EUROPEAN COMMISSION

Directorate-General for Health & Consumers

Scientific Committee on Consumer Safety

SCCS

OPINION ON

Basic Red 51

COLIPA n° B116

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

The SCCS adopted this opinion at its 10th plenary meeting

of 22 March 2011

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 Agency (EMA), 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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ISSN 1831-4767 ISBN 978-92-79-30712-6
Doi:10.2772/97050 ND-AQ-11-005-EN-N

ACKNOWLEDGMENTS

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Keywords: SCCS, scientific opinion, hair dye, Basic Red 51, B116, CAS 77061-58-6, EC 278-601-4, Directive 76/768/EEC

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on Basic Red 51, 22 March 2011

This opinion has been subject to a commenting period of four weeks after its initial publication. Comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged. Revised opinions carry the date of revision.

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1. BACKGROUND

Based on submission I, the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) adopted at its 25th plenary meeting on 20 October 2003 its opinion (SCCNFP/0735/03) on Basic Red 51 with the chemical name 2-[(4-(dimethylamino)phenyl)azo]-1,3-dimethyl-1H-imidazolium chloride. According to this opinion further information is needed on:

- The stability of the test material in the test preparations and in the hair dye formulations
- A percutaneous absorption study in accordance with the Notes of Guidance, if used in an oxidising environment
- Data on the genotoxicity/mutagenicity following the relevant SCCNFP opinions and in accordance with the Notes of Guidance.

Submission II was submitted in July 2005. According to this submission Basic Red 51 is a hair dying ingredient intended be used in direct (non-oxidative) hair dyes at 1.0% concentration and it is also used at a concentration of 1.0% in oxidative hair dye formulations, which after mixing in a 1:1 ratio with hydrogen peroxide just prior to use corresponds to a concentration of 0.5% upon application. The applied hair dye formulations are rinsed off after about 30 minutes and the normal frequency of application being about once per month.

Submission II presents updated scientific data on the above mentioned substance in line with the second step of the strategy for the evaluation of hair dyes (http://europa.eu.int/comm/enterprise/cosmetics/doc/hairdyestrategyinternet.pdf)) within the framework of the Cosmetics Directive 76/768/EEC.

2. TERMS OF REFERENCE

1. *Does the Scientific Committee on Consumer Safety (SCCS) consider Basic Red 51 to be safe for use in non-oxidative hair dye formulations at a maximum on-head concentration up to 1% and in oxidative hair dye formulations at a maximum on-head concentration of 0.5% taken into account the data scientific provided?*

2. *Does the SCCS recommend any restrictions with regard to the use of Basic Red 51 in non-oxidative and oxidative hair dye formulations?*

3. OPINION

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Basic Red 51 (INCI)

3.1.1.2. Chemical names

2-[((4-Dimethylamino)phenyl)azo]-1,3-dimethyl-1H-imidazolium chloride
1H-Imidazolium, 2-[((4-dimethylamino)phenyl)azo]-1,3-dimethyl-, chloride (CAS Name)

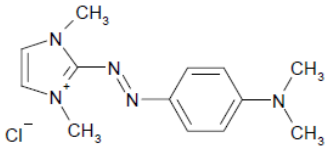
3.1.1.3. Trade names and abbreviations

MIP RED 2985
RED (MIP 2985)
MIP 2985
VIBRACOLOR® Ruby Red

3.1.1.4. CAS / EC number

CAS: 77061-58-6
EC: 278-601-4

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_{13}H_{18}N_5Cl$

3.1.2. Physical form

Blue to dark violet powder containing lumps

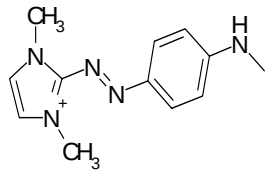
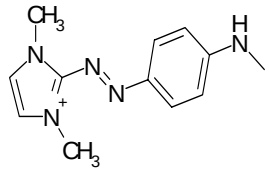
3.1.3. Molecular weight

Molecular weight: 279.8

3.1.4. Purity, composition and substance codes

Basic Red 51 was chemically characterised by IR and UV-Vis spectroscopy.

The composition of the MIP RED batches 2985 (21187FC3 and 28936FC3) used for the toxicological assays is summarized in the table below.

Batch Nr.	Measured purity active* (%)	Impurities* (%)	Volatile matter** (%)	Salt: Sum of ($Cl+SO_4+CH_3SO_4$) as $NaCH_3SO$*** (%)
21187FC3	95.9	0.4 coloured by-product  Molecular Weight =230,29 Exact Mass =230 Molecular Formula =C12H16N5 Molecular Composition =C 62.59% H 7.00% N 30.41%	3.5	<= 5
28936FC3	97.9	0.4 coloured by-product  Molecular Weight =230,29 Exact Mass =230 Molecular Formula =C12H16N5 Molecular Composition =C 62.59% H 7.00% N 30.41%	0.6	<= 5

* Quantification of active ingredient and impurities was performed by HPLC and UV-VIS detection using external standard.
** Determination of volatile matter by IR, after drying
*** Determination of salt content as sum of chloride, sulfate and methyl sulfate, Calculation based on sodium content

Comment
No reference materials were used for identification and quantification of Basic Red 1 and its impurities.

3.1.5. Impurities / accompanying contaminants

See 3.1.4

3.1.6. Solubility

In water: 40 g/l at 30°C

Comment
Solubility is not determined by the EC method A.6

3.1.7. Partition coefficient (Log P_{ow})

Log Pow: -1.97 (OECD method No. 197/1981)

3.1.8. Additional physical and chemical specifications

Melting point: > 200 °C
Boiling point: /

Flash point:	/
Vapour pressure:	/
Density:	/
Viscosity:	/
pKa:	/
Refractive index:	/
UV_Vis spectrum (200-800 nm):	λmax 524 nm

3.1.9. Homogeneity and Stability

The MIP RED 2985 in rodent feed (Batch nr. CGF-F016740/0018) was shown to be homogeneous and stable for a period of at least 21 days (variations ± 7%).

The stability of MIP RED 2985 in the semi-permanent and oxidative hair dye formulations used for dermal absorption study has been assessed during a period of 24 hours at room temperature. The concentrations of active material in both semi-permanent and oxidative formulations were within ± 5% of the nominal concentration.

Ref.: 1, submission II

General comments on analytical and physico-chemical characterisation

- Solubility of Basic Red 51 is not determined by the EC method A.6.
- Stability of Basic Red 51 in typical hair dye formulations has been studied only for 24 hours.
- No reference materials were used for identification and quantification of Basic Red 1 and its impurities.
- The stability in an oxidative environment has not been demonstrated.

3.2. Function and uses

Basic Red 51 is intended for use in direct hair dye formulations at concentrations up to 1 % and in oxidative hair dyes at a final concentration of 0.5%, after mixing with the oxidative agent.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Taken from SCCNFP/0735/03

Guideline:	/
Species/strain:	Rat, Crl:CD (SD)IGS BR
Group Size:	2 rats per sex at the 3 low doses, 5 rats per sex at the highest dose
Test material:	MIP 2985
Batch:	029753 A8AA
Purity:	97.2%
Dose:	500, 1000, 1500, 2000 mg/kg bw
Observ. period:	14 days
GLP:	in compliance

In a dose-limit test, the test substance was dissolved in cell culture grade water and administered by gavage as single doses of MIP 2985 at 2000 mg/kg bw (5 rats per sex). The treatment-related mortality occurred at this dose. Thus additional dose levels (500, 1000, 1500) were tested on 2 rats per sex per dose.
Most animals, except one female and 2 males at the lowest dose (500 mg/kg bw), died within the first 24 h of the experiment. The female died on Day 1. Clinical observations observed prior to death included hypoactivity, recumbency, irregular respiration, ataxia, and squinted eye.

The 2 males from the lowest dose (500 mg/kg bw) survived until the scheduled sacrifice and gained weight during the course of the study. Clinical observations in these animals included hypoactivity, irregular respiration, ataxia, squinted eyes, discoloured urine, faecal stains, and/or few faeces. Most of these findings were resolved by Day 3.

