

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

CONCERNING

BASIC RED 76

COLIPA n° C8

adopted by the SCCNFP during the 23rd plenary meeting
of 18 March 2003

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 :

- * Is Basic Red 76 safe for use in cosmetic products?
- * Does the SCCNFP propose any restrictions or conditions for its use in cosmetic products?

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 name**

Basic Red 76 (INCI)

2.1.2. Chemical names

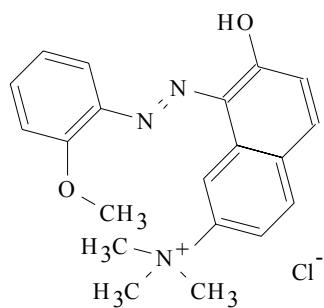
2-Methoxyphenylazo-1-(2-hydroxynaphthyl-7-trimethylammonium chloride)
 2-Naphthalenaminium, 7-hydroxy-8-((2-methoxyphenyl) azo)-N,N,N-trimethyl-, chloride).

2.1.3. Trade names and abbreviations

Arianor Madder
 CI 12245

2.1.4. CAS no. and EINECS no.

CAS no. 68391-30-0
 EINECS no 269-941-4

2.1.5. Structural formula**2.1.6. Empirical formula**

Emp. Formula : C₂₀H₂₂N₃O₂Cl
 Mol weight : 371.5 (as chloride)

2.1.7. Purity, composition and substance codes

Composition : Dye (as chloride) 55.5%

Sugar 16.0%
 volatile matter/water of crystallisation 14.0%
 inorganic salts (chloride, sulphate, etc.) to 100%

Purity of the dye (batch no.: Lot 18/19): >98% area-% (HPLC)

2.1.8. Physical properties

Subst. Code : COLIPA C 8
 Appearance : dark red powder, odourless
 Melting point : 165-175 °C (decomposition)
 Boiling point : No information
 Density : No information
 Rel. vap. dens. : No information
 Vapour Press. : No information
 Log P_{ow} : No information

2.1.9. Solubility

Soluble in water and ethanol

General comments on analytical and physico-chemical characterisation

The following issues do not, or poorly comply with the basic requirements for proper characterisation :

- * Purity of only one batch is described, and this batch is not used for the reported studies.
 The batch no. of the test substance used for several studies has not been reported.
- * Impurities in the test substances have not been reported.
- * Information on the 16% sugar content of the dye has not been reported.
- * Information on the 14.5% inorganic salt content of the dye is inadequate.
- * Data on Log P_{ow} and density of the test substance is not reported.
- * Information on the solubility of the dye does not provide quantitative data.
- * No information is provided on the stability of the test material in cosmetic formulations.

2.2. Function and uses

Basic Red 76 is used in direct hair dye formulations in concentrations up to 2.0%. It is common practice for 35 ml of undiluted formulation to be applied for a period of 30 minutes before washing. It is assumed that application may be repeated weekly.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

Study 1

Guideline	:	/
Species	:	CFY rat
Group size	:	2 male + 2 female
Material	:	Arianor Madder Red in 1% aqueous methylcellulose
Batch no	:	not stated
Dose	:	0, 0.1, 1.0, 4.0, 8.0 and 16.0 g/kg bw in a volume of 1 to 40 ml/kg
Observ. Period	:	14 days
GLP	:	not in compliance

Groups of 2 male and 2 female rats received a single oral dose of Arianor Madder Red at a range of dose levels from 0.1 to 16 g/kg bw. Control animals received vehicle alone. The animals were observed for 14 days for mortality and clinical abnormalities. Body weights and macroscopic observations were recorded, but histological examinations were not performed.

Results

No mortalities were reported. Signs of reaction to treatment included lethargy, piloerection, decreased respiratory rate and abnormal body carriage (hunched posture). Red staining of the urine and faeces was observed in rats treated with the material at dose levels of 8.0 and 16 g/kg body weight.

The acute lethal dose of Arianor Madder Red was reported to be greater than 16 g/kg bw.

Remark

This study predates OECD guideline 401 and does not conform to its requirement for 5 animals of each sex per group. Nevertheless, the study is considered acceptable for evaluation.

