SCCS/1313/10
24 March 2010

EUROPEAN COMMISSION

Directorate-General for Health & Consumers

Scientific Committee on Consumer Safety

SCCS

OPINION ON

Vitamin K1 (Phytonadione)

Scientific Committees
on consumer safety
on emerging and newly identified health risks
on health and environmental risks

The SCCS adopted this opinion at 6th plenary meeting of 23 March 2010

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Evaluation Agency (EMEA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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1. BACKGROUND

Due to a notification procedure by a member state on vitamin K1 triggered by cases of allergic reactions, a scientific evaluation of its use in cosmetic products was requested. The first scientific opinion on vitamin K1 (SCCP/1105/07) was adopted by the SCCP by written procedure on 28 September 2007 with the following conclusion:
"*Because of the inadequate nature of the dossier submitted, the SCCP is unable to provide an adequate safety evaluation for the use of vitamin K1 (phytonadione) and its "oxide" in cosmetic products. However, as such use may cause cutaneous allergy, individuals so affected may be denied an important therapeutic agent."*

A complete dossier was submitted by November 2007. The applicant applied for the use of vitamin K1 - with the CAS 81818-54-4 and EINECS 279-833-9 and the chemical name 2-methyl-3-(3,7,11,15-tetramethylhexadec-2-enyl)-1,4-naphthoquinone - in cosmetic products in a concentration up to a maximum of 1.0%.

A second opinion (SCCP/ 1187/08) was adopted by the SCCP at the 24 June 2008 with the conclusion: "*The studies provided on the allergenic potential of Vitamin K1 did not supersede the concerns stated in opinion SCCP/1105/07. The SCCP maintains the view that use of Vitamin K1 in cosmetic products is not safe, since it may cause cutaneous allergy and individuals so affected may be denied an important therapeutic agent."*

Following this opinion, additional, newly generated data on skin sensitisation was submitted, which has not been evaluated by the SCCS (formerly SCCP) together with some comments of stakeholders.

2. TERMS OF REFERENCE

1. *Does the SCCS consider that the new scientific data submitted supersedes the concern about the allergenic potential of vitamin K1 when used in cosmetic products in a concentration up to 1.0%?*

2. *If yes, does the SCCS consider that vitamin K1 is safe when used in cosmetic product in a concentration up to 1.0%?*

3. OPINION

The data described in this opinion have been taken from three submissions. Submission I, which formed the basis of opinion SCCP/1105/07, did not provide adequate information on the chemical identity of the substance used and did not allow for a safety assessment of the substance. Nevertheless, the data contained in the dossier is included in this opinion for completeness. Submission II and III were submitted by a different applicant.

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

According to the European Pharmacopoeia, Phytomenadione is a mixture of

2-methyl-3-[(2*E*)-(7*R*,11*R*)-3,7,11,15-tetramethylhexadec-2-enyl]naphthalene-1,4-dione (*trans*-phytomenadione),
2-methyl-3-[(2*Z*)-(7*R*,11*R*)-3,7,11,15-tetramethylhexadec-2-enyl]naphthalene-1,4-dione (*cis*-phytomenadione), and
2,3-epoxy-2-methyl-3-[(2*E*)-(7*R*,11*R*)-3,7,11,15-tetramethylhexadec-2-enyl]-2,3-dihydronaphthalene-1,4-dione (*trans*-epoxyphytomenadione).

It contains not more than 4.0 per cent of *trans*-epoxyphytomenadione and not less than 75.0 per cent of *trans*-phytomenadione. The total of the three components is not less than 97.0 per cent and not more than the equivalent of 103.0 per cent.

3.1.1.1. Primary name and/or INCI name

Phytonadione (INCI name)
Phytomenadione (INN)

3.1.1.2. Chemical names

1,4-naphthalenedione, 2-methyl-3-(3,7,11,15-tetramethyl-2-hexadecenyl)-, (r-(r*,r*-(e)))
2',3'-trans-vitamin K1
2-methyl-3-[(7RS,11RS)-3,7,11,15-tetramethyl-2-hexadecenyl]-1,4-naphtoquinone;
2-methyl-3-[(7RS,11RS)-3,7,11,15-tetramethyl-2-hexadecenyl]-naphtalene-1,4-dione;
2',3'-trans-phylloquinone
α-phylloquinone
2-methyl-3-phytyl-1,4-naphthoquinone
phytylmenadione
3-phytylmenadione
antihemorrhagic vitamin

3.1.1.3. Trade names and abbreviations

Aquamephyton	Konakion
Combinal k1	Mephyton
K-ject	Mono-kay
Kativ n	Monodion
Kephton	Synthex p
Kinadion	

-Vitamin K1

- Phytomenadione (all-rac)
- Phytonadione (all-rac)
- α-Phylloquinone (all-rac)
- Vitamin K (all-rac)

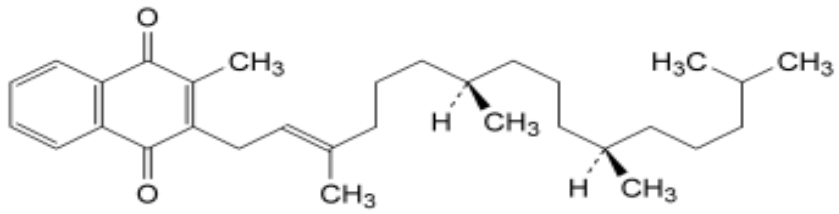
3.1.1.4. CAS / EC number

CAS	EC *	
84-80-0	201-564-2	(phytomenadione)
11104-38-4	234-330-3	(vitamin K1)
81818-54-4	279-833-9	(2-methyl-3-(3,7,11,15-tetramethylhexadec-2-enyl)-1,4-naphthoquinone)

* The names in brackets refer only to the EC numbers

3.1.1.5. Structural formula

The Vitamin K1 molecule has two geometrical isomers (cis-trans or *(Z)-(E)* isomers) plus two asymmetric carbon atoms (C7 and C11), each generating two enantiomers (R or S). Thus, there are eight diastereoisomers (four in the *trans-* and four in the *cis-* configuration). The name Vitamin K is appropriate only for the 2'-Trans-7R, 11R-stereoisomer (the others are not vitamins). The formula indicates the 2'-Trans-7R, 11R-stereoisomer.



3.1.1.6. Empirical formula

Empirical formula: $C_{31}H_{46}O_2$

3.1.2. Physical form

Clear yellow to golden yellow viscous liquid

3.1.3. Molecular weight

Molecular weight: 450.68 g/mol

3.1.4. Purity, composition and substance codes

Submission II/III states that the applicant's
Vitamin K1 preparation meets all requirements of the USP, FCC and Ph. Eur. when tested according to these compendia:
• Purity (HPLC): vitamin K1 content 97.0 - 103.0 %, trans- vitamin K1 min. 75 %, cis-vitamin K1 max. 15.0 %, vitamin K1 epoxyde max. 1.0 %
• Appearance: intense yellow, viscous oil.
• Solution 10% in trimethylpentane: clear

- Refractive index (589 nm, 25 °C): 1.523–1.526
- Reaction: passes test (USP)
- Heavy metals: max. 20 ppm
- Lead: max. 2 ppm
- Sulphated ash (residue on ignition): max. 0.1%
- Acid value: max. 2.0
- Menadione: max. 0.2%
- Other related substances: corresponds (Ph. Eur.)

The applicant's Vitamin K1 preparation is declared to be CEP certified.

Comment
No documentation for the characterisation of batches according to the above mentioned specifications has been provided.

3.1.5. Impurities / accompanying contaminants

According to the Merck Index (1989), commercial preparations may contain up to 20% of the biological inactive *cis* isomer.

Ref.: 1

Phylloquinone occurs in nature only as the 2', 3'-Trans-7R, 11R-stereoisomer. Commercially available phytonadione (phylloquinone) is prepared synthetically and may contain not only 2',3'-trans-phylloquinone (not less than 75%), but also 2',3'-cis-phylloquinone and trans-epoxyphylloquinone (not more than 4.0 percent).

Ref.: 2

In submission I, three HPLC chromatograms were provided without any further explanation or identification of the peaks. After 5 minutes and one hour irradiation with UV, several new unidentified peaks appeared on the chromatograms, which were also not described. No information on the nature of UV exposure of the test substance was given.

According to Eur Ph, Menadione is the principle impurity. No information on its actual content in commercial batches was provided in the dossier.

3.1.6. Solubility

500 mg/l	water (22 °C)
500 mg/l	hydrochloric acid 0.1 N (22 °C)
500 mg/l	sodium hydroxide 0.1 N (22 °C))
500 mg/l	glycerine (22 °C)
4500 mg/l	DMSO (dimethyl sulfoxide) (22 °C)
11000 mg/l	methanol (22 °C)
15000 mg/l	acetonitrile (22 °C)
75000 mg/l	ethanol (22 °C)
1 g/l	n-octanol, diethyl ether, acetone, benzene, ethyl acetate, dichloromethane, chloroform, n-hexane, cydohexane, dioxane (22 °C)

Ref.: 3

Vitamin K1 is insoluble in water, slightly soluble in ethanol, and freely soluble in ether, chloroform, fats and oils.