At necropsy, no visible lesions were noted in the two males that survived in the 500 mg/kg dose group. In the animals that died, macroscopic findings involved the stomach, caecum, ileum, duodenum, jejunum, and/or colon. These were discoloured and/or distended filled with purple, red, or pink fluid.

Lower Dose Study

Guideline:	/
Species/strain:	Rat, Crl:CD (SD)IGS BR
Group Size:	5 male rats at the high dose only, 5 female rats at the low dose only
Test material:	MIP 2985
Batch:	029753 A8AA
Purity:	97.2%
Dose:	250, 500 mg/kg bw
Observ. period:	14 days
GLP:	in compliance

In this small range-finding study, dosages were reduced. 2 males receiving 500 mg/kg bw died on Day 1. All other animals survived until the end of the experiment.
3 males, including the two that died on Day 1, were hypoactive and had roughened haircoat within 4 hours. Macroscopic findings of the 2 males that died showed discoloured and/or distended stomach, caecum, ileum, duodenum, jejunum and bladder filled with purple, red, or pink fluid. 1 female was hypoactive and had liquid and/or mucoid faeces within 4h post-dosing. All surviving animals gained weight and showed no post-mortem anomalies at the end of the study. The acute oral LD5O was considered to be between 250 - 500 mg/kg bw in females and between 500 - 1000 mg/kg bw in males.

Ref.: 1

3.3.1.2. Acute dermal toxicity

Taken from SCCNFP/0735/03

Guideline:	OECD Guideline 402
Species/strain:	Rat, Crl:CD (SD)IGS BR
Group Size:	10 males and females
Test material:	MIP 2985
Batch:	029753 A8AA
Purity:	97.2%
Dose:	2000 mg/kg bw
Observ. period:	14 days
GLP:	in compliance

The hair was clipped the day prior to the experiment. The test material was moistened with distilled water and applied at a dose of 2000 mg/kg bw. It was applied to the clipped area as a thin uniform layer from scapula to iliac crest and half way down the flank on each side of the animal's back. The area was occluded for 24 h. The initial dermal irritation was scored and recorded 30 minutes after bandage removal on Day 1. The untreated skin of each animal served as the control. Additional dermal irritation readings were performed for each animal on Days 3, 7, 10, and 14. All animals were examined for clinical signs of ill health or mortality immediately post-dose and approximately 1, 2.5 and 4 hours post-dose, and daily thereafter. Body weights were recorded pre-dose on the day of dosing (Day 0), and on Days 7 and 14, and prior to sacrifice on Day 15 (fasted). A curtailed gross examination of the cervical, thoracic and abdominal viscera was performed.

Signs of clinical toxicity included chromodacryorrhea and/or red nasal discharge. Findings were first noted 4 hours post-dose and were resolved by Day 2. Signs of dermal irritation included desquamation (slight scaling) in all males on Day 3 and in one male and one female on Day 7. There were no signs of dermal irritation at any observation interval in any of the remaining animals. All animals gained weight during the course of the study. No visible lesions were noted in any of the animals at necropsy.

MIP 2985 was mildly irritating to the skin when applied dermally at a dose of 2000 mg/kg bw. Under the conditions of this study, there were also minor systemic effects. The acute dermal LD5O is greater than 2000 mg/kg bw.

Ref.: 2

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

Taken from SCCNFP/0735/03

Guideline:	OECD 404 (1981)
Species/strain:	albino rabbits, New Zealand
Group size:	1 male, 2 females
Test substance:	MIP 2985
Batch:	029753A8AA
Purity:	97.2% by UV-VIS; 98.8% by HPLC
Dose:	0.5 g applied to 6.25 cm^2 of intact skin
GLP:	In compliance

After clipping the back and flanks, 0.5g of the test material was applied to an area of approximately 6.25 cm^2 under a semi-occlusive dressing. The patches were removed after 4 hours and observations made at 0.5, 1, 24, 48 and 72 hours after removal.

Results
No erythema or oedema was observed. The primary irritation index was 0.0. The test material was considered to be non-irritating to the rabbit skin.

Ref.: 6

3.3.2.2. Mucous membrane irritation

Taken from SCCNFP/0735/03

Guideline:	OECD 405 (1981)
Species/strain:	albino rabbits, New Zealand
Group size:	3 (sex not stated)
Test substance:	MIP 2985
Batch:	029753A8AA
Purity:	97.2% by UV-VIS; 98.8% by HPLC
Dose:	0.0360 g in 0.1ml
GLP:	In compliance

The test material was applied instilled into the lower lid of the right eye of each animal. The left eye served as the untreated control. The eyes of the 3 animals remained unrinsed for approximately 24 hours after instillation of the test material.
1, 24, 48, 72 and 96 hours and 7, 14 and 21 days after instillation of the test material, the treated eyes of the rabbits were observed for signs of ocular irritation. Corneal injury was assessed using sodium fluorescein (followed by a saline wash) on all animals at 24 hours post-instillation.

Results
There were no effects involving the cornea or iris.
Redness of the conjunctiva was noted in all animals from 1 hour to 14 days post instillation and in 1 animal at 21 days post instillation. Chemosis was noted in 2 animals from 1 hour through to 7 days post-instillation and in 1 animal on days 14 and 21. Discharge was noted in 2 animals from 1 hour to day 14 and in 1 animal through to day 21. There was no evidence of corrosion.
A maximum mean score of 7.3 (of max. 110 possible) at 1 and 24 hours post instillation was determined. MIP 2985 was moderately irritating to the eyes of the rabbits under the conditions of the study.

Ref.: 7

3.3.3. Skin sensitisation

Taken from SCCNFP/0735/03

Magnusson and Kligman Guinea pig maximisation test

Guideline:	OECD 406	
Species/strain:	albino guinea pigs	
Group size:	15 females (10 test and 5 control)	
Test substance:	MIP 2985	
Batch:	CGF-F016740/0018	
Purity:	>98%	
Dose:	Intradermal induction:	A 5% aqueous solution with and without Freund's Complete Adjuvant.
	Topical induction:	A 50% preparation of test material under occlusion for 48 hours. Controls received vehicle only.
	Challenge:	14 days later by exposing 25% aqueous dilution of the test substance (24 hours, occlusion).
GLP:	In compliance	

Animals were examined 24 and 48 hours after removal of the patches for signs of erythema and oedema.

Results
None of the animals of the control or test group were observed with skin reactions after challenge with a non-irritating preparation of 25% of the test material. MIP 2985 was considered not to be a sensitizer under the test conditions.

Ref.: 8

3.3.4.	Dermal / percutaneous absorption

Rat and human skin, *in vitro*

Guideline:	OECD 428 (2004)
Tissue:	rat split-thickness skin, 200 µm (2 males, strain: HanBrl: WIST (SPF)) Human split-thickness skin, 200 µm (2 female donors)
Group size:	7 membranes per species (3 or 4 cells/donor)
Skin integrity:	permeability coefficient (Kp) of tritium water Kp < 3.5 x 10^{-3} cm/h (rat) Kp < 2.5 x 10^{-3} cm/h (human)
Diffusion cell:	automated flow-through cell system, 0.64 cm²
Test substance:	Red (MIP 2985)
Batch:	21187FC3 3501-069 (radio-labelled, 2072 MBq/mmol, 56 mCi/mmol)
Purity:	95.9% (contains about 4% inorganic salt) 98.15% (radio-labelled)
Test item:	aqueous solution
Dose:	19 mg/cm²
Dose of test substance:	0.182 mg/cm²
Receptor fluid:	physiological saline, 0.9% NaCl w/v
Solubility receptor fluid:	/
Stability receptor fluid:	/
Method of Analysis:	Liquid Scintillation Counting
GLP:	in compliance
Study date:	18 February – 4 March 2005

The percutaneous penetration of the test substance, formulated as aqueous solution was determined in vitro using split-thickness skin membranes from rat and human skin. The formulated [^{14}C] Red (MIP 2985) was applied onto skin membranes of 200 µm thickness at a concentration of 19 mg/cm² leading to an area concentration of 182 µg/cm². For each species 7 replicates were used.

