RISK PROFILE

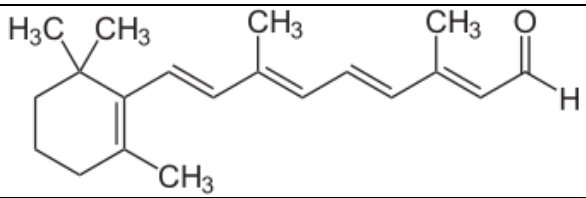
Retinaldehyde

CAS No.116-31-4

Date of reporting 16.04.2012

Content of document

1. Identification of substance

Chemical name (IUPAC):	3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexenyl)-nona-2,4,6,8-tetraenal
INCI	Retinal
Synonyms	Retinaldehyde; RAL[1]
CAS No.	116-31-4
EINECS No.	204-135-8
Molecular formula	$C_{20}H_{28}O$
Chemical structure	
Molecular weight	284.44 g/mol
Contents (if relevant)	
Physiochemical properties	Appearance: orange crystals from petroleum ether Melting point: 61-64 °C Solubility: nearly insoluble in water, soluble in fat

[1] Abbreviations: RAL, retinaldehyde; ROL, retinol; RA, retinoic acid; RE, retinyl esters.

2. Uses and origin

Uses	➢ **Cosmetic products** *Functions according to* • CosIng database: "Skin conditioning: maintains the skin in good condition" • Other: - Rejuvenation of the skin – anti-aging: in cosmetic products this involves mainly *ROL* and retinol esters (e.g. *retinyl palmitate),* with proven effects on the appearance of fine wrinkles, hyperpigmentation and roughness of facial skin (Rolewski, 2003; Council of Europe, 2008; VKM, 2012). For anti-aging activity of topical retinoids, see Annex 2 (Sorg et al., 2006). RAL has been shown (e.g. optical profilometry) to be equally effective as RA in reducing wrinkles and roughness of aged and photoaged skin, with a lower frequency of irritation (Diridollou et al., 1999; Creidi et al., 1998; reviewed in Mukherjee et al., 2006 and in Darlenski et al., 2010). *Concentrations of Retinaldehyde being applied* Example: RAL is typically found at concentrations of 0.025, 0.05% and 0.1%, but example of cosmetic products with RAL up to 2% are available in the market (internal overview based on internet search April 2012). *Frequency of use* The EWG Skin Deep [online] database lists the following 20 cosmetic products[2] containing RAL: – facial moisturizer/ treatment (10 products) – sunscreen: moisturizer (5 products) – anti-aging (7 products) – around-eye cream (1 product) – body firming lotion (1 product) Codecheck [online] contains 12 products with RAL, including facial cream (4), around eye cream (2), and body lotion (4). ➢ **Food** Natural retinoids (vitamin A and its derivatives) are present in all living organisms, either as preformed vitamin A or as carotenoids (referred to as provitamin A), which by conversion in the body provide vitamin A activity. ROL is obtained directly from foods of animal origin (liver, dairy products such as milk and butter) or plants (carotenoids), and in the form of supplements containing vitamin A (Sorg et al., 2006). ROL

[2] Some products appear in more than one of the listed categories.

	can then be converted to RAL and RA by oxidative conversion. β-carotene (in vegetable and fruit) can be cleaved symmetrically forming two molecules of RAL, which can then be either oxidized to RA or reduced to ROL. ➢ **Medicinal products** Topically applied RA (e.g. tretinoin, see Annex 1) is widely used in clinical dermatology for treatment of acne and photo damaged skin. It is also found in prescription treatments for skin diseases such as psoriasis (including brand names Accutane, Retin-A, Renova, Amnesteem, Soriatane, Claravis, Sotret, Tegison, Differin, Tazorac) and certain types of cancer (Vesanoid and Targretin) (Darlenski et al., 2010; Rolewski, 2003; Manriquez et al., 2008). RA is *not allowed in cosmetic products.* ➢ **Other products** Topical retinoids are the main therapy for mild and moderate acne, with RAL being best tolerated (Dreno et al., 2007; Sachsenberg-Studer, 1999). There is evidence that RAL possesses some antibacterial activity (e.g. it kills *P. acnes* bacteria) *in vivo* and *in vitro*, which is likely related to the presence of an aldehyde group in the side chain of RAL (and thus not shared by ROL or RA) (Péchère et al., 2002).
Origin Natural (exo /endo) Synthetic	Retinoids are essential throughout life. Although they were first discovered in the retina with a central role in the biology of vision, they function as key regulators of differentiation and proliferation in various tissues e.g. during fetal development, bone growth and immune function (Council of Europe, 2008; VKM, 2012). The natural retinoids retinoic acid (RA), *retinaldehyde (RAL***)**, all-*trans*-retinol (ROL), retinyl esters (RE) and β-carotene are interrelated through enzymatic conversions (Annex 1). The retinoids define a class of lipophilic molecules consisting of four isoprenoid units [$H_2C=C(CH_3)-CH=CH_2$]. The intake of vitamin A from diet or supplementation is expressed as retinol equivalents (RE)[3]. 1 RE is defined as 1 µg of all-trans-retinol.

3. Regulation

Norway	No regulation[4].
EU	No regulation[5].
Rest of the world	US[6]: The retinoids RAL, ROL, retinyl palmitate, and retinyl acetate may be purchased over the counter (OTC) (Babamiri, 2011; Sorg et

[3] Retinol units or equivalents (see Annex 4).

