Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Adverse local effects of retinol and retinyl esters in the skin

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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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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Assessed by

This opinion has been evaluated and approved by the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics of VKM.

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Summary

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM), Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics has at the request of the Norwegian Food Safety Authority (Mattilsynet) conducted an assessment of local adverse effects, such as skin irritation, induced by vitamin A (retinol and retinyl esters) in cosmetic products. Vitamin A constitutes a group of lipid-soluble compounds including retinyl esters, retinol and retinal. 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. The use of retinol and retinyl esters in cosmetic products has been restricted in the Norwegian cosmetics regulations with maximum allowed concentrations of 0.3 % retinol and 0.55 % retinyl palmitate. However, upon entry into force of the EU Cosmetics Regulation July 11th 2013, the national regulation of these compounds ceased. The Norwegian Food Safety Authority is working on compensatory measures to ensure safe use of these products, and has requested VKM to identify the upper concentrations of vitamin A (retinol and retinyl esters) that may be safely used in cosmetics in relation to the ingredients' ability to irritate the skin. In order to perform this task, a literature search was performed and the relevant scientific papers were evaluated.

VKM concludes that there are not enough relevant studies with the necessary data on association between concentrations and adverse effects in the skin to define safe upper concentrations for the use of retinol and retinyl esters (retinyl palmitate and retinyl retinoate) in cosmetics. However, the present available data indicates that local adverse effects in human skin may appear after application of retinol at concentrations of 0.075 % and above. For retinyl esters, no conclusion can be drawn from the available data.

Norsk sammendrag

Vitenskapskomiteen for mattrygghet (VKM), Faggruppen for tilsetningsstoffer, aroma, matemballasje og kosmetikk, har på oppdrag fra Mattilsynet gjennomført en vurdering av hvilke konsentrasjoner av vitamin A (stoffene retinol og retinyl estere) i kosmetikk som er trygge i bruk, det vil si som ikke fører til negative effekter som for eksempel irritasjon i huden. Vitamin A består av en gruppe fettløselige forbindelser, inkludert retinyl estere, retinol og retinal. På grunn av sine effekter i huden er retinol og retinyl estere mye brukt som ingredienser i kosmetiske produkter, for eksempel i antirynkekremer, fuktighetskremer og solkremer. Retinol og retinyl palmitat er ikke regulert på EU-nivå, men har i Norge vært nasjonalt regulert med en maksimalt tillatt konsentrasjon på 0,3 % retinol og 0,55 % retinyl palmitat. Ved ikrafttredelse av EUs kosmetikkforordning 11. juli 2013, opphørte den nasjonale reguleringen av disse forbindelsene. Det er derfor ikke lenger en øvre tillatt konsentrasjon av vitamin A for bruk i kosmetiske produkter. Mattilsynet arbeider derfor med kompenserende tiltak og har bedt VKM om å identifisere de høyeste konsentrasjonene av retinol og retinyl estere som det er trygt å bruke i forhold til deres mulige negative effekter i huden. Det ble gjort et litteratursøk, og relevante vitenskapelige artikler ble gjennomgått.

VKM konkluderer med at på grunn av mangelfullt datagrunnlag når det gjelder en eventuell sammenheng mellom konsentrasjoner av retinol og retinyl estere (retinyl palmitat og retinyl retinoat) som brukes i kosmetikk og negative effekter på huden kan det ikke settes en trygg øvre grense for bruk av disse forbindelsene. De dataene som er tilgjengelig indikerer at lokale

negative effekter på huden kan forekomme etter applikasjon av retinol ved konsentrasjoner på omtrent 0,075 % og høyere. Når det gjelder retinyl estere er det ikke tilstrekkelig datagrunnlag til å trekke noen konklusjoner.

Keywords

Retinol, retinyl esters, retinyl palmitate, retinyl retinoate, skin, local effects, irritation

Glossary/abbreviations

Retinoids – all synthetic or natural compounds that have biological activity similar to vitamin A

TEWL – trans-epidermal water loss

VKM – Norwegian Scientific Committee for Food Safety

Contents

Background

The Norwegian Scientific Committee for Food Safety published the report "Risk assessment of vitamin A (retinol and retinyl esters) in cosmetics" in 2012. Both systemic and local effects of the total intake of vitamin A were assessed. It was concluded that diet is the most important source of vitamin A in the population, followed by food supplements and cosmetics. The most critical adverse health effect of excess intake of vitamin A is teratogenicity. Regarding local adverse effects in the skin, the reported effects included irritation and erythema. The use of vitamin A (retinol and retinyl esters) in cosmetics was regulated by the Norwegian cosmetics regulations. Upon entry into force of the EU Cosmetics Regulation July 11th 2013, the

national regulation of these compounds ceased. The EU Cosmetics Regulation includes no restrictions on the use of retinol and retinyl esters in cosmetic products and it is therefore legal to put on the Norwegian market products with higher levels than the previous maximum allowed concentrations of 0.3% (retinol) and 0.55% (retinyl palmitate). Since vitamin A may induce local adverse effects such as irritation and erythema in the skin, the Norwegian Food Safety Authority is working on compensatory measures to ensure safe use of these products and needs information on the upper concentrations of vitamin A (retinol and retinyl esters) that is safe to use in cosmetics. Therefore, the Norwegian Food Safety Authority has asked VKM to give a scientific opinion on retinol and retinyl esters and adverse skin effects induced by use of cosmetics, based on available literature.

Terms of reference

The Norwegian Food Safety Authority requests the Norwegian Scientific Committee for Food Safety to evaluate the upper concentrations of vitamin A (retinol and retinyl esters) that may be safely used in cosmetics in relation to the ingredients' ability to irritate the skin.

Assessment

1 Introduction

1.1 Vitamin A and cosmetic products

Retinoids are defined as all synthetic or natural compounds that have biological activity similar to vitamin A. The main retinoids used in cosmetics are the retinyl esters retinyl palmitate and retinyl acetate, and the retinol all-*trans*-retinol (Figure 1). Retinoic acid, the biological active metabolite, is not allowed in cosmetics. Retinal, however, can be found in some cosmetic products (Commission 1976; Regulations 1995). In the present opinion, only adverse effects of retinol and retinyl esters in the skin will be assessed.

Retinol and retinyl esters are used as active ingredients in a large variety of cosmetic products such as anti-wrinkle creams, body lotions, hand creams and sunscreens. 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).

