



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

Adopted 1 July 2005

Comments concerning Henna (*Lawsonia inermis*) for hair-dyeing

SUMMARY

The Norwegian Food Safety Authority (Mattilsynet) has asked the Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) to consider the health risk related to the use of Henna products for hair-dyeing. The case has been assessed by the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food, and Cosmetics.

Henna (CAS no. 84988-66-9) represents a natural material derived from dried leaves of the plant *Lawsonia inermis*. In Europe it is mainly used as a hair dye based on the staining properties of the main active ingredient Lawsone, 2-hydroxy-1,4-naphthoquinone (CAS no. 83-72-7). Lawsone is known to be a natural part of Henna. It has been reported that the concentration of Lawsone in Henna can vary from 0 up to 1.5 %.

The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) has evaluated the safety of both Lawsone and Henna several times in recent years. In their last opinion concerning Lawsone adopted on 16 February 2004 it was concluded that Lawsone has genotoxic/mutagenic potential *in vitro* and *in vivo* and that therefore no safe level for Lawsone can be established. Based on this opinion the EU Commission has recently decided to ban the use of Lawsone as a cosmetic ingredient, while the use of Henna (*Lawsonia inermis*) has been temporary allowed until December 2005.

This assessment from the Norwegian Scientific Committee for Food Safety is limited to a semi-quantitative estimate of a possible cancer risk of Lawsone in Henna products for hair dyeing. The possible carcinogenic risk of *in vivo* mutagens is in general considered to be more critical than germ cell mutagenesis. Since no safe level of exposure for mutagens can be established, the linear relationship with similar numerical values recently demonstrated between the lowest effective dose (LED) after oral administration for *in vivo* genotoxicity and the carcinogen dose descriptor T25 may offer a pragmatic approach for a semi-quantitative cancer risk assessment. Thus LED divided with an extrapolation factor of 25 000 would correspond to a life-time cancer risk of 10^{-5} . The systemic exposure dose (SED) of Lawsone based on a worst case use situation of Henna products was calculated to be 0.4 µg/kg body weight/day.

Since the ratio LED (110 mg/kg bw/day)/25 000 is equal to 4.4 µg/kg bw/day and thus greater than SED (0.4 µg/kg bw/day), it follows that the possible cancer risk associated with the use of Henna products for hair-dyeing containing Lawsone would be negligible. The margin of exposure (MoE) would be approximately 275 000 (LED/SED = 110 000 µg/kg bw/day/ 0.4 µg/kg bw/day).

BACKGROUND

Henna (CAS no. 84988-66-9) represents a natural material derived from dried leaves of the plant *Lawsonia inermis*. It is mainly used as a hair dye in Europe, based on the staining properties of the main active ingredient Lawsone, 2-hydroxy-1,4-naphthoquinone (CAS no. 83-72-7). Lawsone is known to be a natural part of Henna. It has been reported that the concentration of Lawsone in Henna can vary from 0 up to 1.5 % (1).

The use of both Henna (*Lawsonia inermis*) and Lawsone as cosmetic ingredients for hair-dyeing has been discussed by the EU Commission in recent years. Hence, the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) and other scientific experts groups around Europe have assessed if the use of the two substances could be considered as safe.

SCCNFP has due to continuous occurrence of new toxicological data evaluated the safety of Lawsone and Henna several times between 2001 and 2004. The first opinion on Lawsone was adopted during the 16th plenary meeting of 13 March 2001 (2), and two additional opinions on Lawsone and one concerning Henna were adopted during the 19th and 21th plenary meetings of 27 February (3) and 17 September 2002 (4,5), respectively. Their last opinion concerning Lawsone was adopted 16 February 2004 (1).

In the opinion concerning Henna (*Lawsonia inermis*) adopted by the SCCNFP during the 21th plenary meeting of 17 September 2002 (5) it was concluded that:

“The present submission I on Lawsonia inermis is inadequate. Before any further consideration, a full and adequate dossier would be required, including:

- * specifications of the substance tested and marketed, and*
- * adequate in vivo genotoxicity data on natural henna containing the maximum amount of 2-Hydroxy-1,4-naphthoquinone.”*

The last reevaluated opinion concerning Lawsone adopted by the SCCNFP 16 February 2004 (1) concluded that:

“The SCCNFP is aware that some of the genotoxicity/mutagenicity data is equivocal. However, on balance, the SCCNFP considers that Lawsone has genotoxicity/mutagenicity potential in vitro and in vivo and that therefore no safe threshold for Lawsone can be established.”