[4] The use of **retinol and retinyl esters** in cosmetic products is restricted in the current Norwegian cosmetics regulation, with the maximum allowed concentration of 0.3% retinol equivalents (RE). According to the cosmetic industry, retinol and its esters are used in the following concentrations: 0.01% - 0.3% RE in face and hand creams and 0.01% - 0.05% RE in body lotions. **Retinaldehyde** is not regulated, whereas **retinoic acid** is reserved for medicinal products and not allowed in cosmetic products. The Norwegian medicinal products agency considered retinaldehyde extracts medicinal remedies. Because of that up till 2008 topical products containing the substance were considered medicines – meaning a topical product containing it were automatically classified a medicine. Applications for allowance to use the extract for other purposes (cosmetics) were rejected. This regime has since been lifted.

[5] CH: Max. allowed conc. 0.05% (Council of Europe, 2008).

	al., 2006; Darlenski et al., 2010). Canada: No regulation[7].

4. Relevant toxicity studies

Absorption Skin GI tractus	Only limited skin absorption studies for retinoids have been conducted (including ROL and RAL), and the systemic bioavailability is not well understood (Yourick et al., 2008). Although both *in vivo* and *in vitro* data demonstrate that substantial levels of retinoids are loaded in skin after topical application, no significant increase in plasma levels of retinoids could be detected after repeated application of RAL, ROL or retinyl palmitate (Nohynek, 2006; Antille et al., 2004; Sorg et al., 1999; reviewed VKM, 2012). Similarly, Sass et al. (1996) showed that plasma retinoid levels did not change following topical application of 0.025% RAL (Council of Europe, 2008). In a previous assessment of vitamin A in 1997 the Norwegian Institute of Public Health applied a systemic absorption rate for ROL of approx. 7% (Council of Europe, 2008). This was based on data for all-trans-RA, and considered to represent a worst case scenario, since no relevant data was available on the systemic absorption of ROL and retinyl palmitate. For ROL, the VKM Panel in their most recent risk assessment of ROL estimated a dermal absorption rate of 5.7% (VKM, 2012). This was based on *in vitro* (rat, human) and *in vivo* (rat) percutaneous absorption studies of retinol from cosmetic formulations (Yourick et al., 2008) as well as SCCS's guideline (SCCS [online]). According to SCCS criteria, the systemic availability of topical ROL equals mean absorption in viable skin + receptor fluid +2SD (VKM, 2012). Sass et al. (1996) investigated plasma retinoids after topical use of RAL in 10 male volunteers; 40% of the body surface was exposed daily to 7 mg of RAL for 14 days. The skin metabolism did not result in detectable changes of plasma retinoid levels (e.g. ROL, RA, RAL, retinyl ester). Antille et al. (2004) showed that topical retinoids (0.05% RA, RAL, ROL or retinyl palmitate) penetrated well into the epidermis, using *ex vivo* human skin explants (mounted on Franz perfusion cells) and two mouse models (*ex vivo* skin explants and hairless mice *in vivo*). RAL increased endogenous ROL and retinyl ester slightly, and small amounts were converted into RA. ROL and retinyl esters increased endogenous ROL and retinyl esters, but no RAL or RA was detected. RA did not undergo metabolism. Nohynek et al. (2006) reported that repeated topical treatment of ROL, retinyl esters or RA - in contrast to a single *oral* dose - did not affect endogenous levels of RAs in female human subjects of child-bearing age. The extremely low (not measurable to trace) level conversion of RA

[6] Natural (e.g. tretinoin, isotretinoin) or synthetic RA variants (tazarotene and adapalene) are prescription medications (Babamiri & Nassab, 2010; Sorg et al., 2006; Darlenski et al., 2010).

[7] Retinol (vitamin A) (CAS.no. 68-26-8) and its esters; retinyl acetate (Cas.no. 127-47-9), retinyl palmitate (Cas.no. 79-81-2): permitted at concentrations equal to or less than 1%. The Hotlist is a science-based document that is reviewed and updated as new scientific data becomes available (Health Canada [online]).