Retinol and retinyl esters have to be metabolized to retinoic acid by the skin to exert their effects (Figure 2), but the potency of the retinoids is strongly dependent on its metabolic distance to retinoic acid. Therefore, the retinoid-like activity after topical application is increasing in the following order: retinyl esters << retinol < retinal < retinoic acid. After dermal application of retinyl palmitate, increased endogenous retinyl palmitate in the skin has been observed (Antille *et al.* 2004). A large part of the absorbed retinyl palmitate may also be metabolized into retinol (Boehnlein *et al.* 1994). After application, 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 2).

Several studies have demonstrated that topical retinol may induce the same cellular and molecular changes as retinoic acid. Although a 20 times higher dose of retinol is needed, the

local irritation characteristics are less prominent. Retinol has been shown to be effective in the treatment of aging and photoaging. However, the effect was dependent on the vehicle used, as retinol is unstable and easily degraded to biological inactive forms when exposed to light and air (reviewed in (Mukherjee *et al.* 2006). Retinyl palmitate is widely used in cosmetics because of its stability (Mukherjee *et al.* 2006). It is used in sunscreen products because of its antioxidant, stabilizing properties. However, in Europe and USA retinyl palmitate is not allowed to be added as UV-filter as such.

In the Norwegian cosmetic regulations, the use of retinol and retinyl esters in cosmetic products was until July 11th 2013 restricted to maximum allowed concentrations of 0.3% (retinol) and 0.55% (retinyl palmitate). According to Nohynek *et al.* (2006), retinol and retinyl esters are used in cosmetic products at concentrations up to 0.3% (retinol) or 0.55% (retinyl palmitate). Higher concentrations tend to be irritating to the skin and are therefore considered to be unsuitable for cosmetic use (Ries and Hess 1999; Fluhr *et al.* 1999).

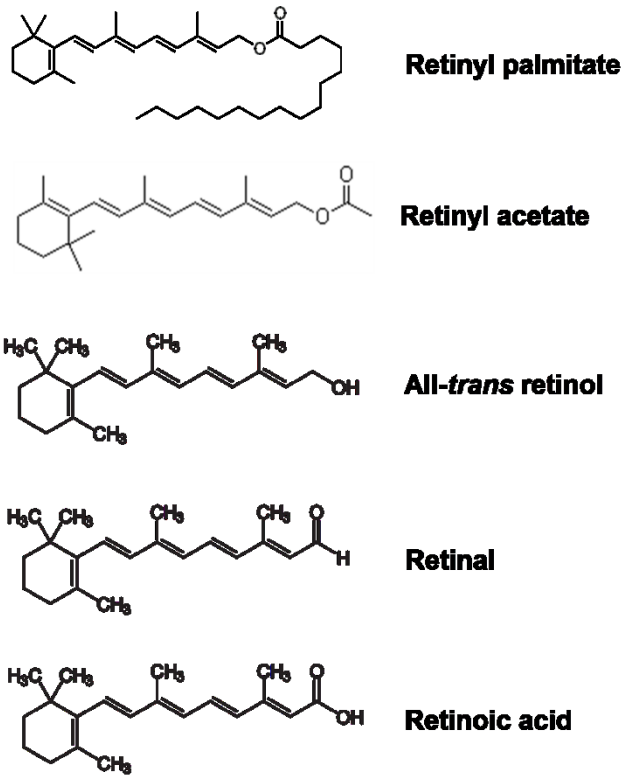

Figure 1: Structural formulas of retinyl palmitate, retinyl acetate, all-trans retinol, retinal and retinoic acid.

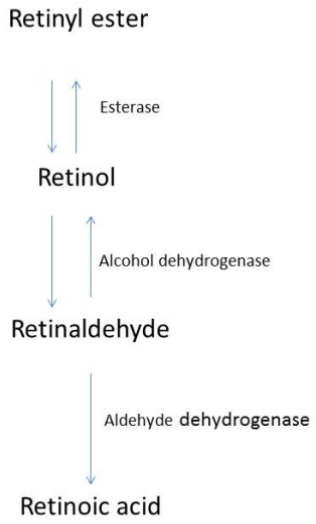

Figure 2: Metabolism of retinyl esters to retinoic acid.

1.2 Retinoids: function and local effects in the skin

1.2.1 Structure and physiology of the skin

The skin is primarily stratified into the epidermis, the dermis and the hypodermis (Figure 3). The epidermis, the upper layer of the skin, is mainly responsible for providing protection against the ingress of chemicals. It consists of distinct strata that reflect different stages of keratinocyte maturation: stratum corneum, stratum lucidum (only palms of hands and sole of feet), stratum granulosum, stratum spinosum and stratum basale. Stratum corneum provides a significant barrier to diffusion. The layers below stratum corneum constitute the viable epidermis. In addition, the epidermis contains melanocytes (produce the sunlight protecting pigment melanin), Langerhans cells (immune function) and Merkel (sensory) cells. The epidermis is subject to a cycle of renewal which takes 5 – 30 days (Chilcott 2008; Fu *et al.* 2007; series 2012).

The dermis is a protein-rich region that support and strengthens the epidermis. Dermis interfaces with the epidermis through a system of upward protrusions of dermal papillae. This papillary dermis contains a network of capillaries that provide a large surface area for the bi-directional transfer of nutrients, oxygen and waste products. The reticular region, the deeper portion of dermis, is lesser vascularized. The dermis also contains hair follicles, sweat glands, sebaceous glands, apocrine glands and lymphatic vessels. Chemicals that cross the epidermis are generally subject to systemic absorption by the papillary dermis (Chilcott 2008).

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

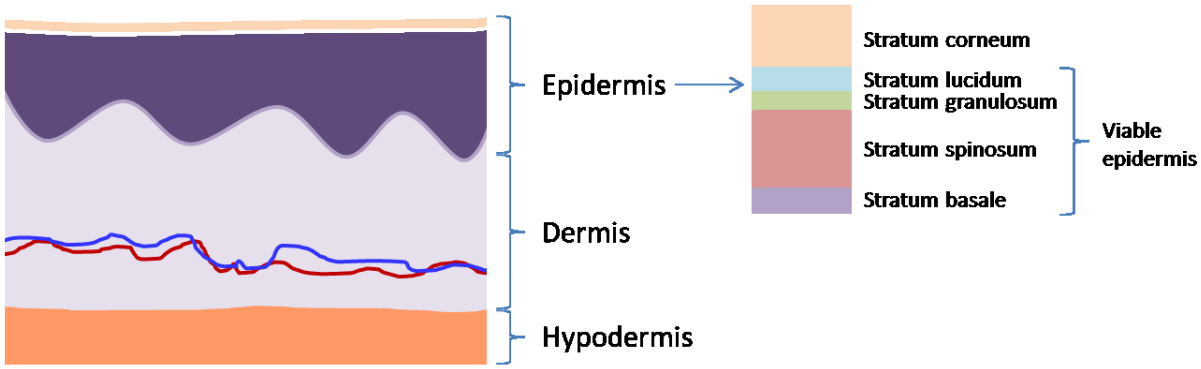

Figure 3: Principal components of the skin.

