) over body weight (BW) ratio between adults and children (SCCS, 2010).

From Table 4 it can be seen that the estimated SED from topical application of body lotions containing 0.05% ranged from 56 µg RE/day in 1-year-old infants to 146 µg RE/day in 9-year-olds.

In the worst case scenario that was based on the maximum allowed concentration of retinol in cosmetic products in Norway (0.3%), the estimated exposure increased to 334 µg RE/day for 1-year-old infants. For 2-, 4- and 9-year-old children, the respective SED-values were estimated to 432, 569 and 876 µg RE/day for topical application of body lotions containing 0.3%.

3.1.3 Estimated exposure to retinol and retinyl esters from the use of cosmetic products for adolescents

The estimated exposure to retinol and retinyl esters from topical application of cosmetic products for 13-year-old adolescents is shown in Table 5. Application of skin care products containing vitamin A, such as body lotions and hand creams, is considered a realistic exposure for Norwegian adolescents.

Different exposure scenarios for adolescents, taking into account topical application of both body lotions, hand creams and a combination of these two skin care products containing retinol and retinyl esters are presented in Table 5. The two scenarios for body lotions are based on the concentration levels of retinol or retinyl esters in such products (0.05%) referred to by the cosmetic industry and BfR and on the maximum allowed concentration of retinol in cosmetic products in Norway (0.3%). For hand creams, the first scenario is based on the maximum allowed concentration in Norway (0.3%), whereas the second scenario is based on information in a letter from the Norwegian Medicines Agency (2009) regarding an application for classification of medicinal products showing that products for cosmetic use could contain 1% retinol.

Table 5: Exposure scenarios for the application of skin care products containing retinol and retinyl esters for 13-year-old adolescents.

Cosmetic product type	Estimated daily amount applied (g)[1]	Concentration of retinol and retinyl esters (%[2])[3]	Dermal absorption (%)[4]	SSA/BW[5]	Systemic exposure dose (SED) (µg RE/day)	UL
Body lotion	6.10	0.05	5.7	1	174	2000
Body lotion	6.10	0.3	5.7	1	1043	2000
Hand cream	1.62	0.3	5.7	1	277	2000
Hand cream	1.62	1.0	5.7	1	923	2000
Body lotion + hand cream	6.10 1.62	0.05 0.3	5.7	1	451	2000
Body lotion + hand cream	6.10 1.62	0.3 1.0	5.7	1	1967	2000

[1]Based on default exposure levels from Table 3 in SCCS Notes of guidance and adjusted for the mean body weight of 13-year-old adolescents (49.5 kg).
[2]Retinyl palmitate and retinyl acetate at corresponding retinol concentrations.
[3]Standard scenario: 0.05% in body lotion and 0.3% in hand cream, Worst case scenario: 0.3% in body lotion and 1.0% in hand cream.
[4]The different scenarios are based on a dermal absorption rate of 5.7% (Yourick *et al*., 1998; SCCS, 2010).
[5]Factor for the difference in skin surface area (SSA) over body weight (BW) ratio between adults and children (SCCS, 2010).

The results in Table 5 show that the SED from topical application of body lotions containing retinol and retinyl esters in 13-year-old adolescents could be estimated to 174 and 1043 µg RE/day for the standard scenario (0.05%) and the worst case scenario (0.3%), respectively.

For topical application of hand creams containing retinol and retinyl esters, the standard scenario based on the maximum allowed concentration of retinol in cosmetic products in Norway (0.3%) resulted in an estimated SED of 277 µg RE/day. In the worst case scenario (1%) (Norwegian Medicines Agency, 2009), a SED of 923 µg RE/day was calculated.

For topical application of both body lotions and hand creams daily, SED-values of 451 µg RE/day and 1967 µg RE/day were estimated for the standard scenario and the worst case scenario, respectively.

3.1.4 Estimated exposure to retinol and retinyl esters from the use of cosmetic products for adults

The estimated exposure to retinol and retinyl esters from topical application of cosmetic products for adults is shown in Table 6. Application of various skin care products containing vitamin A, such as body lotions, face creams and hand creams, is considered as a relevant and realistic exposure for Norwegian adults of both gender.

Different exposure scenarios for adults, taking into account topical application of body lotions, face creams and hand creams and a combination of all these three skin care products containing retinol and retinyl esters are presented in Table 6. The two scenarios for body lotions and hand creams are based on the same concentration levels of retinol or retinyl esters as described in the exposure scenarios for adolescents (section 3.1.3). For face creams, the standard scenario is based on the maximum allowed concentration of retinol in cosmetic products in Norway (0.3%), whereas the worst case scenario is based on information in a letter from the Norwegian Medicines Agency (2009) regarding an application for classification of medicinal products showing that products for cosmetic use could contain 1% retinol.

Table 6: Exposure scenarios for the application of skin care products containing retinol and retinyl esters for adults.

Cosmetic product type	Estimated daily amount applied (g)[1]	Concentration of retinol and retinyl esters (%[2])[3]	Dermal absorption (%)[4]	SSA/BW[5]	Systemic exposure dose (SED) (µg RE/day)	UL
Body lotion	7.82	0.05	5.7	1	223	3000
Body lotion	7.82	0.3	5.7	1	1337	3000
Face cream	1.54	0.3	5.7	1	263	3000
Face cream	1.54	1.0	5.7	1	878	3000
Hand cream	2.16	0.3	5.7	1	369	3000
Hand cream	2.16	1.0	5.7	1	1231	3000
Body lotion + face cream + hand cream	7.82 1.54 2.16	0.05 0.3 0.3	5.7	1	856	3000
Body lotion + face cream + hand cream	7.82 1.54 2.16	0.3 1.0 1.0	5.7	1	3446	3000

[1]Based on default exposure levels from Table 3 in SCCS Notes of guidance.
[2]Retinyl palmitate and retinyl acetate at corresponding retinol concentrations.
[3]Standard scenario: 0.05% in body lotion and 0.3% in hand cream and face cream, Worst case scenario: 0.3% in body lotion and 1.0% in hand cream and face cream.
[4]The different scenarios are based on a dermal absorption rate of 5.7% (Yourick *et al*., 1998; SCCS, 2010).
[5]Factor for the difference in skin surface area (SSA) over body weight (BW) ratio between adults and children (SCCS, 2010).

In Table 6, the SED from topical application of body lotions containing retinol and its esters in adults has been estimated to 223 µg RE/day in the standard scenario (0.05%) and 1337 µg RE/day in the worst case scenario (0.3%).

The two exposure scenarios for face creams resulted in a SED of 263 µg RE/day for products containing 0.3% and 878 µg RE/day for products containing 1.0% retinol. Slightly higher exposures were found for the two scenarios for topical application of hand creams. The standard scenario based on the maximum allowed concentration of retinol in cosmetic products in Norway (0.3%) resulted in a SED of 369 µg RE/day. In the worst case scenario (1.0%) (Norwegian Medicines Agency, 2009), a SED of 1231 µg RE/day was calculated.

For a daily topical application of body lotions, face creams and hand creams, SED values of 856 µg RE/day and 3446 µg RE/day were estimated for the standard scenario and the worst case scenario, respectively. Daily application of all the abovementioned vitamin A-containing products should be considered more common for women than for men.

3.2 Dietary intake from preformed vitamin A

Vitamin A is present in the diet as preformed vitamin A in various animal sources such as liver, milk, eggs, butter and fish liver oil or as provitamin A carotenoids (mainly ß-carotene) in dark green leafy vegetables, and in red or orange coloured fruits and vegetables such as carrot, mango, papaya and sweet potato (Blomhoff *et al*., 2003; EFSA, 2008). In addition, preformed vitamin A is also contained in a number of mono and multivitamin supplements. The dietary intake from vitamin A calculated in this VKM opinion only consider the intake from preformed vitamin A (retinol), as there is no evidence for vitamin A-related toxicity from provitamin A carotenoids.

3.2.1 Methodological considerations and description of the national consumption surveys

The dietary intake estimates from preformed vitamin A presented in this opinion are based on data from the nationally food consumption surveys for infants, children, adolescents and adults. The food consumption data are the most complete and detailed currently available in Norway. However, it should be pointed out that three different methodologies were used in the different surveys and thus direct comparisons between different age groups (1-2-year-olds/ 4-13 year-olds/ adults) can be misleading.