Ref. : 1

Study 2

Guideline	:	/
Species	:	CF1 mouse
Group size	:	3 or 10 males
Material	:	Arianor Madder Red in distilled water
Batch no	:	not stated
Dose	:	1.0, 2.51, 5.01 and 10.0 g/kg bw in a volume of 20 to 40 ml/kg
Observ. Period	:	7 days
GLP	:	not in compliance

Groups of 3 male mice received a single oral dose of Arianor Madder Red at dose levels of 1.0, 2.51 and 5.01 g/kg bw, 10 male mice received the top dose of 10 mg/kg bw. The animals were observed for 14 days for mortality and clinical abnormalities. Body weights and macroscopic observations were recorded, but histological examinations were not performed.

Results

No mortalities occurred. Following treatment lethargy and breathing disorders were noted in the mice in the highest dose group only.

The LD50 value was reported to be greater than 10.0 g/kg bw.

Remark

OECD guideline 401 specifies groups of at least 5 animals of each sex. The observation period should be at least 14 days. This study should have been conducted in compliance with the OECD guideline (or a validated alternative) and GLP.

Ref. : 2

2.3.7. Sub-chronic oral toxicity

Study 1

Guideline	:	/
Species	:	Wistar MuRa Han 67 SPF rat
Route	:	oral
Group sizes	:	20 male and 20 female + 10/10 recovery
Material	:	Commercial grade Arianor Madder Red in aqueous solution
Batch no	:	not stated
Dose levels	:	0 and 200 mg/kg bw in a volume of 10 ml/kg
Exposure	:	5 days per week for 12 weeks by gavage
GLP	:	not in compliance

Commercial Arianor Madder Red, in aqueous solution, was administered by oral gavage daily, 5 days per week for 12 weeks, to groups of 20 male and female Wistar rats at 200 mg/kg bw. The controls were treated with the vehicle alone.

All animals were observed daily for mortality and clinical signs. Body weight and food consumption were recorded at weekly intervals. Haematological, clinical chemistry and urine analyses were performed. At autopsy, organ weights were recorded and the main organs were examined macroscopically and histologically.

The study was conducted as a 'screening study' for dose finding purposes.

Results

No mortalities were observed. The dosed animals showed an aggressive behaviour compared to the control animals. The administration of the test material was difficult. The body weight gain was generally comparable to the control groups for the male animals. Slight but significantly lower mean body weights (95-96% of control) were recorded in female animals on the 5th, 7th, 9th and 12th week and at the end of the study. All dosed animals excreted coloured urine.

An increase of the mean cell volume and a significant increase in the hematocrit value (HT) were found in male animals. Similar trends were seen in some female animals. No clear treatment-related effects were seen following biochemical and urological evaluation.

The absolute organ weights showed a slight increase in the cerebral weights of male rats compared to the controls whereas in the female rats a diminution of the weights of the hearts, kidneys and liver within the respective groups was documented.

No differences between control and treated animals were observed at either the macroscopic or histopathological levels.

The NOAEL was reported to be lower than 200 mg/kg bw/day.

Ref. : 7

Study 2

Guideline	:	OECD 408 (1981)
Species	:	Sprague Dawley CD rat
Route	:	oral
Group sizes	:	10 male and 10 female + 5/5 recovery
Material	:	Commercial grade Arianor Madder Red in aqueous solution
Batch no	:	1077 (purity not stated)
Dose levels	:	0 and 20 mg/kg bw in a volume of 10 ml/kg
Exposure	:	5 days per week for 13 weeks by gavage
GLP	:	in compliance

Commercial Arianor Madder Red, in aqueous solution, was administered by oral gavage daily, 5 days per week for 13 weeks, to groups of 10 male and female Sprague Dawley rats at 20 mg/kg bw. The controls received the vehicle alone. Satellite groups of 5 rats of each sex were kept for a further 28 day treatment free period.

All animals were observed daily for mortality and clinical signs. Body weight and food consumption were recorded at weekly intervals. Haematological, clinical chemistry and urine analyses were performed. Ophthalmological examinations were performed at the start and end of the study. At autopsy, organ weights were recorded and the main organs were examined macroscopically and histologically.

Results

No mortalities occurred. The body weight gain of treated animals was comparable to the control group. There were no indications of treatment-related effects from clinical, macroscopic or microscopic examinations.

