Scientific Committee on Consumer Safety

SCCS

OPINION ON

HC Red No. 18
(B124)

The SCCS adopted this Opinion at its 12th plenary meeting
on 15 December 2015

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.
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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 ISBN
Doi: ND-

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ACKNOWLEDGMENTS

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Keywords: SCCS, scientific opinion, HC Red No. 18 (B124), Regulation 1223/2009, CAS 1444596-49-9

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on HC Red No. 18 (B124), 15 December 2015, SCCS/1569/15

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1. BACKGROUND

Submission I on the new hair dye HC Red No. 18 (B124) (FPK 245) (CAS 1444596-49-9) was transmitted by Cosmetics Europe in December 2014.

The hair dye HC Red No. 18 (B124) (FPK 245) is intended to be used as a hair dye ingredient in oxidative hair colouring products at on-head concentrations of up to 1.5 % under oxidative conditions.

2. TERMS OF REFERENCE

(1) In light of the data provided, does the SCCS consider HC Red No. 18 (BÍ 24) (FPK 245) safe when used as a hair dye ingredient in oxidative hair colouring products at on-head concentrations of up to 1.5 % under oxidative conditions?

(2) Does the SCCS have any further scientific concerns regarding the use of HC Red No. 18 (B124) (FPK 245) in other cosmetic products?

3. OPINION

3.1 Chemical and Physical Specifications

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

HC Red No. 18

3.1.1.2 Chemical names

2-chloro-4-{(E)-[3-(methylthio)-1,2,4-thiadiazol-5-yl]diazenyl}phenol

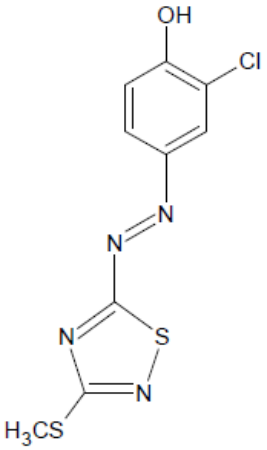
3.1.1.3 Trade names and abbreviations

FPK-245

3.1.1.4 CAS / EC number

CAS: 1444596-49-9
EC: /

3.1.1.5 Structural formula



3.1.1.6 Empirical formula

C9H7ClN4OS2

3.1.2 Physical form

Red powder

3.1.3 Molecular weight

Molecular weight: 286,76 g/mol

3.1.4 Purity, composition and substance codes

Chemical identification of HC Red 18 lot WK 120914 was performed by ^{1}H-NMR, ^{13}C-NMR, IR and UV-vis spectroscopy (in DMSO as diluent solvent). (Ref 18)

Purity and impurities of HC Red 18 were determined by HPLC-PDA. The detection was performed at 254 nm for all the batches.

Table Summarising Composition of FPK-245 for the batches WK120919, WK120914 and 06041404:

	WK120919	WK120914	06041404	Proposed Specification
FPK-245 (10,89 min)	99.82	99.39	99.56	>98.5%
Impurity 1 (4,2min)	0.08	0.22	0	<0.3%
Impurity 2 (4,8min)	Not detected	Not detected	0.17	<0.3%
Impurity 3 (21,6min)	Not detected	Not detected	0.28	<0.5%

*Gradient RP-HPLC method with UV detection at 254 nm + Diode array (Appendix III)

Batches used in respective toxicological studies

WK120919	**06041404**	**WK120914**
-Eye irritation -Skin irritation -BCOP -*In vitro* dermal delivery of cream FPK-245 under oxidative conditions and non-oxidative conditions -Subchronic toxicity 90 day oral toxicity -DRF Teratogenicity -Teratogenicity -Gene mutation assay in Chinese Hamster V79 cells in vitro (V79/HPRT) -*In vitro* Micronucleus assay in human lymphocytes -Medium-term liver carcinogenesis assay -Toxicokinetics (non-labelled)	-Ames Assay -SHE Cell Trans-formation Assay	-Local Lymph Node Assay (LLNA) -DRF 14-day study