Comment
No study is provided, quantitative data on the solubility is not provided.

3.1.7. Partition coefficient (Log P_{ow})

No data submitted

3.1.8. Additional physical and chemical specifications

Melting point: approx. - 20 °C
Boiling point: 140-145 °C
Density: 0.97 g/cm³

3.1.9. Stability

Stable to air and moisture, but decomposes in sunlight. Unaffected by dilute acids, but is destroyed by solutions of alkali hydroxides and by reducing agents.

Ref.: 1

The stability of phytomenadione stored in aluminium cans under nitrogen was investigated under the following conditions:
For long term storage condition studies samples of commercial lots are stored at 25°C + 2°C at 60% RH + 5% RH for 36 months.
For accelerated storage condition studies samples of commercial lots were stored at 40°C + 2°C at 75% RH + 5% RH for 6 months.

Under these storage conditions samples of phytomenadion are described to be stable: *trans*-phytomenadione (84.4-86.4%, epoxy phytomenadione 0.15, total *cis*-, t*rans*- and epoxy phytomenadione (97.8-100.3%)

Vitamin K1 is slowly degraded by atmospheric oxygen, but is fairly rapidly degraded by light. It is relatively stable to heat, but is decomposed by alkalis. Due to these cognitions and the results of the stability test programs the following is recommended:
The product may be stored for 36 months from the date of manufacture in the unopened original container (which is sealed under inert gas) and at a temperature below 15°C.

Ref.: 24

Comment
Above mentioned data is presented in a summary document, but no documentation has been provided for this.
No information is available on the fate of vitamin K1 in cosmetic products under normal use conditions.

General Comments on physico-chemical characterisation

- The data provided on the physico-chemical characterisation of phytonadione is insufficient. Documentation of characterisation, determination of composition, purity and impurities is missing.
- Log Pow and quantitative data on the solubility are not provided.
- Vitamin K1 is slowly degraded by atmospheric oxygen and fairly rapidly degraded by light. No information on stability of phytomenadione in typical cosmetic formulations and under use conditions is available.

- According to the applicant of submission I, since 2005 "oxidised" Vitamin K1 has been used in products. It is not clear from the submission to which chemical substance the term 'phytonadione-oxide' refers to and no data was provided on this substance.

3.2 Function and use

Phytonadione (phylloquinone) is present in plant sources, especially green leafy vegetables. It is also present in small amounts in dairy products. It is synthesised by bacterial flora in the jejunum and ileum. The amount synthesised in the gut contributes significantly towards the daily requirement of the vitamin.

Vitamin K is involved in blood clotting, bone and kidney metabolism. Roles in cell signalling and brain lipid metabolism have also been proposed. Because vitamin K is widespread in the diet and provided by bacteria, deficiency is generally secondary to conditions such as mal-absorption. Newborn babies have low levels of vitamin K, which may result in haemorrhagic disease of the newborn.

There may be decreased utilisation of the vitamin in the production of the vitamin K-dependent clotting factors during any form of acute or chronic liver disease. This is as a result of the destruction of the rough endoplasmic reticulum in the hepatocyte. Patients with hypoprothrombinaemia related to hepatic disorders usually respond to daily parenteral doses of 10 mg of vitamin K for three days. If no response to this treatment is noted this suggests serious hepatocellular damage (Basu and Dickerson, 1996).

Ref.: 4

The physiological activity of phylloquinone is based on its ability to change between its oxidized (quinone and 2,3-epoxide) and reduced (hydroquinone) forms. The major role of phylloquinone is the post-translational addition of a carboxyl-group into the γ-position of glutamate residues of specific proteins. In this respect, the prime physiological relevance of phylloquinone is the synthesis of coagulation proteins (Ferland, 1998; Olson, 1999 and 2000).

Whereas the vitamin K-dependent coagulation proteins are all synthesised in the liver, vitamin K is also essential for the synthesis of a number of proteins produced in extra-hepatic tissues. Examples of the latter group of proteins include:

- the bone Gla-protein, osteocalcin, which is exclusively synthesised by osteoblasts and odontoblasts, and which is a negative regulator of bone formation;
- matrix Gla-protein (MGP), which is synthesised in most soft tissues, but predominantly in cartilage (by chondrocytes) and in vessel wall (by vascular smooth muscle cells) and which is a potent inhibitor of soft tissue calcification;
- growth arrest-specific gene 6 protein (Gas6), which is a ligand for tyrosine kinases and has strong apoptopic activity in cultured cells.

Ref.: 5

Medical uses

Vitamin K1 is indicated in a number of coagulation disorders caused by vitamin K deficiency or interference with vitamin K activity, such as:

- anticoagulant-induced prothrombin deficiency caused by coumarin or indanedione derivatives;
- prophylaxis and therapy of haemorrhagic disease of the newborn;
- hypoprothrombinaemia due to antibacterial therapy;

- hypoprothrombinaemia secondary to factors limiting absorption or synthesis of vitamin K, e.g., obstructive jaundice, biliary fistula, sprue, ulcerative colitis, coeliac disease, intestinal resection, cystic fibrosis and regional enteritis;
- other drug-induced hypoprothrombinaemia where it is definitely shown that the result is due to interference with vitamin K metabolism, e.g., salicylates.

Ref.: 51

In the USA, medicinal products containing Vitamin K1 carry the following warning:

Severe reactions, including fatalities, have occurred during and immediately after INTRAVENOUS injection of phytonadione, even when precautions have been taken to dilute the phytonadione and to avoid rapid infusion. Severe reactions, including fatalities, have also been reported following INTRAMUSCULAR administration. Typically these severe reactions have resembled hypersensitivity or anaphylaxis, including shock and cardiac and/or respiratory arrest. Some patients have exhibited these severe reactions on receiving phytonadione for the first time. Therefore the INTRAVENOUS and INTRAMUSCULAR routes should be restricted to those situations where the subcutaneous route is not feasible and the serious risk involved is considered justified

Ref.: 52

In 2006, the British Committee for Standards in Haematology Guidelines on oral anticoagulation recommended that prothrombin complex concentrates (PCCs), in conjunction with a minimum intravenous (i.v.) dose of vitamin K, 5 mg should use to reverse life-threatening bleeding in patients on warfarin therapy.

Ref.: 53

The US guidelines for warfarin reversal prescribe the injection of Vitamin K (10 mg i.v.) in the case of serious or life-threatening bleeding.

Ref.: 54

Cosmetic uses

Claimed uses for Vitamin K1 in cosmetic products include moisturising, skin lightening, periorbital hyper pigmentation, application on actinic and traumatic purpura and bruising after laser therapy.

The SCCP is aware of cosmetic products on the market with Vitamin K1 concentrations as high as 8%.

The company that provided submission I declared that: "*…no product […] contains Vitamin K since 1 January 2005*". It also stated that from this date, an oxidised form of Vitamin K1 was used instead. No data was provided about the formula of oxidised Vitamin K1.

Ref.: 6

Vitamin K1 was banned from use in cosmetics by Directive 2009/6/EC in Annex II, entry 1371 Phytonadione [INCI], phytomenadione [INN] CAS No 84-80-0 / 81818-54-4 and EC no 201-564-2.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

Vitamin K has been tested for acute toxicity in mouse and rat by different routes of exposure.

Table 1: Summary of Acute Toxicity Results

Species	Route	LD50 (mg/kg bw)	Study ref.	Ref.
Mouse	Oral	> 20000	B-90447	25
Mouse	Oral	> 12000	B-90447	25
Mouse	Intravenous	> 100 mg/kg corresponding to 50 mL/kg (Konakion solution 2 mg K1/mL) Higher injection volumes not feasible)	B-1024418	26
rat	oral	> 4000	B-90447	25

3.3.1.1. Acute oral toxicity

Vitamin K1 has a low acute oral toxicity (see table 1)

The oral toxicity of Vitamin K1 was determined using Konakion solution 2 mg K1/ml (vehicle unknown)

3.3.1.2. Acute dermal toxicity

No data submitted

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.1.4. Acute intravenous toxicity

Intravenous toxicity of vitamin K1 has been tested in the mouse with a Konakion solution containing 10 mg/mL of vitamin K1. The test item was a solution of vitamin K1 for intravenous application containing 80 mM Epikuron 200 (Lecithin) and 80 mM Na-cholate. The LD50 of this solution was determined as 24.3 mL/kg. Calculated for vitamin K1 this corresponds to 243 mg vitamin K1/ kg bw.