The dose of 20 mg/kg bw/day was considered to be a no effect level.

Ref. : 8

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

Test on undiluted material

Guideline	:	/
Species	:	New Zealand white rabbit
Route	:	skin
Group sizes	:	3 male and 3 female
Material	:	undiluted Arianor Madder Red
Batch no	:	9-1294N (purity not stated)
Dose	:	0.5 g/in ²
GLP	:	not in compliance

Arianor Madder Red was applied without any vehicle at the level of 0.5 g/in² to the backs of 3 rabbits of each sex with either shorn intact or scarified skin. The sample was occlusively covered and left in place for 24 hours. Readings were made according to Draize upon removal of the test material and daily for 14 days post administration.

Results

There were no observable reactions to the dye. The test material was considered “not irritant” to rabbit skin.

Ref. : 4

Test on diluted material

Guideline	:	/
Species	:	New Zealand white rabbit
Route	:	skin
Group sizes	:	3 (sex not specified)
Material	:	Arianor Madder Red moistened 1:1 with distilled water
Batch no	:	9-1294 N (purity not stated)
Dose	:	0.5 g
GLP	:	not in compliance

0.5 g of the test material was dampened with 0.5 ml distilled water and applied to an area of 1 in² on the backs of 3 rabbits each with either shorn intact or scarified skin. The sample was covered by an impervious material and left in place for 24 hours. Skin reactions were recorded after 24 and 72 hours.

Results

There were no observable reactions to the dye. The test material was considered as “not irritant” to rabbit skin.

Ref. : 5

2.4.2. Irritation (mucous membranes)

Guideline	:	/
Species	:	New Zealand white rabbit
Route	:	eye
Group sizes	:	3 (sex not specified)
Material	:	0.5% Arianor Madder Red solution in physiological saline

Batch no : not stated
 Dose : 0.1 ml
 GLP : not in compliance

0.1 ml of 0.5% solution Arianor Madder Red was instilled into the conjunctival sac of the left eye of three rabbits. The right eye was treated with 0.1 ml of the vehicle and served as a control. Eye reactions were recorded at 30 and 60 minutes and 1 and 2 days following and evaluated by the Draize method.

Results

The treatment provoked no effects on the cornea or iris in any of the test animals, however, there was a discolouration of the conjunctivae.

Remarks

This test was conducted at a concentration below the intended in-use maximum of 2%. A mild reaction could have been masked by the discolouration caused by the dye. Nevertheless, the study is considered acceptable for evaluation.

Ref. : 3

2.5. Sensitisation

Magnusson and Kligman test

Guideline : OECD 406
 Species : Hartley/Dunkin guinea pigs
 Group sizes : 10 female
 Material : Arianor Madder Red in aqueous solution
 Batch no : 9-1294 N
 Concentrations used :
 Intradermal induction : 0.1%
 Topical induction : 75%
 Challenge : 25%
 GLP : not in compliance

Arianor Madder Red was prepared as a 0.1% w/v solution in water (injection 1). Freund's complete adjuvant was diluted with an equal volume of water (injection 2). A 1:1 mixture of the material solution and Freund's complete adjuvant solution was prepared (injection 3). The induction of sensitisation was made through 3 pairs of 2 intradermal injections. One week after the injections a solution of 75% w/v of the material in distilled water was topically applied. The animals were challenged topically two weeks after the induction period using Arianor Madder Red at a concentration of 25% w/v. Potential skin reactions were recorded 24, 48 and 72 hours after the final challenge.

Results

The intradermal injection caused an irritation response, that was still present at the time of the topical induction. Following the challenge, erythema was observed in 5 of the animals at 24 hours, but had resolved by 48 hours.

The sensitisation potential is equivocal.