Results
The totally absorbed test item was calculated based on the amount penetrated through the skin membrane (perfusate) and the amount in the remaining skin membrane after tape stripping. The total absorption amounted to 1.61% and 0.13% of the applied dose for rat and human skin membranes, respectively. The penetration of [^{14}C] Red (MIP 2985) through rat and human skin membrane was very low. The mean flux value was calculated to be 0.067 µg/cm²/h in rat and 0.031 µg/cm²/h in human.

Ref.: 1, submission II

Comments
Only 2 subjects were used for each study. Only non-oxidative conditions have been studied

Human skin, *in vitro*

Guideline:	OECD 428 (2004)
Tissue:	human post mortem dermatomed skin, 400 µm thickness
Group size:	9 membranes from 6 female donors (oxidative conditions) 9 membranes from 4 female donors (non-oxidative conditions)
Skin integrity:	electrical resistance, > 10 kΩ
Diffusion cell:	glass diffusion cell; 2.54 cm²
Test substance:	Basic Red 51
Batch:	028936FC3 3501-069 (radio-labelled) (50.5 mCi/mmol)
Purity:	97.9% (UV-Vis spectroscopy) 98.15% (HPLC)
Test item:	Basic Red 51 1% formulations oxidative and non-oxidative conditions
Dose:	20 mg/cm² test formulation
Dose of test substance:	0.5% (100 µg/cm²) Basic Red 51 under oxidative conditions 1.0% (200 µg/cm²) Basic Red 51 under non-oxidative conditions
Receptor fluid:	phosphate buffered saline
Solubility receptor fluid:	40 mg/ml at 30 °C
Stability receptor fluid:	/
Method of Analysis:	Liquid scintillation counting
GLP:	in compliance
Study date:	25 February – 8 April 2005

The absorption and distribution of Basic Red 51 from two different and typical nominal 1% w/w formulations, has been measured *in vitro* through human skin

Results
Oxidative conditions
3 cells were excluded from the analysis.

Test Compartment	Amount Recovered ($\mu g_{eq}/cm^2$)												
	Cell 3	Cell 5	Cell 7	Cell 11	Cell 17	Cell 18	Cell 1**	Cell 14***	Cell 15**	Mean	SD	SEM	n
Receptor & Grid	0.022	0.022	0.021	0.018	0.023	0.024	*0.030*	*0.025*	*0.021*	0.022	0.002	0.001	6
Flange	0.133	0.079	0.098	0.011	0.012	0.019	*0.106*	*0.013*	*0.018*	0.059	0.052	0.021	6
Donor Chamber	0.192	0.049	0.036	0.054	0.073	0.207	*0.068*	*0.104*	*0.043*	0.102	0.077	0.031	6
Skin Wash @ 0.5h	97.9	97.3	92.0	95.7	106	101	*116*	*92.0*	*128*	98.2	4.63	1.89	6
Stratum Corneum	2.33	0.368	0.496	1.17	0.423	1.17	*1.89*	*0.584*	*0.399*	0.992	0.748	0.305	6
Remaining Epidermis/Dermis	0.298	0.122	0.298	0.340	0.277	0.690	*0.151*	*0.452*	*1.17*	0.338	0.188	0.077	6
Receptor Fluid	0.017	0.008	0.013	0.018	0.012	0.013	*0.017*	*0.040*	*0.026*	0.014	0.003	0.001	6
Systemically Available*	0.315	0.131	0.311	0.358	0.289	0.703	*0.168*	*0.492*	*1.20*	0.351	0.189	0.077	6
TOTAL	101	97.9	93.0	97.4	106	103	118	93.2	*130*	100	4.73	1.93	6

Test Compartment	Percent of Dose Recovered (%)												
	Cell 3	Cell 5	Cell 7	Cell 11	Cell 17	Cell 18	Cell 1**	Cell 14***	Cell 15**	Mean	SD	SEM	n
Receptor & Grid	0.022	0.022	0.021	0.018	0.023	0.024	*0.029*	*0.025*	*0.021*	0.022	0.002	0.001	6
Flange	0.133	0.079	0.098	0.011	0.012	0.019	*0.106*	*0.013*	*0.018*	0.059	0.052	0.021	6
Donor Chamber	0.191	0.049	0.036	0.054	0.073	0.206	*0.068*	*0.104*	*0.043*	0.102	0.076	0.031	6
Skin Wash @ 0.5h	97.8	97.2	91.9	95.6	105	101	*116*	*91.9*	*128*	98.1	4.63	1.89	6
Stratum Corneum	2.32	0.368	0.496	1.16	0.423	1.17	*1.89*	*0.584*	*0.398*	0.991	0.747	0.305	6
Remaining Epidermis/Dermis	0.298	0.122	0.298	0.340	0.277	0.689	*0.150*	*0.452*	*1.17*	0.337	0.188	0.077	6
Receptor Fluid	0.017	0.008	0.013	0.018	0.012	0.013	*0.017*	*0.040*	*0.026*	0.013	0.003	0.001	6
Systemically Available*	0.315	0.130	0.311	0.357	0.289	0.702	*0.167*	*0.492*	*1.20*	0.351	0.189	0.077	6
TOTAL	101	97.8	92.9	97.2	106	103	*118*	*93.1*	*130*	99.7	4.72	1.93	6

* Systemically Available = Sum of Remaining Epidermis and Receptor Fluid data
** Cell excluded as high wash value indicates error; *** Cell excluded as profile indicates membrane damage at 0.5h
Data shown in italics is not included in the mean calculations

Non-oxidative conditions

Test Compartment	Amount Recovered ($\mu g_{eq}/cm^2$)												
	Cell 81	Cell 82	Cell 83	Cell 84	Cell 85	Cell 87	Cell 88	Cell 89	Cell 92	Mean	SD	SEM	n
Receptor & Grid	0.019	0.023	0.019	0.021	0.022	0.016	0.017	0.021	0.020	0.020	0.002	0.001	9
Flange	0.010	0.029	0.071	0.102	0.028	0.009	0.007	0.049	0.025	0.036	0.032	0.011	9
Donor Chamber	0.063	0.070	0.083	0.075	0.067	0.057	0.077	0.080	0.268	0.093	0.066	0.022	9
Skin Wash @ 0.5h	193	186	191	190	190	191	196	203	200	193	5.43	1.81	9
Stratum Corneum	0.316	0.652	0.312	0.626	0.483	0.601	0.622	0.863	0.591	0.563	0.172	0.057	9
Remaining Epidermis/Dermis	0.207	0.130	0.862	0.696	0.472	0.141	0.386	0.280	0.279	0.384	0.253	0.084	9
Receptor Fluid	0.006	0.008	0.037	0.024	0.013	0.009	0.009	0.016	0.020	0.016	0.010	0.003	9
Systemically Available*	**0.213**	**0.138**	**0.898**	**0.720**	**0.485**	**0.149**	**0.394**	**0.296**	**0.299**	**0.399**	**0.261**	**0.087**	**9**
TOTAL	194	187	192	192	191	191	197	204	201	194	5.51	1.84	9