A short description of the consumption surveys and the different methodologies used is given below:

- 1-year-old infants; Spedkost 2006-2007 is based on a semi-quantitative food frequency questionnaire. In addition to predefined household units, food amounts were also estimated from photographs. The study was conducted in 2007, and a total of 1635 1-year-old children participated (Øverby *et al*., 2009).
- 2-year-old children; Småbarnskost 2007 is based on a semi-quantitative food frequency questionnaire. In addition to predefined household units, food amounts were also estimated from photographs. The study was conducted in 2007, and a total of 1674 2-year-olds participated (Kristiansen *et al*., 2009).
- 4-, 9-, and 13-year-old children/adolescents; Ungkost 2000 is based on a 4-day food intake registration with a precoded food diary. Food amounts were presented in predefined household units or as portions estimated from photographs. The study among 4-year-olds was conducted in 2001, and 391 4-year-old children participated (Pollestad *et al*., 2002). The study among 9- and 13-year-olds was conducted in 2000 and 810 9-year-old children and 1005 13-year-old adolescents participated (Øverby and Andersen, 2002).

- Adults; Norkost 3 is based on two 24-hour recalls by telephone at least one month apart. Food amounts were presented in household measures or estimated from photographs (Totland *et al*., 2012). The study was conducted in 2010/2011 and 925 women and 862 men aged 18-70 years participated.

Daily intake of preformed vitamin A was computed by using food databases in the software system (KBS) developed at the Institute of Basic Medical Sciences, Department of Nutrition, at the University of Oslo. The food databases are mainly based on various versions of the official Norwegian food composition table (Rimestad *et al*., 2000, The Norwegian Food Composition Table, 1995; 2006) and are continuously supplemented with data on new food items. The three dietary surveys that are the basis for this risk assessment were conducted at three different time points, Ungkost-2000 in 2000-2001, Sped- and Småbarnskost in 2007 and the data for Norkost 3 were collected in 2010-2011. The retinol calculations were conducted both with and without vitamin supplements.

In the intake calculations each age group are divided in two, with one group consisting of those taking retinol supplements and the other group consisting of those not taking retinol supplements.

As retinol is found in relatively large quantities in a small number of foods, it is difficult to estimate the intake with few food registration days. To obtain an accurate estimate of individual retinol intakes a large number of repeated measurements are needed (Willett, 1998).

3.2.2 Contribution of major food sources to retinol intake in Norway

The relative contribution of major food sources, including food supplements, to the intake of retinol in different age groups is shown in Table 7. The table also shows the mean intake of retinol for all participants in each age group. The figures presented are based on the intakes reported by all participants in the consumption surveys.

Table 7: Relative contribution (%) of major food sources, including food supplements, to retinol intake based on data from all participants in the Norwegian consumption surveys. For reference, mean intake of retinol in each age group is presented.

	1-year-olds (n=1635)	2-year-olds (n=1674)	4-year-olds (n=391)	9-year-olds (n=810)	13-year-olds (n=1005)	Adults (n=1787)
Mean intake of retinol	*467 µg*	*705 µg*	*848 µg*	*832 µg*	*766 µg*	*808 µg*
Food supplements	22	21	29	23	20	17
Meat, blood, offal (liver pâté)	40 (40)	32 (31)	28 (24)	27 (24)	28 (16)	22 (19)
Butter, oil, margarine	18	21	17	19	16	22
Milk, cream, ice-cream	6	10	12	13	12	10
Cheese	5	8	5	7	8	11

Liver pâté is the single food item contributing most to retinol intake on group level in all age groups (Table 7). Food supplements are also a major contributor of retinol with between 17% and 29% relative to the intake in the different age groups. Milk and milk products, and butter, oil, and margarine with fortified retinol do also contribute to the overall intake.

Worth mentioning is that the retinol content of cod liver oil, decreased from 10000 µg/100 g cod liver oil in year 2000 to 5000 µg/100 g cod liver oil in 2007. It is therefore likely that the retinol intake calculated from cod liver oil in Ungkost 2000 is an overestimation of the intake compared with today's retinol content in cod liver oil.

3.2.3 Estimated intake of preformed vitamin A in the Norwegian population

The estimated intakes (mean and 95 percentile) in different age groups in the Norwegian population are shown in Table 8. The results are presented both for the selection of participants with a reported consumption of vitamin A supplements and for those that did not consume food supplements. For 1-year-old infants, the intakes have been estimated both for the infants that were breastfed and those that were not breastfed at the age of 12 months.

Table 8: Mean and 95 percentile intakes for consumers of preformed vitamin A (retinol) in different age groups (µg RE/day).

Diet without food supplements			Diet including food supplements		
Age group (number of consumers)	**Mean intake of retinol**	**95 percentile intake of retinol**	**Age group (number of consumers)**	**Meam intake of retinol**	**95 percentile intake of retinol**
1-year old infants (breastfed) (n=354)	315	737	**1-year old infants (breastfed) (n=368)**	521	1080
1-year old infants (non-breastfed) (n=485)	394	1005	**1-year old infants (non-breastfed) (n=396)**	644	1297
2-year-old children (n=775)	563	1129	**2-year-old children (n=899)**	827	1485
4-year-old children (n=156)	592	1329	**4-year-old children (n=235)**	1019	1711
9-year-old children (n=450)	651	1581	**9-year-old children (n=360)**	1060	2015
13-year-old children (n=708)	631	1293	**13-year-old children (n=297)**	1087	2184
Adults (women) (n=629)	544	1236	**Adults (women) (n=296)**	983	1983
Adults (men) (n=587)	751	1635	**Adults (men) (n=275)**	1349	2691

The intake of retinol varies between age groups. Those who take supplements have a higher mean and 95 percentile intake compared with those not taking supplements in the same age group. Adult men taking supplements have the highest intake with 2691 µg retinol among the 95 percentile. The 13-year-old adolescents have the second highest intake with 2184 µg retinol among the 95 percentile.

4 Risk characterisation

4.1 Systemic effects of retinol and retinyl esters from food, food supplements and cosmetics

VKM has chosen to base this risk characterisation on the ULs derived by SCF/EFSA and the estimated total exposure from diet, supplements and cosmetics for different age groups in the Norwegian population. For adult women, as considered at greater risk of osteoporosis and bone fracture, the estimated total intake is also discussed in relation to the GL level based on retinol intake and effects on bone health. Diet is the dominating source, but the contribution from supplements is substantial. In addition, dermal absorption of retinol and retinyl esters increases the total exposure proportionately to the use of cosmetics. Dietary intake of preformed vitamin A is estimated from national food consumption surveys for the various age groups and the risk characterisation for this intake in the different age groups has been demonstrated for two different groups of the population. One group is based on the individuals not consuming supplements and where retinol intake from diet only is included. The other group is based on the individuals that have consumed supplements with retinol, and where the intake of retinol from both diet and supplements are combined.

The estimated additional contribution from cosmetics is calculated according to the SCCS's Notes of Guidance for the Testing of Cosmetics Ingredients and Their Safety Evaluation (SCCS, 2010). The estimated values are based on default values for daily exposure to various skin care products and a dermal absorption rate of skin products of 5.7% derived from the study of Yourick *et al*. (2008). The concentrations of vitamin A generally used (body lotion 0.05%, face and hand cream 0.3%) have been chosen for the standard exposure scenario in this opinion. As a worst case scenario, the estimated contribution from cosmetics is based on an assumed increase in the concentration of vitamin A as described in 3.1.1.2 (body lotion 0.3%, face and hand cream 1%). The estimated systemic exposure dose (SED) both for the standard and the worst case scenarios, as well as the SEDs as percentage of the ULs for the different age groups, are presented in Table 9. When exposure to retinol and retinyl esters from cosmetics is calculated as percentage of UL, however, the values may represent an underestimation relative to the contribution from consumption of food and food supplements. This is because UL is based on dietary intake of preformed vitamin A and do not take into account the 70 – 90% absorption rate in the intestine (NNR, 2004).

Table 9: Exposure scenarios for the application of skin care products containing retinol and retinyl esters compared to the tolerable upper intake level (UL).