The conclusions in the opinions on Lawsone adopted by SCCNFP have been considered as controversial as the mutagenic risk is debatable. Scientific experts in France, Germany and Denmark (6,7,8) consider Lawsone not to have a mutagenic potential, and have during the discussions related to the use of Lawsone as a non-oxidising colouring agent for hair dyeing in the recent years forwarded their point of view to the EU Commission.

The SCCNFP opinion on Henna has not yet been reevaluated in the light of their recent opinions on Lawsone so the opinion adopted during the 21th plenary meeting of 17 September 2002 (5) is still valid.

REGULATION

Based on the conclusion from SCCNFP that Lawsone has genotoxic and mutagenic potential *in vitro* and *in vivo* and therefore no safe threshold for Lawsone can be established, the Standing Committee on Cosmetic Products in the EU Commission has at their recent meeting of 14-15 February 2005 decided to ban the use of Lawsone as a cosmetic ingredient (9). Therefore, Lawsone (CAS no. 83-72-7) will be included in Annex II (List of substances which must not form part of the composition of cosmetic products) of the Cosmetics Directive 76/768/EEC.

With regard to Henna (*Lawsonia inermis*) (CAS no. 84988-66-9), it was decided by the Standing Committee on Cosmetic Products that “the submitted request needs to be supplemented in order for the SCCNFP to formulate a final opinion. Until then Henna (*Lawsonia inermis*) should be included in Annex III, Part 2”. This implies that the substance will be temporarily allowed until December 2005. Henna (*Lawsonia inermis*) is only allowed for hair-dyeing and not for dyeing of the skin (9).

TERMS OF REFERENCE

In a first request dated 23 March 2004 the Norwegian Food Safety Authority (Mattilsynet) asked the Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) to consider the mutagenic risk of the two cosmetic ingredients Lawsone and Henna (*Lawsonia inermis*).

The Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food, and Cosmetics discussed the first request from the Norwegian Food Safety Authority in a meeting on 23 February 2005. It was then decided to ask for a revised request from the Norwegian Food Safety Authority due to the recent decision by the EU Commission to ban Lawsone as such as a cosmetic ingredient.

The Norwegian Scientific Committee for Food Safety received a revised request on 26 April 2005. In this new request VKM is asked to consider the health risk related to the use of Henna for hair-dyeing. The assessment should be limited to assess a possible cancer risk by assuming that all Henna products contain 1.5 % of Lawsone as described by SCCNFP in their opinion adopted 17 September 2002 (4). Another calculation should be based on the highest concentration of Lawsone in Henna products for hair-dyeing being 0.3%, as has been found in a recent Danish survey (Hans Jørgen Talberg, personal communication).

ASSESSMENT

The request has been assessed by the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food, and Cosmetics of the Norwegian Scientific Committee for Food Safety.

As it is emphasised in the revised request from the Norwegian Food Safety Authority, Lawsone as such has been banned as a cosmetic ingredient. The comments from the Panel will therefore be on Henna (*Lawsonia inermis*) where Lawsone is the principal colouring ingredient. The last opinion concerning Lawsone adopted by the SCCNFP on 16 February 2004 (1), where Lawsone is considered to have genotoxic/mutagenic potential *in vitro* and *in vivo*, is used as a basis for the Panel's comments.

Hazard characterisation

Much of the discussion concerning whether Lawsone should be considered to have similar properties as mutagens classified as category 3 according to Council Directive 67/548/EEC (10), is based on whether two micronucleus studies in mice (72 h sampling time) from Österreichisches Forschungszentrum from 1989 and 1990 should be concluded as positive or not (11,12). The first study states: "There was a significant increase in the incidence of micronucleated polychromatic erythrocytes (MPE) in the 72 hour test group (combined males and female data) but not at the other harvest times. The positive control agent gave the expected results. The substance was positive in the micronucleus assay." In the second study it is stated: "There were significant increases in the incidence of micronucleated polychromatic erythrocytes at 110 and 250 mg/kg bw (combined males and female data) but not at 25 mg/kg bw. The results show evidence of a positive dose response relationship and were reported to be increased beyond the range of the historical negative control data. The positive control agent gave the expected results. The study confirmed the results of the previous study." The lowest effective dose (LED) after oral administration giving a positive response in the *in vivo* micronucleus test was 110 mg/kg bw.