Ref.: 26

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

No data submitted

3.3.2.2. Mucous membrane irritation

Human Studies

Guideline: /
Species: Human
Group: 31 females
Substance: Bionic Eye Cream
Batch: L#111-154
Purity: /

Concentrations: 'as is'
Dose: normal application
Vehicle: /
GCP: in compliance
Study period: 2006

31 healthy female subjects applied the test eye cream twice daily to the under eye area for four weeks. Eyelids, conjunctivae and cornea and lacrimation were examined by an ophthalmologist at the start of the study, after two and after four weeks.

By 2 weeks 3 subjects showed minor palpebral and/or conjunctival irritation and by 4 weeks 1 subject showed minor palpebral and/or conjunctival irritation

Ref.: 39

Comment
In the original report it is not stated that the cream contained vitamin K. However, as the name and batch number are identical to the HRIPT study (Ref.: 38) it can be taken that it contained 1% vitamin K.

3.3.3. Skin sensitisation

Murine Local Lymph Node Assay (LLNA) for Vitamin K

Guideline: OECD 429
Species/strain: CBA/J mouse, nulliparous and non-pregnant females.
Group size: 7 groups of 4 animals
Test substance: vitamin K
Batch: 20040510
Purity: 98.96% (full isomeric composition not clarified, % active ingredient unknown)
Concentrations: 5, 10, 25, 50, 100% in acetone/olive oil (4/1; v/v)
Positive control: α-hexylcinnamaldehyde (HCA)
GLP: in compliance

Results
Vitamin K1 was non-irritant in the preliminary test. The highest concentration retained for the main test was the maximal practicable concentration (100%).

In the main test, twenty-eight female CBA/J mice were allocated to seven groups: five treated groups of four animals receiving the test item Vitamin K (isomeric mixture) at the concentration of 5, 10, 25, 50 or 100%, one negative control group of four animals receiving the vehicle (mixture acetone/olive oil (4/1; v/v)), one positive control group of four animals receiving alpha-hexylcinnamaldehyde (HCA) at the concentration of 25%.

Proliferation assay
No lymphoproliferation was noted at any of the test concentrations, while significant lymphoproliferation was observed with HCA at 25%.

The results are presented in the following table:

Table 2: LLNA results Vitamin K

Treatment	Concentration (%)	Irritation level	Stimulation Index (SI)
Test item	5	non-irritant	1.78

Treatment	Concentration (%)	Irritation level	Stimulation Index (SI)
Test item	10	non-irritant	2.10
Test item	25	non-irritant	0.80
Test item	50	non-irritant	0.88
Test item	100	non-irritant	1.67
HCA	25	-	11.18

Conclusion
Under these experimental conditions, Vitamin K1 (isomeric mixture) did not induce delayed contact hypersensitivity in the murine Local Lymph Node Assay.

Ref.: 7

Murine Local Lymph Node Assay (LLNA) for Vitamin K hv (irradiated Vitamin K1)

Guideline: OECD 429
Species/strain: CBA/J mouse, nulliparous and non-pregnant females.
Group size: 7 groups of 4 animals
Test substance: vitamin K(hv)
Batch: 20040510
Purity: 98.96% (full isomeric composition not clarified, % active ingredient unknown)
Concentrations: 5, 10, 25, 50, 100% in acetone/olive oil (4/1; v/v)
Positive control: α-hexylcinnamaldehyde (HCA)
GLP: in compliance

Results
Vitamin K1 was non-irritant in the preliminary test. The highest concentration retained for the main test was the maximal practicable concentration (100%).

In the main test, twenty-eight female CBA/J mice were allocated to seven groups: five treated groups of four animals receiving the test item Vitamin K at the concentration of 5, 10, 25, 50 or 100%, one negative control group of four animals receiving the vehicle (mixture acetone/olive oil (4/1; v/v)), one positive control group of four animals receiving α α-hexylcinnamaldehyde (HCA), at the concentration of 25%.

Proliferation assay
No lymphoproliferation was noted at any of the test concentrations, while significant lymphoproliferation was observed with HCA at 25%.

The results are presented in table 3.

Table 3: LLNA results Vitamin K hv

Treatment	Concentration (%)	Irritation level	Stimulation Index (SI)
Test item	5	non-irritant	1.02
Test item	10	non-irritant	0.64
Test item	25	non-irritant	0.80
Test item	50	non-irritant	1.10

Treatment	Concentration (%)	Irritation level	Stimulation Index (SI)
Test item	100	non-irritant	1.10
HCA	25	-	8.54

Conclusion
Under these experimental conditions, the test item Vitamin K hv did not induce delayed contact hypersensitivity in the murine Local Lymph Node Assay.

Ref.: 8

Comment
The batch and the analytical certificate provided was for the non-irradiated form of Vitamin K (isomeric mixture). No data about the isomeric composition of the test substance in both tests are provided. Therefore, no conclusion can be made regarding the sensitising potential in the LLNA of vitamin K1, either irradiated or non-irradiated before use.

Local Lymph Node Assay (LLNA)

Guideline: OECD 429
Species: Mice, CBA/CaOlaHsd
Group: 20 females (4 per group; nulliparous and non-pregnant)
Substance: Vitamin K1
Batch: S107090011
Purity: 101.2% (LC)
Concentrations: 1, 10, 50 and 100% w/v
Dose: 25 µl
Vehicle: acetone:olive oil (4:1 v/v)
Control: α-hexylcinnamaldehyde (July 2008)
GLP: in compliance
Study period: 26 August – 09 September 2008

Each test group of mice was treated by topical application to the dorsal surface of each ear lobe (left and right) with different test item concentrations of 1, 10, 50, and 100% (w/v) in acetone:olive oil (4:1). The application volume, 25 µl, was spread over the entire dorsal surface of each ear lobe once daily for three consecutive days. A further group of mice was treated with an equivalent volume of the relevant vehicle alone (control animals).

Five days after the first topical application, all mice were administered with 250 µl of 79.6 µCi/ml ^{3}H-methyl thymidine (corresponds to 19.9 µCi ^{3}H-methyl thymidine per mouse) by intravenous injection via a tail vein.

Approximately five hours after treatment with ^{3}H-methyl thymidine all mice were killed. The draining lymph nodes were rapidly excised and pooled per group (8 nodes per group). Single cell suspensions (in phosphate buffered saline) of pooled lymph node cells were prepared by gentle mechanical disaggregation through stainless steel gauze (200 µm mesh size). After washing two times with phosphate buffered saline (approx. 10 ml) the lymph node cells were resuspended in 5% trichloroacetic acid (approx. 3 ml) and incubated at approximately +4 °C for at least 18 hours for precipitation of macromolecules.
The precipitates were then resuspended in 5% trichloroacetic acid (1 ml) and transferred to plastic scintillation vials with 10 ml of 'Ultima Gold' scintillation liquid and thoroughly mixed. The level of ^{3}H-methyl thymidine incorporation was then measured on a β-scintillation counter. Similarly, background ^{3}H-methyl thymidine levels were also measured in two 1ml-aliquots of 5 % trichloroacetic acid. The β -scintillation counter expresses ^{3}H-methyl thymidine incorporation as the number of radioactive disintegrations per minute (DPM).

Table 4: LLNA results Vitamin K1

Concentration	Stimulation Index
Test item	
1%	1.59
10%	1.24
50%	1.82
100%	4.03
α-Hexylcinnamaldehyde	
5%	5.24
10%	7.38
25%	9.32

A test item is regarded as a sensitizer in the LLNA if the exposure to one or more test concentration resulted in 3-fold or greater increase in incorporation of ^{3}H-methyl thymidine compared with concurrent controls, as indicated by the Stimulation Index (S.I.). The estimated concentration of test item required to produce a S.I. of 3 is referred to as the EC3 value. The EC3 value calculated was 76.7 %.

Ref.: 44

Comment
According to the potency categorisation given in SCCP memorandum of 20 September 2005 (SCCP/0919/05) vitamin K1 would be categorised as a moderate sensitizer. Other schemes for categorisation exist and that proposed by Kimber at al. (Ref: 56) would class it as a weak sensitizer.

Vitamin K1 allergic reaction after intravenous (i.v) and intradermal (i.d) route.

The two following studies do not concern skin sensitisation from topical exposure:

One study compared sensitisation potential in Guinea pigs of two **Konakion** formulations (10 mg vitamin K1/mL mixed micelles formulation and 10.5 mg vitamin K1/mL commercial cremophor EL containing formulation) given for induction either i.v. or i.d.. The respective control groups received NaCl solution. There were 8 animals per group.

- Induction by the i.v. route consisted of total 4 injections of 4 or 2 mL/kg of undiluted test material once a week for 4 weeks.
- For induction by the i.d. route animals received totally 10 intradermal inductions of 0.1 mL of undiluted test material 3 times a week.