Ref. : 6

2.6. Teratogenicity

Guideline	:	/
Species	:	Sprague Dawley CD rat
Route	:	oral
Group sizes	:	Control group: 20; test group : 25
Material	:	Arianor Madder Red in distilled water
Batch no	:	KS 1077 (purity not stated)
Dose levels	:	0 and 50 mg/kg bw/day in a volume of 10 ml/kg
Administration	:	days 6-15 of gestation
GLP	:	in compliance

Arianor Madder Red was administered by gavage daily to 25 pregnant rats at a dose 50 mg/kg bw/day from days 6 to 15 of gestation. Twenty control animals were given the vehicle alone (distilled water). The dams were observed and weighed daily. On day 20 post coitum the dams were sacrificed and Caesarean sections were performed. The number of implantation sites, resorptions, living foetuses and the number of corpora lutea were counted in each litter. The weight of placenta, uterus, foetuses and the sex of the foetuses were recorded. About one-third of each litter was prepared and examined for soft tissue anomalies. The remaining foetuses were examined for skeletal abnormalities after staining with alizarin red S.

Results

Dams : Treatment with 50 mg/kg bw/day Arianor Madder Red resulted in no adverse effects in the dams. Bodyweight gain was comparable to controls.

Foetuses : There were no changes in reproduction data or malformations of the foetuses. The level of skeletal variation and/or ossification in the test and control groups were similar.

Thus, the test material was considered to produce no embryo-toxic or teratogenic effects under the employed test conditions up to a dose of 50 mg/kg bw/day.

Ref. : 9

2.7. Toxicokinetics (incl. Percutaneous Absorption)

Skin absorption study in human volunteers

Guideline	:	/
Method	:	Human volunteer study
Group size	:	10 males
Material	:	1 mM dye content in 40% aqueous isopropanol
Batch no	:	not stated
Application levels:	:	20 µl on 5.3 cm ² skin of the inner forearm
GLP	:	not in compliance

20 µl of a 1 mM solution of the test material, in 40% aqueous isopropanol, were applied to five separate skin areas (5.3 cm² s/c) of the inner forearm. After 10 minutes, and 24, 48 and 72 hours,

the dye stains of one treatment area after the other were removed by ten repeated strippings with Tesafilm-Spezial^R tape. During the intervals between sampling the skin areas were protected by a special non-occlusive mould. The stripping-tapes were glued on to a white cardboard and kept in the dark until they were used for densitometry. The amounts of the dye that possibly penetrated the skin were estimated from the recovery rates.

Results

It was reported that, using this method, the dye did not diffuse into the horny layer, according to the corrected recovery rates. It was concluded that Arianor Madder Red was not absorbed by the skin.

Remark

This study did not measure percutaneous absorption directly. The used methodology has been published.

Ref. : 14, 15

In vivo study in rats, study 1

Guideline	:	/
Species	:	Sprague Dawley rat
Group sizes	:	3 (sex not specified)
Route	:	topical
Material	:	¹⁴ C-Arianor Madder Red in a setting lotion formulation
Batch no	:	not stated
Dose levels	:	200 µl formulation
GLP	:	not in compliance

200 µl of a hair setting lotion formulation containing 0.1 % ¹⁴C-labelled Arianor Madder Red, was applied to the clipped dorsal skin of the rats. The application was made over an area of 1.5" x 1.5" (about 200 mg formulation/6.4 cm² corresponding to 31.3 mg/cm² skin or 31.3 µg Arianor Madder Red/cm² skin). The animals were lightly anaesthetised for the first hour, after which they were fitted with collars to prevent licking of the application site. Excretion of radioactivity via urine and faeces was measured for 24 hours after application.

Results

The recoveries of radioactivity in urine and faeces from the rats after topical administration were very low. The percentage of radioactivity recovered in the faeces was less than 0.2% and in the urine less than 0.3% of the applied dose. From this a maximum total absorption of 0.5% was deduced, corresponding to a maximum of 0.15µg per cm² of skin.

No further conclusions can be drawn from this study because of the absence of information on the amount of radioactivity retained in the carcass at the end of the study.

Ref. : 16

In vivo study in rats, study 2

Guideline	:	/
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Species	:	Wistar rat
Group sizes	:	5 male and 5 female
Route	:	topical
Material	:	¹⁴ C-Arianor Madder Red in a setting lotion formulation
Batch no	:	not stated
Dose levels	:	0.2%
GLP	:	not in compliance

The cutaneous absorption of radiolabelled ¹⁴C-Arianor Madder Red contained in a setting lotion was measured in a simulation of in-use conditions. A trimmed but not completely shaven area of the back was shampooed with 25 mg of a cream shampoo, dried with swabs and then 100 µl of a setting lotion containing 0.2% Arianor Madder Red (25 µg of test material/cm² skin) was applied on the moistened hair. The treatment site was covered with a non-occlusive protective glass capsule which contained small holes and was exposed for 24 hours to the test material. Radioactivity was determined in urine, faeces and in the carcass.