The relevance of the positive effects at 72 hours is disputed (6,7,8). If, however, the results at 72 hours are accepted as positive it implies that Lawsone has similar properties as mutagens classified as category 3.

No quantitative or semi-quantitative hazard characterisation methods are currently in use for regulatory purposes of mutagens. Mutagens demonstrated to be carcinogenic are in general regulated on the basis of their carcinogenicity since the carcinogenic effect is considered to be more critical than germ cell mutagenesis. In the case of mutagens where no carcinogenicity studies are available, no safe level of exposure can be established. However, the recent publication of Sanner and Dybing (13) demonstrating a linear relationship between LED after oral administration for *in vivo* genotoxicity and the carcinogen dose descriptor T25, with similar numerical values within a factor 5-10, may offer a pragmatic approach for a semi-quantitative cancer risk assessment.

Exposure characterisation

The Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation (SCCNFP/0690/03 Final) (14) indicate a weekly use of 35 ml for a semi-permanent hair dye, with a retention factor of 0.1. The area of skin related to the use of hair dyes is 580 cm².

According to the terms of reference a worst case exposure scenario is calculated assuming that the concentration of Lawsone in Henna products is 1.5%. The Panel has also estimated the exposure to Henna products for hair-dyeing based on the highest concentration of Lawsone (0.3%) detected in a recent survey carried out in Denmark (Hans Jørgen Talberg, personal communication).

With reference to the Notes of Guidance from SCCNFP the systemic exposure dosage (SED) of Lawsone in a finished cosmetic product can be calculated according to two different models for dermal absorption, depending on whether the dermal absorption is reported in $\mu\text{g}/\text{cm}^2$ or as a percentage of the substance applied (14).

In the SCCNFP Opinion of 16.02.04 the absorbed amount of Lawsone was reported to be $2.6 \pm 1.8 \mu\text{g}/\text{cm}^2$ based on a study with human dermatomed skin and the Franz diffusion cell method. In this experiment 2 % Lawsone in a hair dye formulation was used (1).

In a recent abstract from Kraeling *et al.* (15) the absorption of Lawsone was assessed in the case of two hair colour pastes using non-viable human skin mounted in flow-through diffusion cells. For the Henna paste products 0.29 and 1.4%, respectively, of the applied doses were absorbed into the receptor fluid in 24 h after the hair colour paste had remained on the skin for 1 h.

Below, the systemic exposure dose has been calculated both on the basis of μg absorbed per cm^2 using $2.6 \pm 1.8 \mu\text{g}/\text{cm}^2$ (1) and on percent absorbed using the highest percentage (1.4%) reported by Kraeling *et al.* (15).

Exposure based on μg absorbed per cm^2

$$\text{SED} = \frac{\text{DA}_a (\mu\text{g}/\text{cm}^2) \times 10^{-3} \text{ mg}/\mu\text{g} \times \text{SSA} (\text{cm}^2) \times \text{F} (\text{day}^{-1}) \times \text{R}}{60 \text{ kg}}$$

SED = (mg/kg bw/day) =	Systemic Exposure Dosage
DA _a ($\mu\text{g}/\text{cm}^2$) =	Dermal Absorption reported as amount/ cm^2
SSA (cm^2) =	Skin Surface Area expected to be treated with the finished cosmetic product
F (day^{-1}) =	Frequency of application of the finished product
60 kg =	default human body weight (bw)

The following values were used:

Dermal Absorption (DA _a)	= $2.6 \mu\text{g}/\text{cm}^2$ (1)
Skin Surface Area (SSA)	= 580 cm^2
Frequency of application of the finished product (F)	= $[1/7] \text{ day}^{-1}$
Retention factor (R)	= 0.1

$$\begin{aligned} \text{Systemic exposure dose (SED)} &= \frac{2.6 \mu\text{g}/\text{cm}^2 \times 10^{-3} \text{ mg}/\mu\text{g} \times 580 \text{ cm}^2 \times 1 \text{ day}^{-1} \times 0.1}{60 \text{ kg} \times 7} \\ &= 0.0004 \text{ mg/kg bw/day} \\ &= \mathbf{0.4 \mu\text{g}/\text{kg bw/day}} \end{aligned}$$

Exposure based on percent absorbed

$$\text{SED} = \frac{A \text{ (g/day)} \times 1000\text{mg/g} \times C \text{ (\%)} / 100 \times \text{DA}_p \text{ (\%)} / 100 \times R}{60 \text{ kg}}$$

SED = (mg/kg bw/day) =	Systemic Exposure Dosage
A (g/day) =	Amount of the cosmetic product applied daily
C (%) =	the Concentration of the ingredient under study in the finished cosmetic product on the application site
DA _p (%) =	Dermal Absorption expressed as a percentage
60 kg =	default human body weight (bw)

The following values were used:

Maximum absorption through the skin (DA _p)	= 1.4% (15)
Exposure to hair dye formulation (A)	= 35 g/week x [1/7]
Retention factor (R)	= 0.1
Concentration of dye in the formulation (C)	= 1.5 %

$$\begin{aligned} \text{Systemic exposure dose (SED)} &= \frac{35 \text{ (g/day)} \times 1000\text{mg/g} \times 1.5 \text{ (\%)} / 100 \times 1.4 \text{ (\%)} / 100 \times 0.1}{60 \text{ kg} \times 7} \\ &= \mathbf{0.0018 \text{ mg/kg bw/day}} \\ &= \mathbf{1.8 \text{ }\mu\text{g/kg bw/day}} \end{aligned}$$

A similar calculation based on the highest concentration of Lawsone (0.3%) detected in Henna products in the recent survey carried out in Denmark (Hans Jørgen Talberg, personal communication) will result in an estimated SED of **0.4 μg/kg bw/day**.

Based on the dermal absorption model reported in μg/cm² a SED of 0.4 μg/kg bw/day is calculated. If the model based on percentage absorbed is used, and assuming a concentration of Lawsone in Henna products of 1.5 %, a SED of 1.8 μg/kg bw/day is estimated. Generally the latter model would overestimate the dermal absorption since the absorption experiment (15) was performed with a product on the market likely to contain a lower concentration of Lawsone. Using a concentration of 0.3 % which was the highest amount found in the Danish study, a SED of 0.4 μg/kg bw/day was calculated in this model. In the following risk characterisation we will therefore use the exposure based on μg absorbed per cm² (= 0.4 μg/kg bw/day) as used by SCCNFP in their last opinion (1).

Risk characterisation

The possible carcinogenic risk of *in vivo* mutagens is in general considered to be more critical than germ cell mutagenesis. The linear relationship with similar numerical values between the lowest effective dose (LED) showing *in vivo* genotoxicity after oral administration and the carcinogen dose descriptor T25 demonstrated in a recent publication of Sanner and Dybing (12) may offer a pragmatic approach for a semi-quantitative cancer risk assessment. Thus LED divided with an extrapolation factor of 25 000 would correspond to a lifetime cancer risk of 10⁻⁵. The magnitude of the extrapolation factor to be used may depend on a number of

factors. In the case of Lawsone there are different views as to whether the substance fulfills the criteria for classification as a mutagen category 3. Since the ratio LED (110 mg/kg bw/day)/25 000 is equal to 4.4 µg/kg bw/day and thus greater than SED (0.4 µg/kg bw/day), it follows that the possible cancer risk associated with the use of Henna products for hair-dyeing containing Lawsone would be negligible. The margin of exposure (MoE) would be approximately 275 000 (LED/SED = 110 000 µg/kg bw/day/ 0.4 µg/kg bw/day).

CONCLUSION

The Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food, and Cosmetics of the Norwegian Scientific Committee for Food Safety is of the opinion that if Lawsone is considered as an *in vivo* mutagen and using an estimated systemic exposure dose based on a worst case use situation, the possible cancer risk associated with the use of Henna products for hair-dyeing containing Lawsone would be negligible.

ASSESSED BY:

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