Test Compartment	Percent of Dose Recovered (%)												
	Cell 81	Cell 82	Cell 83	Cell 84	Cell 85	Cell 87	Cell 88	Cell 89	Cell 92	Mean	SD	SEM	n
Receptor & Grid	0.009	0.011	0.010	0.010	0.011	0.008	0.008	0.011	0.010	0.010	0.001	0.000	9
Flange	0.005	0.015	0.035	0.051	0.014	0.004	0.003	0.025	0.012	0.018	0.016	0.005	9
Donor Chamber	0.032	0.035	0.042	0.038	0.034	0.029	0.038	0.040	0.134	0.047	0.033	0.011	9
Skin Wash @ 0.5h	96.6	92.8	95.4	95.2	95.1	95.3	98.0	101	100	96.7	2.72	0.905	9
Stratum Corneum	0.158	0.326	0.156	0.313	0.242	0.301	0.311	0.432	0.295	0.281	0.086	0.029	9
Remaining Epidermis/Dermis	0.103	0.065	0.431	0.348	0.236	0.070	0.193	0.140	0.139	0.192	0.126	0.042	9
Receptor Fluid	0.003	0.004	0.018	0.012	0.007	0.004	0.004	0.008	0.010	0.008	0.005	0.002	9
Systemically Available*	**0.106**	**0.069**	**0.449**	**0.360**	**0.243**	**0.075**	**0.197**	**0.148**	**0.149**	**0.200**	**0.131**	**0.044**	**9**
TOTAL	96.9	93.3	96.1	95.9	95.6	95.7	98.5	102	101	97.2	2.60	0.865	9

* Systemically Available = Sum of Remaining Epidermis and Receptor Fluid data
** Cell excluded as high wash value indicates error
*** Cell excluded as profile indicates membrane damage at 0.5h

The results obtained in this study indicate that the absorption of Basic Red 51 through human skin from the semi-permanent or oxidative formulations is slow. 0.200% (0.399/μgeq/cm²) and 0.351% (0.351/μgeq/cm²) of the applied Basic Red 51, semi-permanent and oxidative formulations respectively, was regarded as being systemically available.

Ref.: 2, submission II

Comment
The mean + 1 SD, 0.660 (0.399 ± 0.261) μg/cm² can be used to calculate the MOS under non-oxidative conditions and 0.540 (0.351 ± 0.189) μg/cm² under oxidative conditions.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral toxicity

Taken from SCCNFP/0735/03

Guideline:	OECD Guideline 407
Species/strain:	Wistar Hanlbm (SPF) rat
Group Size:	10 rats per sex: control and the high dose 5 rats per sex: mid and low doses
Test material:	MIP 2985
Batch:	CGF-F016740/0018
Purity:	98%
Dose:	15, 50 and 150 mg/kg bw
Treatment Period:	28 days
Observ. period:	14 days
GLP:	in compliance

MIP 2985 was administered in feed for 28 days at theoretical dose levels of 15, 50 and 150 mg/kg body weight/day while the control group received the normal diet. The corresponding effective daily intake for males was 12.25, 39.59 and 135.46 mg/kg body weight, and for females, 13.16, 40.50 and 132.54 mg/kg body weight for low, mid and high dose groups respectively. A 14-day recovery period was allowed for 5 animals per sex in the control and high dose groups.

The animals were examined for clinical signs daily and checked twice daily for mortality. Food consumption and body weight were recorded once pre-test, and weekly thereafter and body weight at necropsy. A functional observational battery (modified Irwin screen test), grip strength and locomotor activity were performed during week 4. Urine and blood for haematology and clinical biochemistry were collected from all animals. All animals were killed and descriptions of all macroscopic abnormalities were recorded. The major organ weights (absolute and relative) were recorded on the date of necropsy. Samples of major organs from control and top dose groups, as well as liver and thyroid glands and all gross lesions from all animals were examined by light microscopy. Only liver, thyroid gland and gross lesions were examined microscopically from rats of mid and low dose groups.

All animals survived during the study. No quantitative or qualitative differences of clinical parameters from the control values were noted. A dark discoloration of the faeces was observed in all animals of the high-dose group on Day 3 dosing to Day 3 recovery. In the high dose group, urine of 9 males and 3 females was a deep yellow colour after 4 weeks but by week 6, the urine colour was normal. A lack of appetite was observed in females of the high-dose group only during the treatment period. The relative food consumption was similar in all groups. Mean body weight and body weight gain were slightly increased in the mid and low dose group males, and reduced in high-dose group females when compared with the control. There were no dose-related effects in the functional observational battery, grip strength measurement, and locomotor activity.

Minor clinical laboratory changes were recorded after 4 weeks treatment between the high dose group and the controls. At the high dose, there was a slight increase in circulating lymphocytes (relative) as well as a slight decrease in segmented neutrophils (relative and/or absolute) in both sexes ($p<0.05$). At end of the treatment-free recovery period, these haematological parameters were found to be similar to the controls, indicating reversibility. Clinical biochemistry results showed the following findings in the high dose group:

Total cholesterol level slightly increased in both males and females ($p<0.01$).
Triglyceride level slightly increased in both males ($p<0.01$) and females ($p<0.05$); a slight increase was also recorded in the mid-dose group females ($p<0.05$)
Phospholipid level slightly increased in both males and females ($p<0.0l$)
Albumin level slightly increased in both males ($p<0.05$) and females ($p<0.0l$)
Globulin level slightly reduced in both males ($p<0.05$) and females (statistically not significant)
Albumin to globulin ratio slightly increased in both males and females ($p<0.05$).

At the end of the treatment-free recovery period, there was an indication of reversibility for most parameters. However, slightly higher significant ($p<0.05$) values were still observed in the triglyceride, phospholipid and albumin concentrations in male rats. The authors considered these findings to primarily reflect adaptive changes in lipid metabolism. Histopathological correlates to these hepatic metabolic changes were not found. Urinalysis parameters were not adversely affected by treatment and only the reversible deep-yellow urine discoloration was considered test article-related. Organ weights and organ to body weight ratios were higher in the mid-dose group males, but were attributed to the higher terminal body weights of the rats. Macroscopically, there was a reddish brown discoloration of the thyroid gland observed at 4 weeks in 80% of the males and in all the females of the high-dose group, 40% of the males in mid-dose group and one control female. At 6 weeks,

all the high dose recovery group animals showed thyroid discoloration. All other lesions recorded were considered to be within the normal range of background findings commonly seen in rats of this strain and age.

Based on these results, the NOAEL of MIP 2985 was estimated as 12.25 mg/kg body weight/day.

Ref: 4

3.3.5.2. Repeated Dose (14 days) dermal toxicity

Taken from SCCNFP/0735/03

Guideline:	OECD Guideline 402
Species/strain:	Albino Guinea pig, Ibm: GOHI, SPF
Group Size:	4 males and 4 females
Test material:	MIP 2985
Batch:	CGF-F016740/0018
Purity:	98%
Dose:	1.0, 0.5, 0.1%w/w
Observ. period:	14 days
GLP:	in compliance

A 14-day repeated dose dermal toxicity study to assess the cumulative irritation potential with MIP 2985 was applied daily at concentrations of 1.0, 0.5 and 0.1% w/w in double-distilled water. The skin was shaved prior to the study and 2 circular areas 7 cm^2 were marked. The animals were shaved four times during the first week and 3 times in the second week. A depilatory cream was used on Day 15 after the final application. One male and female served as controls and received double distilled water. No grading scores were recorded from test day 2-14, as no depilation was used during this period. However after depilation on test day 15, no skin reaction was observed. These data were confirmed by histopathology. Under the experimental conditions, the study authors considered MIP 2985 to be a non-irritant when repeatedly applied to guinea pigs skin.

Ref.: 3

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

Taken from SCCNFP/0735/03

Guideline:	OECD 408 (1981) and Directive 96/54/EEC
Species/strain:	Wistar rat, Hannover (SPF)
Group Size:	10 males + 10 females per dose
Test material:	MIP 2985
Batch:	CGF-F016740/0018
Purity:	98%
Dose:	0, 10, 50 and 250 mg/kg bw/day
Exposure period:	13 weeks
GLP:	in compliance

MIP 2985 was administered in feed at theoretical dose levels of 10, 50 and 250 mg/kg bw/day while the control group received the normal diet. The corresponding effective daily intake, based on food consumption and body weight for males was 9.8, 49.5 and 253.4 mg/kg body weight, and for females 10.1 51.2 and 247.3 mg/kg. bw for low, mid and high dose groups respectively.