1.2.2 Function and local effects of retinoids

Vitamin A plays a critical role in homeostasis of various epithelia, including epidermis, and is important for sustaining normal growth and differentiation. Although epidermis is avascular it contains significant quantities of retinoids (Randolph and Siegenthaler 1999).

Topically applied retinoids may reverse dermatological disorders most likely by interfering with local retinoid functions. Therefore, topical retinoids have been used for clinical treatment of psoriasis, hyperkeratosis, acne, early aging and photodamage (Orfanos *et al.* 1997). The retinoids may play a role in the aging process of the skin, since many of the changes can be reversed by topical application (Darlenski *et al.* 2010). Topically applied retinoids may increase synthesis and inhibit degradation of collagen in the dermis, 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 are changes that are associated with increased smoothness of the skin.

Retinol and retinyl esters are not as potent as retinoic acid, but they may induce local irritation in the skin. A similar ranking as above (see section 1.1.) is seen for their capacity to induce skin irritation, where retinoic acid is the most irritating retinoid. For all topical retinoids the skin irritation effect is dose-dependent.

1.3 Definition of adverse effects in the skin

As described above, there are several expected skin effects after the use of retinol and retinyl esters in cosmetics. The desired positive changes after use of retinol and retinyl esters leading to less wrinkling and smoother skin as described above (section 1.2.2.), are not considered as adverse effects by VKM.

Irritated skin after the application of retinol and retinyl esters is characterized by redness, dryness and flaking of the skin at the treated site (Varani *et al.* 2008). Thus, in the present opinion, adverse local effects in the skin refers to symptoms described by the authors in the evaluated papers as erythema, rash, itching, stinging, prickling, swelling/oedema, scaling, burning, tightness and/or irritation.

2 Search strategy

The search was performed to retrieve publications addressing the skin irritating ability of vitamin A in cosmetics. The strategy for the literature search was discussed by the project group and then set up together with a librarian. Test searches were conducted to find relevant terms and search words and controlled vocabulary (MeSH and EMTREE). Literature searches were conducted in Ovid Medline and Embase. Both databases were used to ensure comprehensive study retrieval. The searches were conducted in May 2013 using a combination of both controlled vocabulary and text words as shown in Appendix 1. The search was limited by omitting abstracts and by limiting the languages to Danish, English, French, Norwegian or Swedish.

2.1 Publication selection

The literature search resulted in 909 publications. The titles and abstracts of all publications identified in the search process were assessed for relevance by two reviewers (members of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics of VKM) as shown in Figure 4. A publication was excluded if only retinoic acid (RA) and/or synthetic retinoids were used, if vitamin A was given orally, if only *in vitro* studies were performed, if the study was performed on damaged skin (e.g. used for treatment of psoriasis, hyperkeratosis, acne, or cancer), if skin irritation was not assessed, or if the used dose was not reported. In this process, 806 publications were excluded. The full text versions of the remaining 103 papers were assessed by the two reviewers (working alone or together). The publications evaluated to be not relevant was assessed by a third reviewer (also a member of the scientific panel) to ensure that no relevant publications were excluded. Of the 103 reviewed full text papers, 12 publications were found relevant according to the terms of reference and were subjected to further evaluation.
The search period covered publications from Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) from 1946 to May 06, 2013 and publications in Embase from 1974 to May 06, 2013.

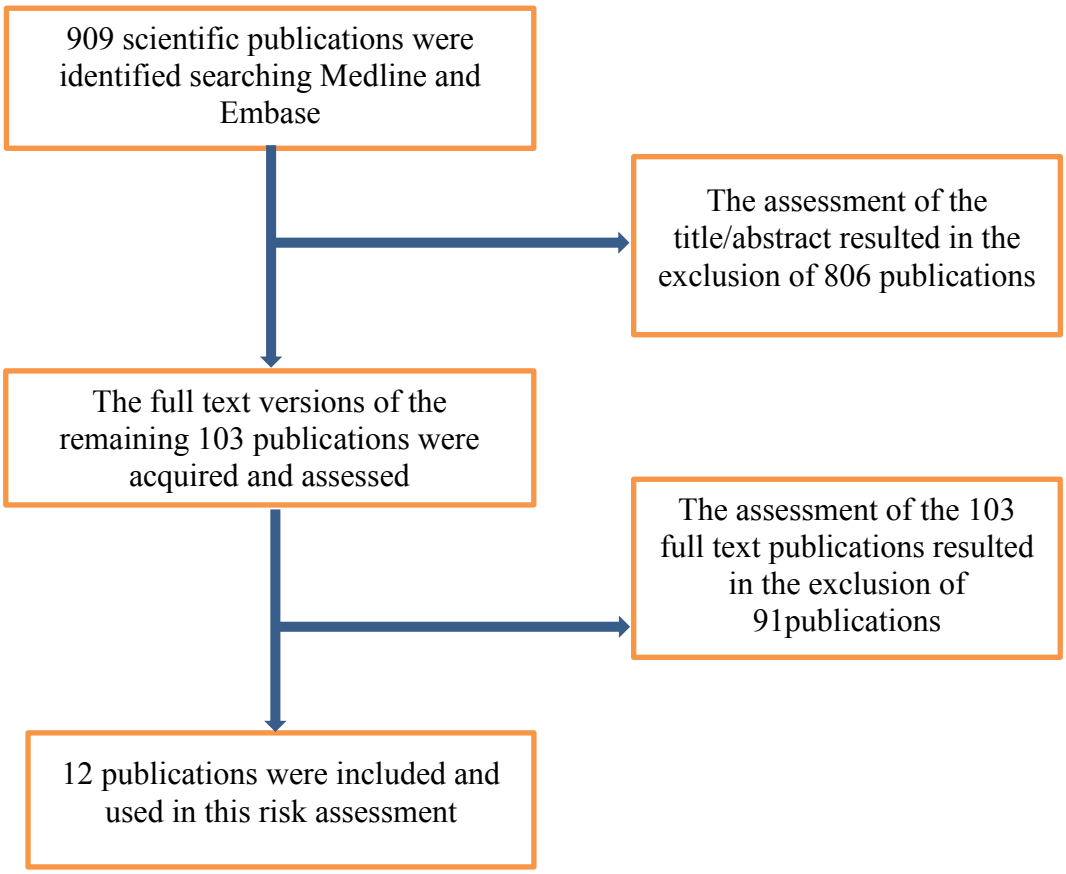

Figure 4: Overview of the literature search and the selection of publications

2.2 Data extraction, relevance and quality assessment

The finally selected publications reported the usage and the concentration(s) of retinol and/or retinyl esters as treatment agents. They contained, with varying levels of details and quality, descriptions of the study design and discussions of local effects in the skin that was relevant for this opinion.

