OPINION OF THE SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD PRODUCTS INTENDED FOR CONSUMERS

CONCERNING

LAWSONIA INERMIS, HENNA

COLIPA n° C169

adopted by the SCCNFP during the 21st Plenary meeting
of 17 September 2002

1. Terms of Reference

1.1 Context of the question

The adaptation to technical progress of the Annexes to Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products.

1.2 Request to the SCCNFP

The SCCNFP is requested to answer the following questions :

* Can Lawsonia inermis derived from the dried leaves of the mentioned plant be safely used in cosmetic hair dye formulations?

* Does the SCCNFP propose any restrictions or conditions for its intended use?

1.3 Statement on the toxicological evaluation

The SCCNFP is the scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food products intended for consumers.

The Commission's general policy regarding research on animals supports the development of alternative methods to replace or to reduce animal testing when possible. In this context, the SCCNFP has a specific working group on alternatives to animal testing which, in co-operation with other Commission services such as ECVAM (European Centre for Validation of Alternative Methods), evaluates these methods.
The extent to which these validated methods are applicable to cosmetic products and its ingredients is a matter of the SCCNFP.

SCCNFP opinions include evaluations of experiments using laboratory animals; such tests are conducted in accordance with all legal provisions and preferably under chemical law regulations. Only in cases where no alternative method is available will such tests be evaluated and the resulting data accepted, in order to meet the fundamental requirements of the protection of consumer health.

2. Toxicological Evaluation and Characterisation

2.1. General

2.1.1. Primary names

Here are considered in practice two different cosmetic ingredients :

- *Lawsonia inermis* with EINECS n° 284-854-1 corresponding to extracts and their physically modified derivatives from *Lawsonia inermis*, Lythraceae,

Henna with EINECS n° 201-496-3 corresponding to 2-hydroxy-1,4-naphthoquinone, the main active ingredient present at 1 to 2 % in the dried leaves of the plant.

2.1.2. Chemical names

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2.1.3. Trade names and abbreviations

Henna extract Hennapulver Henna Rot
Henna powder Lawsonia alba

2.1.4. CAS no.

84988-66-9 (*Lawsonia inermis*)
83-72-7 (Henna)

2.1.5. Structural formula

Not applicable

2.1.6. Empirical formula

Emp. Formula : /
Mol weight : /

2.1.7. Purity, composition and substance codes

Specifications (source, form, preparation and chemical characteristics) are incomplete.

2.1.8. Physical properties

Subst. Code	:	/
Appearance	:	greenish powder
Melting point	:	/
Boiling point	:	/
Density	:	/
Rel. vap. dens.	:	/
Vapour Press.	:	/
Log P_{ow}	:	/

2.1.9. Solubility

No data available

2.2. Function and uses

Lawsonia inermis, syn. Henna, represents a natural material derived from powdered dried leaves of the specified plant. It is mainly used as a hair dye, based on the staining properties of the main active ingredient Lawsone.

A hair dye formulation contains a maximum of 20% Henna powder suspended in 80 % water.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

An acute oral toxicity study of Henna Rot was performed in the Sprague-Dawley Rat according to the OECD Guideline N°401 (1981).
The calculated oral median lethal dose was > 2000 mg/kg bw.

Ref. : 1

2.3.2. Acute dermal toxicity

An acute dermal toxicity study of Henna Rot was conducted in the Wistar Rat according to OECD Guideline N°402 (1987).
Median lethal dose for Henna Rot was > 2000 mg/kg bw.

Ref. : 2

2.3.3. Sub-chronic oral toxicity

A 13-week oral toxicity study was conducted in Sprague-Dawley rats (4 groups of 10 rats per sex) with a 0.5 % aqueous methylcellulose solution of Henna Rot administered once daily by gavage according to the OECD Guideline N° 408. The treated animals received the test substance corresponding to daily dosage of 40, 200 and 1000 mg/kg body weight. Control animals received the vehicle alone under the same conditions. In addition, 10 males and 10 females were included in the control and high dose group for a 4-week recovery period.