The animals were examined for clinical signs daily and checked twice daily for mortality/viability. Food consumption and body weight were recorded once pre-test, and weekly thereafter and body weight at necropsy. Ophthalmoscopic examination was

performed at pre-test and at week 13 (control and high dose animals). A functional observational battery (modified Irwin screen test) was performed during pre-test and at week 12 on all rats and grip strength and locomotor activity were evaluated. At week 13, blood samples were collected for haematology and clinical biochemistry from all animals and urine samples were collected for analysis. After 13 weeks, all animals were weighed and killed and descriptions of all macroscopic abnormalities were recorded. The major tissues and organ were collected from all animals and absolute and relative weights were recorded at necropsy for adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, thyroid, and thymus. Samples of major organs from control and high-dose and all gross lesions from all animals were processed as haematoxylin-eosin slides and examined by light microscopy.

Deaths did not occur during the study. No clinical signs considered related to the test substance occurred. In the high dose group, a reduction in mean food consumption was 25% in males and 20% in females. An 8% reduction in the males of the mid dose group was noted. These data were considered test substance-related.

No effect of MIP 2985 was observed in any group on food consumption relative to body weight. The body weight and body weight gain in males and females of the high-dose group showed a significant ($p<0.05$) reduction. In the mid-dose group males, a non-significant reduction was observed. The low dose group was not affected.

Ophthalmologic findings were noted in a small proportion of animals from all groups during the study.
The following findings, considered unrelated to treatment, occurred at similar incidences in the high and in the control group: persistent pupillary membranes, comeal opacities, anterior synechia and persistent hyaloid vessels in the vitreous body. From the functional observational battery, grip strength measurement and locomotor activity, some incidental findings were recorded: decreased pupil diameter and contraction response in one male of the high-dose group, increased aggressiveness and fearfulness in females of the high-dose group; a statistically significant decreased grip strength of both forelimb and hind limb in group 4 was attributed to the reduced body weight of the animals.

Haematology results showed a significant ($p<0.05$) increase in methaemoglobin levels in males and females of the high dose group. A dose-related decrease of the number of white blood cells ($p<0.01$) was noted in high dose males along with a significantly ($p<0.01$) reduced absolute number of lymphocytes; these changes were considered dose-related. The clinical biochemistry parameters showed a significant ($p<0.01$) increase of gamma-glutamyl transferase levels in rats of the high dose group (212 % and 202 % in males and females, respectively). The high dose group animals also showed significant ($p<0.01$) decreases in creatinine levels, in amount of total protein accompanied by disproteinemia, and in urea and uric acid levels. Glucose, total bilirubin and phospholipids levels were decreased in high dose males; total cholesterol, creatine kinase and alkaline phosphatase were increased in high dose females. The authors considered these findings to be metabolic adaptations to MIP 2985 and of no toxicological relevance. No abnormalities were revealed by urinalysis results.

Decreased ($p<0.05$) absolute organ weights and/or organ weight ratios of most organs were recorded for males in the mid and high dose groups and for females in the high dose group. Macroscopically, there was a reddish discoloration of the forestomach mucosa, the thymus was reduced in size (high dose), and the thyroid gland was discoloured red-brown (mid dose) or black (high dose). The microscopic findings occurred mainly in the high dose group and included the following: Minimal to slight diffuse hepatocellular hypertrophy associated with moderately increased incidence of focal necrosis, and decreased haematopoiesis. The spleen showed a decreased incidence of increased haematopoiesis. In the kidneys an increased incidence of intratubular granular casts and transitional cell hyperplasia occurred in females. Non-glandular stomach dilation was observed in one male

and one female in the high dose group and most of the high dose animals showed congestion; a low incidence of glandular stomach erosion also occurred.
In the thymus, the observed cortical atrophy was considered as a condition of stress rather than an immunotoxic effect. The ovaries showed an interstitial cell hyperplasia considered as a nonneoplastic proliferation of cellular components of the interstitial gland. An increased diffuse fatty change was observed in the adrenal cortices of males and females of the high dose group. Thyroid of mid- and high-dose males and females showed an increased incidence of follicular cell hypertrophy, pigmented colloid, and pigmented follicular cells. The pituitary gland of males of these two groups showed an increased incidence of TSH/ACTH cell hypertrophy.

Ref.: 5

Comment
The study authors estimated the NOAEL to be 50 mg/kg bw/d, and the NOEL, 10 mg/kg bw/day, the lowest dose used. However, in light of the effects on the thyroid and pituitary at 50 mg/kg bw/d and higher, The SCCNFP concluded that the NOAEL should be 10 mg/kg bw/day.

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

Taken from SCCNFP/0735/03

Bacterial Reverse Mutation Test

Guideline:	OECD 471
Species/strain:	*S. typhimurium* TA98, TA100, TA1535 and TA1537; *E. coli* WP2 uvrA
Replicates:	Triplicate plates, 2 independent tests
Test substance:	MIP 2985
Batch:	029753A8AA
Purity:	98.8% by HPLC
Concentrations:	*S. typhimurium* Without metabolic activation Test #1: 3.33 – 333 µg/plate With metabolic activation (rat liver) Test #1: 3.33 – 500 µg/plate With reductive metabolic activation (hamster liver) Test #1: 3.33 – 500 µg/plate *E. coli* With and without metabolic activation (normal & reductive systems) Test #1: 3.33 – 333 µg/plate *S. typhimurium* Without metabolic activation Test #2: 3.33 – 200 µg/plate (the last 2 concentrations are toxic) With metabolic activation (rat liver) Test #2: 3.33 – 333 µg/plate With reductive metabolic activation (hamster liver) Test #2: 10 – 500 µg/plate

E. coli
Without metabolic activation (normal & reductive systems)
Test #2: 3.33 – 333 µg/plate (the last 2 concentrations are toxic)
With metabolic activation (normal & reductive systems)
Test #2: 3.33 – 333 µg/plate (the last concentrations were toxic)
GLP: In compliance

MIP 2985 was investigated for gene mutation in *Salmonella typhimurium* and *Escherichia coli* using the direct plate incorporation method both with and without S9-mix. S9-mix from different origin was used. Standard: Sprague-Dawley rats injected i.p. with Aroclor™ 1254; reductive: uninduced male Golden Syrian hamsters.
Negative and positive controls were in accordance with the OECD guideline.

Results
Test # 1
In the absence of activation, no dose related and biologically relevant increase in revertant numbers was observed, in all but one tester strains (strain TA 98). The increase is according to the OECD criteria.
In the presence of rat (commonly used S9) activation: No dose related and biologically relevant increase in revertant numbers was observed, in any of the tester strains used (*S. typhimurium* or *E. coli*).
In the presence of hamster (reductive S9) activation: an increase in revertant numbers was observed for TA 98 - a frameshift tester strain - at the dose of 100 µg/plate. There is a trend for a dose relationship until cytotoxicity that could have prevented the expression.
For the other strains (including *E. coli*), no statistically or biologically relevant increase of mutant frequencies have been observed as compared to the controls. Positive controls showed the expected response.

Test # 2
In the absence of activation, no dose related and biologically relevant increase in revertant numbers was observed, in any of the tester strains.
In the presence of rat activation, no dose related and biologically relevant increase in revertant numbers was observed, in any of the tester strains.
In the presence of hamster (reductive S9) activation, a statistical and dose related significant increase in revertant numbers was observed for TA 98.