3 Results

Of a total of 12 publications, 10 human *in vivo* studies and 2 animal *in vivo* publications (one study and one report on a safety assessment) of relevance to this opinion were identified.

3.1 Human studies

The 10 selected human studies are summarized in Table 1.

In the Final Report on the Safety Assessment of Retinyl Palmitate and Retinol (1987), several human studies were assessed (mostly unpublished reports). In a Repeated Insult Patch Test (RIPT) (n=100), 1% retinyl palmitate in a moisturizer were applied to the upper arm (0.3 ml sample) using an occlusive patch (pages 305-306 in the cited report). The patch size was not stated. Patches remained in place for 24 hours and were scored upon removal. Patches were applied on alternate days to the same site for a total of 10 applications. Similar 24 hour challenge patches were applied to the same as well as untreated sites after a rest period of 2-3 weeks. One subject had erythema after the 8th and 9th application. No reactions were observed after challenge.

Furthermore, 0.1% retinyl palmitate in a body lotion was tested in a Shelanski/Jordan RIPT in 210 subjects (cream applied on alternate days under occlusion, total of 10 applications) (page 306 in the cited report). Applied amount of test solution and patch size were not stated. After a rest period of 10-14 days, a 48 hours challenge was applied. Seven to ten days later, the subjects received an additional 48 hour challenge. Two of the subjects experienced erythema and papules during the study period. No reactions were observed after challenge.

In a 21-day cumulative irritation test, occlusive patches containing 0.1% retinyl palmitate were applied to the back of 12 subjects for 23 hours (page 307 in the cited report). Applied amount of test solution and patch size were not stated. Reactions were graded at 24 hours. Patches were applied daily for 21 consecutive days. Patches containing the base were applied on the back of 10 subjects. Retinyl palmitate gave a total score of 70 (max=756) whereas the base gave a score of 58 (max=630). Thus there was evidence for a slight potential for a very mild cumulative irritation. A 24 hour challenge was given after a two week rest period where reactions were graded after 48 and 96 hours (page 307 in the cited report). Exposure to both retinyl palmitate and the base gave a total score of 0 (max=1260 and 630, respectively).

In a study from 1974, 0.1% retinyl palmitate was evaluated using a modified Draize-Shelanski RIPT where occlusive patches were applied to the upper back of the subjects for 48 hours (n=189) (page 307 in the cited report). Applied amount of test solution and patch size were not stated. Sites were graded upon removal. Patches were applied on alternate days for a total of 10 applications. After a rest period of 10-14 days, similar challenge patches were applied for 48 hours. None of the subjects experienced adverse local effects.

In a similar study using a modified Draize-Shelanski RIPT (n=108) (page 307 in the cited report), 0.1 g of a moisturizer containing 0.1% retinyl palmitate were applied. Patch size was not stated. One subject had erythema after the challenge patch.

Both RIPT and 21-day cumulative irritation test are standardised tests used for evaluation of skin irritation and/or skin sensitization. Thus VKM regards these studies as relevant for evaluating adverse local effects of retinyl palmitate.

Kang *et al.* (1995) investigated the clinical and histological response of normal skin to all-*trans*-retinol application. Retinol in concentrations ranging from 0.002 to 1.6% where dissolved in an ethanol:propylene glycol vehicle (0.02 – 16 mg retinol/ml). The test solution were applied to buttock skin (100 µl/18 cm^2) on adults and occluded for 4 days (corresponding to 0.11 – 89 µg retinol/cm^2). The number of human volunteers included in the different retinol groups varied from 5-10, whereas 16 were included in the vehicle group. There is no information on gender or age. On day 4, the degree of erythema present at each site was scored according to a 10-point scale (0: none; 1-3: mild; 4-6: moderate; 7-9: severe). Local effects varied from none to only trace erythema, which was clinically and statistically insignificant relative to the vehicle. However, compared to the vehicle, ROL induced statistically significant epidermal thickening in a dose dependent manner from 0.05% retinol.

The study design includes only one application of test solution followed by four days of occlusion. VKM regards the study design not to mimic the anticipated use of body lotions.

In a study by Duell *et al.* (1997), clinical and histopathological changes in epidermis of human skin was recorded in response to increasing concentrations of retinoic acid (RA, 0.001 – 0.05%), retinol (0.025 – 1%), retinaldehyde (0.01 – 1%) and retinyl palmitate (0.1 – 0.6%). Retinoids were dissolved in a vehicle containing 95% ethanol:propylene glycol (7:3 vol/vol) with 0.5 mg butylated hydroxytoluene per ml of solvent except for retinyl palmitate which was dissolved in 95% ethanol with butylated hydroxytoluene. One site (not reported, but probably buttock as described in the referred paper by Duell *et al*. (1992) was treated with one application of the test substance or vehicle and occluded for 4 days, another site was treated daily with the test substance or vehicle for 4 days and kept unoccluded. Retinoid activity of all test substances was demonstrated as increased activity of retinoic acid 4-hydroxylase. The authors report statistically significant ($P < 0.05$, n = 8) increases in epidermal thickness four days after a single application of 0.05% retinaldehyde, 0.05% retinol or 0.3% retinyl palmitate (data not shown). It is also reported that erythema, as observed in response to retinoic acid, was not detected after application of retinaldehyde, retinol or retinyl palmitate (data not shown). The study design includes a single application with occlusion and daily application for four days without occlusion. VKM regards the repeated treatment-part of the study design to mimic an anticipated use of body lotion.