	following topical ROL application suggests that systemic RA (derived from ROL) from cosmetics would be trivial compared to diet-derived microgram quantities of ROL normally present in blood (Underwood, 1994, cited in Kang et al., 1995). In summary, these data are consistent with the notion that absorbed topical retinoids (e.g. ROL, RAL, retinyl esters) from cosmetics do not result in measurable changes in systemic retinoid plasma levels.
Distribution	In the *skin*, RAL and RA are normally below the detection limit (10 pmol/g), whereas ROL and retinyl esters account for more than 99% of total retinoids (i.e. approx. 1 nmol/g) in the epidermis (Sorg et al., 2006, and references therein). In the *blood and tissues,* ROL and retinyl esters (ROL esterified with fatty acids) are the predominant forms; approx. 90% of the body's reserve of vitamin A is stored in the liver as retinyl esters. ROL is very unstable and degrades easily into inactive metabolites (Darlenski et al., 2010).
Metabolism	*Enzymatic conversion of RAL into ROL or RA:* Bailly et al (1998) found that in explants of human skin ROL can be converted by oxidative metabolism to RAL followed by further oxidation to RA. The major metabolite in the epidermis was in the form of retinyl esters. Human keratinocytes convert RAL enzymatically predominantly into either ROL or retinyl-esters (i.e. storage forms of vitamin A in peripheral target cells or liver), whereas a relatively low amount of bioactive RA is formed (fig. 1, Annex 1) (Sorg et al., 2006; Didierjean et al., 1996, 1999; Vahlquist, 1999; Roos et al., 1998 Council of Europe, 2008). The metabolism of RAL to RA appears to take place at a defined stage of keratinocyte differentiation, leading to a more controlled release of active RA and weaker adverse effects of RAL than RA (Mukherjee et al., 2006). By bypassing the first of two rate-limiting oxidation step of ROL into RA, RAL is directly oxidized by the skin to form biologically active RA (Annex 1, Sorg et al., 2006; Roos et al., 1998). Reduction of a surplus of RAL in the form of biologically inactive ROL and retinyl esters is regarded as advantageous in cosmetic applications (Vahlquist, 1999). Once RAL has been converted to RA, the reaction is not reversible. Likewise, topically applied RA is not converted into RAL or ROL. *Receptor-mediated events:* The main effect of topical retinoids is believed to be the receptor-mediated gene activation induced by the ligand RA (VKM, 2012). Thus, the other retinoids (retinyl ester, ROL, and RAL) have to be converted into RA in order to activate the receptor. In line with this, topically applied ROL and retinyl palmitate have been shown to cause biochemical and histological changes in the skin expected from perturbations of retinoid homeostasis (VKM, 2012). Finding at most trace levels of RA in ROL-treated skin does not contradict the need for conversion to RA, but rather indicates tight regulation of ROL oxidation to RA in human epidermis (Kang et al., 1995). Moreover, the human CRABP-II gene contains a RA responsive element in its upstream region, indicating *in vivo* conversion of ROL to RA (via RAL) since ROL is unable to directly activate RA receptor-

	mediated gene transcription. This was also supported by in vitro studies with cultured human keratinocytes. Application of ROL to skin has been reported to cause molecular changes that are similar to those seen after treatment with RA (Kang et al., 1995), but much higher concentrations of ROL than RA was required to produce similar results; e.g. epidermal thickening and enhanced expression of CRABP-II and CRBP genes. Based on some studies, *ROL is about twentyfold less potent than RA* in processes involving RAR receptor-mediated events; e.g. in CRABP-II mRNA in vivo bioassay (Kang et al., 1995). See also letter SLV.
Excretion	RA is catabolized either by phase I or phase II enzymes, giving rise to retinoyl glucuronide or 4-oxoretinoic acid (Sorg et al., 2006, and references therein).
Local toxic effects Irritation Sensitivity	Irritated skin is characterized by redness, dryness and flaking of the skin at the treated site. Epidermal hyperplasia and features of abnormal differentiation are seen at the histological level, but the molecular events that underlie retinoid-elicited irritation is not clear (VKM, 2012). Retinoids are known to be irritating in sensitive individuals, tending to define a *practical upper use level.* Irritation may partly be related to an overload of non-physiological amounts of exogenous RA in the skin. For all topical retinoids the skin irritation effect is dose dependent. Nohynek et al. (2006) found that the adverse effect may be avoided with a topical dose below 0.3% (ROL) or 0.55% retinyl ester. The implied benefits of retinoids such as ROL and RAL in cosmetics are related to their conversion into bioactive RA after adsorption into skin cells (Rolewski, 2003). RAL and ROL seem to display a better tolerance profile than RA, which cause frequent irritation of the skin (Fluhr et al., 1999; Sachsenberg-Studer, 1999). While less irritating, ROL and retinyl palimitate tend to be considerably less efficient than RA and directly acting retinoids (Manriquez et al., 2008). The skin irritation potential of topical retinoids inversely reflects their ranking order with regard to biological activity mediated by the nuclear retinoid receptors (RAR, RXR): RE < ROL = RAL << RA (Sorg et al., 2006). RAL can be converted either into RA or to ROL (and further into retinyl palmitate) in just one step (see Annex 1). It is believed that treating the skin with RAL could produce the biologically active RA metabolite more efficiently than by using ROL, while reducing the risk of side effects associated with RA excess (e.g. skin irritation). Saurat and co-workers treated 229 volunteers with various skin problems with 1.0, 0.5, 0.1 or 0.05% RAL once daily for 1-3 months. 30% of individuals showed intolerance at a conc. of 1%, and performed only slightly better at 0.5%. *However, the 0.05% and 0.1% preparations were well tolerated and allowed prolonged use (up to 3 years) on facial skin in patients with inflammatory dermatoses* (Saurat et al., 1994; cited in Council of Europe, 2008). This indicates that RAL may be used as a topical agent on human skin. Fluhr et al (1999) found that RAL (0.05%) and ROL (0.075%) both had equally low irritation potential, whereas a more pronounced irritant effect was observed with RA. RAL and RA induced more exfoliative skin scaling than ROL, whereas ROL and RA tended to induce more burning/pruritus than RAL (non-significant). This study concluded that