No mortality was observed during the study. In the high dose group, 2/40 animals occasionally presented signs of poor clinical condition (loud breathing, piloerection) and 6/20 males presented ptyalism from week 9 or 11 onwards. Brown urine and/or tail was noted in almost all animals. All clinical signs were reversible after 4-weeks recovery period, except for brown-coloured tail.

In the high dose group, the hair and body extremities as well as the fore-stomach and the mucosa of the bladder were coloured orange related to the staining properties of the test substance. Mean food consumption and body weight gain of the treated males were in the range of controls ; mean body weight gain of the females given 40 or 1000 mg/kg/day was slightly lower than that of control but this finding was not dose-dependent and was not considered by investigators to be treatment-related. Neither treatment-related ophthalmological abnormalities nor effects in clinical chemistry (blood biochemistry, urinalysis) were noted in any treated group.
Concerning haematological parameters, slightly lower erythrocyte count and haemoglobin were noted in the high dose group when compared to the control values, these differences were considered of no toxicological importance by the investigators. In the highest dose group, statistically significant higher mean kidney and spleen weights were noted.
All these findings were reversible after 4-weeks recovery period except for the kidney weight of the females, but it was considered to be of no toxicological importance by the investigators as no relevant microscopic findings were noted.

Concerning microscopic examinations, no findings of toxicological relevance were noted at the low dose level. In the 200 mg/kg/day group, minimal to slight hemosiderosis was noted in the spleen. In the high dose group, minimal to moderate accumulation of acidophilic globules in the cortical tubular epithelium of the kidneys were recorded and were considered to be treatment-related. In the 200 mg/kg/day and in the 1000 mg/kg/day groups, minimal to slight hemosiderosis and some extramedullary hemopoiesis were noted in the spleen. Except for the hemosiderosis in the spleen and the dying effects in the high dose group, all findings were reversible during the recovery period.

Based on these results, the NOAEL (No-Observed-Adverse-Effect-Level) of Henna Rot was established to be 40 mg/kg bw.

Ref. : 3

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

Guideline	:	OECD 402 (1987)
Species/strain	:	Wistar rat
Group size	:	5 male and 5 female
Test substance	:	Not given, report not available
Batch no	:	Not given
Dose	:	2 g/kg bw
GLP	:	Not given

The test was performed to assess acute dermal toxicity. No skin irritation was noticed during the observation period.

Ref. : 2

2.4.2. Irritation (mucous membranes)

Guideline	:	OECD guideline 405 (1987)
Species/strain	:	New Zealand white rabbit
Group size	:	3 rabbits, 6 not specified
Test substance	:	Henna Rot
Batch no	:	830.72
Dose	:	0.1 ml of the test material, approximately 58 mg
GLP	:	QA statement included

The test substance was applied into the conjunctival sack of the right eye of 3 rabbits, without rinsing. The left eye served as control and was untreated. Ocular reactions were recorded at 1 hour and 1-3 days after instillation.

No adverse corneal affects were noted. Iridial inflammation and moderate conjunctival irritation was observed up to a maximum of 48 respectively 72 hours. All treated eyes appeared normal on day 7. The test material produced a maximum mean score of 17.0 and was classified as a moderate irritant (class 5 on a 1-8 scale) to the rabbit eye. The results were interpreted and classified as “a non-irritant” to the rabbit eye.

Ref. : 4

2.5. Sensitisation

Buehler-delayed contact hypersensitivity study

Guideline	:	OECD 406 (1981)
Species/strain	:	Dunkin Hartley guinea pigs
Group size	:	4 guinea pigs were used for pilot study to select the concentration used for topical induction and topical challenge. Main study included 20 tests and 10 control animals
Test substance	:	Henna Rot
Batch no	:	830.72

Concentration	:	Topical induction	:	50% (w/w) in petrolatum jelly B.P.
		Topical challenge	:	50% (w/w) in petrolatum jelly B.P.
GLP	:	QA statement included		

A topical application (0.5 ml) of the test material was applied on absorbent lint (approximate size 15 mm x 35 mm) which was occluded for 6 hours under a strip of Blenderm tape and further secured with an elastic adhesive bandage wound in a double layer around the torso of each animal. The induction procedure was repeated on the same site on days 7 and 14 for a total of three 6-hour exposures.