Cosmetic product type	Concentration of retinol and retinyl esters (%[1])[2]	Systemic exposure dose (SED) (µg RE/day)	Percentage of UL
1-year-old infants			
Body lotion	0.05	56	7
Body lotion	0.3	334	42
2-year-old children			
Body lotion	0.05	72	9

Body lotion	0.3	432	54
4-year-old children			
Body lotion	0.05	95	9
Body lotion	0.3	569	52
9-year-old children			
Body lotion	0.05	146	10
Body lotion	0.3	876	58
13-year-old adolescents			
Body lotion + hand cream	0.05 0.3	451	23
Body lotion + hand cream	0.3 1.0	1967	98
Adults			
Body lotion + face cream + hand cream	0.05 0.3 0.3	856	29
Body lotion + face cream + hand cream	0.3 1.0 1.0	3446	115

[1]Retinyl palmitate and retinyl acetate at corresponding retinol concentrations.
[2]Standard scenario: 0.05% in body lotion and 0.3% in hand cream and face cream, Worst case scenario: 0.3% in body lotion and 1.0% in hand cream and face cream.

4.1.1 Total exposure to retinol and retinyl esters in Norwegian children

The total estimated exposures to vitamin A for the different age groups of the population, as well as the ULs, are presented in Figures 4-11. The exposure scenarios presented in the figures are all based on the chosen standard concentrations of vitamin A (retinol and retinyl esters) in cosmetics (see section 3.1.1.2). Exposures are shown separately for the groups with a diet without food supplements and those who include supplements in their diet. The additional exposure from cosmetics is illustrated in the figures for both groups. The proportion of each age group that exceeds its respective UL is presented in Table 10. Values are given as the closest 5%.

For all age groups of children (1-, 2-, 4- and 9-year-olds), daily exposure to vitamin A (retinol and retinyl esters) from cosmetics is ascribed to the use of body lotion. The additional contribution of vitamin A (retinol and retinyl esters) from cosmetics to the total intake from diet without and with supplement, based on the concentrations in the standard exposure scenario (body lotion 0.05%, face and hand cream 0.3%) is illustrated in Figures 4 - 8.

In the standard scenario for 1-year-old breastfed infants (Figure 4B), 15% of those taking supplements exceed the UL of 800 µg RE/day (Table 10). The children not using supplements will not reach the UL even when cosmetics is added (Figure 4A and Table 10). The vitamin A content in breast milk is not known and therefore not taken into account. When it comes to

non-breastfed children (Figure 5), the UL may be reached or exceeded by more than 10% if supplements are not included in the diet (Figure 5A and Table 10) and by 30% among those who receive food supplements (Figure 5B and Table 10). For 1-year-old infants, the exposure from cosmetics in the standard scenario represents 7% of the UL (Table 9).

In a worst case scenario, based on an increase in the concentration of vitamin A in body lotions from 0.05% to 0.3% (current maximum allowed concentration in Norway), the estimated proportion of breastfed children that would exceed the UL would be 20% for those without and 50% for those with supplements in their diet. For non-breastfed, the corresponding estimates are 30% and 65%, respectively. The estimated exposure from cosmetics would constitute 42% of the UL (Table 9).

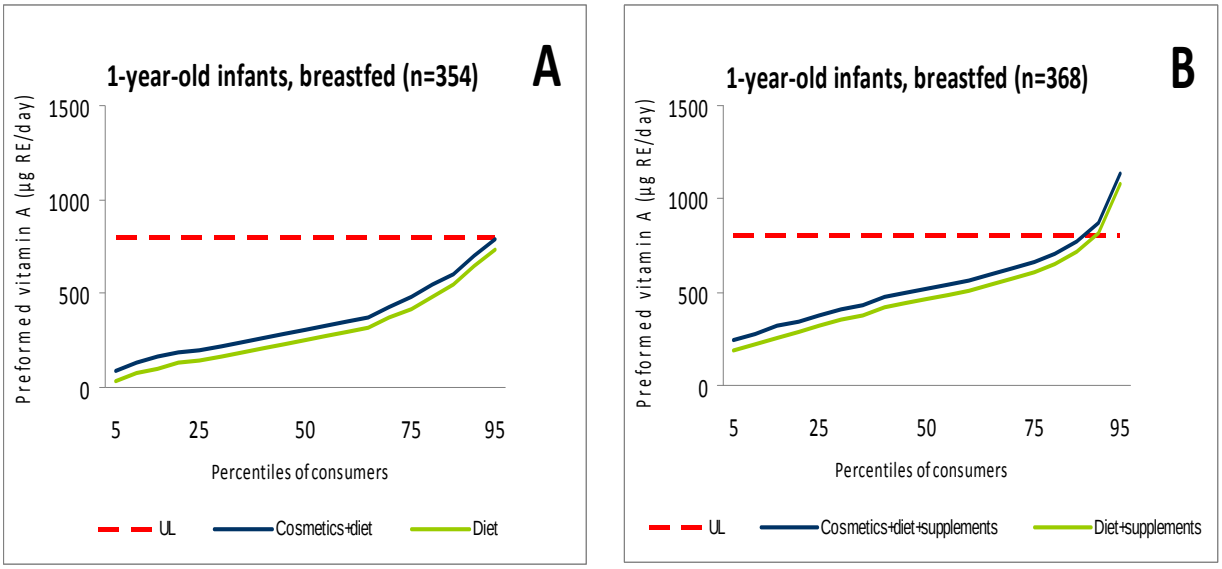

Figure 4: Additional contribution of preformed vitamin A from cosmetic products (body lotion 0.05%) to the intake from diet only (A) and to the intake from diet and food supplements (B) in Norwegian 1-year-old children (breastfed) compared to the UL of 800 µg RE/day.

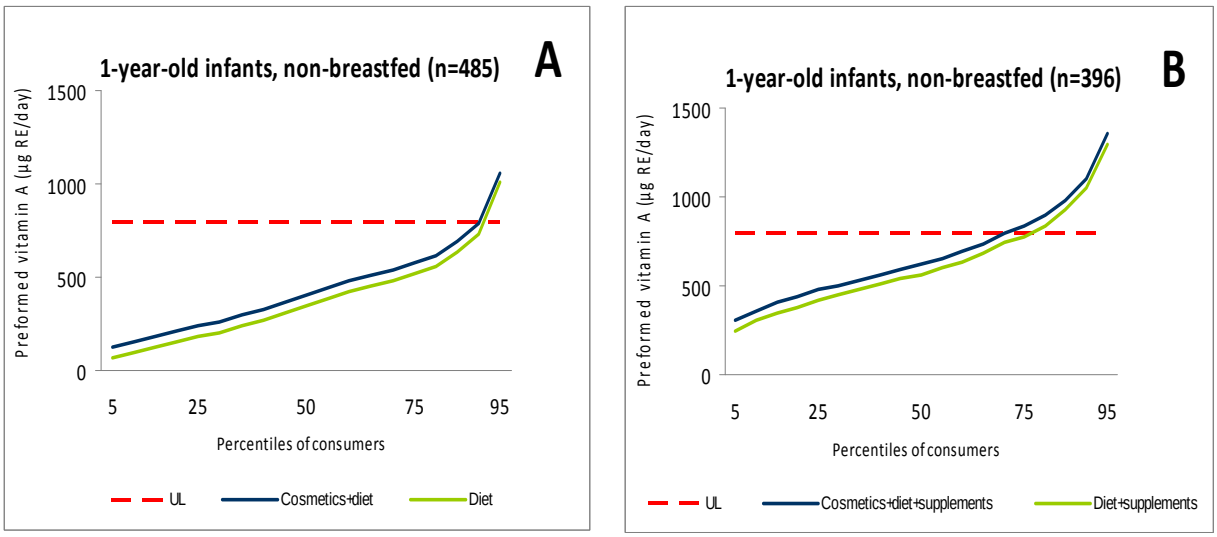

Figure 5: Additional contribution of preformed vitamin A from cosmetic products (body lotion 0.05%) to the intake from diet only (A) and to the intake from diet and food supplements (B) in Norwegian 1-year-old children (non-breastfed) compared to the UL of 800 µg RE/day.

The total exposure to retinol and retinyl esters for 2-, 4- and 9-year old children, based on the concentrations in the standard scenario for cosmetic products is presented in Figures 6 – 8. Among 2- and 4-year-old children without supplements in their diet (Figures 6A and 7A), the daily exposure reaches or exceeds the ULs of 800 µg RE/day and 1100 µg RE/day, respectively, for 20% and 15% in these age groups (Table 10). For children in the same age groups that use supplements (Figures 6B and 7B), 55% and 45% exceeds their ULs (Table 10). The total exposure for the 9-year-old children is shown in Figure 8, and it is estimated that 10% of those not using and 20% of those using supplements would exceed the UL of 1500 µg RE/day (Table 10). It should be mentioned, however, that the retinol level in cod liver oil had been reduced by the time of the food consumption survey for 2-year-old children compared to the survey for the 4- and 9-year old children. In this standard scenario, the contribution from cosmetics to the total exposure represents 9% of the referred age-related ULs for both 2- and 4-years old children and 10% of the UL for 9-year-old children (Table 9).