Results

After topical administration without rinsing and under non-occlusive conditions, most of the applied radioactivity was recovered on the hair (more than 80%, both for male and female animals) and on the skin (about 10% for both sexes). The radioactivity in urine and faeces was below the detection limits (for urine and faeces 0.07 and 0.16% respectively of the applied radioactivity). No radioactivity was detected in the carcasses. The assumed maximum absorption of 0.23% would have resulted in 0.06 µg of the Arianor Madder Red/cm² skin being absorbed cutaneously.

The results were published in a peer reviewed journal.

Ref. : 17

In vivo study in rats, study 3

Guideline	:	/
Species	:	Wistar rat
Group sizes	:	5 male and 5 female
Route	:	topical
Material	:	¹⁴ C-Arianor Madder Red in a shampoo formulation
Batch no	:	not stated
Dose levels	:	0.5%
GLP	:	not in compliance

The cutaneous absorption of radiolabelled ¹⁴C-Arianor Madder Red contained in a shampoo was measured in a simulation of in-use conditions. A trimmed but not completely shaven area of the back was pre-washed for 2 minutes with 70 µl of the shampoo formulation containing 0.5% of Arianor Madder Red, rinsed off 10 minutes later with a defined amount of lukewarm water and then dried with swabs. Then the hair was washed again with 140µl of the shampoo and treated in the same way as in the pre-wash step. The treatment side was covered with a non-occlusive protective glass capsule which contained small holes and was exposed for 24 hours to the remaining test material.

Radioactivity was determined in urine and faeces.

Results

After topical administration of the radio-labelled material in shampoo, including rinsing during the treatment procedure and conducted under non-occlusive conditions, most of the applied radioactivity was recovered in the rinsings (93 to 102 %). On the hair 5.6 and 8.3 % were found for male and female animals respectively, and 2.1 and 1.7 % on the treated skin. The radioactivity in urine was less than 0.007 % for male and 0.002% for female animals. In faeces less than 0.10 % of the applied radioactivity was found for both sexes.

Ref. : 17

Conclusion

The studies on percutaneous absorption are inadequate. The test concentrations were well below the expected use concentration.

2.8. Mutagenicity/Genotoxicity

Bacterial Reverse Mutation Test

Guidelines	:	OECD 471
Species/strain	:	<i>Salmonella typhimurium</i> , TA98, TA100, TA1535, TA1537, TA 1538
Substance	:	C8
Batch no	:	not given
Purity	:	not given
GLP	:	in compliance

Liver S9 fraction from liver rats pre-treated with Aroclor 1254 was used as the exogenous metabolic activation system.

Results

C8 has induced mutation in the strain TA1537 (with metabolic activation) and in the strain 1538 (in the presence and in the absence of metabolic activation).

Ref. : 13

In Vitro Mammalian Cell Gene Mutation Test

Guideline	:	OECD 476
Species/strain	:	Chinese hamster lung cells V79
Replicates	:	2 independent experiments
Substance	:	C8
Batch no	:	not given
Purity	:	not given
GLP	:	in compliance

Liver S9 fraction from male Wistar rats pre-treated with Aroclor 1254 was used as the exogenous metabolic activation system.

Results

C8 did not induce gene mutation in the V79 VHL cells.

Ref. : 14

DNA Damage and Repair-Unscheduled DNA Synthesis-Mammalian Cells *In Vitro*

Guideline : OECD 482
 Species/strain : HeLa cells
 Replicates : No
 Test substance : C8, purity not given
 GLP : in compliance

Results

C8 did not induce significant increase in the incorporation of 3H-thymidine into the hepatocytes. The method adopted (scintillation counting) is the least sensitive to be used in this type of test, according to the scientific literature). The study is considered inadequate.