3.1.5 Impurities / accompanying contaminants

Peak purity and impurities at 254nm

Overall Purity (HPLC 254nm) (FPK-245 retention time: 10.89)	greater than 98.5%
Impurity 1 (retention time: 4.2)	less than 0.3%
Impurity 2 (retention time: 4.8)	less than 0.3%
Impurity 3 (retention time: 21.6)	less than 0.5%
Water Content	less than 1%

Ash Content	less than 1%
Heavy Metal Content	Arsenic less than 5ppm Antimony less than 5ppm Lead less than 20ppm Cadmium less than 10ppm Mercury less than 5ppm

SCCS comments on purity and impurity:

-Study report on analytical methodology and information on the validation of the HPLC method used for purity/impurity testing was not submitted.
- IR, ^{13}C-NMR and ^{1}H-NMR data for the chemical identification of the main compound was submitted only for the batch WK 120914. IR, ^{13}C-NMR and ^{1}H-NMR data for the batches WK120919 and 06041404 must be submitted.
-The HPLC purity was based on the detection at 254 nm, but the λ_{max} of HC Red 18 is ca. 526 nm in the mobile phase. Chromatograms were obtained using PDA detection, so a reprocess of the LC- chromatograms using λ_{max} ~ 526 nm will give a realistic purity of HC Red 18 and it will provide further information on the impurities related with the synthesis of HC Red 18.
- In the absence of the analytical report, it is not known how the peak purity of the major peak was calculated before determination or whether a reference standard was used for the determination of the purity of HC Red 18 in various batches.
- None of the impurities were chemically characterised. The synthetic route was provided in additional data. The applicant provided one possible impurity according to the synthetic route. All impurities should be characterised by LC/MS and GC/MS. Any impurity belonging to CMR (carcinogenic, mutagenic and reproduction toxic) classes must be quantified. The SCCS has been informed by the applicant that details on impurities will be submitted at a later date as the work is still on-going.
- Methodology used for the determination of water content, ash content and heavy metal content was not described and the study reports were not submitted.
- On the basis of the above-mentioned shortcomings, reported purity and impurity data for the batches WK120919, WK120914 and 06041404 cannot be accepted.

3.1.6 Solubility

Water: insoluble (less than 0.001%)
DMSO: 12.5%
Ethanol: Less than 0.1%

SCCS comment:
The study report of water solubility determination was not submitted. It is not known whether the water solubility was determined by the EU Method A.6.

3.1.7 Partition coefficient (Log P_{ow})

Log P (ACD): 0.90 ± 1.00 (monoanion): +4.05 ± 0.62 (neutral form)

SCCS comment:
The study report for Log Pow determination was not submitted. It is not known whether the water solubility was determined by the EU Method A.8.

3.1.8 Additional physicochemical specifications

Melting point: /
Boiling point: /
Flash point: /
Vapour pressure: /
Density: /
Viscosity: /
pKa: /
Refractive index: /
pH: /
UV-Vis spectrum (200nm-800 nm): λ_{max} ca. 545 nm in DMSO as diluent solvent (Ref 18), and λ_{max} ca. 526 nm in mobile phase (0.1% CH_3COOH, 0.1% TEA in methanol - 0.1% CH_3COOH, 0.1% TEA in water: 90:10, v/v).

3.1.9 Homogeneity and Stability

A 0.5% FPK-245 solution (dissolved in MEA buffer pH 10, containing 4.5% monoethanolamine and 5% isopropanol in water) was shown to be stable for 45 min when mixed with 6% hydrogen peroxide in a ratio 1:1, v/v.
The HPLC chromatograms, taken at two different wavelengths, clearly show that FPK-245 is stable to alkaline peroxide over a 45min period. There is no change in retention time, peak shape or peak area over 45mins and no additional peaks (i.e. due to degradation products) can be detected. This is also confirmed in the following table where the exact peak areas are shown to remain constant.

Table 1: Comparison of FPK-245 Peak Areas at time = 0min and time = 45min as quantification of stability

Sample Name	Ret.Time	Area	Area
	min	mAU*min	mAU*min
		530nm	254nm
FPK-245 0,5% MEA buffer 1:1 H_2O_2 6% t=0	12,767	649,9516	78,1203
FPK-245 0,5% MEA buffer 1:1 water (100%)	12,767	646,4885	77,5517
FPK-245 0,5% MEA buffer 1:1 H_2O_2 6% t=45	12,758	643,8065	77,6164
Stability at 45min		**99.1%**	**99.4%**

It is concluded that FPK-245 is stable in alkaline peroxide 6% over a 45min period.