A worst case scenario based on an increase in concentration of retinol and retinyl esters in body lotion from 0.05% to 0.3% would represent a 75% and 45% exceedance of the respective UL for 2- and 4-year-old children not using supplements and a 100% and 90% exceedance for children in the same age groups who use supplements. For the 9-year old children the estimated portions that exceed the UL would reach 35% for the group without and 80% for the group with supplements in their diet. This worst case scenario would correspond to an estimated increase in percentage of the age-related contribution from cosmetics to the UL from 9% to 54 % and 52% for 2- and 4-year-old children, respectively (Table 9). For the 9-year-old children, the worst case scenario would represent an estimated increase from cosmetics to the UL from 10% to 58% (Table 9).

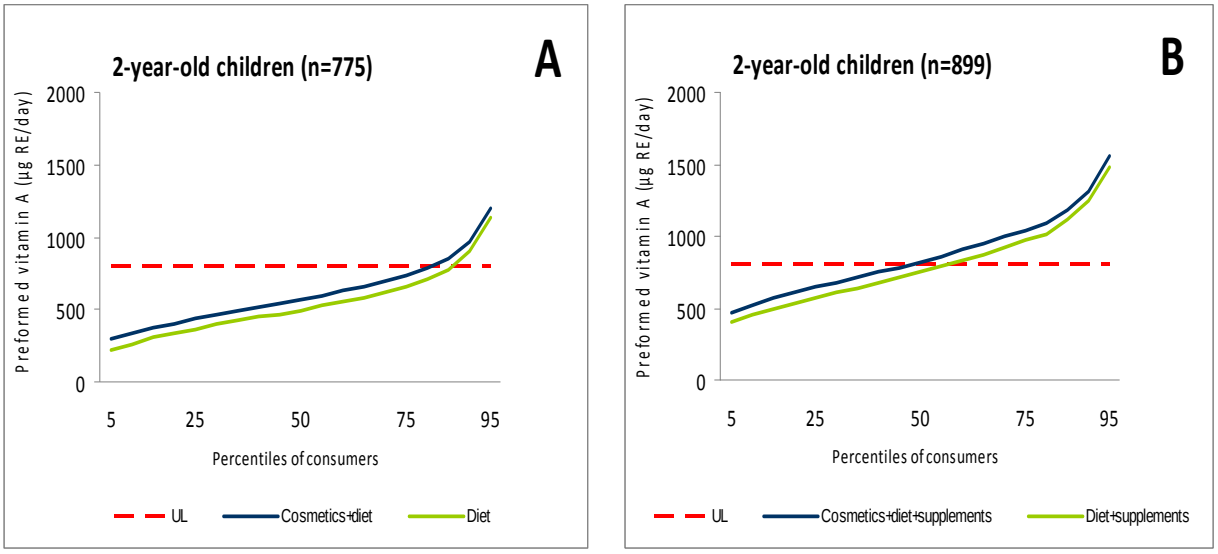

Figure 6: Additional contribution of preformed vitamin A from cosmetic products (body lotion 0.05%) to the intake from diet only (A) and to the intake from diet and food supplements (B) in Norwegian 2-year-old children compared to the UL of 800 µg RE/day.

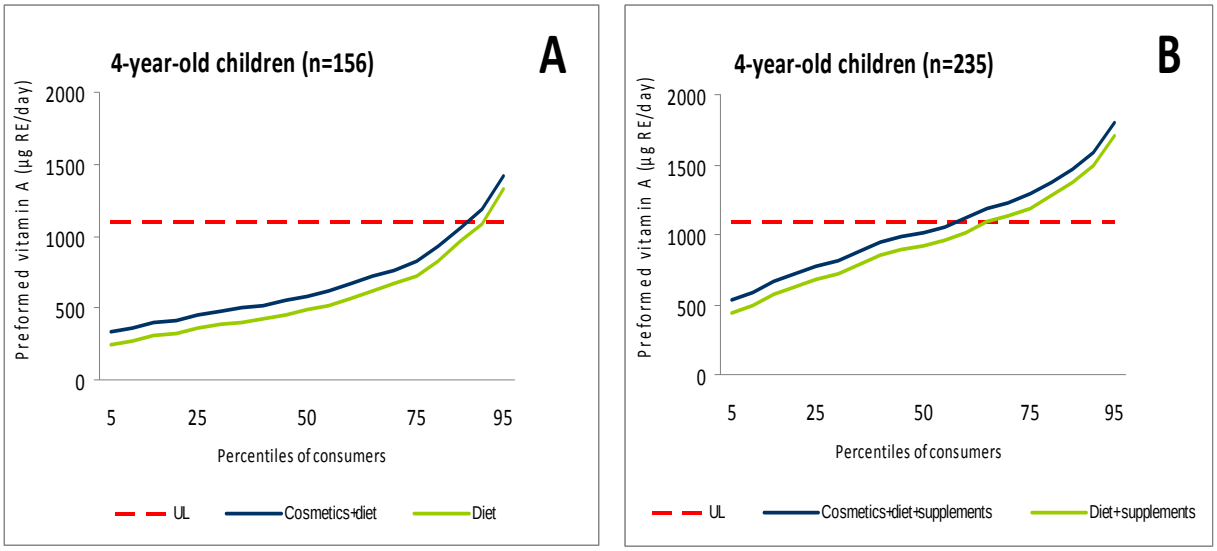

Figure 7: Additional contribution of preformed vitamin A from cosmetic products (body lotion 0.05%) to the intake from diet only (A) and to the intake from diet and food supplements (B) in Norwegian 4-year-old children compared to the UL of 1100 µg RE/day.

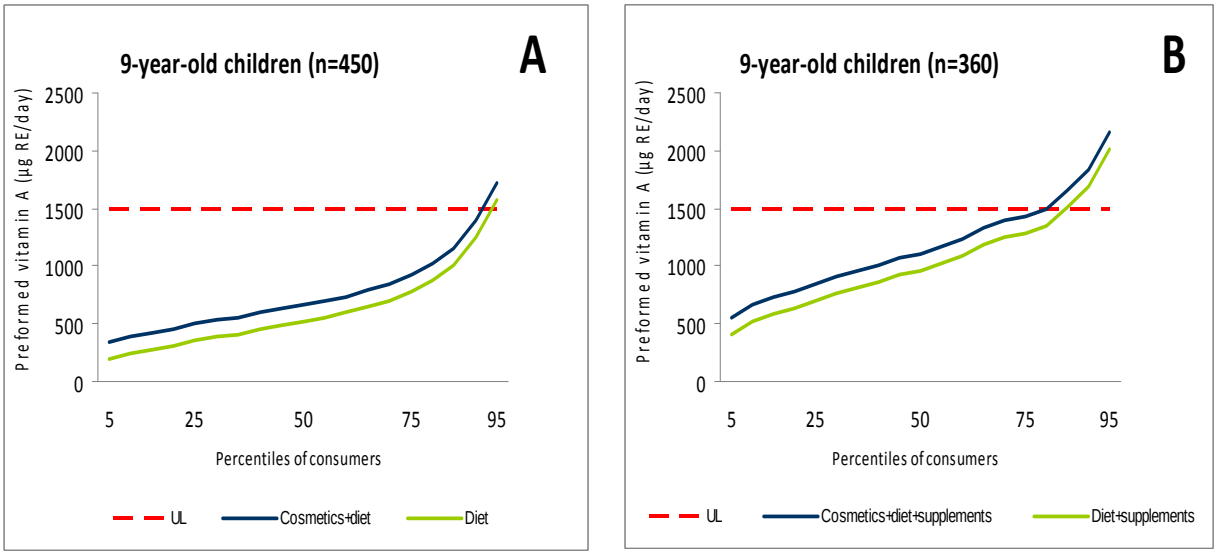

Figure 8: Additional contribution of preformed vitamin A from cosmetic products (body lotion 0.05%) to the intake from diet only (A) and to the intake from diet and food supplements (B) in Norwegian 9-year-old children compared to the UL of 1500 µg RE/day.