	both ROL and RAL demonstrated a good tolerance profile. RAL was much better tolerated than RA (both at 0.05%) and vehicle cream for treatment of photo-aging during 18 and 44 weeks (Creidi et al. 1998). Both substances significantly reduced fine and deep wrinkles compared to vehicle controls. None of 45 patients had developed toxic allergic reaction or photosensivity after long-term (median: 48.6 months) use of RAL (Sachsenberg-Studer, 1999). The good tolerance of topical RAL has been confirmed by cosmetic vigilance data: for cream and emulsion they were below 1 of 10000. Complaints have been reported to be rare also for gel formulation - used especially for red faces in France (2.6 for 10000 sold products) (cited in Sachsenberg-Studer, 1999). No allergic reaction was recorded or published in this cohort. Two studies of the combination of 0.1% RAL and 6% glycolic acid (GA) in acne patients demonstrated very good tolerance (Dreno et al., 2005, 2007), with few complaints about side-effects; in one of the studies 3 patients in the treatment group (out of 71) and 1 in the control group (out of 74 patients) experienced cutaneous adverse effects (Dreno et al., 2007 and references therein). This could be related to the presence of RAL, some of the other ingredients (GA), or a combination.
Systemic toxic effects	There is no direct evidence for systemic adverse effects of topically applied RAL. This is also consistent with findings in the most recent VKM opinion for vitamin A, in which it was stated that “there are no available data or sufficient information demonstrating that topically applied vitamin A can contribute to systemic adverse effects” (VKM, 2012). With regard to RAL, the VKM Panel also listed that more data is needed on the use of this substance in cosmetics. For example, Sass et al (1996) found that skin metabolism of topically applied RAL did not result in detectable alterations of constitutive levels of plasma retinoids (e.g. all-trans RA, RAL, ROL, retinyl palmitate) in 10 healthy male volunteers on a restricted vitamin A diet. 14 grams of 0.05% RAL cream corresponding to 7 mg of RAL were applied daily on 40% of the body surface for 14 days. These data indicate that 0.05% topical RAL does not result in detectable systemic loading. Furthermore, in vitro experiments demonstrated that topical RAL is mainly converted into storage forms (i.e. 80% retinyl esters) and 11% ROL (itself further metabolized to retinyl esters) in the epidermis, whereas ca. 8% was converted into RA (Chatellard-Gruaz et al., 1994, cited in Sass et al., 1996). Thus, the constitutive levels of plasma ROL (ca. 500 ng/ml) appear not be altered by the small amount originating in the skin. Topical application of the natural RA analog tretinoin (single dose or repeated long-term exposure), did not result in detectable changes in systemic levels of retinoid metabolites (Mukherjee et al., 2006; Darlenski et al., 2010; Latriano et al., 1997). Daily application of 5 mg of RA (0.025% cream) did not alter the plasma retinoids and appears unlikely to induce systemic effects (Buchan et al., 1994). In summary, these observations support the experience that topical application of RAL does not affect systemic retinoid levels, neither directly, or in the form of converted ROL (or retinyl esters).

Acute	No further data retrieved, cf. RAL monograph (Council of Europe, 2008).
Repeated dose	No data retrieved.
Mutagenicity /genotoxicity	No data retrieved.
Carcinogenicity	No data retrieved.
Reprotoxicity / teratogenicy	No data retrieved for RAL. During pregnancy and lactation, vitamin A (ROL or retinyl ester) has a particularly important role in the healthy development of the child (Grune et al., 2010). However, teratogenicity of vitamin A is biologically and physiologically possible, yet its real occurrence in humans seems limited (Azais-Braesco & Pascal, 2000; Grune et al., 2010). Topical application of RAL does not result in changes in constitutive plasma levels of other retinoid metabolites (see above). There is also no evidence that *topical* RA treatment is associated with congenital abnormalities. (e.g. Darlenski et al., 2010). A similar argument has been made for the retinyl ester All-Trans-Retinyl Palmitate [CAS.no. 79-81-2] by National Institute of Environmental Health Sciences (NIEHS) (NIEHS, 2000 [online]). In a preliminary safety assessment report by Paulsen et al. (1998) at the Institute of Public Health, Norway, the short term teratogenic risk and local side-effects of topical ROL and retinyl palmitate were considered safe, whereas data were not available to evaluate long-term effects. However, in order to exclude individual uncertainties with respect to vitamin A and pregnancy, the use of topical retinoids during (the first trimester of) pregnancy is not recommended and should be avoided. In 2002 the Scientific Committee on Food (SCF) derived an upper safe limit (UL) for preformed vitamin A (ROL and retinyl esters) of 3000 µg retinol equivalents/day for all women of child-bearing age, based on teratogenic potential of vitamin A (VKM, 2012). The UL set by SCF is still appropriate today (VKM, 2012). Although teratogenicity is only relevant for women of child-bearing age, SCF considered that 3000 µg/day is appropriate for men and for infants and children after correction for metabolic rate (VKM, 2012).
Other effects	In 2008, EFSA considered that a maximum intake of 1500 µg/day would serve as a guidance level for individuals at risk of osteoporosis and bone fracture (particularly post-menopausal women) (cited in VKM, 2012).

5. Exposure estimate and critical NOAEL / NOEL

NOAEL/NOEL critical	None available for RAL, cf. Council of Europe, 2008. The UL for preformed vitamin A (ROL and retinyl esters) has been set at 3000 µg retinol equivalents/day, based on the teratogenic potential of vitamin A as the limiting adverse effect (Annex 5; VKM, 2012). The UL (for ROL) is not changed with regard to increased risk of bone fracture in postmenopausal women, but a guidance level (GL) of1500