Two challenges were performed all by i.d. route with 2 suitable dilutions: 1:100 and 1:300 for the mixed micelles formulation and 1:30 and 1:100 for the commercial cremophor EL formulation. The first challenge was given 2 weeks after the last induction and the second after a further two weeks. Elicitation was performed 2 weeks after the second challenge by i.v. administration at the same dose as used in the i.v. induction phase.

With the mixed micelles formulation no reaction was observed independent of whether induction was performed by the i.v. or the i.d. route. However with the commercial formulation containing cremophor EL (PEG-35 Castor Oil) there was a positive challenge test after i.d. induction but not after i.v. induction. Positive i.v. elicitation reactions were observed after both i.d. and i.v. induction with the cremophor EL formulation.

Ref.: 27

The anaphylactogenic potential of Konakion Ro 01-6772/120 (mixed micelles formulation containing 10 mg vitamin K1/mL) was investigated in female guinea pigs with induction either by i.v. or i.d. application. Induction by the i.v. route consisted of total 4 injections of 3 mL/kg of undiluted test material once a week for four weeks. For induction by the i.d. route animals received totally 10 intradermal inductions of 0.1 mL of undiluted test material over a period of 22 days. Control groups received physiological saline. Two challenges were performed all by i.d. route with a 1:10 dilution of the test material. The first challenge was given 2 weeks after the last induction treatment and the second after further two weeks. Elicitation was performed 2 weeks after the second challenge by i.v. application at the same dose as used in the i.v. induction phase (3 mL/kg).

In the test group that received intravenous induction one animal showed clinical signs indicative of anaphylaxis at elicitation. No responses were noted in the control group.

In the test group which received intradermal induction no effect was noted upon challenge, whereas at elicitation 2 animals showed minimal clinical signs indicative of anaphylaxis at elicitation. No responses were noted in the control group.

Ref.: 28

3.3.4. Dermal / percutaneous absorption

Dermal penetration of ^{3}H-labeled vitamin K1 was determined *in vitro* using excised rat and pig skin.

Incubations were performed for 1, 6 and 16 hours. Unabsorbed test material was removed from the skin. Amount of test material in the stratum corneum, in the stripped skin and in the chamber liquid was determined.

The total skin penetration rate of vitamin K1 is time- as well as species-dependent and reached 101.45 µg/cm² on naked rat skin after an exposure time of 16 hours while 20.31,µg/cm2 were measured on pig skin. Taking into account also the amount in the stratum corneum, the amount passing into and partly through the skin after 16 hours was 56.4% for rat skin and 11.2% for pig skin. Whereas pig skin represents closer the penetration in human skin, rat skin produces an overestimation of dermal absorption (SCCP/0970/06).

Ref.: 29

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity

4 week study, see also section 3.3.9. toxicokinetics

Toxicity of Konakion MM (Ro 01-6722/131) after oral administration was investigated in Beagle pups. Approximately 2-week old male and female Beagle dogs (6 pups/group) were treated orally (by gavage) with 2 mg/pup, 20 mg/pup and 100 mg/pup for 4 weeks (2 administrations/week). This corresponds to a dose of 0, 1.8, 18 and 106 mg/kg bw in the first week and 0, 1.3, 13 and 77 mg/kg bw in the last week of dosing. Extensive clinical, laboratory (haematological, clinical chemistry, urine analyses) and pathology examinations (necropsy, organ weights, histopathology) were performed and said to be described in details in Research Report No. 161'220, which was not included in the current submission. It was stated that repeated oral doses of Konakion MM were tolerated without noticeable toxicity and no biologically relevant findings were observed in the pups treated with doses up to 50 times the prophylactic dose.

Ref.: 34

Comment
The study report of the toxicological examination was not provided for evaluation

No adverse effects were recorded when daily oral doses of up to 2000 mg phylloquinone/kg body weight were administered to rats for 30 days

Ref.: Molitor and Robinson, 1940, cited in Ref. 5

3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity

No data submitted

3.3.5.3. Chronic (> 12 months) toxicity in mice

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

Bacterial Reverse Mutation Test

In order to determine the mutagenic potential of vitamin K1 (batch 01092 56), the standard and preincubation versions of the Ames test was used. Deviating from actual OECD 471 recommendations only 4 concentrations were tested. The study was GLP compliant. Ro 01-6722 (vitamin K1) was tested for mutagenic activity in the standard Salmonella/mammalian microsome plate incorporation assay (Ames test). No mutagenic effect of Ro 01-6722 could be detected at concentrations of 4250, 425, 42.5 and 4.25 µg/plate, neither in presence nor in absence of a Phenobarbital-induced rat liver homogenate fraction (S9-mix).
A positive control with cyclophosphamide shows the validity of the test procedure and the activity of the S-9 mix. At concentrations of 200 µg cyclophosphamide/plate, the number of TA 1535 revertant colonies is higher than the control values without cyclophosphamide or without S-9 mix activation.
The results obtained in the Ames test show that neither Ro 01-6722 (formulated vitamin K1) per se, nor one of its metabolites being formed under the described in vitro conditions, induce DNA damage.

Ref.: 30

In order to determine the mutagenic potential of vitamin K1 (batch 301382, purity: 95.6% cis+trans vitamin K1), the standard and preincubation versions of the Ames test following the OECD 471 guidelines were used. The study was GLP compliant.
Vitamin K1 (Ro 01-6722), the active ingredient of Konakion MM, was tested for mutagenic activity using two versions of the Ames test: a standard plate incorporation and a preincubation modification method. Six Salmonella typhimurium tester strains (TA1535, TA1537, TA97, TA98, TA100 and TA102) and one E. coli tester strain (WP2 uvrA) were employed. The experiments were performed in absence as well as in presence of an exogenous metabolic activation system (S9). Its activity and the responsiveness of the strains were verified by including appropriate positive controls into each experiment.
Vitamin K1 was dissolved in ethanol. After dilution the same volume of a Tween 80 (10%) solution was added. The following dose ranges were evaluated 46 to 5'000 µg/plate and 50 to 5'000 µg/plate in the standard and preincubation assay, respectively. Upon addition to the aqueous medium milky suspensions were formed starting at 455 µg/plate (standard assay) and an oily precipitation starting at 1'666 µg/plate (preincubation assay). No toxic effects were observed up to 5'000 µg/plate, the highest generally recommended test concentration for non-toxic compounds.

No increase in the number of revertant colonies was observed after treatment with vitamin K1. Thus it can be concluded that neither vitamin K1 per se, nor any of the metabolites formed under the described experimental conditions are mutagenic in the Ames test.

Ref.: 31

Konakion MM (10 mg/mL) and mixed micelle vehicle alone were classified as non-mutagenic in the Salmonella/mammalian microsome assay (Ames test).

(Schüpbach 1982, Albertini 1990)

***In vitro* Mammalian Chromosome Aberration Test**

Vitamin K1 (batch no. 301382, purity ≥98.5%) was assessed for its ability to induce structural and numerical chromosome aberrations in human peripheral blood lymphocytes in vitro. In the presence of a metabolic activation mix prepared from livers of phenobarbital/betanaphtoflavone treated rats. Doses between 10 and 750 µg/mL were tested after short-term treatment of 3 h using normal (21 h) or delayed (44 h) sampling time (recovery time). Without metabolic activation doses between 100 and 750 µg/mL were tested after long-term treatment (24 and 48 h).
The upper dose level was selected on the basis of solubility of vitamin K1. The sensitivity of the test system and the activity of the metabolic activation were demonstrated by using the directly acting mutagen bleomycin and the promutagen cyclophosphamide as positive controls. Both substances increased significantly the rate of structural chromosome aberrations. The spindle poison colcemid was used as positive control for numerical chromosome changes at the delayed sampling time. It increased significantly the rate of polyploid cells.
Vitamin K1 did not increase the number of cells with structural aberrations in the experiments using metabolic activation as well as without metabolic activation. The number of polyploid cells was not increased over the control value. In conclusion, vitamin K1 is neither clastogenic nor aneuploidogenic in human lymphocytes under the described experimental conditions.

Ref.: 32

3.3.6.2. Mutagenicity / Genotoxicity *in vivo*

An increase in sister chromatid exchange (SCE) after vitamin K1 treatment was reported in the literature (Cornelissen et al. 1991). However, in a randomised clinical trial with neonates, intramuscular application of 1 mg/kg vitamin K1 did not induce chromosomal aberrations or SCE in peripheral blood lymphocytes (Havel et al. 1987; Venitt et al. 1987; Silverman and Andrews 1977; Israels et al. 1983).