4.1.2 Total exposure to retinol and retinyl esters in Norwegian adolescents

The total exposure to retinol and retinyl esters in the standard scenario for 13-year-old adolescents is shown in Figure 9. The total exposure will keep below the UL of 2000 µg RE/day for those who do not use supplements, irrespective of the use of cosmetics (Figures 9A and Table 10). For the diet plus supplement group, however, the contribution from cosmetics increases the proportion that exceeds the UL from 10% to 20% (Figures 9B and Table 10). It should be noted that the retinol concentration in cod liver oil has been reduced since the consumption survey was performed. The use of hand cream and body lotion with the concentrations of retinol and retinyl esters in the standard scenario results in a contribution from cosmetics of 23% of the UL for this age group (Table 9).

In a worst case scenario for the 13-year-old adolescents, the estimates represent a 100% exceedance of the UL both for the group without and with supplements in their diet. The estimated contribution from cosmetics alone would correspond to an increase in percentage of the UL from 23% to 98% (Table 9).

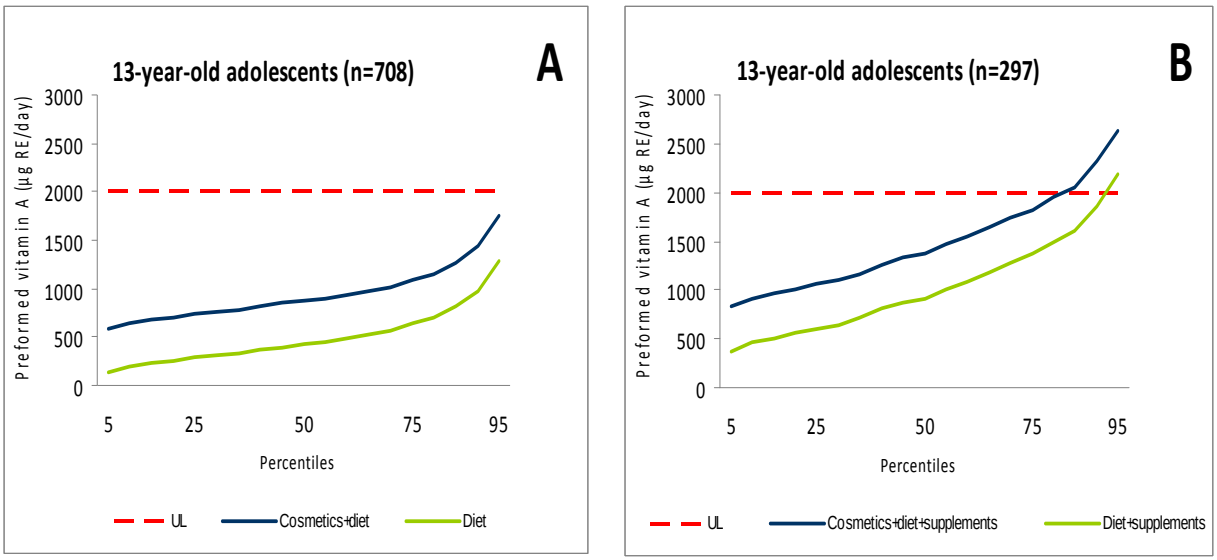

Figure 9: Additional contribution of preformed vitamin A from cosmetic products (body lotion 0.05%) to the intake from diet only (A) and to the intake from diet and food supplements (B) in Norwegian 13-year-old adolescents compared to the UL of 2000 µg RE/day.

4.1.3 Total exposure to retinol and retinyl esters in Norwegian adults

The total exposure to retinol and retinyl esters in the standard scenarios for adults is shown in Figures 10 and 11. The exposure characterisation shows that both women and men who do not use supplements will keep below the UL of 3000 µg RE/day (Figures 10A and 11A, Table 10). Among those who have an intake of preformed vitamin A from diet and supplements, an estimated portion of 10% of the men will exceed the UL when cosmetics are included, whereas the women will keep below (Figures 10B and 11B, Table 10). The estimated contribution from cosmetics constitutes 29% of the UL for adult women and men with the retinol concentrations in the standard exposure scenario (Table 9). The relationship between the total exposure to vitamin A and the GL for individuals at risk of osteoporosis and bone fracture is discussed under vulnerable groups (section 4.3).

In a worst case scenario for adults, based on the assumed increase in retinol concentrations described previously, the estimated contribution from cosmetics would correspond to an increase in percentage of the UL from 29% to 115% (Table 9). Thus, this worst case estimate for adult women and men represents a 100% exceedance of the UL both for the group without and with supplements in their diet.

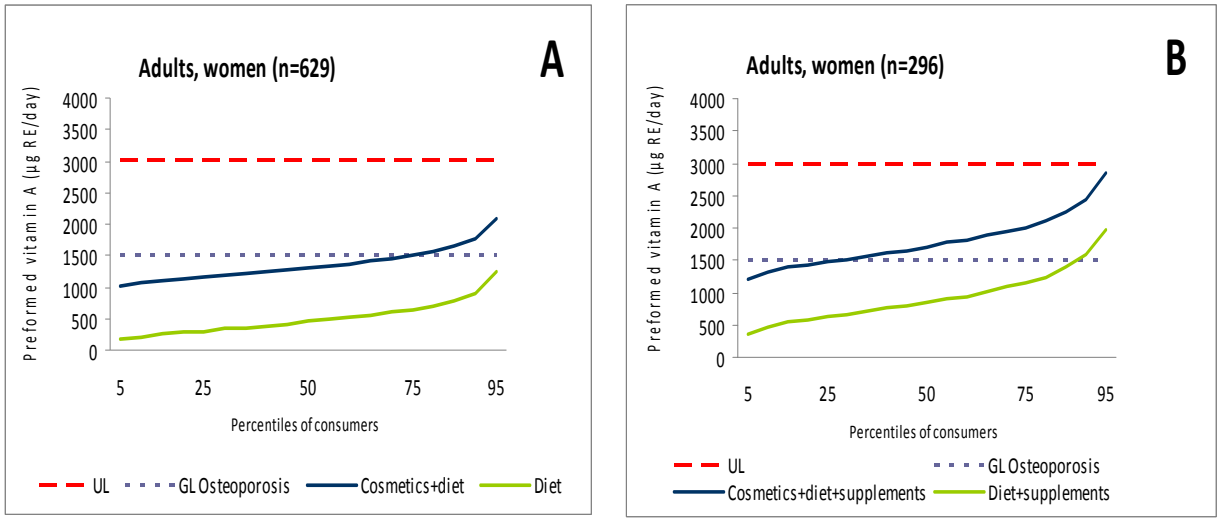

Figure 10: Additional contribution of preformed vitamin A from cosmetic products (body lotion 0.05%, face cream 0.3% and hand cream 0.3%) to the intake from diet only (A) and to the intake from diet and food supplements (B) in Norwegian adults (women) compared to the UL of 3000 µg RE/day and GL for osteoporosis of 1500 µg RE/day.

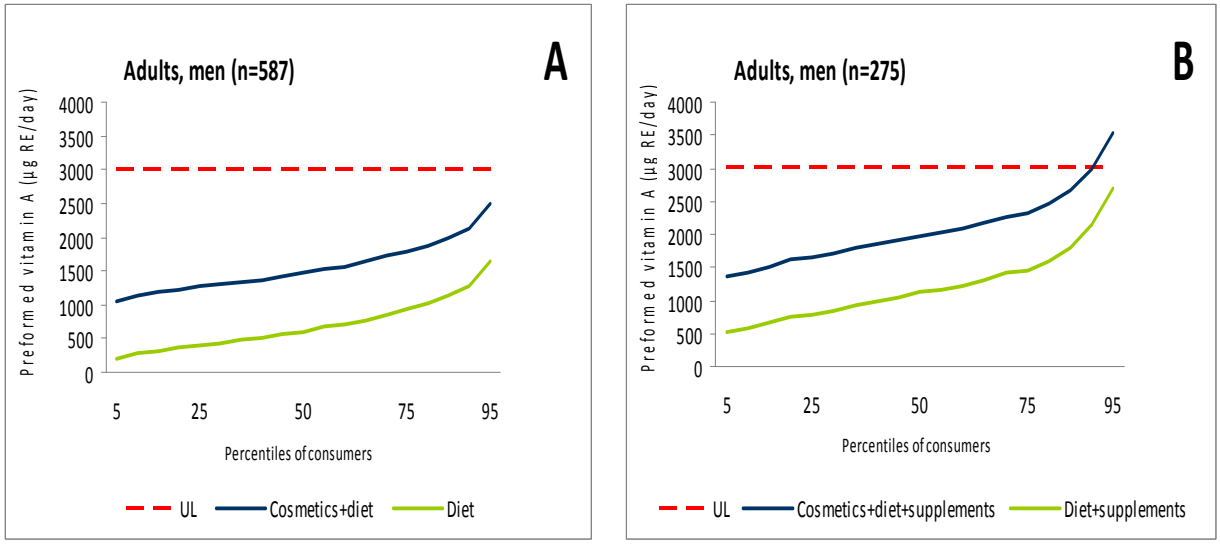

Figure 11: Additional contribution of preformed vitamin A from cosmetic products (body lotion 0.05%, face cream 0.3% and hand cream 0.3%) to the intake from diet only (A) and to the intake from diet and food supplements (B) in Norwegian adults (men) compared to the UL of 3000 µg RE/day.