The clinical and histological effects of 0.15% retinyl propionate cream on extrinsic photo-aged human skin were assessed in a double-blind, randomized and vehicle-controlled study (Green *et al.* 1998). After an initial 4-week placebo run-in period, subjects were instructed to apply a pea-sized amount of cream twice daily to the face and dorsa of both forearms and hands. Of the 80 included subjects (25 - 75 years) 75 completed a 24-week period; two of these withdrawals were because of adverse cutaneous reactions (week 1 and week 5). The second 24-week-period started with 60 subjects, one withdrew because of skin irritation. Clinical assessments of the skin, including any adverse effects (erythema, burning, rash, dryness or flaking) were made by two physicians. Additionally, subject self-assessment, photography of face and forearm, measurements of wrinkle depths and histology were performed. No statistically significant differences at any time point for any site between the active and placebo groups were found. With respect to adverse effects, eight subjects experienced skin irritation during the initial 4-week placebo period. In the first 12-week period, 10 subjects in the placebo group (including five from the run-in period) and eight subjects in the treatment group (two withdrawals) reacted with irritation, redness, dryness or flaking of the face and forearm (only treatment group). Between week 12 and 24, five subjects continued to react in both groups and three in the active and four in the placebo group reacted for the first time. In the final 24 weeks, all the reactions largely settled but one subject in the active group withdraw due to irritation. VKM regards the study design as such acceptable to mimic the use of face cream and body lotion. However, the adverse reactions were found to a similar degree in both the placebo and the treatment group. It is therefore not possible to ascribe the observed effects to the use of the tested retinyl propionate cream.

In a study by Fluhr et al. (1999), the local skin tolerance of retinol (0.07%), retinaldehyde (0.05%) and retinoic acid (0.05%) in three marketed products was studied in a maximization test (n = 6). Retinaldehyde and retinoic acid were also tested in a clinical study (n = 350). In the maximization test, 100 mg of the test product was applied daily for 14 days on the ventral forearms, with occlusion. Test sites were chosen according to randomization schedule.

Scoring of erythema, scaling and burning/pruritus was performed by a trained dermatologist, with intensity graded from 0 – 3 (0 = absent, 1 = slight, 2 = moderate, 3 = severe). Instrumental evaluations of barrier function and irritation were performed by measurement of transepidermal water loss (TEWL) and perfusion unit (PU) at baseline and end of treatment according to standard guidelines. Results on erythema, scaling and burning/pruritus from the maximization test are presented (in a bar chart) as the sum of clinical scores for each parameter in the study population (n = 6). Erythema with retinol (15 - 16) and retinaldehyde (14 - 15) was significantly lower than with retinoic acid (> 20). Retinol showed low effects on scaling (~ 2), with both retinaldehyde and retinoic acid being significantly higher. Burning/pruritus tended to increase more frequently with retinol (5 – 6) and retinoic acid (3 – 4) than with retinaldehyde (the quoted scores given in the brackets are not provided by the authors but read from the bar chart by VKM). Treatments with retinol resulted in little (non-significant) changes in TEWL and PU from baseline to end of treatment. TEWL was a little more increased with retinaldehyde and retinoic acid (no significant differences between treatments), whereas PU was significantly higher with retinoic acid compared to retinol and retinaldehyde. The clinical study confirmed a good tolerance profile of retinaldehyde compared to retinoic acid (retinol was not included). VKM regards the study design to mimic an anticipated use of body lotions.

In a double-blinded, randomized, vehicle-controlled study, 20 slightly to moderately overweight women (28-45 years) with moderate degrees of cellulite were included (Kligman et al. 1999). Vehicle and 0.3% retinol were applied twice daily on opposite lateral thighs for 6 months. The amount applied was approximately 3 mg/cm^2 corresponding to 9 µg retinol/cm^2. Total area of application was not given. A dermatologist examined the volunteers every month to note improvement and adverse event, especially irritation. No adverse events were observed in any of the volunteers. VKM regards the study design to mimic an anticipated use of body lotions.

In a study by Nohynek *et al.* (2006) two groups of 14 female volunteers (20-38 years) 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). No vehicle group was included in the study. Daily, 1 mg cream/cm^2 was applied comprising 3 µg retinol/cm^2 (9 mg/day) or 5.3 µg retinyl palmitate/cm^2 (16 mg/day). Adverse local effects were defined as rash, itching and other dermal manifestations (not specified by the authors) at treatment site. After one week, 13/28 participants had skin reactions such as rash and itching, and the regimen was temporally adjusted for 9 subjects to partial treatment or treatment on affected sites was suspended for one or several days. In the retinol group, skin reactions were observed in 9/14, in which one subject with moderate to severe reactions was discontinued for 4 days. In the retinyl palmitate group, 2/14 reported itching, but the treatment was continued as scheduled. In all subjects, the skin reactions stabilized and/or subsided after approximately 10-12 treatment days. A total of 109 adverse events were reported in 25 subjects: 107 mild cases, 37 moderate cases, 2 severe cases. A possible or probable relationship to the treatment was considered for 44 of the adverse events. In 25 of the reported adverse events, over-the-counter medication was applied. VKM regards the study design to mimic an anticipated use of body lotions.

The effectiveness of topical retinol in improving clinical signs of naturally aged skin was evaluated in a study population of 36 elderly subjects (mean age 87 years) (Kafi *et al.*

2007).Topical 0.4% retinol lotion (Norwegian Formula Neutrogena Body Moisturizer prepared with retinol in polysorbate) or its vehicle (Norwegian Formula Neutrogena Body Moisturizer prepared with polysorbate) was applied to either right or left arm up to 3 times a week for 24 weeks. Evaluations were performed by dermatologists at baseline and at weeks 2, 4, 8, 16 and 24. Clinical effects on the arm (tactile roughness, fine wrinkling and overall severity) were graded semi-quantitatively from 0 – 9 (0 – none; 1 – 3, mild; 4 – 6, moderate; 7 – 9; severe). At each visit, signs of irritation (erythema, peeling, pruritus, burning and/or stinging and dryness was evaluated on a similar 0 – 9 scale. Photographs of treatment areas were obtained at baseline and all visits, skin biopsies for biochemical analysis were taken at baseline and at 24 weeks. Statistically significant changes in score on the clinical effects (reduction), as well as on biochemical results (induction of glycosaminoglycan expression; increase in type I procollagen staining) from baseline to week 24 was seen in the retinol treated group. 13 of the 36 subjects withdrew prior to completion, five of these because of cutaneous irritation and/or pruritus. By week 24, most subjects reported some degree of cutaneous irritation on the retinol-treated arm, including erythema (n = 18), peeling (n = 16), pruritus (n= 12), dryness (n = 14) and burning and/or stinging (n =3). Most adverse reactions were rated as mild; however, 3 subjects had severe reactions. VKM regards the study design to mimic an anticipated use of body lotions.