Conclusions
Based on the reversion rate, and under the conditions of the 2 assays performed, it could be concluded that the test agent Basic Red 51, in the presence of reductive S9-mix, shows clear evidence of mutagenic activity in tester strain TA 98. Such positive results may be the consequence of the metabolizing properties (azo-reduction) of the S9-mix fraction from hamster.
The higher amount of aromatic amines released, which are metabolised to electrophilic molecules that may react with DNA, might explain the positive results observed in TA 98.
Ref.: 13

In Vitro Mammalian Cell Gene Mutation Test

Guideline: OECD 476
Cells: Chinese Hamster V-79 cell line (mutation at the *hprt* locus)
Replicates: 2 independent tests
Test substance: MIP 2985 in serum free medium
Batch: 11R-1
Purity: 60 %
Concentrations: Test #1
Without metabolic activation: 3 – 300 µg/ml (6 doses)

With metabolic activation: 3 – 400 µg/ml (6 doses)
Test #2
Without metabolic activation : 3 – 150 µg/ml (6 doses)
With metabolic activation : 3 – 200 µg/ml (6 doses)
GLP: In compliance

MIP 2985 in serum free medium was investigated for gene mutation at the *hprt* locus in V79 Chinese hamster cell line.
S9-mixes from different origins were used: Standard: Sprague-Dawley rats injected i.p. with Aroclor™ 1254 in the second test; reductive: uninduced male Golden Syrian hamsters in the first test.

Results
Toxicity occurred at dose of 100 µg/ml and above with and without activation in both assays.

No visible precipitation was observed.

In test #1 and in test # 2 with S9-mix, no statistically or biologically significant increase in mutant frequency was observed over the concurrent solvent controls for any doses in all conditions.
In test # 2 without S9-mix, the negative control value was extremely low (1.4 per million cells). The treated doses showed an increase in mutant frequency without dose dependence. Therefore, the increases observed are thought to be devoid of biological relevance.

Conclusions
MIP 2985 did not demonstrate mutagenic potential on the *hprt* gene of V79 cells.

Ref.: 15

In Vitro Mammalian Chromosomal Aberration Test

Guideline: OECD 473
Species/strain: Human lymphocytes (pooled cultured blood samples)
Replicates: Duplicate cultures, 2 independent experiments
Test substance: MIP 2985 in cell culture grade water
Batch: 029753A8AA
Purity: 98.8% by HPLC
Concentrations: Test #1
3 h without S9-mix: 20.2, 28.8, 41.2 µg/ml
3 h with S9-mix: 41.2, 58.8, 84, 120 µg/ml
Test # 2
3 h with S9-mix: 22.5, 45, 90, 120 µg/ml
22.2 h without S9-mix: 17.5, 22.5, 30, 35 µg/ml
GLP: In compliance

MIP 2985 was investigated for induction of chromosomal aberrations in human pooled lymphocytes. The test concentrations were established from a preliminary toxicity study.
Liver S9 fraction from Aroclor 1254-induced rats was used as the exogenous metabolic activation system.

Results
pH was not significantly changed at the maximum dose tested of 4990 µg/ml(pH =7.5).
Molarity for the maximum dose tested in the assay (2990 µg/ml) was found to exceed slightly the maximum value recommended (10 mM = 2798 µg/ml). However, the small deviation (2990-2798 = 192) is devoid of biological relevance.

No statistically or biologically significant increase in the number of aberrant cells was observed as compared to the corresponding solvent control.

No significant increase of aneuploidy and/or endoreduplicated cells was noted.

Conclusions
Basic Red 51 is considered negative for clastogenic and/or aneugenic activity in human lymphocytes in the presence or the absence of activation under the conditions of the test.
Ref.: 14

Comment
The tests have been performed only with the standard metabolic activation system (rats injected i.p. with Aroclor™ 1254). No definitive conclusions can be made on the basis of this test.

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Taken from SCCNFP/0735/03

Mammalian Erythrocyte Micronucleus Test

Guideline:	OECD 474 (1983)
Species:	NMRI mice
Group sizes:	6 male and 6 female
Material:	MIP 2985
Batch:	0017
Purity:	> 88%
Dose levels:	MIP 2985 was administered by 1 single oral dose of: 10, 33 and 100 mg/kg bw for the 24 h sacrifice time 100 mg/kg bw for the 48 h sacrifice time.
GLP:	In compliance

MIP 2985 was investigated for induction of micronuclei in the bone marrow cells of male or female mice. Dose levels were determined by a preliminary range finding study in which observable toxic effects were seen at doses of 1000 and 2000 mg/kg bw. The substance was administered by a single intragastric gavage and the groups of animals sacrificed 24, 48 and 72 hours after administration. Negative and positive controls were in accordance with the OECD guideline.

Results
A total of at least 1000 erythrocytes were examined from each animal; the incidence of micronucleated erythrocytes and the ratio of polychromatic erythrocytes to normochromatic erythrocytes were calculated.
In a pre-experiment for toxicity, all mice treated with 250 mg/kg bw died within 6 h. Consequently, 100 mg/kg bw was chosen as the highest dose. At this dose in the pre-experiment clinical signs (reduction of spontaneous activity, eyelid closure, apathy, abdominal position and occasionally tumbling movement and convulsions up to at least 6 h) were observed which may indicate to systemic bioavailability.

The mean number of NCE (mature differentiated cells) was not significantly increased after treatment as compared to controls; this reflects the lack of cytotoxicity of the test agent.
Both at the 24 h and 48 h sampling time, no statistically significant or biologically relevant increase in the incidence of micronucleated polychromatic erythrocytes over the concurrent vehicle control values was observed for any dose levels.

Conclusion

Under the conditions of the test it can be concluded that MIP 2985, at doses at which some signs of clinical toxicity were recorded, does not induce statistically significant increase in the frequency of micronucleated PCE.
Therefore, MIP 2985 is not clastogenic and/or aneugenic in this mouse bone marrow micronucleus test.

Ref.: 17

Comment
Although the mean number of NCE was not significantly increased after treatment as compared to controls reflecting a lack of cytotoxicity of the test agent, clinical signs found in the pre-experiment in treated animals up to 6 h after the highest dose may point to systemic bioavailability of MIP 2985.

Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *in vivo*

Guideline:	OECD draft guideline 486
Species/strain:	Wistar rat, HanIbm: WIST (SPF) strain
Group size:	4 male rats
Test substance:	MIP 2985
Batch:	CGF-F016740/0018
Purity:	> 98%
Dose levels:	0, 75 and 300 mg/kg bw, by single oral gavage
Exposure time:	16 hours: all dose groups; 2 h: high dose group
GLP:	In compliance

MIP 2985 was investigated for induction of unscheduled DNA synthesis in rat hepatocytes at 2 doses 75 and 300 mg/kg bw.
Positive controls were in accordance with OECD guideline for 60 hours treatment and UDS analysed by autoradiography. 3 males were used per dose/time sampling.

Results
The viability of the hepatocytes was not substantially affected by the treatments. Treatment with MIP 2985 at doses of 75 & 300 mg/kg yielded group mean NNG values less than 0 for both experiment time and caused no significant increases, as compared to control, in the mean nuclear grain counts.

Conclusion
The study is inadequate for evaluation. According to OECD guidelines, a positive control should have been included for the short time exposure.

Ref.: 16

Submission II, 2005

Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *in vivo*

Guideline:	OECD 486 (1997)
Species/strain:	rat, Wistar Hanlbm: WIST (SPF)
Group size:	3 males per dose and time
Test substance:	Red MIP 2985
Batch:	21187FC3
Purity:	95.5%
Vehicle:	deionised water
Dose levels:	125 and 250 mg/kg bw
Volume:	10 ml/kg bw
Route:	oral, once

Exposure time: 2 and 16 hours
Positive control: N,N'-dimethylhydrazinedihydrochloride (DMH): 2h preparation interval
2-acetylaminofluorene (2-AAF): 16h preparation interval
GLP: in compliance
Study period: 8 July - 10 September 2004

The test item RED MIP 2985 was assessed in the *in vivo* UDS assay for its potential to induce DNA repair (UDS) in the hepatocytes of rats. The highest dose used in the main experiment was estimated in a pre-experiment to be the maximum applicable dose at which clinical signs of toxicity occurred without affecting the survival rates. Appropriate positive controls were included in the assay.