4.1.4 Summary of total exposure scenarios

Table 10 summarizes the estimated proportions within each age group that may exceed their respective age-related UL. The percentages are higher for the groups with a reported consumption of supplements compared to the groups who do not use supplements. The estimates also demonstrate that the contribution from cosmetics increases the proportion of individuals that exceeds the UL by up to 10% in the diet plus supplement groups (Table 10).

Table 10: Estimated percentiles of consumers that reach or exceed their respective UL in different age groups based on the concentrations of retinol and retinyl esters in the standard exposure scenario for cosmetics (body lotion 0.05%, face and hand cream 0.3%).

	Percentile of consumers exceeding UL			Percentile of consumers exceeding UL	
Age group (number of consumers)	**Diet**	**Diet + cosmetics**	**Age group (number of consumers)**	**Diet + food supplements**	**Diet + food supplements + cosmetics**
1-year old infants (breastfed) (n=354)	Below UL	Below UL	**1-year old infants (breastfed) (n=368)**	10%	15%
1-year old infants (non-breastfed) (n=485)	10%	10%	**1-year old infants (non-breastfed) (n=396)**	25%	30%
2-year-old children (n=775)	15%	20%	**2-year-old children (n=899)**	45%	55%
4-year-old children (n=156)	10%	15%	**4-year-old children (n=235)**	35%	45%
9-year-old children (n=450)	5%	10%	**9-year-old children (n=360)**	15%	20%
13-year-old adolescents (n=708)	Below UL	Below UL	**13-year-old adolescents (n=297)**	10%	20%
Adults (women) (n=629)	Below UL	5%	**Adults (women) (n=296)**	Below UL	Below UL
Adults (men) (n=587)	Below UL	Below UL	**Adults (men) (n=275)**	Below UL	10%

As demonstrated in the exposure characterisation, the total intake of vitamin A may be high in some groups of the Norwegian population. The use of food supplements contributes significantly. The groups at highest risk of exceeding their respective UL are 1-year-old infants (non-breastfed) and 2- and 4-year-old children who use food supplements (Table 10). The additional contribution from cosmetics results in increased exposure to vitamin A (retinol and retinyl esters) and a further increase in the proportion of the population that exceeds the UL. When the estimated exposures from cosmetics are related to the respective age-related ULs, the contribution is most prominent for 13-year-old adolescents (23%) and adults (29%) (Table 9).

For other toxic effects than teratogenicity and bone health, e.g. liver toxicity, the reported lowest effect dose is 7500 µg RE/day (Table 2). This value is 2 times higher than the estimated 95 percentile intake for adult men, which represents the highest intake within all age groups (Table 8). Thus, the estimations presented in this opinion indicate that the intakes of preformed vitamin A should not pose a risk of other adverse health effects such as liver toxicity for any of the age groups.

4.2 Local adverse effects of retinoids

The retinoid effect in cosmetics is primarily against aging of the skin. In contrast to retinoic acid, the weaker retinoids retinol and retinyl esters are not classified as drugs and are currently used as active ingredients in various cosmetic products. Although not as potent as retinoic acid, the retinoids used in cosmetics may also induce local irritation. The concentrations of these retinoids in cosmetic products are to a high degree determined and limited by these immediate adverse effects. On the other hand, as ingredients in cosmetics, they may be used daily throughout the life without medical control and examination, also by vulnerable groups, such as atopics. VKM has not found reported information indicating that long-term use of topical retinoids may induce local adverse effects other than irritation and erythema.

A NTP study indicates that retinol and retinyl palmitate may be photocarcinogenic in mice. However, VKM concludes that these data do not provide sufficient information for a risk assessment of this effect of retinol and retinyl esters in cosmetics. More studies are needed to clarify mechanisms of possible retinoid photocarcinogenesis as well as characteristics needed for the extrapolation from mouse skin to human skin.

4.3 Vulnerable groups

The UL is based on the teratogenic effects of vitamin A and is therefore of special significance for women of fertile age. The contribution from supplements and cosmetics to the total exposure should be given special attention in this group.

In addition to the UL, a guidance level (GL) of 1500 µg RE/day is established for groups with increased risk of reduced BMD, osteoporosis and fractures. This is especially applicable for postmenopausal women. The estimated contribution of vitamin A exposure from cosmetics leads to an increase in exceedance of the GL from 0% to 25% for those who do not use supplements and an increase in exceedance from 10% to 75% in the group where supplements are included (Figures 10A and 10B). The estimated use of cosmetics in the standard exposure scenario results in a substantial exposure to retinol and retinyl esters in postmenopausal women with an increased risk of osteoporosis. A further increase in concentrations of retinol and retinyl esters in cosmetic products would aggravate this situation.

The estimated exposures from cosmetics presented for the different age groups are based on normal skin and healthy populations. There are, however, conditions in the skin that may lead to increased absorption of cosmetic products. In infants and small children the use of diapers may often lead to sore and irritated skin. In order to rectify this, the use of cosmetic cream may increase both in amount and number of applications per day. Assuming that the impaired skin is more easily penetrated and the cream contains vitamin A, this will contribute to an increased uptake and a subsequent increased exposure. Occlusion under the diaper is also likely to further increase the absorption.

An increased dermal uptake and a subsequent increased exposure will also be the case for people with diagnosed skin disorders, such as atopic dermatitis which constitute a prevalence of 10-12% of the schoolchildren, and 1-2% of the adult population in most western countries (Horii *et al.*, 2007). These situations, in which the skin barrier is disrupted, lead to frequent use of hand cream, and with a dysfunctional epithelial barrier the absorption through the skin will increase.

Large groups of the population in the Nordic countries suffer from dry skin due to air with very low humidity during the winter season. A subsequent enhanced absorption and a more frequent use of cosmetic products may result in increased dermal exposure.

Patients treated with oral drugs containing retinoids (isotretinoin etc.) receive contribution of the more active forms of vitamin A. This additional exposure to retinoids increases the risk of these patients to reach or exceed the UL for vitamin A.

5 Uncertainty

5.1 Dermal absorption and systemic availability

VKM has used the SCCS's Notes of Guidance for the Testing of Cosmetic ingredients (SCCS, 2010) when estimating the dermal absorption of retinol and the SED for the different exposure scenarios. However, some human *in vivo* studies did not find any increase in plasma levels of retinoids after daily applications to the skin. As discussed in 2.3.5, the lack of an increase in plasma levels may be due to the small application areas and low doses in these studies. Short-term studies show that the metabolism of retinol into retinol esters is the primary route for disposition of retinol in the skin. In addition, there is a tightly controlled low-level conversion of retinol to retinoic acid. The tightly controlled levels of retinol and retinyl esters may result in an overestimation of the systemic exposure dose after topical application.

The studies on skin metabolism of retinol and retinol esters are short-term studies. How long-term exposure by daily applications of retinol and retinyl esters affects the systemic or local doses of retinoids are not known. Thus the estimated systemic exposure doses in the present opinion may be either over- or underestimated.

In the different exposure scenarios presented in this opinion, VKM has assumed daily applications of body lotion covering the entire body surface (except the face) for all age groups. In addition, adolescents are also assumed to use hand cream and adults hand and face cream daily. Furthermore, it has been assumed that all product types contain retinol or retinyl esters. In situations where fewer product types have been used or not all products contain retinol or retinyl esters, the systemic exposure dose will be overestimated. Similarly, application on a smaller surface area or a less frequent application of products containing retinol or retinyl esters than used in the exposure scenarios, would result in a lower systemic exposure dose from cosmetic products.

The contribution from cosmetics calculated as percentage of UL may be underestimated relative to the contribution from consumption of food and food supplements since the UL is based on dietary intake of preformed vitamin A and do not take into account the 70 – 90% absorption rate in the intestine (NNR, 2004).