	µg retinol equivalents/day has been recommended (VKM, 2012). It is unlikely that RAL derived from beta-carotenoids in food would reach toxic levels, since conversion into ROL is regulated via feedback mechanisms depending upon vitamin A levels. *Because retinoids are irritating to skin, defining skin-tolerated doses clinically is a key to the safety of these substances*.
Exposure cosmetic products	Skin penetration[8] data for RAL is not available, and there is also not enough data to estimate the (systemic) exposure of RAL from cosmetic products (Council of Europe, 2008; VKM, 2012). For exposure calculations we have used a dermal absorption rate[9] of 5.7% also for RAL, based on the latest risk assessment of ROL in cosmetics (VKM, 2012). The concentration of RAL in cosmetic products has been reported to be between 0.025 - 0.1%, but concentrations up to 1% have been used in some investigations. 2% RAL is the highest amount available in the market (Annex 3). Systemic exposure dose (SED) was estimated for the most relevant cosmetic product types according to *Colipa[10] data* (SCCS [online]), using 0.1% RAL as an illustrative example: • **Body lotion** 0.1% RAL (cream, body lotion) Calculated relative daily exposure of product: 123.20 mg/kg bw/day Concentration of ingredient in the product: 0.1% = 0.001 Dermal absorption[11]: 5.7% = 0.057 SED = A (mg/kg bw/day) x C(%)/100 x DAp (%)/100 = 123.20 mg/kg bw/day x 0.001 x 0.057 x 1000µg/mg = **7 µg/kg bw/day** For a 60 kg person, this corresponds to: 7 x 60 = <u>420 µg RAL/day</u> Conversion of RAL into "ROL equivalents": Based on some bioassays, the biological activity of RA (and by approximation RAL) has been estimated to be approx. 20 fold higher than ROL (Kang et al., 1995;SLV, letter). Using this conversion factor for illustrative purposes: ➔ 420 µg RAL x 20 = <u>8400</u> µg ROL "equivalents" The value for total body application is greater than UL (3000 µg/day).

[8] <u>Skin Penetration</u> represents the amount of a topically chemical that exists between the top layer (stratum corneum) and the bottom layer (stratum basale.) During penetration, the body does not yet absorb the chemical, and it cannot affect the body systems. <u>Skin Absorption</u> occurs when the topically applied chemical breaks the skin barrier to reach the bloodstream. Whether this chemical becomes a risk is determined by what occurs after absorption. The chemical substance can be drained into the circulation, or build up (i.e. bioaccumulation) in tissues.

[9] According to SCCS criteria, the systemic availability of topical ROL equals mean absorption in viable skin + receptor fluid +2SD (VKM, 2012)

[10] 'Colipa' has changed name to 'Cosmetics Europe' (from 2012).

[11] Based on data for ROL, cf. Yourick et al. (2008) and VKM (2012).

	To comply with the recommended UL and GL, RAL should not be present at concentrations higher than: 3000/8400 = 0.36 x 0.1% = 0.036% = 0.04% (teratogenesis) 1500/8400 = 0.18 x 0.1% = 0.018% = 0.02% (osteoporosis) • **Face cream** 0.1% RAL (cream, body lotion) Calculated relative daily exposure of product: 24.14 mg/kg bw/day Concentration of ingredient in the product: 0.1% = 0.001 Dermal absorption[12]: 5.7% = 0.057 SED = A (mg/kg bw/day) x C(%)/100 x DAp (%)/100 = 24.14 mg/kg bw/day x 0.001 x 0.057 x 1000µg/mg = **1.4 µg/kg bw/day** For a 60 kg person, this corresponds to: 1.4 x 60 = 83 µg RAL/day Using a conversion factor of 20 for illustrative purposes: ➔ 83 µg RAL x 20 = 1651 µg ROL "equivalents" For usage of RAL in facial cream/moisturizer the estimated SED is below the UL (3000 µg/day), i.e. 1651/3000= 55% of UL (RAL, 0.1%). To comply with the recommended GL (osteoporosis), RAL should not be present at concentrations greater than: 1500/1651 = 0.9 x 0.1% = 0.09% • **Hand cream** 0.1% RAL (cream, body lotion) Calculated relative daily exposure of product: 24.14 mg/kg bw/day Concentration of ingredient in the product: 0.1% = 0.001 Dermal absorption[13]: 5.7% = 0.057 SED = A (mg/kg bw/day) x C(%)/100 x DAp (%)/100 = 32.70 mg/kg bw/day x 0.001 x 0.057 x 1000µg/mg = **1.9 µg/kg bw/day** For a 60 kg person, this corresponds to: 1.9 x 60 = 112 µg RAL/day Using a conversion factor of 20 for illustrative purposes: ➔ 112 µg RAL x 20 = 2240 µg ROL "equivalents" This value for facial skin is below the UL (3000 µg/day): i.e. 2240/3000 = 75% of UL (RAL, 0.1%). To comply with the recommended GL (osteoporosis), RAL should not be present at concentrations greater than: 1500/2240 = 0.67 x 0.1% = 0.067%
Margin of Safety (MoS)	*Because a NOEL value is missing, it is not possible to calculate a margin of safety for RAL.*

[12] Based on data for ROL, cf. Yourick et al. (2008) and VKM (2012).
[13] Based on data for ROL, cf. Yourick et al. (2008) and VKM (2012).