The EFSA Scientific Committee on Food (SCF) considered in its 2003 opinion the following data on Mutagenicity / Genotoxicity of Vitamin K:

Phylloquinone was reported to reduce the mutagenicity of six heterocyclic amines in the Ames Salmonella typhimurium assay. There was no evidence of mutagenicity of phylloquinone in the absence of the amines (Edenharder et al., 1999).Conflicting results have been obtained in studies on the ability of phylloquinone to induce sister chromatid exchanges (SCE) in human or animal leucocytes. When 5 foetal sheep were given a dose of 1 mg phylloquinone via the femoral vein, the mean number of SCEs rose from 3.94 (±0.15) prior to injection to 5.4 (±0.23) 24 hours after injections. This increase was stated to be statistically significant (Israels et al., 1987). In an in vitro study of the concentration response for SCE induction, foetal or adult sheep leucocytes were incubated with phylloquinone at concentrations of 0.1 nM to 1 µM. At 0.1 nM the number of SCEs was increased in foetal cells but the increase in adult cells was only observed at 10 nM and above.

With human leucocytes taken from adult and placental blood, an increase in the mean number of SCEs per metaphase was reported in the presence of 1 µM phylloquinone. The SCEs rose from 3.32 ±0.219 to 5.76 ± 0.219 in placental leucocytes and from 5.13 ±0.273 to 7.81 ±0.326 in adult cells (Israels et al., 1987).

Conversely, negative results were obtained when human neonates were injected with 1 mg phylloquinone i.m. No significant difference in the mean number of SCEs and chromosomal aberrations in peripheral blood lymphocytes between treated and untreated controls was observed 24 hours after injection (Cornelissen et al. 1991).

The EFSA Scientific Committee on Food concluded on basis of the data described above that the limited data presently available do not allow an adequate evaluation of the genotoxic potential of phylloquinone at the gene or chromosome level.

Ref.: 5

3.3.7. Carcinogenicity

The applicant stated that since no mutagenic properties were observed with vitamin K1 and Vitamin K is used extensively in medicinal practice, therefore, a carcinogenicity study is considered not necessary.

The EFSA Scientific Committee on Food (SCF) stated in its 2003 opinion that no experimental animal studies on carcinogenicity of vitamin K have been found. One epidemiological study indicated that there was a significant association between intramuscular injection of vitamin K and childhood cancer, especially leukaemia (Golding et al., 1992). No significantly increased risk was associated with oral administration (Huysman and Sauer, 1994). Several other population studies have failed to confirm an association between vitamin K administration to children and cancer. A nested case-control study using data from a large, multicentre prospective study of 54,795 children showed no association between vitamin K administration and risk of any childhood cancer, or of all cancers combined (Klebanoff et al., 1993). A study of associations between leukaemia and prenatal or neonatal administration of vitamin K did not show any increased risk in neonates receiving vitamin K i.m. (Ansell et al., 1996). The latter results were confirmed in other studies (McKinney et al., 1998; Parker et al., 1998; Passmore et al., 1998). The SCF concluded that the evidence for an association between administration of phylloquinone to neonates and childhood cancer is therefore not convincing.

Ref.: 5

3.3.8. Reproductive toxicity

3.3.8.1. One generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

In an evaluation of the toxicological profile of Vitamin K 1, the following statement can be found:
Pregnant rats were treated from day 9 to day 20 of gestation with i.m. injection of vitamin K1 at10 mg/kg/day. There were no malformations or hemorrhages.
No further information on this study was provided.

Ref.: 33

According to the applicant, Konakion has not undergone adequate animal testing to evaluate impairment of fertility.

3.3.9. Toxicokinetics

Toxicity of Konakion mixed micelles (MM) (Ro 01-6722/131) after oral administration was investigated in Beagle pups (see also section 3.3.5.1). Approximately 2-week old male and female Beagle dogs (6 pups/group) were treated orally (by gavage) with 2 mg/pup, 20 mg/pup and 100 mg/pup for 4 weeks (2 administrations/week). For toxicokinetic determination, blood samples were taken before treatment and in week 1 and 4 and were measured by HPLC with electrochemical detection.
High plasma vitamin K1 concentrations were measured 2 hours after dosing (this is approximate the Tmax in humans) and plasma levels were significantly decreased 3-4 days after dosing.

Table 5: Pharmacokinetic parameters of Vitamin K1 after oral dosing of Konakion MM

Dose mg/pup	Treatment Day 2 AUC $_{2\text{-}96h}$ µg*h/mL	Treatment Day 7 AUC 2-72h µg*h/mL
2	34.4	5.3
20	133.2	39.8
100	249.1	99.8

The increase in vitamin K1 was dose-dependent and plasma vitamin K1 levels were higher when the pups were younger (study week 1) than when older (study week 4). Apart from the higher respectively increased body weights of the older animals (Konakion MM doses were calculated as mg/pup and, therefore, older pups received a lower mg/kg dose) further parameters, e.g. differences in distribution, metabolism, elimination rate, might be responsible for the reduced plasma vitamin K1 levels in study week 4 (no details on these parameters provided).
It is concluded that in male and female Beagle pups, repeated oral doses of Konakion MM up to 50 times the human prophylactic dose yielded significant dose-dependent vitamin K1 plasma exposure.

Ref.: 34

The EFSA Scientific Committee on Food (SCF) stated in its 2003 opinion that under normal physiological conditions, lipid soluble K-vitamins are absorbed in cooperation with bile acids and pancreatic enzymes. The efficacy of absorption (10-90% depending on the food matrix) (Schurgers and Vermeer, 2000) can be reduced by long-chain polyunsaturated fatty acids and badly absorbed lipid-soluble substances and hydrocarbons, like mineral oils and squalene. Vitamin K1 and K2 are stored in the liver. The total body pool of vitamin K (1.5 µg/kg body weight) is small compared to other fat-soluble vitamins and its turnover is rapid. Under normal conditions, 30-40% of the absorbed vitamin K is excreted via the bile into the faeces, while approximately 15% is excreted in the urine as water soluble metabolites. Alimentary deficiency, disturbance of fat absorption, increased excretion, presence of antagonists, disturbance of bile function and liver disease, lead to decreased bioavailability of vitamin K (Suttie, 1996; Elmadfa and Leitzmann, 1998).

Ref.: 5

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

***In vitro* assessment of phototoxic potential using human reconstructed epidermis**

Guideline: /
Tissue: human reconstituted epidermis [SKINETHIC™]
N° of chambers: 6
Test substance: vitamin K1 phytonadione
Batch: 03.581
Purity: /
Concentrations: 100% and diluted to 10 and 1% in paraffin oil
GLP: /

A toxicity assay was carried out using SKINETHIC cultures (0.63 cm^2 epidermis). The test product was tested as supplied (i.e, 100%) and diluted to 10 and 1% (v/v) in paraffin oil. Reconstituted epidermis (REp) was treated overnight (approximately 18h). The toxic effect was assessed by using the MTT assay. No significant cytotoxicity was observed in epidermis. Taking into account these results, the test substance was to be applied undiluted on the culture surface, for a 24h incubation period prior to UV exposure.

Test product (6.3µl /epidermis) was applied directly onto the surface of tissues. Control and treated REps were incubated at 37°C for 24 hr and subsequently washed with HBSS. REps were irradiated with UV_A dose 6 J/cm^2 (3 biopsies/UV_A dose), while 2 REps of each experimental group were kept at room temperature in the dark during UVA exposure (dark control). After further incubation at 37° C for 24h, viability was assessed by a MTT test.

Table 6: Results *in vitro* Phototoxicity

	CONTROL		**TREATED**	
	0	**6 J/cm2**	**0**	**6 J/cm2**
OD_{570} (mean) St. Dev.	0.899 0.005	0.871 0.008	0.861 0.011	0.323 0.011
Viability	**100%**	**97%**	**100%**	**38%**
Cytotoxicity		**3%**		**62%**

Conclusion
Vitamin K1 (phytonadione) can be considered as phototoxic *in vitro*.

Ref.: 9

Comments
In this study, no positive controls were used. The isomeric composition of the test compound was not reported.
The method which was used is not a validated *in vitro* method for the assessment of phototoxic potential. The 3T3 NRU Phototoxicity test (OECD 432) should have been used instead.

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

Guideline: /
Species: Human
Group: 100 completed study; males and females
Substance: NS Bionic Eye Cream (containing 1% vitamin K)

Batch: L#111-154
Purity: /
Concentrations: 'as is'
Dose: 0.2g on 2.5cm x 2.5 cm square
Vehicle: /
GCP: in compliance
Study period: 2005

A semi-occlusive patch test with an eye cream (NeoStrata® Bionic Eye cream) containing 1% vitamin K (not otherwise defined/described in the original report) on the upper back was conducted with 100 healthy volunteers. The patches with the test product were applied three times a week for a total of 9 applications in the induction phase followed by a challenge patch two weeks later. Patches were removed after 24 hours, and the sites were scored immediately before re-application during induction and in the challenge phase 24 and 72 hours after application.

Under the conditions of the study the tested eye cream did not show potential for dermal irritation or allergic contact sensitisation.