SCCS comment
Stability study under alkaline peroxide conditions was performed using HPLC-PDA analysis at 254 and 530 nm.
There is a drift in the retention time of the test compound in the HPLC-PDA chromatograms provided for the stability study (12.76 min) in relation to the retention time of HC Red 18 in the HPLC-PDA chromatograms provided for the impurity test (10.89 min) because of the different gradient HPLC method used. Typically impurity and stability studies are performed

using the same HPLC method; the applicant should provide an explanation of why this was not done in this case.
The SCCS has no information on the batch used for the stability study of HC Red 18 under alkaline peroxide conditions. Information on the batch number, purity and impurity of this batch must be provided.

General Comments to physicochemical characterisation

- Chemical characterisation data should be provided regarding IR, ^{13}C-NMR and ^{1}H-NMR for all the batches used in the studies.
- The purity and impurity data of the HC Red 18 batches WK120919, WK120914 and 06041404 cannot be accepted in the absence of a study report describing analytical methodology, validation of the method, reference standard used for the determination, etc.
- None of the impurities were chemically characterised. An overview of impurities should be provided by both HPLC-PDA chromatogram and/or GC chromatogram of HC Red 18. The impurities should be characterised by a hyphenated technique such as LC/MS or GC/MS and any impurity belonging to CMR (carcinogenic, mutagenic and reproduction toxic) must be quantified.
- The SCCS has been informed by the applicant that details on impurities will be submitted at a later date as the work is still on-going.
- The SCCS has no information on the batch used for the stability study of HC Red 18 under alkaline peroxide conditions. Information on the batch number, purity and impurity of this batch must be provided. An explanation of the different HPLC-PDA method used for stability studies in relation to the HPLC-PDA method used for impurity studies is needed.
- It is not known whether water solubility and Log Pow were determined by respective EU methods as the study reports for their determination were not submitted.
- Several physicochemical properties such as melting point, pka, etc. of HC Red 18 are not reported.

3.2 Function and uses

FPK-245, a non-reactive dye, is used as a direct hair colouring agent up to on-head concentration of 1.5 % in oxidative hair dye formulations.

3.3 Toxicological evaluation

3.3.1 Acute toxicity

No acute oral toxicity studies with HC Red No. 18 were submitted.

In the 14-day dose-range finding study in rats administered FPK-245 by oral gavage (0.5% in methylcellulose) at dose levels of 0, 30, 100, 300(200) or 1000(600) mg/kg bw/day, deaths occurred at 1000(600) mg/kg bw/day (2 males on day 8 and all 5 females on days 6-13). The remaining 3 males at 1000(600) mg/kg bw/day and 2 females at 300(200) mg/kg bw/day were subjected to moribund sacrifice on day 10, and day 6-8, respectively. No deaths were reported at the lower dose levels (30 and 100 mg/kg bw/day). Prior to death / sacrifice, these animals exhibited deterioration of conditions such as a decrease in locomotor activity, abnormal gait, and decreases in body weight and food consumption.

Ref. 14

SCCS comment
Based on the mortalities in the 14-day dose range-finding rat study from 300(200) mg/kg bw/day, HC Red No. 18 is considered to possess a moderate acute toxic potential following oral administration.