Challenge was performed on day 28, an area approximately 50 mm x 70 mm on the right flank of each animal was clipped, and 0.5 ml of the test material was applied on absorbent lint (approximate size 15 ml x 30 ml) occluded with Blenderm tape and an adhesive bandage. Approximately 24 and 48 hours after dressing removal test reactions were quantified using a 4-point ranking scale.

No adverse reactions were noted, and the test material was classified as a non sensitiser to guinea pig skin.

Ref. : 5

Repeated insult patch test in humans

Repeated insult patch testing according to a modified protocol as described by Shelanski and Shelanski, Proc. Sci. T.G.A., 19, 46-49, 1953; Ludwig J. Soc. Cosm. Chem. 27, 345-349, 1976 was performed with Henna Rot, Batch no 830.72, on a panel of 10 volunteers. Test concentration was 10% in petrolatum. No skin changes were observed in the test area of any of the volunteers at any time during the 3 weeks of testing and challenge. Further details on the test procedure were not given. The investigators concluded that the test preparation is not irritating and is not likely to cause allergic reactions.

Ref. : 6

Experience under specific conditions in humans

The submission includes 3 references on side effects experienced in humans under intended usage of henna.

A beautician with known allergy to house dust experienced rhinoconjunctivitis, asthma, and once generalised urticaria after exposure to henna. The symptoms increased in severity after continued exposure. Scratch tests with henna powder were strongly positive. By thin-layer-chromatography the red colour and 2-hydroxy-1,4-naphthoquinone were isolated from the extract. These materials gave negative scratch tests. The result showed that the antigen is neither the quinone nor the red colour but an undertermined impurity.

Ref. : 7

As reported in the literature, Henna is widely used as a hair dye in some countries like India and Egypt and also for skin paintings in ceremonies. Under such excessive conditions of skin contact

the reported effects of contact allergies are considered very rare by the authors. Two case reports from India describe allergic contact dermatitis from henna.

Ref. : 8 , 9

A literature search of Pub Med, April 2001, using the search phrase: "henna and allergy" revealed a number of other scientific reports on immediate type reactions and contact dermatitis caused by henna:
A case is reported of a hairdresser who developed an immediate type hypersensitivity with urticaria, rhinitis, and bronchial asthma on exposure to henna. Prick tests with henna 1% in aqua and in ethanol showed positive reactions. Both patch tests and prick tests performed with the dye in henna, Lawsone or 2-hydroxy-1.4-naphthoquinone, which is supposed to be an allergen gave negative results. These data suggest that not only 2-hydroxy-1,4-naphthoquinone, but also other still undetermined ingredients of the henna powder should be considered as possible allergens.

Ref. : 10

Poisoning by a mixture of henna dye and para-phenylenediamine dyes caused hospitalisation of 31 Sudanese children between 1984 and 1989. There was a characteristic clinical presentation. All children presented with an acute and severe angioneurotic oedema and 15 of the cases required emergency tracheostomy for respiratory obstruction. Acute renal failure occurred in 5 children who recovered after peritoneal dialysis. Mortality was high, in all 13 deaths occurred within 24 hours of presentation. Hypotensive shock gave a poor prognosis. It is possible that similar cases may be occurring unrecognised where henna is traditionally used. A program of public education and restriction of use of para-phenylenediamine is urgently required in the Sudan and other affected nations. Injection was accidental in 12 children, deliberate in 1 and homocidal in 3 cases. Cutaneous absorption was likely in the remaining 6 cases.