Ref. : 16

***In Vitro* Mammalian Chromosome Aberration Test**

Guideline : OECD 473
 Species/strain : Chinese hamster lung cells V79
 Replicates : 2 independent experiments
 Substance : C8
 Batch no : not given
 Purity : not given
 Treatment : 4 h in first experiment; 18 and 28 h (in absence of S9)
 4 h in first and second experiment (in presence of S9)
 Doses : 62.5 – 125 – 250 mg/ml (in absence of S9) for 18 h
 250-500-1000 mg/ml (in absence of S9) for 28 h
 50 – 100 – 200 mg/ml for 18 h (in absence of S9)
 62.5-125-250 mg/ml in first experiment and 31.3-62.5-250 mg/ml
 for the second experiment (in presence of S9)
 GLP : in compliance

Liver S9 fraction from male Wistar rats pre-treated with Phenobarbital and β -naphthoflavone was used as the exogenous metabolic activation system.

Results

Under all conditions C8 was found non-clastogenic, except at the 1000 mg/ml dose, in the absence of S9 mix, which produced a slight but significant increase in the frequency of chromosome aberrations, over the control.

Ref. : 18

***In Vivo* Mammalian Erythrocytes Micronucleus Test**

Guideline	:	OECD 474
Species/strain	:	Mice, CFW 1
Group size	:	5 male + 5 female per group/harvest time
Test substance	:	C8
Batch no	:	not given
Dose levels	:	5000 mg/kg bw
Sacrifice times	:	24, 48 and 72 h
Administration	:	gavage
GLP	:	in compliance

Results

C8 did not induce micronuclei at all conditions. The ratio on normochromatic to polychromatic erythrocytes was not different from the control. However, there is no clear evidence that the test agent has reached the target organ.

Ref. : 15

2.10. Special investigations

Excretion following i.v. administration

Guideline	:	/
Species	:	Wistar rat
Group sizes	:	3 male
Route	:	intravenous
Material	:	¹⁴ C-Arianor Madder Red in physiological saline
Batch no	:	not stated
Dose levels	:	2.5 mg/kg bw in a volume of 1.0 ml
GLP	:	not in compliance

The excretion of radiolabelled ¹⁴C-Arianor Madder Red was examined in 3 male rats following application of a single i.v. dose of 2.5 mg/kg bw. The amount of radioactivity excreted in the urine, faeces and expired air during the study, and the amount of radioactivity in the carcass at the end of the observation period (24 hours) was measured. Urine was examined for metabolites by thin layer chromatography.

Results

Most of the dose was recovered in the faeces (mean value 63%). The urine contained about 15% of the dose, whilst the radioactivity in the expired air was below 1% of the administered radioactivity. The level of radioactivity detected in the carcasses 24 hours after administration was about 9%. Only the parent material was found in the urine.

Ref. : 17

Excretion after subcutaneous administration

Guideline	:	/
Species	:	Wistar rat
Group sizes	:	5 male and 5 female
Route	:	subcutaneous
Material	:	¹⁴ C-Arianor Madder Red in physiological saline
Batch no	:	not stated
Dose levels	:	2.5 mg/kg bw in a volume of 1.0 ml
GLP	:	not in compliance

The excretion of radiolabelled ¹⁴C- Arianor Madder Red was examined in 5 male and 5 female rats following a single subcutaneous dose of 2.5 mg/kg bw. The amount of radioactivity excreted in the urine and faeces during the study, and the amount of radioactivity in the carcass at the end of the observation period (24 hours) was measured. Urine was examined for metabolites by thin layer chromatography.

Results

Most of the dose was recovered in the faeces (mean value 46% for male and 41% for female rats). The urine contained about 20% (male rats) or 24% (female rats) of the dose. The level of radioactivity detected in the carcasses 24 hours after administration was about 15% for the male rats and 16% for the female rats. Only the parent material was found in the urine.

Ref. : 17

Organ distribution after i.v. administration

Guideline	:	/
Species	:	mouse
Group sizes	:	3 per time point (sex not specified)
Route	:	intravenous
Material	:	¹⁴ C-Arianor Madder Red in physiological saline
Batch no	:	not stated
Dose levels	:	5 mg/kg bw in a volume of 0.5 ml
GLP	:	not in compliance

Two mice were used at each of the points of time for the determination of radioactivity in the examined organs and one mouse per time point for whole body autoradiography.