Kikuchi *et al.* (2009) performed a double-blinded study in 57 healthy Japanese women (40-60 years) using a 0.075% retinol cream and its vehicle cream for 6 months. Pea-size amounts of retinol cream were applied to one side of the face and the vehicle on the other side (from the outer canthus to the cheek, and the eyelid and temple) (exact amount of cream applied and size of the total application area were not stated by the authors). The volunteers either applied the test solutions once daily or once every second day for the first two weeks. A dermatologist performed a clinical evaluation at weeks 0, 4, 8, 12, 18 and 26. The clinical evaluation included erythema, swelling and scaling as adverse events. Three volunteers that applied test solutions daily withdrew from the study due to irritation. A higher level of erythematous colour was noted on the retinol-treated side, but the differences were not statistically significant compared to the vehicle-treated side. At the retinol-treated side, swelling was noted at a slightly higher rate. Fine scaling were noted after four weeks and reached a significantly higher level compared the vehicle at eight weeks. The adverse effects disappeared during the study period. In a second study, 36 of the volunteers participated in a double-blinded study with a 0.04% retinol cream for 3 months. No erythema, swelling or scaling were observed in these volunteers. VKM regards the study design to mimic an anticipated use of face creams.

Healthy Korean women (n=46, 34-53 years) were included in a double-blinded, randomized and controlled study (Kim *et al.* 2010a). The volunteers had periorbital wrinkles and mild to moderate photo-damaged skin. In the first study, 12 volunteers in group A applied 0.06% retinyl retinoate to the left periorbital area, whereas the right periorbital area was treated with placebo. In group B (n=12), retinyl retinoate and placebo were applied to the opposite side of the face compared to group A. The test solutions were applied twice daily for 12 weeks. In the second study, 11 volunteers in group P applied 0.06% retinyl retinoate and 0.075% retinol to the left and right periorbital area, respectively. In group Q (n=11) the test solutions were applied on opposite side as in group P. The test solutions were applied twice daily for 8 weeks. Exact amount of cream applied and size of the total application area were not stated by the authors. Subject self-assessments via questionnaires were made at weeks 0, 4, 8 and 12.

Adverse effects such as erythema, edema, scaling, itching, stinging, burning, tightness and prickling were examined by the investigator. According to the self-assessment, none of the participants experienced allergic or irritant contact dermatitis. Possible findings made by the investigators were not stated by the authors. VKM regards the study design to mimic an anticipated use of face creams.

In order to identify the efficacy of retinyl retinoate as an antiaging agent of cosmetics, 11 Korean women over 30 years, with periorbital wrinkles, were included in a prospective, double-blind, randomized and controlled study (Kim *et al.* 2011). The subjects were divided into two groups. One group was tested on the left periorbital area with 0.06% retinyl retinoate cream (cream A), the other group was tested on the right periorbital area with 0.075% retinol cream (cream B). Cream was applied morning and night daily for 12 weeks. Clinical evaluations were performed at pretreatment, 4, 8 and 12 weeks. The subjects' periorbital wrinkles were scored by two dermatologists and adverse effects such as erythema, edema, scaling, itching, stinging, burning, tightness and prickling were analysed on the tested skin. Based on the subjects' self-assessment, all subjects noted an improvement with the two creams. Investigators assessment of wrinkles based on photo-damaged skin score showed statistically significant improvement in both groups. With respect to adverse effects, topical cream A did not show any skin irritation responses whereas topical cream B resulted in adverse skin reactions of stinging and burning. VKM regards the study design to mimic an anticipated use of face creams.

Table 1: Relevant human *in vivo* studies

Treatment	Design	Outcome	Comment	Reference
Retinyl palmitate: 0.1 1%	Tests (several tests with slightly different design): • Repeated Insult Patch Test • 21-day Cumulative Irritation test • Patches applied to arm or back	Slightly irritating and/or non-irritating Non-sensitising	Strengths: • Standardised tests for irritation/sensitisation Limitations: • Mostly unpublished data • Patch size and/or applied amount are unknown	Final Report on the Safety Assessment of Retinyl Palmitate and Retinol, 1987 (Evaluation of several human studies, mostly unpublished)
Retinol: 0.002 – 1.6%	Adults (n=5-16) Application on buttock, occlusion for 4 days	No statistical significant difference with regard to erythema between retinol and vehicle	Strengths: • Dose-response design • Amount applied in µg/cm^2 is known Limitations: • Only one application • Occlusion • No information on gender and age	Kang *et al.* (1995)
Retinol: 0.001 – 1% Retinyl palmitate: 0.1 – 6%	a) A single application, occlusion for 4 days (n = 8) b) Daily applications for 4 days (n = 8)	No observation of erythema	Strengths: • Dose-response design • Repeated daily applications in part of the study Limitations: • No information on gender and age • Short test period	Duell *et al.* (1997)

Treatment	Design	Outcome	Comment	Reference
Retinyl propionate: 0.15%	Adults (> 18 years) with photo-damaged skin (n=80, 11 males and 69 females) Application of pea-sized amount twice daily to face and dorsa of both forearms and hands (24 or 48 weeks)	Placebo run-in period (4 weeks): Irritation (8) 0-12 weeks: Irritation (8 in treatment group, 8 and 10 in placebo group) 2-24 weeks: Irritation, redness, dryness or flaking (8 in treatment group and 9 in placebo group) 24 – 48 weeks: Irritation (1 in treatment group) No significant difference in response between placebo and treatment groups	Strengths: • Long test period • Repeated daily applications • Double-blind study Limitations: • Size of application area is unknown	Green *et al.* (1998)
Retinol: 0.07%	Male volunteers (n=6, 29 – 36 years) Daily applications of 100 mg on the ventral forearm for 14 days, occlusion	Erythema, scaling and burning/pruritus described as rather low	Strengths: • Repeated daily applications Limitations: • Occlusive conditions • Size of application area is unknown	Fluhr *et al.* (1999)
Retinol: 0.3%	Women (n=20) Daily applications on lateral thighs for 6 months	No adverse events reported	Strengths: • Long test period • Repeated daily applications • Amount in µg/cm^2 is known Limitations: • Size of application area is	Kligman *et al.* (1999)

Treatment	Design	Outcome	Comment	Reference
			unknown	
Retinol: 0.30% Retinyl palmitate: 0.55%	Women (n=28) Daily applications on back and upper legs for 21 days	1 week: 13/28 had skin reactions (rash, itching) Retinol group: Skin reaction in 9/14. One with moderate to severe reactions Retinyl palmitate group: 2/14 with itching All subjects: Skin reactions stabilized and/or subsided after approximately 10-12 treatment days	Strengths: • Repeated, daily applications • Size of application is known • Amount applied in µg/cm^2 is known Limitations: • No vehicle group included	Nohynek *et al.* (2006)
Retinol: 0.4%	Elderly men > 80 years old (n = 36) Application of 2 ml to right or left upper inner portion of arm up to 3x per week for 24 weeks (mean 1.6x per week). Retinol and vehicle were applied to opposite arms	24 weeks: Erythema (n = 18), peeling (n = 16), pruritus (n= 12), dryness (n = 14) and burning and/or stinging (n =3)	Strengths: • Long test periods • Repeated applications • Double blinded studies Limitations: • Size of application area is unknown	Kafi *et al.* (2007)
Retinol: 0.075% 0.04%	Japanese women 0.075% retinol: 26 week study (n=57) 0.04% retinol: 13 week study (n=36)	0.075% retinol: 3/54 dropped out just after start due to irritation Swelling and fine scaling were noted but disappeared during the study period	Strengths: • Long test periods • Repeated applications • Double-blinded studies Limitations: • Amount of applied test	Kikuchi *et al.* (2009)