Ref. : 11

2.6. Teratogenicity

The product Henna Rot was administered by daily gavage to 100 pregnant female Sprague Dawley rats on day 6 through 15 of gestation at the dose levels of 40, 200 and 1000 mg/kg/day body weight and at a constant dose volume of 10 ml/kg/day according to the OECD N°414 (1981). A control group was administered with the vehicle alone, a 0.5 % aqueous solution of methylcellulose. Pregnant animals were killed on day 20 of gestation ; macroscopic examinations of the dams were performed, visceral and skeletal malformations were recorded on the foetuses. No clinical signs, no abortions and no mortalities were recorded in any female of any group during the study. However, a very slight but significant decrease of body weight gain and food consumption was observed with the dams receiving 1000 mg/kg/day and was considered by investigators treatment-related. Pre and post-implantation loss, foetal body weight and sex-ratio were similar between control and all treated groups. At the external examination, no treatment-related anomalies or malformations were observed. In the highest dose group, two foetuses revealed dilatation of cerebral ventricles (lateral ones or 3rd one) and one foetus revealed cleft palate. At the skeletal examinations, reduced ossification of the pubic bone and cleft palate in one foetus were noted in the 200 mg/kg/day group ; a significant reduced ossification of caudal vertebra and unossification of the 5th sternebra and the caudal vertebra, an

increase in reduced ossification of the 1st to 4th metatarsals and of the pubic bone were noted in the 1000 mg/kg/day group. These foetal findings recorded at 200 and 1000 mg/kg/day were considered by investigators to be probably treatment-related.

Under the experimental conditions adopted, the NOAEL of the test product, Henna Rot, was established at 200 mg/kg/day for the pregnant female rats and at 40 mg/kg/day for the rats foetuses.

Ref. : 12

2.7. Toxicokinetics (incl. Percutaneous Absorption)

Percutaneous absorption in vitro

Guideline	:	Not available
Tissue	:	Isolated pig skin, frozen samples (-20°)
Method	:	Permeation chambers (flow through system)
Test substance	:	2-hydroxy-1,4-naphthoquinone (C146) (purity 98%) and 25% Lawsonia powder in aqueous preparation
Batch no	:	Not given
Dose levels	:	2% C146 ethanol 1% C146 in Koleston 2000 (16 hours) 10% C146 in Koleston 200 25% C146 in aqueous preparation 25% Lawsonia powder in aqueous preparation
Replicate cells	:	5 experiments with a total of 30 permeation chambers
GLP	:	Study not in compliance

The preparation of the skin pieces is not described in detail in the report. The skin pieces were fixed in permeation cells and 0.1 g/cm2 of the formulation was exposed for 30 minutes. Then the residues were removed by a spatula and the skin was washed using water and no shampoo. After an incubation time of 72 hours percutaneous permeation was determined. After extraction, the amounts of 2-hydroxy-1,4-naphthoquinone were measured by HPLC.

In the experiments using aqueous 25% *Lawsonia inermis* to resemble intended use conditions, 2-hydroxy-1,4-naphthoquinone (Lawsone) permeated though pig skin in vitro. After an exposure time of 30 minutes and a follow-up period of 72 hours only 0.28% of the applied dose of 2-hydroxy-1,4-naphthoquinone was found in receptor fluid and 0.006 ± 0.003% in the skin. The results correspond to a permeation of 703 ± 232 µg/cm2.
The efficiency of the method was documented by experiments using 2-hydroxy-1,4-naphthoquinone. However, the study did not include determination of recovery of the test substance. Water was used as a receptor fluid, which may not be adequate for a relatively lipophilic substance.
The study is considered inadequate according to the SCCNFP Notes of Guidance.

Ref. : 13

Percutaneous absorption *in vivo*

Guideline	:	None available
Test animal	:	Sprague Dawley rats (HIM: OFA, SPF), 5 males and 5 females
Test substance	:	1.5% of 14C labelled 2-hydroxy-1,4-naphthoquinone mixed with 23.5% henna powder and 75% de-ionised water and formulated into a paste shortly before application, and in a third study 25 mg/ml of ^{14}C labelled 2-hydroxy-1,4-naphthoquinone dissolved in DMSO/water 3:1.
Batch no	:	Not given
Dose levels	:	Cutaneous formulations : 0.509 and 0.517 g/animal, and 0.85 and 0.86 mg/cm²
		Cutaneous solutions of test substance 0.318 g/animal, 0.88 mg/cm²
GLP	:	not given

Two experiments were performed with the formulation, one with a sampling time of 72 hours and one with a sampling time of 24 hours. The formulation was spread with a spatula to an area of 3 x 3 cm to the dorsal, median thoracic to lumbar area. The solution was applied and spread evenly until the skin was wetted. The formulation or the solution was left for 40 minutes and then rinsed off. The animals were anaesthetised and held tightly during the contact period.
The treated areas were covered, and the rats subsequently placed into the metabolism cages.
Animals in both studies were sacrificed after 72 hours and samples were drawn from rinsing water, treated skin, urine, faeces, organs, carcasses.