The distribution of ¹⁴C- Arianor Madder Red was examined 2 and 20 min, and 1, 6 and 24 hours after administration a single i.v. dose of 5 mg/kg bw. Two mice were used at each time point for the determination of radioactivity in the examined organs (liver, kidneys, heart, lungs, spleen, stomach, small and large intestine, caecum, testes, brain, skin, muscle, intestinal fat, blood) and one mouse per time point for whole body autoradiography.

Results

At two minutes, liver and kidneys contained the largest amount of radioactivity (about 31% respectively) followed by the small intestine (about 9%) and the lungs (about 1.3%). Until the end of the study (24 hours after administration) the radioactivity in the liver, kidneys and lungs decreased to about 33.7% of the administered dose. Later on the radioactivity in the small intestine decreased sharply whereas the radioactivity in the caecum and large intestine increased

to about 16% at 6 hours but contained only < 0.4% of the applied dose at 24 hours. At the end of the study, the specific radioactivity was highest in the caecum and large intestine, followed by the liver and spleen (1.2 µg-equivalents/g dried organ and 0.93 µg-equivalents/g dried organ respectively). The blood level decreased from 0.93 µg-equivalents/g dried blood two minutes after administration to 0.15 µg-equivalents/g dried blood at the end of the study.

The autoradiographs confirmed the results of the organ distribution study. In addition, the autoradiograph from the mouse exposed to the test material for one hour clearly showed a high radioactivity in the gall bladder.

Ref. : 17

2.11. Safety evaluation

NOT APPLICABLE

CALCULATION OF THE MARGIN OF SAFETY

(Basic Red 76)
(Direct Hair Dyes)

Based on a usage volume of 35 ml, containing at maximum

Maximum absorption through the skin	A (µg/cm ²)	=	µg/cm ²
Typical body weight of human		=	60 kg
Skin Area surface	SAS (cm ²)	=	cm ²
Dermal absorption per treatment	SAS x A x 0.001	=	mg
Systemic exposure dose (SED)	SAS x A x 0.001/60	=	mg/kg
No observed adverse effect level (mg/kg) (species, study)	NOAEL	=	mg/kg
Margin of Safety	NOAEL / SED	=	

2.12. Conclusions

Commercial grade dye of different batches has been used for various tests, but purity (>98%) of the dye has been described only in one case. The impurities in the dye should be described. The test substance is an azo-dye, therefore, free aromatic amine (2-methoxy aniline) content in the dye (in all batches) are required for the evaluation of carcinogenic potential of the dye. The dye formulation contains 16% sugar and 14.5% inorganic salts. A complete description of the sugar and salts is required. Following physical properties are also required: density and Log P_{ow}.

The sensitisation data in the dossier used a concentration too low for intradermal induction.

The studies on percutaneous absorption are inadequate. The test concentrations were well below the expected use concentration.

Basic Red 76 has been tested for the induction of gene mutation in bacterial cells (positive results) and in the mammalian cells, *in vitro* (negative results), and for chromosome aberration in mammalian cells *in vitro* (negative results). The *in vitro* UDS study was inadequate. The *in vivo* micronucleus test gave negative results; no firm evidence that the bone marrow was reached by the test agent was noted. No conclusion can be drawn.

2.13. References

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3. Opinion of the SCCNFP

The SCCNFP is of the opinion that the information submitted is insufficient to allow an adequate risk assessment to be carried out. Accordingly, the SCCNFP considers that it is not possible to assess the safe use of the substance.

Before any further consideration, the following information is required :

- * The NOAEL has to be defined based on a subacute or subchronic toxicity study according to current guidelines including investigations on haematotoxicity and of thyroid function.
- * A study on percutaneous absorption according to the Notes of Guidance (SCCNFP/0321/00);
- * data on the genotoxicity/mutagenicity following the SCCNFP-opinion “Proposal for a Strategy for Testing Hair Dye Cosmetic Ingredients for their Potential of Genotoxicity / Mutagenicity”, doc. n° SCCNFP/0566/02 of 4 June 2002, and in accordance with the Notes of Guidance, regularly updated by the SCCNFP (doc. n° SCCNFP/0321/00).

4. Other considerations

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5. Minority opinions

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