Ref.: 38

Comment
The SCCS does not consider human studies for determining sensitisation potential to be ethical.

Patch tests with two isomers of Vitamin K were applied on the back of 107 healthy volunteers. No reaction was observed after 48 and 72 hours (only statement, no data provided).

Ref.: 10

Post-marketing data (Consumer complaints)

Table 7: Post marketing data for three Vitamin K1-containing cosmetic products

Vitamin K	Product type	Units sold	Adverse skin reactions reported	Reference
1% vitamin K	Eye cream	211 180	3 "local irritation"	36
< 1% vitamin K1 (DSM, formerly Roche)	Face and body cream	50 000	0	35
< 0.1%	Face and body cream	318 800	"No medically confirmed cases of allergic reaction"	37

Contact dermatitis due to topical cosmetic use of Vitamin K1

There have been unspecified adverse skin reactions reported from the topical use of vitamin K containing products (6 case reports in France, 2 in Belgium, 1 in Spain and 1 in Italy). The product manufacturer considered these adverse reactions to be secondary to the use of the products on injured skin, which is not recommended. The estimated frequency was 2.5 to 4 cutaneous intolerance for 100 000 sold products (Vitamin K1, 1 and 2%).

Ref.: 13

The French authorities, in a report of the Commission de Cosmétologie, noted 11 cases of adverse effects after topical application of Vitamin K1 containing products in France, including the 6 cases reported in the cosmetovigilance report from the manufacturer (Ref. 13). No

further detailed information is available on the cases reported to French authorities mentioned in the SCCP opinion of 2007. However SCCP noted that amongst these cases there were 2 cases (severe according to AFSSAPS) reported with products containing oxidised vitamin K1.

Ref.: 14

Published clinical cases of adverse reactions to vitamin K1 (Table 8)

A 45 year old woman developed a severe eczematous reaction on her face where she had been applying a clarifying cream. A repeated open application test (ROAT) on her forearm was positive. Patch testing of the cream's ingredients showed positive reactions to vitamin K1. At 1% and 10% in petrolatum; controls were negative.
The authors commented "*The cosmetic use of topical vitamin K is infrequent and this may account for the rarity of cases of sensitivity to date*".

Ref.: 16

A 27-year-old woman developed dermatitis of the face, particularly in the periorbital area. For 4 months, she had used a cosmetic treatment of the face to decrease periorbital hyper-pigmentation, Auriderm K2® cream, a mixture containing vitamin K1 and retinol. Patch tests 2 months later showed a positive reaction to Auriderm K2® cream. Patch tests with the ingredients of the cosmetic cream were positive to vitamin K1 2% pet. (D2+/ D3++) and *trans*-vitamin K 2% pet (D2+/D3+++).

Ref.: 17

Two cases of eyelid dermatitis caused by Vitamin K1 in a cosmetic cream (Ureadin® facial contour) were reported in women using the cream for 3-4 weeks. Patch tests to the Ureadin® cream (+++) and Vitamin K1 0.1% aq. (+++) were positive but the cream without the vitamin was negative; 15 control subjects showed no reactions.
The authors commented "*With the increasing use of vitamin K1 in cosmetic creams and the subsequent amplification of the exposition to this vitamin, we will probably see a higher frequency of allergic reactions to vitamin K1 in the near future. Vitamin K1 must be investigated as a new cause of eyelid ACD*".

Ref.: 20

A 55 year old woman presented with dermatitis affecting the eyelids and zygomatic regions. She related her symptoms to the local application of Ureadin facial contour cream. A patch test to the cream 'as is' was positive on D2 (+) abd D4 (++). A ROAT was positive. There was a positive patch test reaction to phytomenadione 1% pet. supplied by the manufacturer. The concentration of the cream's components was not disclosed, so patch test with Konakion MM (Roche), containing 10mg phytomenadione in 1 mL colloidal aqueous solution was performed; there was an intense positive reaction on D3.

Ref.: 46

A 28 year old woman presented with an acute eczema affecting her lower eyelids. She had been using Auriderm K5 cream for some weeks before the onset of symptoms. A patch test with the cream 'as is' was positive (++) on D4. a ROAT was positive by D3. Individual components of the cream were not provided by the company. Patch tests to phytomenadione (1% pet.) and Konakion MM were positive on D2 and D4.

20 controls tested with vitamin K1 (1-10% pet.) and Konakion MM were negative.

The authors commented "*The reason that skin hypersensitivity to these substances is becoming more and more frequent is yet to be determined. It might have to do with the concentrations used in cosmetics and their combination with other components (such as urea or retinol, which are well known irritants) that might foster the development of skin reactions. It would be interesting if manufacturers specified the concentration of the*

components on the information leaflet of the creams and if they avoided the combination of irritant substances, as previously mentioned".

Ref.: 46

Table 8: Published clinical cases of adverse reactions to vitamin K1

Reference	Product	% vitamin K1 in product	Use	Effect	Time course	Testing
Ref. 16	Clarifying cream containing vitamin K1	not stated	Treatment of rosacea	Acute eczematous reaction at application site.	After few days use	ROAT to cream positive. Positive patch test to vitamin K1 at 1%, 10% pet. Controls tested with 1% and 10% Vitamin K, negative.
Ref. 17	Auriderm K2® cream containing vitamin K1 and *trans*-vitamin K	not stated	Decreasing periorbital hyper-pigmentation.	Facial dermatitis, particularly on peri-orbital area.	4 months use.	Positive patch test to Auriderm K2®, vitamin K1 and *trans*-vitamin K at 2% pet.
Ref. 20	Ureadin® facial eye contour containing vitamin K1	not stated	Reducing peri-orbital pigmentation.	2 patients. Acute eczematous reaction of peri-orbital skin.	3 and 4 weeks use, respectively	Positive patch test to Ureadin® and vitamin K1 at 0.1% aq. 15 controls negative to Ureadin and Vitamin K1 0.1% aq.
Ref. 46	Ureadin® facial eye contour containing vitamin K1	not stated	Eyelid lifter cream.	Eczematous reaction affecting per-orbital skin and zygomatic areas	not stated	Positive patch test to Ureadin®. Positive ROAT with product. Positive patch test to vitamin K1 1% pet. and Konakion MM 0.1mL (1% active). 20 controls negative to vitamin K1 (1-10% pet.) and Konakion MM.
Ref. 46	Aurderm K5® cream	not stated	not stated	Eczematous reaction on lower eyelids.	Some weeks before onset	Positive patch test to Aurderm K5®. Positive ROAT with product. Positive patch test to vitamin K1 1% pet. and Konakion MM 0.1mL (1% vitamin K1).

Eczematous hypersensitivity from aqueous vitamin K injection

A case was reported of an eczematous reaction following injection with vitamin K1 for treatment of warfarin-induced hypoprothrombinaemia.

Ref.: 18

Type IV hypersensitivity to vitamin K

The day after intramuscular injection of vitamin K1 (phytomenadione) into her thigh, a 27-year-old-woman with normal liver function developed a relapsing and remitting eczematous reaction localized to the injection site, and later a further eczematous reaction under an adhesive dressing (Duoderm®). On patch testing, she was positive to vitamin K1 and cross-reacted to vitamin K4; she was also positive to colophonium and to ester gum rosin, the dressing adhesive. Recurrent angio-oedema persisted for several months and, 2 years later, symptoms were still occurring over the injection sites. Structure-activity relationships among vitamin K allergens are discussed.

Ref.: 19

Between 1964 and 1994, at least 52 patients with cutaneous adverse effects of vitamin K were described in the European and North American literature, with 94 cases in the Japanese literature. In this publication, a review of the details of these patients, primarily from therapeutic use of Vitamin K1, is given and 2 new therapeutic use cases reported. Adverse effects were seen not only in patients with liver-function disturbances but also in patients without liver diseases, and occur mostly after intramuscular or subcutaneous administration of vitamin K1, independent of the total dose. Patch and intracutaneous tests often give positive reactions. The mechanism of action was considered in many patients to be a delayed-type hypersensitivity reaction.

Intramuscular and subcutaneous injections of Vitamin K1 have induced three types of cutaneous reactions:

- 10-14 days after injection eczematous reactions. The allergic mechanism was confirmed in several patients by intradermal and/or epicutaneous testing. There were positive patch test reactions with Vitamin K1; Vitamin K3 was negative
- Scleroderma-like patches at site of former injection several months or year after injection. Sensitization was proven in 4 patients by intradermal tests.
- Urticaria

Ref.: 15

Bruynzeel et al (Ref. 15) stated that "No cases were found in which orally administered K1, K2 or K3 resulted in skin disease, not even in patients with a positive allergic reaction to i.m. vitamin K1, which had led before to a dermatitis". However, Robinson & Odom (Ref. 55) suggested that dietary vitamin K may have been responsible for provoking exacerbations of dermatitis in their patient.