The mean percutaneous absorption of Lawsone was 0.20% of the administered activity for the formulation and 5.5% for the solution. The ^{14}C labelled substance was excreted mainly via urine (86-89% of the eliminated radioactivity) and to a lesser extent via faeces (11-14% of the eliminated radioactivity). Within the first 24 hours the mean excretion was 91-93% of the eliminated radioactivity. In the group exposed to the paste mean radioactivity levels of blood and the 14 analysed organs were all near or below the detection limit at 72 hours after application.
Relatively highest concentrations were found in blood, kidneys and ovaries ; lowest in muscle, heart and femur.

The study seems to be acceptable. However, the full report should be provided.

Ref. : 14

2.8. Mutagenicity/Genotoxicity

2.8.1. Mutagenicity/Genotoxicity in vitro

Bacterial Gene Mutation test

Henna Rot has been tested for mutagenicity potential *in vitro* on *Salmonella typhimurium* 5 strains (TA98, TA100, TA1535, TA1537, TA1538) according to OECD 471 (1983), using a plate incorporation protocol. Liver S9 fraction from rats (pre-treated with Phenobarbital and β-Naphthoflavone) was used as the exogenous metabolic activation system. The concentration range was selected following a preliminary study which showed no toxicity up to a dose of 5000 µg/plate.

There were no significant increases in revertants in any of the tested strains, with or without S9 metabolic activation. The positive control agents gave the expected results. Concentrations were between 50 – 500 µg/plate ± S9.
No information was provided about the composition of the test substance.
No conclusions can be drawn about the potential mutagenicity *in vitro* of this hair dye on the bacterial gene mutation assay.

Ref. : 15

Henna Rot was tested on *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538. (S9 mix from rat liver + Aroclor) TA98, TA100, TA1535, TA1537, TA1538, and streptomycin resistant strains because the test material was contaminated with bacteria at concentrations higher than 5000 µg/plate.
Concentrations used were 50 to 5000 µg/plate ± S9 mix.
There were no significant increases in revertants in any of the tester strains and protocols, with or without metabolic activation. The positive control agents gave the expected results.
No information was provided about the composition of the test substance and its bacterial contamination.

No conclusions can be drawn about the potential mutagenicity *in vitro* of this hair dye on the bacterial gene mutation assay.

Ref. : 16

Mammalian Cell Gene Mutation assay

Henna Rot was tested for mutagenicity potential *in vitro* on Chinese Hamster V79 cells according to OECD 476 (1984) for the induction of gene mutation at the locus HPRT in the presence and absence of S9 metabolic activation system provided by rat livers pre-treated with Aroclor.
Concentrations were : 1, 5, 10, 50, 100, 200 µg/ml (-S9), 10-1000 µg/ml, 10-3000 µg/ml (+S9).
Henna was toxic at 200 µg/ml in the absence of a metabolic activation system ; no toxicity was observed in the presence of a metabolic activation system.
There were no increases with statistical significance on the mutation frequency observed in all evaluable doses, although in the presence of a metabolic activation system in a replicate experiment some increases were observed without a dose related effect.
No information was provided about the composition of the test substance.
No conclusions can be drawn about the potential mutagenicity *in vitro* of this hair dye on the *in vitro* mammalian gene mutation assay.

Ref. : 17

Henna Rot was tested for mutagenicity potential *in vitro* on Mouse lymphoma L5176Y cells according to OECD 476 and EEC/87/302 for the induction of gene mutation at the locus TK in the presence and absence of a metabolic activation system provided by rat livers pre-treated with Aroclor.
Concentrations doses were from 78.13 to 1250 µg/ml in both two experiment.
There was a dose-related response in the induced mutant frequencies both with and without S9 in the presence of Henna rot in two repeated independent experiments.
Positive controls gave the expected results.
It can be concluded that the batch 830.72 of Henna rot, whose composition is unknown, is mutagenic in the *in vitro* mammalian gene mutation assay.