Results
The viability of the hepatocytes was not substantially affected by the *in vivo* treatment with the test item.
No dose level of the test item revealed UDS induction in the hepatocytes of the treated animals as compared to the current vehicle controls. Neither the nuclear grains nor the resulting net grains were distinctly enhanced due to the *in vivo* treatment of the animals with the test item for 2 hours or 16 hours, respectively. Therefore, the net grain values obtained after treatment with the test item were consistently negative. In addition, no substantial shift to higher values was obtained in the percentage of cells in repair. *In vivo* treatment with DMH or 2-AAF revealed distinct increases in the number of nuclear and net grain counts.

Conclusion
Under the experimental conditions reported, the test item did not induce DNA-damage leading to increased repair synthesis in the hepatocytes of the treated rats. Therefore, RED MIP 2985 is considered to be non-genotoxic in this *in vivo* UDS test system.

Ref.: 3, submission II

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

Taken from SCCNFP/0735/03

Guideline: OECD 414
Species/strain: Wistar rat, Hanlbm (SPF)
Group Size: 22 mated females per dose
Test material: MIP 2985
Batch: CGF-F016740/0018
Purity: 98%
Dose: 0, 20, 60 and 180 mg/kg bw/day
Treatment period: Days 6 to 17 post coitum
GLP: in compliance

The animals were dosed with 10ml /kg by gavage once daily. The control group received only the vehicle (double distilled water).

Food consumption was recorded for the following periods: days 0-6, 6-12, 12-18 and 18-21 post coitum; body weight was recorded daily from day 0 until day 21 post coitum. Clinical observations and mortality were recorded at least twice daily. At post mortem, on day 21, necropsy, all internal organs were examined with emphasis on the uterus, uterine contents, position of foetuses in the uterus and number of corpora lutea. The uteri of all females with live foetuses were weighed at necropsy on day 21 post coitum; the foetuses were removed from the uterus, weighed, sexed, and examined for gross external abnormalities.

Maternal deaths did not occur during the study and clinical signs of toxicity or reactions to treatment did not occur in any group. A dose-dependent reduction of the food consumption was observed during the treatment period in the mid- and high-dose groups (-7.6% and -23.5% respectively); an increase of + 5.5 % was observed in the high dose group after the treatment period.
The mean body weight gain was reduced only in the high dose group, these data being correlated with the decreased food consumption. Mean post-implantation loss and mean number of foetuses per dam were similar between treated and control dams in the low- and mid-dose groups. The increased post-implantation loss observed only in the mid dose group was considered to be incidental. No abnormal findings were noted in any female of any treated group.

The mean foetal body weights were similar in all groups except for a slight increase observed in the mid-dose group, which was attributed to the slightly reduced mean number of foetuses per dam. The sex ratio for foetuses was similar in all groups. Some abnormal findings were noted during foetal examination: externally, one cleft palate was observed in the low dose group. Skeletal changes included a small number of foetuses in each group with abnormally shaped sternebrae. These were not considered related to the test substance, as they were within the range for historical controls.

Under the experimental conditions, MIP 2985 was not toxic to embryo or foetus and was not teratogenic. The study authors considered the NOEL for the maternal effects is 20 mg/kg bw/day and for foetal effects the NOEL is 180 mg/kg bw/day.

Ref.: 11

3.3.9.	Toxicokinetics

Bioavailability after oral administration, mice

Guideline:	OECD 417 (1984)
Species/strain:	Hybrid mice, NMRI, SPF-quality
Group Size:	15 females
Test material:	Vibracolor® Red (MIP 2985)
Batch:	21187FC3
	3501-069 (radio-labelled) (2072 MBq/mmol; 56 mCi/mmol)
Purity:	95.9% (containing about 4% inorganic salts)
	98.15% (radio-labelled)
Vehicle:	water (MilliQ)
Dose:	33 mg/kg bw
Administration:	oral, single gavage
GLP:	in compliance
Study period:	1 – 14 September 2004

The radiolabelled Vibracolor® Red (MIP 2985) test material had a final specific radioactivity of 846 kBq/mg (22.86 µCi/mg).
Fifteen female mice were dosed by gavage at a nominal dose level of 33 mg/kg body weight based on a target volume of 0.5 ml per 100 g body weight.

At intervals of 0.5, 1, 2, 4, and 24 h after dosing, three animals were killed by CO2. Blood and femurs from these animals were taken. Radioactivity was measured by liquid scintillation of plasma and both femurs of each animal.

Results
The test item was rapidly absorbed from the gastrointestinal tract. In plasma, the highest concentration of radioactivity 2.016 ppm (µg Red (MIP 2985) equivalents/g) was found 0.5 h after dosing. After reaching the maximum, the concentration in plasma decreased rapidly with an initial half-life of 1.3 h (initial phase, 1-2 h). Between 4 and 24 h after dosing, a second, slower depletion phase (2-24 h) was seen with a depletion half-life of 8 hours. By 24 hours, the plasma concentration had decreased to 0.117 ppm. The AUC was 14.12 µg h/g.
In the femur, the highest concentration of radioactivity 4.23 ppm (µg Red (MIP 2985) equivalents/g) was found 1 h after dosing. By 24 h post-dosing the concentration in femur decreased to 0.228 ppm. The depletion kinetics in the femur was similar to that seen in the plasma, with an initial half-life of 1.3 h and a depletion half-life of 10 h. It was assumed that the bone marrow of the femur was the main site of radioactivity.

Ref.: 4, submission II

ADME after oral and dermal administration, rat

Guideline:	OECD 417 (1984)
Species/strain:	Rat, Wistar HanBrl:WIST (SPF): outbred,
Group Size:	17 females (oral: 9; dermal: 8)
Test material:	Vibracolor ® Red (MIP 2985)
Batch:	21187FC3 3501-069 (radio-labelled) (2072 MBq/mmol; 56 mCi/mmol)
Purity:	95.9% (containing about 4% inorganic salts) 98.15% (radio-labelled)
Vehicle:	water
Dose:	50 mg/kg bw (gavage) 0.2 mg/cm² (dermal administration)
Exposure period:	30 minutes (dermal administration)
GLP:	in compliance
Study period:	21 August 2004 – 11 January 2005

Oral study
Nine females were dosed by gavage at a nominal dose level of 50 mg/kg body weight with [^{14}C] labelled RED (MIP 2985) with a final specific radioactivity of 204 kBq/mg (5.5 µCi/mg). Blood was taken at 0.5, 1, 2 4, 6, 8 h from selected animals. Terminal blood was taken at the three post-dosing sacrifice times of 24, 48 and 96 h (3 animals). Urine and faeces were collected over 0-24, 24-48, 48–72, and 72-96 h. At the end of the study period, the cagewash was also collected for analysis.

Results
Red (MIP 2985) was rapidly and almost completely absorbed from the gastrointestinal tract. The apparent extent of absorption, based essentially on the amount renally excreted, was calculated to at least 62 % of the dose. There appeared to be some bilary recirculation.
The maximum concentration (C_{max}) in blood and plasma was reached within 1 hour after administration accounting to 3.933 ppm and 4.785 ppm Red (MIP 2985 equivalents), respectively. A second lower maximum in blood and plasma was observed 6 hours post-dosing. After reaching the second maximum, blood and plasma showed a biphasic depletion half-life of 15 and 9 h for the initial phase and 206 and 40 h for the second slower phase, respectively.
The oral dose was rapidly eliminated. Within 24 hours, 74 % of the dose was totally excreted, (53 % in urine, 17 % in the faeces and 4 % collected in the cage wash). By 96

days post-dosing, 93 % of the dose was eliminated. Only 0.5 % of the dose is still remaining in tissues and carcass.
The highest residue levels in tissues and organs were observed 24 hours post-dosing in the kidney (5.3643 ppm) and liver (4.0287 ppm), with low levels in fat (0.3267 ppm). The depletion kinetics in tissues and organs was similar to that seen in blood and plasma. The initial half-life was calculated to be in the range of 11-17 hours followed by a second slower phase with half life of 29-56 hours.
Two major metabolites, 1,3-dimethyl-imidazole-2-amine (about 50 % of the dose) and 1,4-diacetoaminobenzene (about 20% of the dose) and 6 minor metabolites were seen in urine and faeces. Unchanged Red (MIP 2985) (1.5 % of the dose) was found in the faeces. Due to the structure of the identified metabolites, the metabolic pathway suggested that the 1,3-dimethyl-imidazole-2-amine was a cleavage product of Red (MIP 2985) and that the 1,4-diacetoaminobenzene was the di-acetylation product of Red (MIP 2985) after demethylation of the (4-dimethylamino)phenyl site and cleavage of the azo bond.