3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

Guideline:	OECD 439 (July 2010)
Test system:	EpiDerm™ SIT (EPI-200) Reconstructed Human Epidermis Model
Replicates:	3 tissues per condition
Test substance:	FPK-245
Batch:	WK120919
Purity:	99.85% (HPLC)
Dose:	24-27 mg neat test substance wetted with 25 µl of deionised water on the tissue
Treatment period:	60 minutes: 35 minutes at 37 °C followed by 25 minutes at room temperature
Post-treatment incubation time:	about 41 hours
Positive control:	5% sodium lauryl sulphate (SLS) in deionised water
Negative control:	Dulbecco's Phosphate Buffered Saline (DPBS)
Direct interaction with MTT:	negative
Colouring of tissue:	nothing mentioned in study report
GLP:	in compliance
Study period:	January 2013

24 - 27 mg of test substance was applied to each set of three tissues, wetted with 25 µl of deionised water, and spread to match the surface of the tissue. 30 µl of either the negative control or positive control were applied to each tissue. After 35 minutes treatment at 37 °C and 25 minutes at room temperature, the tissues were rinsed with DPBS. Following about a 41-hour post-treatment incubation period, cell viability was assessed by the MTT assay. Hereto, 300 µl of MTT solution was added to the tissues. After a 3-hour incubation period at 37.0 ± 1.5 °C and 5.0 ± 0.5% CO_2, the MTT solution was aspirated from the wells and the tissues were rinsed three times with DPBS. 2 ml isopropanol was added to each well and formed formazan salt was extracted for 69 hours at room temperature. OD was read in a microplate reader at 570 nm.

Results
The quality and suitability of the tissues was assured: after treatment with the negative control the absorbance values were well within the required range of the acceptability criterion of mean OD > 1.0 and < 2.5 for the 60-minutes treatment interval. Treatment with the positive control induced a sufficient decrease in the relative absorbance as compared to the negative control to 4.0%, thus ensuring the validity and suitability of the test system. Compared to the negative control, the mean relative absorbance value did not decrease (113.1%) after exposure to the test item.

Conclusion
FPK-245 was shown to possess no irritant potential to the skin *in vitro* under the experimental conditions of the study.

Ref. 7

SCCS comment

The SCCS notes that an unusually high number (at least 15 times) of washes were performed before the post-treatment incubation period, indicating that red colouring of the tissues by the test item might have occurred. Due to red colouring of the tissues by the test item, interference with the colour formation in the MTT cell viability evaluation may occur leading to false estimates of the skin irritation potential. Therefore, a different technique involving HPLC separation prior to quantification should be used.
Under the conditions of this study, a skin irritation potential of FPK-245 cannot be excluded.

3.3.2.2 Mucous membrane irritation / Eye irritation

Human Cornea Model Test

Guideline:	test kit manual (2013)
Test system:	EpiOcular™ human cornea tissue model
Replicates:	2 tissues per condition
Test substance:	FPK-245
Batch:	WK120919
Purity:	99.85% (HPLC)
Dose:	100 to 106 mg neat test substance wetted with 100 µl deionised water on the tissue
Treatment periods:	3, 30 and 60 minutes (test item), 60 minutes (negative control), 15 and 30 minutes (positive control)
Positive control:	0.3% Triton X-100 in deionised water
Negative control:	deionised water
Direct interaction with MTT:	negative
Colouring of tissue:	yes
GLP:	in compliance
Study period:	January - February 2013

Approximately 100 mg test item was applied to each tissue, wetted with 100 µl deionised water, and spread to match the surface of the tissue. In parallel, 100 µl of the negative and positive control were handled in the same manner for comparison. Tissues were in duplicate treated with the test item and placed in the incubator at 37.0±1.5 °C and 5.0±0.5% CO_2 for 3, 30 and 60 minutes. The cells for the negative control were treated for 60 minutes and the positive control for 15 and 45 minutes, each in duplicate. After incubation, the tissues were rinsed with PBS to remove any residual test material. Cell viability was next measured with the MTT assay. Hereto, 300 µl of MTT solution was added to the tissues. After a 3-hour incubation period at 37.0 ± 1.5 °C and 5.0 ± 0.5% CO_2, the MTT solution was aspirated from the wells and the tissues were rinsed three times with PBS. This rinsing step was followed by a 25-minute post-exposure incubation in fresh medium. 2 ml isopropanol was added to each well and formed formazan salt was extracted for 18 hours at room temperature. OD was read in a microplate reader at 570 nm.
After the extraction period, the isopropanol was red in colour. This fact indicated that the test item could most probably not be removed from the tissues completely.