Ref. : 18

Mammalian cytogenetic assay in human lymphocytes

Henna Rot was tested for mutagenicity/genotoxicity potential *in vitro* on human lymphocytes according to OECD 473 (1981) and EEC 84/449 for the induction of chromosome aberrations ; in the absence and presence of S9 metabolic activation system by rat livers pre-treated with Aroclor.
There was a dose-related inhibition of the mitotic index at 20 hours of harvest. Henna Rot induced a statistically significant increase in the frequency of cells with chromosome aberrations (excluding gaps) in the presence of S9 in both cultures at the maximum evaluable dose.
Positive controls gave the expected results.
Concentrations were 78 – 625 µg/ml in the absence of S9 ; 12501 µg/ml in the presence of S9.
It can be concluded that the batch 830.72 of Henna rot, whose composition is not known, is mutagenic in the *in vitro* human lymphocytes cytogenetic assay.

Ref. : 19

***In vitro* Sister Chromatid Exchange Assay in Mammalian Cells**

Henna Rot of unspecified purity was tested for genotoxicity potential *in vitro* on Chinese hamster cell line (CHO) according to OECD (1986) for the induction of Sister Chromatid Exchange, in the absence and presence of S9 metabolic activation system provided by rat liver pre-treated with Aroclor.
Concentrations were 25 – 100 µg/ml in the absence of S9, 200 – 800 µg/ml in the presence of S9.
In the absence of S9 24h and 3h exposed cells were analysed ; in the presence of S9 3h exposed cells only was analysed. There was a reduction of the mitotic index at the high concentration tested in the two performed experimental procedures.
There was no increase of the SCE frequencies in all treated conditions of Henna rot tested on CHO cell line.
The positive controls gave the expected results. No information was provided about the composition of the test substance.
No conclusions can be drawn about the potential genotoxicity *in vitro* of this hair of dye on the *in vitro* mammalian Sister Chromatid Exchange.

Ref. : 20

2.8.2. Mutagenicity/Genotoxicity in vivo

Mouse bone marrow micronucleus test

Henna Rot was tested *in vivo* on mice treated via i.p. for mutagenicity/genotoxicity potential for the induction of micronucleated cells in the bone marrow cells three times after treatment (24, 48, 72 hours) according to OECD 474 (1983).
Dose levels of 0 and 300 mg/kg bw were injected i.p to 5 CD1 mice of each sex.
A significant increase in the Normochromatic polychromatic erythrocytes ratio was observed in Henna rot treated mice.
The positive control gave the expected results (cyclophosphamide 50 mg/kg).
There was no increase in the number of PCE with micronuclei in the bone marrow cells of the treated mice sacrificed 24, 48 and 72 hours.
No information was provided about the composition of the test substance.
No conclusions can be drawn about the potential mutagenicity/genotoxicity *in vivo* of this hair dye on the induction of micronuclei in the bone marrow cells of mice, due to the inadequacy mentioned.

Ref. : 21

2.9. Carcinogenicity

No data available

2.10. Special investigations

No data available

2.11. Safety evaluation

CALCULATION OF THE MARGIN OF SAFETY

()

(Non-oxidative)

NOT APPLICABLE

Based on a usage volume of … ml, containing at maximum … %

Maximum amount of ingredient applied	**I (mg)**	**=**	**mg**
Typical body weight of human		**=**	**60 kg**
Maximum absorption through the skin	**A (%)**	**=**	**%**
Dermal absorption per treatment	**I x A**	**=**	**mg**
Systemic exposure dose (SED)	**I x A / 60 kg**	**=**	**mg**
No observed adverse effect level (mg/kg) (species, route of application)	**NOAEL**	**=**	**mg/kg bw**

Margin of Safety	**NOAEL / SED**	**=**

2.12. Conclusions

Lawsonia inermis (Henna Rot) is not properly characterised. It is derived from dried powdered leaves of the specified plant and is intended to be used as a hair dye at a maximal concentration of 20 %, based on the staining properties of the main active ingredient 2-hydroxy-1,4-naphthoquinone.