There are products on the market containing higher concentrations of retinol and retinyl esters than in the standard scenario used for estimations of the systemic exposure doses. However, VKM has in the worst case scenarios assumed daily applications of products containing 0.3% (body lotion) and 1% (face and hand cream) retinol. The likelihood for daily use of products containing these concentrations is however small. Thus, the systemic exposure doses in the worst case scenarios will be overestimated.

5.2 Dietary exposure assessment

Every dietary assessment are connected with uncertainty. A description of the most important uncertainties and assumptions in the dietary intake calculations is described below.

Three concepts are fundamental to understanding the limitations of dietary assessement: habitual consumption, validity and precision (Livingstone and Black, 2003).

The habitual consumption of an individual is the person's consumption averaged over a prolonged period of time, such as weeks and months rather than days. However, this is a largely hypothetical concept; the consumption period covered in a dietary assessment is a compromise between desired goal and feasibility. In the Norwegian dietary surveys the time period covered are 14 days among the 1- and 2-year olds (Sped- and småbarnskost 2007), four consecutive days among the 4-, 9- and 13-year-olds (UNGKOST 2000) and 2 none-consecutive days among the adults (Norkost 3). Both large within person and between person variations in retinol intake are found in the consumption surveys. Retinol is found in a relatively limited number of foods, and has large day-to-day variation (Willett, 1998). To obtain an accurate estimate of individual retinol intakes a large number of repeated measurements are needed (Willett, 1998). There is a higher uncertainty associated with the 95 percentile than the mean value, especially among the age groups with a low number of participants.

The validity of a dietary assessment method refers to the degree to which the method actually measures the aspect of diet that it was designed to measure (Nelson and Margetts, 1997). Lack of validity is strongly associated with systematic errors (Burema *et al*., 1988). With systematic errors all respondents in a dietary study or each subgroup in a population produce the same type of error, like systematic under- or overestimation of intake. All the three different dietary assessment methods used in this opinion have limitations when it comes to validity. Results from validation studies among 9- and 13-year-olds indicate an underestimation of energy intake of around 20% when the precoded food diary, used in UNGKOST 2000, is compared with energy expenditure (Andersen *et al*., 2005; Lillegaard and Andersen, 2005). The validation studies among 1- and 2-year-olds were performed on a previously established questionnaire, but the results showed a significantly higher estimate of vitamin A with the FFQ than with the weighed record reference method (Andersen *et al*., 2003; Andersen *et al*., 2004; Andersen *et al*., 2009). The Norwegian 24-hour recall method used among adults in Norkost 3 has not been validated. However, other similar 24-hour recall methods have been validated and show an underestimation in energy intake of around 15% (Subar *et al*., 2003; Poslusna *et al*., 2009). Underestimation of energy intake indicates that not all foods eaten are reported, but not which foods are underreported. It has been shown that foods perceived as unhealthy such as fats, sweets, desserts and snacks tend to be underreported while foods perceived as healthy tend to be overreported (Olafsdottir *et al*., 2006). However, among children and adolescents there have been studies were this selective underreporting was not shown (Sjøberg *et al*., 2003, Lillegaard and Andersen, 2005). As shown in Table 7, consumption of supplements and liver pâte are the diet sources that contribute most to the vitamin A intake. There are not specific studies on the reporting accuracy for these food items, but they will, by most, be regarded as healthy rather than unhealthy.

The precision of a technique is one that yields the same answer on repeat administrations (Livingstone and Black, 2003). Poor precision derives from large random errors in the techniques of dietary assessment. The effect of random errors can be reduced by increasing the number of observations, but cannot be entirely eliminated (Rothman, 2002).

Among the 1-year-olds who still are breastfed it is important to notice that the vitamin A contribution from breast milk is not included in the calculations. The vitamin A intake for this age group is thereby underestimated.

In Norkost 3 the number of registration days is only two. The impact of the short registration period is neither evaluated for single food items nor for nutrients yet.

The retinol content of cod liver oil were halved from year 2000 when the Ungkost 2000 study was performed to the studies, Sped- and Småbarnskost and Norkost 3 conducted in 2006/2007 and 2010/2011. It is therefore likely that the retinol intake from cod liver oil in Ungkost 2000 is an overestimation of the intake compared with the retinol content in cod liver oil in studies from 2006/2007 and 2010/2011.

Since retinol is present in large quantities in a few foods, a correct portion size assessment of for instance cod liver oil consumption is important. The size of the spoons used among the participants varies, and this will be a source of uncertainty in the retinol intake on individual level. The direction of the uncertainty is difficult to estimate without further studies.

The food data bases (KBS) used to calculate retinol have only one given value per food item. There is a rather high variation in the retinol content in different food items. To take liver pâté as example, the variation is in three levels. The first level is the variation in analysed liver, where the variation could be between 6700-29000 µg retinol/100 g in ox liver (Blaker, 1991). The second level is that different brands use different recipes, and during the data collection similar liver pâtés are aggregated into one food code in the food data base. Thirdly, analysed/calculated retinol values for liver pâté will also differ between the databases used to calculate the different diet surveys. A low fat liver pâté used in Ungkost 2000 have 4351 µg retinol/100 g while in Norkost 3 a low fat liver pâté had 8200 µg retinol/100 g.

It is unclear to which extent a low participation rate will influence the assessment of retinol intake. It has been shown is that health conscious people are more likely to participate in a dietary survey. This can indicate a somewhat different dietary pattern and pattern of supplement use among the participants than among the whole population. The direction of the uncertainty is difficult to estimate.

5.3 Summary table of uncertainties

An evaluation of the overall effect of identified uncertainties is presented in Table 11, highlighting the main sources of uncertainty and indicating whether the respective source of uncertainty might have led to an over- or underestimation of the exposure and/or the resulting risk (EFSA, 2006b).

Table 11: Qualitative evaluation of influences of uncertainties on the assessment of vitamin A exposure.

Source of uncertainty	Direction and magnitude
Dermal absorption and systemic availability	
Dermal application of retinol and retinyl esters less than assumed in the exposure scenarios (i.e. smaller application area, less frequent applications, use of fewer products containing retinol/retinyl esters)	+
Long term storage capacity of retinol and retinyl esters in the skin and conversion rate into retinoic acid are not known	+/-
Worst case scenario: small likelihood for all products, assumed to be used by the consumers, to contain the chosen concentrations of retinol and retinyl esters	+
Contribution from cosmetics calculated as percentage of UL may be underestimated relative to the contribution from consumption of food and food supplements	-
Dietary exposure assessment	
Analysed/calculated retinol values in the food data base	+/-
Retinol intake difficult to measure, and needs large number of days with repeated measures	+/-
Different dietary assessment methods	+/-
Portion size	+/-
Sped- and småbarnskost 2006/2007	
Use of 95 percentile	+/-
FFQ time span is 14 days	+/-
Retinol from breast milk not included	-
Ungkost 2000	
Retinol content in cod liver oil have decreased since 2000	+
Use of 95 percentile - the number of participants among 4-year-olds is only 391	+/-
Four registration days	+/-
Norkost 3, Adults	
Participation rate 37%	+/(-)
Two registration days	+/-
Use of 95 percentile - the number of men and women taking retinol supplements are only 275 and 296	+/-
Qualitative evaluation of overall effect of identified uncertainties:	+

+: uncertainty likely to cause over-estimation of exposure
-: uncertainty likely to cause under-estimation of exposure

In spite of the limitations outlined above in evaluating the dermal application and systemic availability and assessing the food consumption, leading to uncertainties related to estimating the exposure to vitamin A, VKM conclude that the exposure to retinol and retinyl esters presented in this report can be considered realistic for the different age groups. The risk assessment of vitamin A according to the standard scenario is likely to be conservative, i.e. more likely to overestimate than to underestimate the risk. The worst case scenario is an overestimation of the exposure to vitamin A.