Occupational exposure

According to the applicant, there has been no case of sensitisation to vitamin K1 at the manufacturing site of the raw material. In addition, no further health effects were observed.
Ref.: 40

The 5 occupational cases after occupational cutaneous exposure, cited by Bruynzeel (Re. 15), were not with vitamin K1 but with vitamin K3 which lacks the isoprenoid side chain.
Ref.: 15

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

Not applicable

3.3.14. Discussion

Physico-chemical properties
Claimed uses for Vitamin K1 as a cosmetic include moisturising, skin lightening, periorbital hyper pigmentation, application on actinic and traumatic purpura and bruising after laser therapy.
Although the current submission only supports the use of Vitamin K1 up to 1%, the SCCP is aware of cosmetic products on the market with Vitamin K1 concentrations as high as 8%.

Vitamin K1 is used therapeutically by parenteral injection and orally to treat coagulation disorders.

The data provided on the physico-chemical characterisation of phytonadione is insufficient. Documentation of characterisation, determination of composition, purity and impurities is missing. In several studies, the test substance is described as Vitamin K. However, the actual content of vitamin K1 in these test articles was not given.

Log Pow and quantitative data on the solubility are not provided. Vitamin K1 is slowly degraded by atmospheric oxygen and fairly rapidly degraded by light. No information on stability of phytomenadione in typical cosmetic formulations (and under use conditions) is available.

According to the applicant of submission I, since 2005 "oxidised" Vitamin K1 has been used in their products. It is not clear from the submission to which chemical substance the term 'phytonadione-oxide' refers to and no data was provided on this substance.

General toxicity
EFSA concluded in its opinion of 2003, that there are no appropriate data from which to set a numerical upper limit for Vitamin K, however, Vitamin K1 was considered safe as a food supplement, based on absence of evidence for adverse effects

Based on the results of all the safety studies and literature data provided in this assessment, vitamin K1 has a low acute (oral) toxicity. No repeated dose, no mutagenic, no teratogenic, and no embryotoxic effects were demonstrated in the studies performed. However, the database, especially for genotoxicity and developmental/reproductive toxicity, is not considered adequate.

Since the database is not complete, and no reliable NOAEL is available, it is not possible to perform a risk assessment deriving a margin of safety for the use of Vitamin K1 in cosmetic products.

Dermal absorption
The total skin penetration rate of vitamin K1 was time- as well as species-dependent and reached 101.45 µg/cm² (56.4%) on naked rat skin after an exposure time of 16 hours while 20.31 µg/cm² (11.2%) were measured on pig skin.

Skin Irritation and Sensitisation
A cosmetic product containing 1% Vitamin K was not irritating on human volunteers.

Vitamin K1 was phototoxic in an *in vitro* test using reconstituted human epidermis.

Two LLNA studies on sensitisation showed no evidence of either Vitamin K or Vitamin K(Hv) being sensitizers. However, there was no data on the isomeric composition of the Vitamin K used and, therefore, no conclusion on sensitising potential of vitamin K1 is possible.

A more recent LLNA indicated that Vitamin K1 is a skin sensitizer ('moderate' using SCCP scheme or 'weak' according to Kimber et al).

There are 6 published cases of contact allergy and dermatitis from use of Vitamin K1 containing cosmetic products. No information on the concentration of Vitamin K1 in the cosmetic products is available. Affected individuals have shown allergic reactions on diagnostic patch tests using Vitamin K1 (aq. and pet.) at dilutions of 0.1-2%.

A cosmetovigilance report contained in submission I listed 10 unspecified adverse skin reactions reported from the topical use of vitamin K containing products in Europe. The

product manufacturer considered these adverse reactions to be secondary to the use of the products on injured skin, which is not recommended, but no data supporting this assumption have been provided. The estimated frequency was 2.5 to 4 cutaneous intolerance for 100 000 sold products (Vitamin K1, 1 and 2%).

The French authorities recorded 11 cases of adverse effects after topical application of Vitamin K1 containing products in France, including the 6 cases reported in the cosmetovigilance report from the manufacturer. No further detailed information is available on these cases reported to French authorities. However, amongst these cases there were 2 cases (severe according to AFSSAPS) reported with products containing oxidised vitamin K1.

Interestingly, a toxicity assessment (Ref. 32) by the manufacturer of an injectable medicinal product containing Vitamin K1 states that a reformulation of the medicinal product has resulted in a substantial reduction of the sensitizing potential and has greatly diminished, but not totally eliminated, the risk of anaphylactoid reactions. It points out that it should be remembered in this connection, that Vitamin K1 alone has also been implicated in allergic reactions, albeit mostly of the delayed cutaneous type.

Post-marketing surveillance data collected by the applicant of submission II/III has identified few complaints from Vitamin K containing cosmetics.

Conclusions on sensitisation

Vitamin K1 has been demonstrated to be a contact allergen in man and well documented case reports illustrate that its use in cosmetic products has caused allergic contact dermatitis. Such case reports are illustrative as the burden can only be determined when it is known how many individuals being evaluated for eczematous skin conditions have been patch tested to Vitamin K1 and how many have used Vitamin K1-containing cosmetics.

Vitamin K1 has been used in a wide concentration range (mostly 1-2%, but up to 8%) in cosmetics products, but no specific information is available about the extent of consumer exposure to Vitamin K1-containing cosmetics. Sensitisation potential may be influenced by sites of exposure, frequency of applications as well as concentrations of Vitamin K1 present in cosmetic products and their formulation characteristics.

From the data currently available, the risk for consumers to become sensitised by cosmetic products containing 1% Vitamin K1 cannot be quantified.

Vitamin K1 is an important therapeutic agent. In cases of pre-existing sensitisation acquired by topical application of Vitamin K1 in cosmetics, an individual may experience an allergic reaction to Vitamin K1 treatment and/or might not be able to receive Vitamin K1 therapy should it be required.

4. CONCLUSION

The SCCS consider that the new data submitted does not supersede concerns about the allergenic potential of vitamin K1 when used in cosmetic products in a concentration up to 1.0%.

Although the risk for sensitisation from cosmetic products containing 1% Vitamin K1 cannot be quantified from the available data, case reports show that Vitamin K1 is a contact allergen in man. In cases of pre-existing sensitisation acquired by topical application of Vitamin K1 present in cosmetics, an individual might not be able to receive Vitamin K1 therapeutically or experience allergic reactions upon Vitamin K1 treatment. Therefore, the

SCCS considers that vitamin K1 is not safe when used in cosmetic product in a concentration up to 1%.

In addition, the database, especially for genotoxicity and developmental/reproductive toxicity, is not considered adequate. No reliable critical effect level can be derived for a risk assessment of Vitamin K1 in cosmetic products.

5. MINORITY OPINION

Not applicable

6. REFERENCES

References taken from SCCP/1105/07

1. Budavari, S. (ed.). The Merck Index - Encyclopedia of Chemicals, Drugs and Biologicals. Rahway, NJ: Merck and Co., Inc., 1989, p. 1580
2. IARC. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Geneva: World Health Organization, International Agency for Research on Cancer, 1972-present. (Multivolume work)., p. V76 424 (2000)
3. HSDB - Hazardous Substances Data Bank (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB)
4. Expert Group on Vitamins and Minerals. Revised review of vitamin K. doc. n° EVM/01/09, revised aug 2002
5. EFSA (2003). Scientific Committee on Food. Opinion on the Tolerable Upper Intake Level on Vitamin K. doc. n° SCF/CS/NUT/UPPLEV/32 Final. 4 April 2003.
6. AURIGA International, letter to Mrs J. Meunier. Service Denrées alimentaires, aliments pour animaux et autres produits de consommation. Brussels,11 July 2006
7. Sire G. Vitamin K. Evaluation of skin sensitization potential in mice using the local lymph node assay. CIT, France. Study number 31769 TSS, 26 January 2007
8. Sire G. Vitamin K hv. Evaluation of skin sensitization potential in mice using the local lymph node assay (LLNA). CIT, France. Study number 31771 TSS; 26 January 2007
9. Boue-Grabot, M., Halaviat, B. Vitamine K1 Phytonadione. *In vitro* assessment of phototoxic potential using human reconstructed epidermis. Bio HC, France. Report PTC_E 03.581. 13 July 2006
10. Heenen M. Rapport sur les réactions d'irritation observées après la pose de patch tests de 2 isomères de la vitamine K. ULB, Brussels. Ref. adm/heenen/1102. 21.11.2002
11. Cesarini J.P. Rapport d'essai clinique du protocole Auriderm k5® sur les plaies provoquees, standardisees chez l'homme. Unpublished report. 5.10.1999
12. Cesarini J.P. Rapport d'essai clinique avec Auriderm® K5. unpublished report. 27.02.1999
13. AURIGA International. Cosmetovigilance. unpublished report
14. Avis de la Commission de cosmetology n° 25. France. 29 September 2005
15. Bruynzeel I., Hebeda CL, Folkers E., Bruynzeel DP. Cutaneous hypersensitivity reactions to vitamin K: 2 case reports and a review of the literature. Contact Dermatitis. 1995 Feb; 32(2):78-82
16. Serra-Baldrich E., Dalmau J., Pla C., Muntanola A.A. Contact dermatitis due to a clarifying cream. Contact Dermatitis 2005 53(3), 174-175
17. Veneziano L., Simonetta S., Voudouris S., Tosti A. Contact dermatitis due to topical use of vitamin K. Contact Dermatitis 2005. 52(2), 113-114