* *Lawsonia inermis* is not irritant for the rabbit skin and eye. *Lawsonia inermis* 10 % in petrolatum was considered not irritating on human skin after repeated insult patch tests.

* *Lawsonia inermis* can be considered a weak sensitiser in the conditions of use. Few case reports on contact allergies are published.

* Based on the results obtained from the sub-chronic toxicity study conducted by oral route in the rat, the NOAEL of 40 mg/kg/day for embryo-toxicity and of 200 mg/kg/day for maternal toxicity was obtained.

* Several mutagenicity/genotoxicity assays have been performed. Positive reactions were observed only with mouse lymphoma assay and with metaphase analysis in human lymphocytes assay. Negative results have been observed with all other *in vitro* tests and with the *in vivo* micronucleus test in mice. However, Henna Rot contains 1,4-naphthoquinone which appears to be a genotoxic chemical. Moreover, the sample of Henna Rot which has been submitted to all mutagenicity assays (batch n° 830.72) has not been properly characterised.

Further *in vivo* genotoxicity tests are needed.

2.13. Opinion

Henna Rot contains normally 1 to 2 % of 2-Hydroxy-1,4-naphthoquinone. This chemical has been evaluated separately by the SCCNFP (doc. n° SCCNFP/0583/02, final).

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.

2.14. References

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2. RCC N° Tox Project 090236 (02.04.1993) Assessment of acute dermal toxicity with Henna Rot in the rat (Report RT 930112)
3. Centre International de Toxicologie (C.I.T.) Lab Study N°: 11297 TCR (12.5.1995) 13-week toxicity study by oral route in rats with a 4-week recovery period (Report R 9600381)
4. Safepharm Laboratories Limited, Project Number: 338/10 (14.11.1990) Henna Rot : Acute eye irritation test in the rabbit (Report R 9600379)
5. Safepharm Laboratories Limited, Project Number: 338/7 (23.11.1990) Henna Rot : Buehler-delayed contact hypersensitivity study in the guinea pig (Report R 9600380)
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13. Cosmital SA, Projekt-Nr. 7256/46 (12.2.1992) Die Kutanpermeation von 2-hydroxy-1,4-naphtochinon nach Applikation auf Schweinehaut in vitro (Report R 9600407)
14. Forschungszentrum Seibersdorf, OEFZS-A-2498 (04.02.1993) Toxicokinetics of 2-hydroxy-1,4-naphthoquinone (Report R 9600393)
15. FhG-ITA (Fraunhofer Institute for Toxicology and Aerosol Research, Hannover) study N° G90/18 (28.11.1990) Salmonella / Microsome mutagenicity test with Henna Rot (Report R 9600382)
16. FhG-ITA (Fraunhofer Institute for Toxicology and Aerosol Research, Hannover) study N° G91/19 (24.2.1992) Salmonella / Microsome mutagenicity test with Henna Rot (Report R 9600383)
17. FhG-ITA (Fraunhofer Institute for Toxicology and Aerosol Research, Hannover) study N° G94/12 (18.10.1994) In Vitro Mammalian Cell HRPT-Test (V79 Chinese Hamster Cells)

(Report R 9600384)
18. Safepharm Laboratories Limited, Project Number: 297/5 (25.2.1992) Henna Rot: OECD 476, Mutation of L5178Y mouse lymphoma cells at the thymidine kinase $TK^{+}/^{-}$locus; Fluctuation Assay (Report R 9600385)
19. Safepharm Laboratories Limited, Project Number: 297/6 (17.2.1992) Henna Rot: Metaphase analysis in human lymphocytes in vitro (Report R 9600389)
20. FhG-ITA (Fraunhofer Institute for Toxicology and Aerosol Research, Hannover) study N° 94/4 CM (1995) In Vitro Sister chromatid exchange assay in mammalian cells with Henna Rot (Report R 9600387)
21. Safepharm Laboratories Limited, Project Number: 436/4 (26.2.1992) Henna Rot : Micronucleus test in the mouse (Report R 9600390)
22. Annex : "Produktspezifikation für Lawsonia Folium" "Plant preparations used as ingredients of cosmetic products"