Dermal study
Eight females were exposed dermally to [^{14}C] labelled RED (MIP 2985) at 0.2 mg/cm^2 (the highest achievable water solubility of the test material) for 30 min. The final specific radioactivity was 241 kBq/mg (6.52 µCi/mg at pH 7.7). At 24 and 96 h post-application, four animals were killed. The exposure site was washed and tape stripped to remove the unabsorbed test material.

Results
After 30 min dermal exposure to [^{14}C] Red (MIP 2985), only 1.29% of the applied dose was systemically absorbed; 93-94% of the dose could be dislodged from the application site. After the skin washing, 2% of the dose remained in/on the treated skin area. The remaining radioactivity in/on the application site was found in the stratum corneum. Less than 0.01% of the dose was determined in the dermis and subcutis. The quantity of test substance in the stratum corneum was virtually constant throughout the 96 h study period, indicating low penetration into deeper skin layers. The systemically absorbed test substance was rapidly excreted with the urine (1.08 %) and the faeces (0.14%).

Conclusion
Red (MIP 2985) was rapidly and almost completely absorbed after oral dosing. The systemically absorbed test substance was metabolized and eliminated with urine and faeces. Dermal absorption was very low.

Ref.: 5, submission II

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

Taken from SCCNFP/0735/03

Guideline:	OECD draft (1995) "Acute dermal photoirritation dose-response test"
Species/strain:	Himalayan spotted guinea pigs
Group size:	15 males (10 test and 5 control)
Test substance:	MIP 2985
Batch:	CGF-F016740/0018
Purity:	> 98%
Dose:	0.025 ml/2cm2 of 50%, 25%, 15% and 10% aqueous dilutions. Skin at test sites treated 30 minutes before application with 2% DMSO in ethanol.
GLP:	In compliance

MIP 2985 was applied epicutaneously to skin areas of 2 cm2 on both flanks. 30 minutes after application of the test materials, the left flank was exposed to 20J/cm2 UVA. The right

flank remained unexposed to light after treatment and served as a reference site. Control animals were exposed to UVA similarly but treated with solvent only. Cutaneous reactions were evaluated at 24, 48 and 72 hours after application.

Results
There were positive reactions in all animals at the sites treated with 50% of the test material, both with and without UVA. The reactions were considered to be an irritant effect unrelated to UVA exposure. There were no other reactions and it was concluded that under the test conditions, MIP 2985 does not exhibit a phototoxic potential in the guinea pigs of the strain and age.

Ref.: 9

Photo-allergy

Guideline:	CTFA Safety Testing Guideline	
Species/strain:	Himalayan spotted guinea pigs	
Group size:	30 males (20 test and 10 control)	
Test substance:	MIP 2985	
Batch:	CGF-F016740/0018	
Purity:	>98%	
Dose:	Induction:	nuchal skin of the test group shaved. Test site of 6-8 cm² defined by four 0.1ml intradermal injections of Freund's Complete Adjuvant and physiological saline 1:1 into the corners. 0.1ml of 50% MIP 2985 applied to area of 8 cm². The site was then exposed to 1.8J/cm² UVB and 10J/cm² UVA. The application and irradiation (after shaving) was repeated on days 3, 6, 8 and 10.
	Challenge:	3 weeks after the start of the induction procedure, test sites of 2 cm² were marked and 0.025 ml/2cm2 of 50%, 25%, 15% and 10% were applied to the left flank and then irradiated with 10J/cm² UVA. After irradiation of the left flank, the right flank was treated with the test materials without irradiation.
GLP:	In compliance	

3 hours prior to the first readings, the application sites were depilated with a depilatory cream. Each animal was assessed for reactions at 24, 48 and 72 hours after challenge.

Results
A very slight red discoloration produced by the test material at the application sites were observed from test days 2 to 22. No skin reactions were observed in the test animals treated with 50% test material during the induction phase. No reactions were observed on the irradiated or non-irradiated flanks of the control and test animals treated with 50, 25, 15 and 10% test material. The data indicated that MIP 2985 does not exhibit photoallergic potential.

Ref.: 10

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

No data submitted

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Basic Red 51

(non-oxidative)

Absorption through the skin	**A (mean + 1SD)**	**=**	**0.66 µg/cm²**
Skin Area surface	**SAS (cm^2)**	**=**	**580 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**0.383 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.006 mg/kg**
No Observed Adverse Effect Level (subchronic, oral, rat)	**NOAEL**	**=**	**10 mg/kg bw**
MOS		**=**	**1567**

3.3.14. Discussion

Physico-chemical specification
Basic Red 51 is intended for use in direct hair dye formulations at concentrations up to 0.2% and in oxidative hair dyes at a final concentration of 0.1%, after mixing with the oxidative agent. Stability of Basic Red 51 in typical hair dye formulations has been studied only for 24 hours.
No reference materials were used for identification and quantification of Basic Red 1 and its impurities.
The stability in an oxidative environment has not been demonstrated.

General toxicity
The acute oral LD_{50} is considered to be between 250 - 500 mg/kg bw in females and 500 - 1000 mg/kg bw in males. The acute dermal LD_{50} is greater than 2000 mg/kg bw.
The NOAEL was considered to be 12.25 mg/kg bw/day in the repeated dose oral toxicity study. In the sub-chronic oral toxicity study, due to the effects on the thyroid and pituitary, the NOAEL was set at 10 mg/kg bw/day.
Basic Red 51 was neither embryotoxic nor foetotoxic nor teratogenic in the performed assays. The NOEL for the maternal effects was considered to be 20 mg/kg bw/day and 180 mg/kg bw/day for foetal effects.
Basic Red 51 was rapidly absorbed after oral dosing. The systemically absorbed test substance was almost completely metabolized and eliminated with urine and faeces. Dermal absorption was very low.

Irritation, sensitisation
Basic Red 51 is considered as a non skin irritant in a skin irritation study; however, the acute dermal toxicity study shows that the substance is mildly irritating.
Basic Red 51 is moderately irritant to rabbit eye and a non sensitizer.

Dermal absorption
The dermal absorption of Basic Red 51 was 0.399 µg/cm² and 0.351 µg/cm² in non oxidative and oxidative conditions, respectively.
The mean + 1 SD, 0.660 (0.399 ± 0.261) µg/cm² can be used to calculate the MOS under non-oxidative conditions and 0.540 (0.351 ± 0.189) µg/cm² under oxidative conditions.

Mutagenicity
Basic Red 51 has been tested for the three genetic endpoints: gene mutation, structural and numerical chromosomal aberrations. It induced frame shift mutations in bacteria (strain TA98) when reductive metabolic activation was used. In mammalian cells with different metabolic activation, including reductive activation, no gene mutations were induced. An *in vitro* chromosomal aberration study was inconclusive due to inadequate metabolic activation. However, the test substance was not clastogenic or aneugenic in an *in vivo* micronucleus assay and no DNA repair was induced in an adequate performed *in vivo* UDS assay. Therefore, it is concluded that Basic Red 51 does not have genotoxic potential *in vivo*

Carcinogenicity
No data submitted

4. CONCLUSION

Based on the data provided, the SCCS is of the opinion that the use of Basic Red 51 with a maximum on-head concentration of 1% in non-oxidative hair dye formulations does not pose a risk to the health of the consumer. For a final assessment of the use of Basic Red 51 with a maximum on-head concentration of 0.5% in oxidative hair dye formulations, data on the stability in an oxidative environment should be provided.

5. MINORITY OPINION

Not applicable

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