6. **Other sources of exposure than cosmetic products**

Food stuffs	Endogenous retinoids in humans are derived from the intake of ROL and its esters in food of animal origin, and from carotenoids in plants (Underwood, 1994, cited in Didierjean et al., 1996). β-carotene cleavage enzyme catalyzes the conversion of all-*trans*-β-carotene to two molecules of RAL (Grune et al., 2010). There is insufficient evidence to establish a UL for β-carotene for supplemental use, but high intakes can cause yellowing of the skin and may be harmful to smokers. A UL for β-carotene from food does not need to be established, based on an absence of adverse effects (NRV [online]). A WHO expert group recommended that daily intakes of vitamin A in excess of 3000 µg (10000IU) = UL should not be taken at any period during gestation (WHO [online])[14].
Pharmaceuticals	RAL is an intermediate metabolite between ROL and RA. In most countries, including Norway, RAL is not regulated, whereas RA is reserved for medicinal products and not allowed in cosmetic products.
Other sources	
Adverse side effects - from uses other than cosmetics	There are no data for adverse effects of RAL from other uses than cosmetics, since it is not known to be present in food (other than indirectly derived from beta-carotene) or medicinal products. Moreover, there are no available data or sufficient information demonstrating that topically applied vitamin A can contribute to systemic adverse effects (VKM, 2012). The side effects of retinoids related to oral intake of RA variants are well known, some of which are listed below. However, RA cannot be converted into RAL (because the enzymatic reaction is irreversible), so the direct relevance for RAL is not clear. Oral treatments containing retinoids have been directly linked to miscarriages and birth defects. Congenital malformations due to *oral* isotretinoin (i.e. RA variant) exposure during pregnancy consists of craniofacial, cardiac, thymic and central nervous system malformations, with a relative risk of approx. 25% (comparable to the relative risk for thalidomide), cited in Crijns et al., 2011. For a description of pharmacological properties and reported adverse effects of oral tretinoin (all-trans retinsyre, ATRA) and isotretinoin ("Roaccutan"), see Norsk Legemiddelhåndbok [online]; Medline Plus [online];SLV [online]; Hathcock et al., 1990). Although research suggests that topical retinoids (even for prescription drugs such as Retin-A, Renova, a RA variant) do not pose the same risk, enough concerns exist regarding their safety during the early period of human pregnancy. Other effects: Topical retinoids has been reported to enhance the penetration of other drugs (Gollnick et al., 2003).

[14] A level of 1500 µg RE vitamin A in the diet (1500 µg RE) would require more than 800 g liver and is probably well beyond the levels expected in daily intake of food.

7. Assessment

Retinoids are irritating to the skin in sensitive individuals, but the effect is dose dependent. Thus, *defining skin-tolerated doses is a key to the safe use of these substances*. The skin irritation potential is ranked in the following order: RA >>RAL=ROL>retinyl ester, which is inversely related to their biological activities mediated by nuclear retinoid receptors (i.e. RAR, RXR). Because topical RA induces most severe irritation of the skin, which precludes its use in some skin conditions, RAL and ROL have been pursued as alternatives by the cosmetic industry. RAL is much less frequently used than ROL, but is biologically more potent than ROL and appears to be equally well tolerated.

General toxicity:
There is still not sufficient data to estimate the effect level (NOEL) for RAL, and hence it is not possible to calculate a margin of safety (MoS).

A short monograph on the safety of RAL has been produced by the Council of Europe (2008). Moreover, in a recent risk assessment report of vitamin A, the VKM Panel identified several data gaps and stated that "there are no available data or sufficient information demonstrating that topically applied vitamin A can contribute to systemic adverse effects", and further that "more data is needed on the use of retinaldehyde in cosmetics" (VKM, 2012).

There are also no available data demonstrating that topically applied RAL can contribute to systemic adverse effects. There is no evidence that beta-carotenoids in food would reach toxic levels, and accordingly, it is unlikely that RAL derived from beta-carotene would be toxic.

Thus, the assessment of the potential systemic toxicity of RAL in the present preliminary risk assessment leans to a large extent on the available information for ROL. The UL for preformed vitamin A (ROL and retinyl esters) has been set at 3000 µg retinol equivalents/day, based on the production of birth defects in embryos and fetuses (teratogenesis) as the limiting adverse effect (VKM, 2012). Furthermore, the UL (for ROL) is not changed with regard to increased risk of bone fracture in postmenopausal women, but a guidance level (GL) of 1500 µg retinol equivalents/day has been recommended (VKM, 2012).

Cosmetics:
Several studies indicate that RAL (and to a lesser extent ROL) exhibits cutaneous effects similar to RA, but with less skin irritation (Kang et al., 1995; Sass et al., 1996).

In an attempt to establish safety guidelines for RAL, we have used the UL and GL for ROL as reference levels to estimate maximum safe use levels also for RAL in humans. Because the activity of ROL is about 20 times less than RA (RAL) in some bioassays, a conversion factor of 20 was used to give corresponding ROL "equivalents"[15]. Using 0.1% RAL as an illustrative example and a skin penetration rate of 5.7% (also based on ROL), we calculated SED values for some relevant product categories: body lotion, facial cream, and hand cream. The calculated SED values were compared to the reference values based on UL for ROL (i.e. teratogenesis as the limiting adverse effect).

It should be noted that systemic adverse effects of topically applied RAL (or other retinoids) have yet not been demonstrated. Rather, the bioactivity of topical RAL (i.e. following conversion to RA) appears to be restricted to the skin (Sorg et al., 1999). Tissue-specific expression of enzymes catalysing conversion of RAL into RA is one possible mechanism that might limit the tissues that can metabolize ROL to RA to initiate retinoid signalling (Duester et al., 2003).

The utilization of topical retinoids is advantageous over systemic retinoids from a toxicological perspective, as systemic retinoids can be associated with various adverse effects such as teratogenesis (first trimester of pregnancy), hepatotoxicity, elevated triglycerides, depression, musculoskeletal symptoms, and altered night vision (Rolewski, 2003; cf. section 6 above). With topical

[15] "ROL equivalents" should not be confused with RE ("retinoid equivalents"), as it relates to the conversion factor of 20 (based on relative RA and ROL potencies in certain bioassays). As an approximation, we have set the potency of RAL equal to that of RA, as direct evidence is lacking.

retinoids, the possibility of toxicity and side effects are minimized while still contributing to localized effects in the skin.