18. Keough G.C., English J.C., Meffert J.J., Eczematous hypersensitivity from aqueous vitamin K injection. Cutis 1998 Feb; 61(2): 81-83
19. Sommer S., Wilkinson S.M., Peckham D., Wilson C. Type IV hypersensitivity to vitamin K. Contact dermatitis 2002. 46(2), 94-96
20. Ruiz-Hornillos FJ, Prieto A, De Castro FJ et al. Allergic contact dermatitis due to vitamin K1 contained in a cosmetic cream. Contact Dermatitis 2006. 55: 246-247

Additional references from submission II of November 2007

21. DSM Nutritional Products Ltd (2007). Technical Information File Vitamin K1. Version number I, 16.11.2007
22. DSM (2004) Vitamin K1CEP confirmation
23. DSM (2004) Vitamin K1 Specifications and Tests
24. DSM (2007) Vitamin K1 Stability Data
25. Bächtold (1980) Acute toxicity of Menadiol and Vitamin K1, some intermediates and by products of synthesis, degradation products and Metabolites, (Study No. B-90447), F. Hoffmann-La Roche Ltd., Basle, Switzerland.
26. Bächtold (1976), KONAKION Lösung zur intravenözen Applikation (Study No. B-102418), F. Hoffmann-La Roche Ltd., Basle, Switzerland.
27. Geleick H and Klecak G (1984) Determination of allergenic and anaphylactogenic potential of KONAKION suspensions in guinea pigs. - Study on KONAKION mixed micelles ampoule formulation (Ro 01-6722/120) in comparison with commercial KONAKION ampoule formulation (Ro 01-6722/92) by two different induction procedures (i.v. and i.d.) (Study No. B-105507), F. Hoffmann-La Roche Ltd., Basle, Switzerland
28. Geleick H (1985) Determination of anaphylactogenic potential of KONAKION mixed micelles ampoules Ro 01-6722/120 in guinea pigs. (Study No. B-119795), F. Hoffmann-La Roche Ltd., Basle, Switzerland
29. Klecak G (1985) Penetration studies "in vitro" on intact excised naked rat and pig skin with 3% ethanolic solution of Ro 01-6722/102 - Vitamin K1 3H. (Study No. B-119794), F. Hoffmann-La Roche Ltd., Basle, Switzerland
30. Chételat A (1982) Mutagenicity evaluation of Ro 01-6722 (Vitamin K1) in Salmonella typhimurium by means of the standard plate incorporation assay (Ames test) (Study No. B-95576), F. Hoffmann-La Roche Ltd., Basle, Switzerland
31. Albertini S (1993) Mutagenicity evaluation of Ro 01-6722 (vitamin K1 for Konakion MM) in the Ames test (Study No. 182M93), F. Hoffmann-La Roche Ltd., Basle, Switzerland, RDR No. B-161'912
32. Miller B (1994) Chromosome analysis in human peripheral blood lymphocytes treated in vitro with Ro 01-6722 (Vitamin K1 used for Konakion MM) in presence and in absence of a metabolic activation system (study No. 191M93) F. Hoffmann-La Roche Ltd., Basle, Switzerland, RDR No. B-161'110
33. Stenger E (1992). The toxicological profile of vitamin K1 (Konakion®) in laboratory animals. F. Hoffmann-La Roche Ltd., Basle, Switzerland, RDR No. B-156444
34. R. Strobel and B. Schläppi (1996) Toxicokinetic Monitoring of the 4-Week Oral Toxicity Study in Male and Female Beagle Puppies with Konakion MM (Addendum to Study No. 136P94) (Study performed by Vitamin K Diagnostic Laboratory, Haemophilia Center, St. Thomas' Hospital , London, UK) F. Hoffmann-La Roche Ltd., Basle, Switzerland, RDR No. B-164'591(Corrected version of the original report dated November 24, 1995)
35. Pangea, attestation of Vitamin K1 in cosmetic use at less than 1% in skin facial and body cream, November 7, 2007.
36. Green B, NeoStrata Company Inc (2007) Attestation of vitamin K1 in cosmetic use at 1% in skin facial and eye cream, November 2, 2007.
37. Bagshaw K, Fine Fragrance & Cosmetics Ltd. (2007) Attestation of vitamin K1 in cosmetic use at less than 0,1% in skin facial and body cream, November 8, 2007.

38. Eisenberg R (2005) Repeated Insulted Patch Test, NS Bionic Eye Cream, Consumer Products Tested Co. for NeoStrata Company Inc., December 19, 2005.
39. Kanengiser BE (2006) In use safety evaluation to determine the ocular Irritation potential of an eye area product, Clinical Research Laboratories Inc. for NeoStrata Company Inc, Princeton (NJ), 10 April 10, 2006.
40. Bieli Urs, Memo/Note, Health effects related to Occupational Handling of Vitamin K1 in Sisseln, October 2007.

Additional references from submission III of March 2009

41. DSM Nutritional Products Ltd (2009). Technical Information File Vitamin K1. Version number II, 24.03.2009
42. Api AM, Basketter DA, Cadby PA, Cano M-F, Ellis G, Gerberick GF, Griem P, McNamee PM, Ryan CA and Safford R. (2008) Dermal sensitisation quantitative risk assessment (QRA) for fragrance ingredients. Regul Toxicol Pharmacol 52: 3-23.
43. Felter S P, Ryan CA, Basketter DA, Gilmour NJ, and Gerberick GF. 2003. Application of the risk assessment paradigm to the induction of allergic contact dermatitis. Reg Toxicol Pharmacol 37: 1-10.
44. Honarvar N (2008) Local Lymph Node Assay (LLNA) in mice with vitamin K1. (Study No. 1209300), RCC Cytotest Cell Research GmbH (RCC-CCR), Rossdorf, Germany.
45. Mehling A, Eigler D, Ball N, Certa H, Esch H, Haux C, Kreiling R and Veenstra GE (2007). Discordant results between the LLNA and Guinea Pig tests: Is the LLNA overestimating the sensitisation potential for certain chemicals? Toxicol Lett 172S: S8.
46. Ramirez Santos A, Fernandez-Redondo V, Perez Perez L, Cao JC, Toribio J. 2008. Contact allergy from vitamins in cosmetic products. Dermatitis 19(3):154-6.
47. SCCP/0919/05. Memorandum. Classification and categorization of skin sensitisers and grading of test reactions. Adopted by the SCCP during the 5th plenary meeting of 20 September 2005.
48. SCCP 2006. Scientific Committee on Consumer Products SCCP. The SCCP's notes of guidance for the testing of cosmetic ingredients and their safety evaluation. 6th Revision. 19 December 2006. European Commission, Health & Consumer Protection, Directorate-General.
49. SCCP (2007). Opinion on Vitamin K1, phytonadione (INCI name). SCCP/1105/07, 28 September 2007.
50. SCCP (2008). Opinion on Vitamin K1 (Phytonadione). SCCP/1187/08, 24 June 2008.

SCCP/0970/06SCCP Opinion on Criteria for the in vitro assessment of dermal absorption of cosmetic ingredients – updated 2006

Additional References by SCCS

51. RX list, the internet drug index, Vitamin K1 drug description. http://www.rxlist.com/vitamin-k1-injection-drug.htm#, accessed Nov. 2009
52. Drugs.com, drug information online, Vitamin K1. http://www.drugs.com/pro/vitamin-k1.html, accessed Nov 2009
53. Baglin TP, Keeling DM, Watson HG; British Committee for Standards in Haematology (2006). Guidelines on oral anticoagulation (warfarin): third edition--2005 update. Br J Haematol. 2006 Feb;132(3):277-85.
54. Ansell J, Hirsch J, Dalen J, et al. (2001). Managing oral anticoagulant therapy (sixth ACCP consensus conference on antithrombotic therapy). Chest 119:22S–38S.
55. Robinson JW, Odom RB. Delayed cutaneous reaction to phytonadione. Arch Derm 1978; 114: 1790-1792.
56. Kimber I., Basketter D. A., Butler M., Gamer A., Garrigue J.-L., Gerberick G.F., Newsome C., Steiling W., and Vohr H.-W (2003): Classification of contact allergens according to potency: proposals. Food and Chemical Toxicology, 41, 1799-1809.)