Treatment	Design	Outcome	Comment	Reference
	Repeated applications daily/every second day in the face	0.04% retinol: No dropouts No erythema, swelling or scaling	solution and size of application area are unknown	
Retinyl retinoate: 0.06% Retinol: 0.075%	Korean women Study 1 (n=24): 0.06% retinyl retinoate/placebo Twice daily applications for 12 weeks. Retinyl retinoate lotion and placebo applied on opposite sides of the face Study 2 (n=22): 0.06% retinyl retinoate lotion 0.075% retinol lotion Twice daily application for 8 weeks. Retinyl retinoate and retinol lotion applied on opposite sides of the face	Subject self-assessment: Retinol and retinyl retinoate did not lead to allergic or irritant contact dermatitits	Strengths: • Long test periods • Repeated, daily applications • Double blinded studies Limitations: • Amount of applied test solution and size of application area are unknown	Kim *et al.* (2010a)
Retinyl retinoate (cream A): 0.06% Retinol (cream B): 0.075%	Korean women 35 – 56 years (n = 11) Application on aged skin morning and night for 12 weeks	Cream A: no irritation responses. Cream B: adverse irritation responses (stinging and burning)	Strengths: • Long test periods • Repeated, daily applications • Double blinded studies Limitations: • Amount of applied test solution and size of application area are unknown	Kim *et al.* (2011)

3.2 Animal studies

The two selected animal studies are summarized in Table 2.

In the Final Report on the Safety Assessment of Retinyl Palmitate and Retinol (unknown 1987), several studies using experimental animals were assessed (unpublished data). A 0.5 g sample of a moisturizer containing 0.1% retinyl palmitate were applied daily for four days to one side of the shaved back of three albino rabbits (page 294, section Dermal Irritation in the cited report). A control was applied to the other side of the back. The size of application area was not stated. There were insignificant differences in dermal irritation between the test solution and the control.

In a similar study, 0.5 ml sample containing 0.1% retinyl palmitate in a body lotion were applied daily for four days to the back of shaved albino rabbits (n=3) (page 294, section Dermal Irritation in the cited report). The size of application area was not stated. Within 48 hours a well-defined erythema and edema developed and persisted throughout the 7-day study.

In a study (pages 297-298, section Dermal – Specific Studies in the cited report), a body lotion containing 0.1% retinyl palmitate was applied daily for 90 days at a dose of 6 mg/cm^2 to a shaved area on the flank skin of 10 New Zealand albino rabbits (5 males, 5 females). A similar control group was included. All the treated animals developed slight to moderate erythema and oedema during the first week of the study. The symptoms persisted throughout the study and were accompanied by slight to moderate desquamation in all animals. Repeated applications were used in the cited studies, thus mimicking an anticipated use of body lotions. The skin of rabbits differs from human skin. The results may, therefore, not be directly extrapolated to humans.

In a study by Kim *et al.* (2010b) groups of female hairless mice (n = 4) were topically treated once daily for 3 – 5 days with one of the agents 0.05% retinaldehyde, 0.05% retinyl retinoate, 0.05% all-trans retinoic acid, 0.05% retinol or vehicle only (propylene glycol : ethanol = 7 : 3 v/v). TEWL was measured before exposure and daily for 3 days. Changes in TEWL were used to monitor the degree of skin irritation, and the values increased after topical application of retinoid. Retinoic acid and retinaldehyde showed strong signs of irritation after 2 days, whereas TEWL values were increased after 3 – 5 days with retinol and retinyl retinoate application. The ranking order of retinoid-like irritancy was retinyl retinoate < retinol < retinoic acid = retinaldehyde. Expression of hyaluronan, a glycosaminoglycan known to retain water, maintain extracellular space, facilitate transport of solutes and nutrients, and be involved in a wide range of cellular functions, was also measured in the epidermis and dermis of the retinoid-treated mice (after 3 days) and in normal human epidermal keratinocyte cells. The ability to induce hyaluronan production, regarded a positive effect in the skin, was increased to relatively comparable levels for all treatments. It was therefore concluded that retinyl retinoate was comparable to the other well-known retinoids in its ability to induce hyaluronan production without the induction of skin irritation. VKM regards the study design to mimic an anticipated use of body lotions. However, the skin of hairless mice differs from human skin and the results may therefore not be directly extrapolated to humans.

Table 2: Relevant animal *in vivo* studies

Treatment	Design	Outcome	Comment	Reference
Retinyl palmitate: 0.01%	Rabbits (n=3), daily applications for 4 days on shaved back Rabbits (n=3), daily applications for 4 days on shaved back Rabbits (n=10), daily applications for 90 days	Insignificant differences in dermal irritation between test and control group Erythema and edema developed within 48h. Symptoms persisted throughout the study. Subsequent dehydration and desquamation All animals developed slight to moderate erythema and edema the first week. Symptoms persisted throughout the study. Slight to moderate desquamation.	Strengths: • Repeated, daily applications Limitations: • Different physiology of the skin in rabbits and humans	Final Report on the Safety Assessment of Retinyl Palmitate and Retinol, 1987 (Evaluation of several animal studies, mostly unpublished)
Retinyl retinoate: 0.5% Retinol: 0.5% Retinaldehyde: 0.5% Retinoic acid: 0.5%	Female hairless mice, topically treated once daily for 3 – 5 days	Retinoid-like irritancy rank order: retinyl retinoate < retinol < retinaldehyde = retinoic acid (based on increasing TEWL values after exposure to retinyl retinoate, retinol, retinaldehyde and retinoic acid)	Strengths: • Repeated, daily applications Limitations: • Different physiology of the skin in hairless mice and humans	Kim *et al.* (2010b)

3.3 Summary of relevant published data

Of the human studies, the papers by Kang *et al.* (1995) and Green *et al.* (1998) were not assessed further. In these papers, adverse effects were observed. However, there were no statistical differences between exposure and vehicle groups, making it impossible to determine whether the observed effects were caused by retinol/retinol esters or the vehicle.