Data gaps

- There are relatively few studies on absorption of vitamin A in human skin. In addition, none of the cited *in vitro* studies in the present opinion fulfilled the SCCS basic requirements for *in vitro* dermal absorption. Studies on vitamin A fulfilling these requirements are needed.
- There are short-term studies on metabolism of topically applied retinol and retinyl esters. However, information on storage and/or metabolism of retinol and retinyl esters after long-term use of cosmetic products containing vitamin A is lacking.
- There are data on local adverse effects after short-term use of vitamin A containing cosmetic products, whereas information on local adverse effects after long-term use is missing.
- Data demonstrating if topically applied vitamin A can contribute to systemic adverse effects is lacking.
- Further research on the relationship between retinol and bone health is required in order to reach clear conclusions.
- More data is needed to understand the interaction between retinol and vitamin D with respect to fracture risk.
- More studies are needed to clarify mechanisms of possible retinoid photocarcinogenesis as well as characteristics needed for the extrapolation from mouse skin to human skin.
- More information on the frequency of use and concentrations of retinal in cosmetics is needed.
- More information on the frequency of use and concentrations of retinol and retinyl esters in sun screen products on the European market is needed.
- More data is needed to understand under-/over-reporting of food consumption in dietary surveys.
- Further research is needed to get more accurate portion size estimation in the dietary surveys.
- Further research is needed to evaluate the impact of variation in number of registration days in the dietary surveys.

Conclusions

- The critical adverse health effect of excess intake of vitamin A is teratogenicity. This effect is the basis for the tolerable upper intake level (UL) of 3000 µg RE/day.
- The most important source of vitamin A in the population is diet, followed by food supplements and then cosmetics.
- The dietary intake of preformed vitamin A is high in parts of the Norwegian population. Consumption of food supplements contributes significantly to the total intake of preformed vitamin A in all age groups and will increase the proportion of the population exceeding the UL.
- 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) in all age groups.
    - The estimated contribution of retinol and retinyl esters from cosmetics is most prominent for 13-year-old adolescents (23% of UL) and adults (29% of UL).
- In the worst case scenarios based on the assumed increased concentrations in cosmetics (0.3% in body lotions and 1% in face and hand cream), the contribution from cosmetics would further increase the total exposure to vitamin A (retinol and retinyl esters).
    - The estimated contribution of retinol and retinyl esters from cosmetics would reach 42-58% of the ULs for children, 98% of the UL for 13-year-old adolescents and exceed the UL for adults (115%).
- The contribution from cosmetics is of special concern for women of fertile age, and total exposure above the UL before and during pregnancy will increase the risk of birth defects.
- For persons who are at higher risk for reduced bone mineral density, osteoporosis and fractures, especially post-menopausal women, a lower guidance level (GL) than the UL has been set, i.e. 1500 µg RE/day. About 10% of adult women in Norway exceed this GL by intake of vitamin A from food and food supplements alone. The additional contribution from cosmetics increases this proportion to approximately 75%. An increased exposure due to higher concentrations of vitamin A (retinol and retinyl esters) in cosmetic products would further augment the proportion of women at risk of osteoporosis.
- Impaired skin may result in increased absorption of cosmetic products. This can occur in persons with diagnosed atopic dermatitis, in persons that suffer from dry skin and in small children with irritated skin in the nappy area.
- Regarding local adverse effects in the skin, no information was found indicating that long-term use of topical retinoids may induce other effects than irritation and erythema. A NTP study indicates that retinol and retinyl palmitate may be photocarcinogenic in mice. However, these data do not provide sufficient information for a risk assessment of this effect of retinol and retinyl esters in cosmetics.

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Appendices

Appendix I

The following information is based on the basic requirements for *in vitro* dermal absorption studies described in the SCCS' Notes of Guidance for the Testing of Cosmetic ingredients (SCCS, 2010).

Dermal/percutaneous absorption

A) Major guidelines for dermal/percutaneous absorption

Human exposure to cosmetic ingredients occurs mainly via the skin. In order to reach the circulation (blood and lymph vessels) cosmetic ingredients must cross a number of cell layers of the skin, where the rate-determining layer is considered to be the stratum corneum (SC). A number of factors play a key role in this process, including the lipophilicity of the compounds, the thickness and composition of the SC (body site), the duration of exposure, the amount of topically applied product, the concentration of target compounds, occlusion, etc. (for review see Schaefer *et al*., 1996; ECETOC, 1993; Howes *et al*., 1996). The dermal/percutaneous absorption has been described by several international bodies (ECETOC, 1993, US EPA, 1996a, OECD, 2004) using a wide variety of terms and it is recognised that confusion is possible. Therefore, it seems appropriate to define some important terms in this particular field (SCCS/1358/10).

The dermal/percutaneous absorption process is a global term which describes the passage of compounds across the skin. This process can be divided into three steps:

- *penetration* is the entry of a substance into a particular layer or structure such as the entrance of a compound into the stratum corneum;
- *permeation* is the penetration through one layer into another, which is both functionally and structurally different from the first layer;
- *resorption* is the uptake of a substance into the vascular system (lymph and/or blood vessel), which acts as the central compartment.

Dermal/percutaneous absorption studies can be performed *in vivo* or *in vitro*. Today, however, *in vivo* dermal/percutaneous absorption testing is not an option any more for cosmetic ingredients in the European context, as the animal testing deadline of 11 March 2009 has passed (2003/15/EC).

Both *in vivo* and *in vitro* testing protocols form part of the lists of official EU and OECD test methods (EC B.44, 45; OECD 427, 428), accompanied by more detailed guidance on their performance (DG SANCO, 2004; OECD, 2004). Whereas the first version of above-mentioned OECD Guideline 428 was issued in 2000, the SCCNFP already adopted its first set of basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients in 1999 (SCCNFP/0167/99). This opinion, most recently updated in 2010 (SCCS/1358/10), focuses on the *in vitro* testing of cosmetic ingredients, whereas the general EU and OECD Guidance (DG SANCO, 2004; OECD, 2004) addresses percutaneous absorption from a much broader point of view by mentioning *in vivo* methods besides *in vitro* testing and by providing specifications for agricultural products and industrial chemicals besides cosmetics.

As a result, the SCC(NF)P/SCCS has always considered *a combination of the EU/OECD Guidelines* and its own *"Basic criteria" as essential for dermal/percutaneous absorption studies*.

B) The SCCS "Basic criteria"

The purpose of *in vitro* dermal absorption studies of cosmetic ingredients is to obtain qualitative and/or quantitative information on the substances that may enter, under in-use conditions, into the systemic compartment of the human body. The quantities can then be taken into consideration to calculate the margin of safety (MOS) using the no observed adverse effect level (NOAEL) of an appropriate repeated dose toxicity study with the respective substance.

In these relatively complex in vitro studies, there are a number of points that require special attention:

1) The design of the diffusion cell (technicalities and choice between static and flow through system).
2) The choice of the receptor fluid (physiological pH, solubility and stability of chemical in receptor fluid should be demonstrated, no interference with skin/membrane integrity, analytical method, etc.).
3) The skin preparations should be chosen and treated with care (human skin from an appropriate site remains the gold standard).
4) Skin integrity is of key importance and should be verified.
5) Skin temperature has to be ascertained at normal human skin temperature.
6) The test substance has to be rigorously characterised and should correspond to the substance that is intended to be used in the finished cosmetic products.
7) Dose and vehicle/formulation should be representative for the in-use conditions of the intended cosmetic product. Several concentrations, including the highest concentration of the test substance in a typical formulation, should be included.
8) Dose, volume and contact time with the skin have to mimic in-use conditions.
9) Regular sampling is required over the whole exposure period.
10) Appropriate analytical techniques should be used. Their validity, sensitivity and detection limits should be documented in the report.
11) The test compound is to be determined in all relevant compartments:
    - product excess on the skin surface (dislodgeable dose),
    - stratum corneum (e.g. adhesive tape strips),
    - living epidermis (without stratum corneum),
    - dermis,
    - receptor fluid.
12) Mass balance analysis and recovery data are to be provided. The overall recovery of test substance (including metabolites) should be within the range of 85-115%.
13) Variability/validity/reproducibility of the method should be discussed. The SCCS considers that for a reliable dermal absorption study, eight skin samples from at least four donors should be used.

The amounts measured in the dermis, epidermis (without stratum corneum) and the receptor fluid will be considered as dermally absorbed and taken into account for further calculations.

When studies correspond to all of the basic requirements of the SCCS, the mean + 1SD will be used for the calculation of the MOS. The reason for not using the mean *per se* is the frequently observed high variability in the *in vitro* dermal absorption assays. In case of

significant deviations from the protocol and/or very high variability, the mean + 2SD will be used as dermal absorption for the MOS calculation. In case the results are derived from an inadequate *in vitro* study, 100% dermal absorption is used. However, in case MW > 500 Da and log Pow is smaller than -1 or higher than 4, the value of 10% dermal absorption is considered.

Appendix - references

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