Food and dietary supplements:
RAL is not present as such in food and dietary supplements, but β-carotene (in vegetable and fruit) can be cleaved symmetrically forming two molecules of RAL, which can then be either oxidized to RA or reduced to ROL. However, there is no evidence for vitamin A-related toxicity from pro-vitamin A carotenoids (VKM, 2012).

The tolerable Upper Intake Level (UL) for vitamin A has been set at 3000 µg/day for adults age 19 and older (including pregnancy and lactation)[16]. The UL is the highest level of intake that is likely to pose no risk of harmful effects.

Intake of preformed vitamin A has been estimated for different age groups in the Norwegian population (VKM, 2012). In 2010-2011, the estimated intake of preformed vitamin A (ROL) from food and dietary supplement in adult Norwegian women was 983 µg RE/day (1983 µg RE/day, 95 percentile).

Medicinal products:
RAL is present in OTC products (in the U.S.), but is not a registered medicinal product.

Total exposure:
When considering total exposure of retinoids, there is no evidence for vitamin A-related toxicity from carotenoids in food (β-carotene is converted into 2 molecules RAL). Moreover, RAL is not used as a medicinal product.

The biological meaningful contribution to biological effects of RAL therefore seems to be mainly related to the amount of RAL converted into RA locally in the skin (i.e. derived from cosmetic products).

[16] UL for infants and children from birth to 3 years, 600 mcg/day (2000 units); children 4 to 8 years, 900 mcg/day (3000 units); 9 to 13 years, 1700 mcg/day (6000 units); 14 to 18 years (including pregnancy and lactation), 2800 mcg/day (9000 units); adults age 19 and older (including pregnancy and lactation), 3000 mcg/day (10,000 units). http://www.nlm.nih.gov/medlineplus/druginfo/natural/964.html

8. Conclusion

Retinoids are irritating to the skin in sensitive individuals, but the effect is dose dependent. In addition to topical tolerance data, a crucial point in the safety assessment of topical retinoids (including RAL) is whether or not systemic circulating levels are influenced by topical RAL treatment. Presently there is no such data available. Thus, for a more detailed safety assessment, more research on the use of RAL in cosmetics is needed.

In an attempt to establish safety guidelines for RAL, we have used UL (3000 µg/day for ROL) as the highest level of intake that is likely to pose no risk of harmful effects (because a NOAEL value for RAL is not available). Assuming that ROL is 20 times less active than RAL (with reference to the retinoid activity mediated by nuclear retinoid receptors), the systemic exposure dose (SED) was calculated using a cream containing 0.1% RAL as an illustrative example.

The table lists the estimated SED and maximum usage limits for RAL (%), in relation to percentages of UL (teratogenesis) and GL (osteoporosis) (based on human data):

Source	Conc. RAL	Systemic exposure do	Systemic exposure do	Max allowed	Max allowed	Comments
	in the product (%)	(SED) - RAL (µg)	(SED) - ROL eq*	conc. RAL (% UL)	conc. RAL (% GL)	
Food + suppl						
Hand cream	0.1	112	2240	0,1	0,07	75% of UL for 0.1% RAL
Facial cream	0.1	83	1651	0,1	0,09	55% of UL for 0.1% RAL
Body lotion	0.1	420	8400	0,04	0,02	
Total						
UL			3000			

In summary:
Hand cream: 0.1%
Facial cream: 0.1%
Body lotion: 0.04%
Overall exposure from cosmetics: 3000 /12271 = 0.25 x 0.1% = 0.025%

An earlier preliminary risk assessment report by the Council of Europe stated that: “Tolerance tests indicate that *concentrations of RAL at 0.05% are in compliance with requirements that normal use shall not involve any irritation incidents at all”* (Council of Europe, 2008).

Thus, we propose a maximum limit of:
0.05% RAL in hand care and facial cream products
0.025% RAL in body lotion products

Remarks:
Pregnant and breastfeeding women should not use cosmetic products with retinoids, including RAL, as enough concerns exist regarding their safety during the early period of human pregnancy.

Although research suggests that topical retinoids do not pose the same risk as oral retinoids for birth defects, there is not enough information to establish a safe threshold of retinoid consumption during the early (first trimester) of human pregnancy (Azais-Braesco & Pascal, 2000). A precautionary label for these conditions should be displayed on the package /ingredient list.

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10. Annexes

Annex 1A. Structure of retinoids

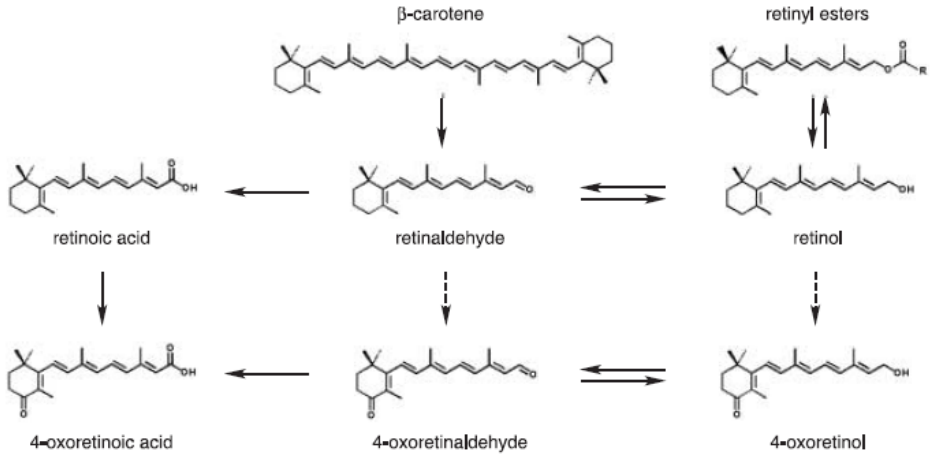

FIG. 1. Structure of natural retinoids. The arrows show the enzyme-catalyzed conversions. The dotted-lined arrows are probable conversions, although they have not been confirmed.