In the studies on retinol, local adverse effects in the skin were observed in six studies (concentrations ranging from 0.075 – 0.4%), whereas three studies did not observe adverse effects (concentrations ranging from 0.025 – 1%) (Table 3). Furthermore, three studies observed local adverse effects of retinyl palmitate (0.1% and 0.55%). In the studies where no local effects in the skin used were observed, retinyl palmitate in the concentration range of 0.1 – 0.6% or retinyl retinoate at the concentration of 0.06% were used.

The animal studies show adverse effects after the use of retinol or retinyl esters. However, the studies include only one treatment concentration and are therefore not suitable to show a dose-response relationship. Furthermore, the skin of the experimental animals (rabbits, hairless mice) and humans differ and are thus not regarded as directly comparable when it comes to setting concentration limits with respect to adverse local effects in human skin. Therefore, the present assessment is based on human data.

Table 3: Summary of selected results used in the final evaluation

	Retinol (%)	**Adverse effects****	**Retinyl esters (%)**	**Adverse effects****	**Reference**
Body*	-	-	0.1%, 1%	None to low	Final Report (1987)
	0.025 – 1%	No	0.1 – 0.6%	No	Duell *et al.* (1997)
	0.07%	Low	-	-	Fluhr *et al.* (1999)
	0.3%	No	-	-	Kligman *et al.* (1999)
	0.3%	Yes	0.55%	Low	Nohynek *et al.* (2006)
	0.4%	Yes	-	-	Kafi *et al.* (2007)
Face*	0.075%	Yes	-	-	Kikuchi *et al.* (2009)
	0.04%	No	-	-	Kikuchi *et al.* (2009)
	0.075%	Yes	0.06%	No	Kim *et al.* (2010a)
	0.075%	Yes	0.06%	No	Kim *et al.* (2011)

* Based on study design

** Yes, persistent; Low, transient or slight/weak

4 Discussion

None of the studies described in this opinion are dose-response studies primarily designed to register local adverse effects in human skin. Among the studied used for this evaluation (Table 3), it is only in the study by Duell et al. (1997), in which the aim was to compare retinoid activity in response to retinoic acid, retinol, retinaldehyde and retinyl palmitate at increasing concentrations that dose-response results are reported. In this study, however, no adverse effects in the skin were demonstrated at any of the used concentrations. Among the other included studies that mimic the use of skin care products, application of 0.3% retinol

(Nohynek et al. 2006) and 0.4% retinol (Kafi et al. 2007) to the body resulted in local adverse effects. However, such effects were not seen at 0.3 % retinol in the study of Kligman et al. (1999). In another study (Fluhr et al. 1999) adverse effects were recorded as the sum of scores of clinical parameters. Application of 0.07% retinol resulted in increased scores for each of these parameters, but the increases were described as rather low. With respect to application of retinyl esters to the body, the use of 0.1 – 6% retinyl palmitate showed no adverse effects (Duell et al. 1997), whereas the use of 0.55% lead to some adverse effect (itching) (Nohynek et al. 2006). When retinol or retinyl esters are applied to the face, two separate studies report effects defined as adverse after application of 0.075% retinol (Kikuchi et al. 2009; Kim et al. 2011). In contrast, another study published by one of these groups, reports no adverse effects at 0.075% retinol in a comparable study. With respect to adverse effects following the application of retinyl esters to the face, no such effects are reported at the concentration of 0.06 % (Kim et al. 2010a; Kim et al. 2011). Taken together, these data may indicate that a concentration of about 0.075% retinol may be able to induce adverse local effects in the skin. With respect to retinyl esters, the number of studies is low and application to the body and the face is reported for different retinyl esters (retinyl palmitate and retinyl retinoate, respectively). Thus, the data is insufficient to indicate a concentration level for the onset of adverse local effects.

5 Conclusions

Based on the reported studies identified in the literature search, VKM concludes that there are not enough relevant studies with the necessary data on association between concentrations and adverse effects to define safe upper concentrations for the use of retinol and retinyl esters (retinyl palmitate and retinyl retinoate) in cosmetics. However, the present available data indicates that local adverse effects in human skin may appear after application of retinol at concentrations about 0.075% and above. For retinyl esters, no conclusion can be drawn from the available data.

6 Data gaps

- There is a lack of studies designed especially to examine adverse local effects.
- There are few dose-response studies.
- There are no studies addressing adverse effects on the skin of infants and children.

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Appendices

Appendix 1

The search was performed by a librarian at the Norwegian Institute of Public Health the 6th May 2013 using Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) 1946 to 2013 May 06 and Embase 1974 to 2013 May 06. The search resulted in 909 scientific publications.

Search terms

1. vitamin a/
2. vitamin a.mp.
3. retinol/
4. retinol*.mp.
5. retinol ester/
6. retinol ester*.mp.
7. retinolester*.mp.
8. retinylester*.mp.
9. retinyl ester*.mp.
10. retinol palmitate/
11. retinol palmitate*.mp.
12. retinolpalmitate*.mp.
13. retinylpalmitate*.mp.
14. retinyl palmitate*.mp.
15. retinoids/
16. retinoid/
17. retinoid*.mp.
18. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17
19. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14
20. skin/
21. skin*.mp.
22. skin cream/
23. skin cream*.mp.
24. dermal*.mp.
25. cosmetics/
26. cosmetic/
27. cosmetic*.mp.
28. 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27
29. irritants/
30. irritant agent/

31. irritant*.mp.
32. irritat*.mp.
33. dermatitis/
34. dermatitis.mp.
35. hypersensitivity/
36. hypersensitiv*.mp.
37. skin allergy/
38. skin allerg*.mp.
39. allergy.mp.
40. allergic reaction/
41. allergic reaction*.mp.
42. reaction*.mp.
43. sensibili?ation.mp.
44. sensitization/
45. sensiti?ation.mp.
46. toxic effect*.mp.
47. toxicity/
48. toxicity.mp.
49. risk assessment/
50. risk assessment*.mp.
51. skin irritancy tests/
52. skin irritancy test*.mp.
53. skin irritation/
54. skin irritation*.mp.
55. local skin reaction/
56. local skin reaction*.mp.
57. 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48 or 49 or 50 or 51 or 52 or 53 or 54 or 55 or 56
58. 19 and 28 and 57
59. exp human/
60. exp humans/
61. human*.mp.
62. 59 or 60 or 61
63. 58 and 62
64. conference abstract.pt.
65. 63 not 64
66. limit 65 to (danish or english or french or norwegian or swedish)
67. remove duplicates from 66