Scientific Committee on Consumer Safety

SCCS

OPINION ON

Vitamin K1
(Phytonadione)

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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http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm
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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-[(2E)-(7R,11R)-3,7,11,15-tetramethylhexadec-2-enyl]naphthalene-1,4-dione (trans-phytomenadione),
- 2-methyl-3-[(2Z)-(7R,11R)-3,7,11,15-tetramethylhexadec-2-enyl]naphthalene-1,4-dione (cis-phytomenadione), and
- 2,3-epoxy-2-methyl-3-[(2E)-(7R,11R)-3,7,11,15-tetramethylhexadec-2-enyl]-2,3-dihydrornaphthalene-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
Combinal k1
K-ject
Kativ n
Kephton
Kinadion

Konakion
Mephyton
Mono-kay
Monodion
Synthex p

-Vitamin K1
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- Phytomenadione (all-rac)
- Phytonadione (all-rac)
- α-Phylloquinone (all-rac)
- Vitamin K (all-rac)

### 3.1.1.4. CAS / EC number

<table>
<thead>
<tr>
<th>CAS</th>
<th>EC</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>84-80-0</td>
<td>201-564-2</td>
<td>phytomenadione</td>
</tr>
<tr>
<td>11104-38-4</td>
<td>234-330-3</td>
<td>vitamin K1</td>
</tr>
<tr>
<td>81818-54-4</td>
<td>279-833-9</td>
<td>2-methyl-3-(3,7,11,15-tetramethylhexadec-2-enyl)-1,4-naphthoquinone</td>
</tr>
</tbody>
</table>

* 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.

![Structural formula of Vitamin K1](image)

### 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, cyclohexane, 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

<table>
<thead>
<tr>
<th>Property</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point</td>
<td>approx. - 20 °C</td>
</tr>
<tr>
<td>Boiling point</td>
<td>140-145 °C</td>
</tr>
<tr>
<td>Density</td>
<td>0.97 g/cm³</td>
</tr>
</tbody>
</table>

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 phytomenadione are described to be stable: trans-phytomenadione (84.4-86.4%), epoxy phytomenadione 0.15, total cis-, trans- 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.
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- 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 (phyloquinone) 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 apoptotic 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;
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- 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

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

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: /
Opinion on Vitamin K1 (phytonadione)

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

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (%)</th>
<th>Irritation level</th>
<th>Stimulation Index (SI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test item</td>
<td>5</td>
<td>non-irritant</td>
<td>1.78</td>
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</tbody>
</table>
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<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (%)</th>
<th>Irritation level</th>
<th>Stimulation Index (SI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test item</td>
<td>10</td>
<td>non-irritant</td>
<td>2.10</td>
</tr>
<tr>
<td>Test item</td>
<td>25</td>
<td>non-irritant</td>
<td>0.80</td>
</tr>
<tr>
<td>Test item</td>
<td>50</td>
<td>non-irritant</td>
<td>0.88</td>
</tr>
<tr>
<td>Test item</td>
<td>100</td>
<td>non-irritant</td>
<td>1.67</td>
</tr>
<tr>
<td>HCA</td>
<td>25</td>
<td>-</td>
<td>11.18</td>
</tr>
</tbody>
</table>

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-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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (%)</th>
<th>Irritation level</th>
<th>Stimulation Index (SI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test item</td>
<td>5</td>
<td>non-irritant</td>
<td>1.02</td>
</tr>
<tr>
<td>Test item</td>
<td>10</td>
<td>non-irritant</td>
<td>0.64</td>
</tr>
<tr>
<td>Test item</td>
<td>25</td>
<td>non-irritant</td>
<td>0.80</td>
</tr>
<tr>
<td>Test item</td>
<td>50</td>
<td>non-irritant</td>
<td>1.10</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (%)</th>
<th>Irritation level</th>
<th>Stimulation Index (SI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test item</td>
<td>100</td>
<td>non-irritant</td>
<td>1.10</td>
</tr>
<tr>
<td>HCA</td>
<td>25</td>
<td>-</td>
<td>8.54</td>
</tr>
</tbody>
</table>

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 ³H-methyl thymidine (corresponds to 19.9 µCi ³H-methyl thymidine per mouse) by intravenous injection via a tail vein.

Approximately five hours after treatment with ³H-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 ³H-methyl thymidine incorporation was then measured on a β-scintillation counter. Similarly, background ³H-methyl thymidine levels were also measured in two 1ml-aliquots of 5 % trichloroacetic acid. The β -scintillation counter expresses ³H-methyl thymidine incorporation as the number of radioactive disintegrations per minute (DPM).
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%.

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 et 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.

---

**Table 4: LLNA results Vitamin K1**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Stimulation Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test item</td>
<td></td>
</tr>
<tr>
<td>1%</td>
<td>1.59</td>
</tr>
<tr>
<td>10%</td>
<td>1.24</td>
</tr>
<tr>
<td>50%</td>
<td>1.82</td>
</tr>
<tr>
<td>100%</td>
<td>4.03</td>
</tr>
<tr>
<td>α-Hexylcinnamaldehyde</td>
<td></td>
</tr>
<tr>
<td>5%</td>
<td>5.24</td>
</tr>
<tr>
<td>10%</td>
<td>7.38</td>
</tr>
<tr>
<td>25%</td>
<td>9.32</td>
</tr>
</tbody>
</table>

Ref.: 44

---

Concentration Stimulation Index

1% 1.59
10% 1.24
50% 1.82
100% 4.03
α-Hexylcinnamaldehyde
5% 5.24
10% 7.38
25% 9.32
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 microscopic 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.

Ref.: 30
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/betanaphthoflavone 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.
Opinion on Vitamin K1 (phytonadione)

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

<table>
<thead>
<tr>
<th>Dose mg/pup</th>
<th>Treatment Day 2 AUC 2-96h µg*h/mL</th>
<th>Treatment Day 7 AUC 2-72h µg*h/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>34.4</td>
<td>5.3</td>
</tr>
<tr>
<td>20</td>
<td>133.2</td>
<td>39.8</td>
</tr>
<tr>
<td>100</td>
<td>249.1</td>
<td>99.8</td>
</tr>
</tbody>
</table>

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

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

In vitro assessment of phototoxic potential using human reconstructed epidermis

Ref.: 5
Opinion on Vitamin K1 (phytonadione)

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 cm² 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 UVλ dose 6 J/cm² (3 biopsies/UVλ 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

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>TREATED</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD₅₇₀ (mean)</td>
<td>0.899</td>
<td>0.861</td>
</tr>
<tr>
<td>St. Dev.</td>
<td>0.005</td>
<td>0.011</td>
</tr>
<tr>
<td>Viability</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Cytotoxicity</td>
<td>3%</td>
<td>62%</td>
</tr>
</tbody>
</table>

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)
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

<table>
<thead>
<tr>
<th>Vitamin K</th>
<th>Product type</th>
<th>Units sold</th>
<th>Adverse skin reactions reported</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% vitamin K</td>
<td>Eye cream</td>
<td>211 180</td>
<td>3 &quot;local irritation&quot;</td>
<td>36</td>
</tr>
<tr>
<td>&lt; 1% vitamin K1</td>
<td>Face and body cream</td>
<td>50 000</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>(DSM, formerly Roche)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 0.1%</td>
<td>Face and body cream</td>
<td>318 800</td>
<td>&quot;No medically confirmed cases of allergic reaction&quot;</td>
<td>37</td>
</tr>
</tbody>
</table>

**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.: 14

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 hyperpigmentation, 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.: 16

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.: 17

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.: 20

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

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

**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

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.

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

**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 cospetrovigilance 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

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