Annex 1B. Natural and synthetic vitamin A variants

Table 1. Natural and Synthetic Vitamin A Derivatives

Natural	Synthetic
Retinol	*Tazarotene*
Retinyl-palmitate	*Adapalene*
Retinyl-acetate	
Retinaldehyde	
Tretinoin	
Isotretinoin	
Alitretinoin	

Prescription-only preparations (which are not addressed in this article) have been italicized.

http://aes.sagepub.com/content/30/1/74.abstract

Annex 2: Topical retinoids and anti-aging activity

Table 1. Topical retinoids: effects relevant for anti-aging for activity

Epidermal cells renewal
Epidermal differentiation modulation
Extracellular matrix production
Inhibition of UV-induced extracellular matrix degradation
Cytokine modulation – angiogenesis
Melanocyte function modulation
Oxidant/antioxidant
Prevention of UV-induced vitamin A deficiency
Sunscreen effect
So-called surface effects

Annex 4: Retinol units or equivalents

Vitamins

Vitamin A includes preformed vitamin A (retinol) and provitamin A carotenoids expressed as beta-carotene activity. International Units (IU) or Retinol Equivalents (RE) have been traditionally used to describe total vitamin A activity. A more recent definition, Retinol Activity Equivalents (RAE), reduces by half the vitamin A activity of the carotenoids.

Vitamin A values are analyzed or are calculated from the following:

RE Vitamin A = mcg retinol + (mcg beta-carotene equivalents/6)
IU Vitamin A = (mcg retinol/0.3) + (mcg beta-carotene equivalents/0.6)
RAE Vitamin A = mcg retinol + (mcg beta-carotene equivalents/12)

Beta carotene equivalents include vitamin A activity from the provitamin A carotenoids: beta-carotene, alpha-carotene, and beta-cryptoxanthin. Beta-carotene equivalents are calculated from the following:

mcg beta-carotene equivalents = mcg beta-carotene + ½(mcg alpha-carotene + mcg beta-cryptoxanthin)

Values are expressed in micrograms. 6 mcg beta-carotene equivalents provide 0.5 RAE vitamin A, 1 RE vitamin A, or 10 I.U. vitamin A.

Retinol is preformed vitamin A found only in animal products. Values are given in micrograms. 1 mcg retinol provides 1 RE (or RAE) vitamin A or 3.33 I.U. vitamin A.

http://www.ncc.umn.edu/products/databaseNUTvitamins.html

Annex 5: LOEL adverse effects

Table 3: 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.

Table 4: 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

(Annex 6: Retinoids - Mechanisms of action and biological effects)

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Retinoids exert many of their biological effects by binding and activating nuclear receptors, thereby modulating expression of genes involved in cellular differentiation and proliferation. Retinoids are used in cosmetic products due to their effectiveness at regulating epithelial cell growth and differentiation (Babamira & Nassab, 2010).

Because RAL (or ROL/RE) does not bind (directly) to retinoid nuclear receptors (RAR, RXR), its biological activity should result from enzymatic conversion into ligands for these receptors (e.g. RA), a so called “intracrine” process. The ranking order of retinoid-like activity following topical application is as follows: RA > RAL > ROL >> RE (Sorg et al., 2006). Topical application of RAL has been shown to result in measurable biological activity in the skin, including proliferation, differentiation, and modulation of metallo-proteinases and inhibitors (Didierjean et al., 1999). Further, it has been shown that topical RAL is converted *in vivo* into all-*trans* RA (*at*-RA) by mouse epidermis. Although the concentrations of RA resulting from conversion of RAL in the skin were much lower compared to topical application of *at*-RA itself, the induced biological effects were similar. Thus, topical RAL loads epidermal cells with *low amounts of ligands* that are *as biologically significant* as those resulting from *much higher tissue loading* of *at*-RA. This selectively delivers low concentrations of RA (converted from RAL), which prevents an excess of RA in the skin, and might be a strategy to overcome the problems related to topical RA.(Didierjean et al., 1996).

Topical retinoids display several other properties relevant to improve aging- or photodamaged skin (Sorg et al., 2006; see also Table 1 in Annex 3). Some of these effects may be independent of nuclear receptors; i.e. non-genomic actions, such as those linked to UV adsorption, anti-oxidant and anti-pigment actions.

RAL is bifunctional: in addition to activities shared with other retinoids (mediated by nuclear receptor activation), RAL also exhibits antibacterial properties (i.e. gram-positive) related to the presence of aldehyde in the side-chain; i.e. not shared with RA and ROL

RAL - a carotenoid constituent of visual pigments. It is the oxidized form of retinol which functions as the active component of the visual cycle. It is bound to the protein opsin forming the complex rhodopsin. When stimulated by visible light, the retinal component of the rhodopsin complex undergoes isomerization at the 11-position of the double bond to the cis-form; this is reversed in "dark" reactions to return to the native trans-configuration.