Results
Both acceptability criteria of the assay were met. The absorbance values for the negative control (1.499 and 1.438) were well above the required acceptability criterion of mean optical density (OD) ≥ 0.8, and the positive control induced a sufficient decrease in the relative absorbance after both treatment intervals (ET_{50} ≤ 30 minutes). No irritating effects were observed following incubation with FPK-245 up to the maximum required duration of 60 minutes. An ET_{50} value could not be calculated for the test item due to the lack of cytotoxicity.

Conclusion
It can be stated that in this study and under the experimental conditions reported, the test item FPK-245 does not possess any eye-irritating potential.

Ref. 6

SCCS comment
Neither individual values nor standard deviations are presented in the study report; only mean values of duplicates are given. The ODs measured for the positive control after both 15 and 45 minutes treatment are not within the defined historical acceptance ranges.
The SCCS notes that due to the red colouring of the tissues by the test item, interference with the colour formation in the MTT cell viability evaluation may occur leading to false estimates of the eye irritation potential. Therefore, a different technique involving HPLC separation prior to quantification should be used. Under the conditions of this study, an eye irritation potential of FPK-245 cannot be excluded.

Bovine Corneal Opacity and Permeability (BCOP) Test

Guideline:	OECD 437 (September 2009), Commission Regulation (EU) 1152/2010, B.47
Test system:	fresh bovine corneae
Replicates:	3 corneae per test condition (9 in total)
Test substance:	FPK-245
Batch:	WK120919
Purity:	99.85% (HPLC)
Test item:	20% (w/v) suspension of FPK-245 in saline
Test volume:	750µl
Treatment period:	240 minutes
Positive control:	10% (w/v) Benzalkonium chloride in saline
Negative control:	saline
GLP:	in compliance
Study period:	February - March 2013

Freshly isolated bovine eyes from at least 9 month old donor cattle were collected from the slaughterhouse and macroscopically examined for defects. Those presenting defects such as vascularization, pigmentation, opacity and scratches were discarded. The corneae were carefully removed from the eyes and mounted in a holder. After a first basal opacity measurement of the fresh bovine corneae (t0), 750 µl of the test item, the positive, and the negative controls were applied onto the corneae and incubated for 240 minutes at 32 ± 1 °C. After the incubation phase, the test item, the positive and the negative controls were each rinsed from the corneae and the opacity was measured again (t240). Thereafter, permeability of the corneae was determined by measuring spectrophotometrically at 490 nm the transfer of 0.5% (w/v) sodium fluorescein upon incubation in a horizontal position for 90 minutes at 32 ± 1 °C.

Results
The negative and positive controls confirmed the suitability and sensitivity of the test system as the negative control did not lead to an increase of opacity or permeability of the corneae (mean IVIS=2.71), while the positive control caused clear opacity (mean IVIS=229.67). Relative to the negative control, the test item FPK-245 at a concentration of 20% (w/v) caused an increase of the corneal opacity. Permeability effects could not be observed. The calculated mean IVIS was 22.07.
Since the test item coloured the corneae during the exposure, and since the colouring could not be rinsed completely, the opacity effect caused by the test item can be most probably set considerably lower than the measured values, which also indicates an even lower IVIS.

Conclusion
FPK-245 showed some irritation potential at the tested concentration of 20% but was not corrosive nor a severe irritant *in vitro* under the experimental conditions investigated. According to OECD 437, the test item is classified as not corrosive / not severe irritant to the eye.

Ref. 4

SCCS comment
The SCCS notes that due to the red colouring of the corneae by the test item FPK-245, interference with both the opacity and the permeability measurement endpoints of the BCOP assay may occur leading to false estimates of the eye irritation potential. Therefore, different endpoints not involving optical quantification methods should be envisaged. Under the conditions of this study, an eye irritation potential of FPK-245 cannot be excluded.

3.3.3 Skin sensitisation

Local Lymph Node Assay (LLNA)

Guideline:	OECD TG429, Commission Directive 2004/73/EC, B.42
Species/strain:	Female mice, CBA/CaOlaHsd
Group size:	2 (pre-test) 4 per dose group (main study)
Test substance:	FPK-245
Batch:	WK120914
Purity:	99.69% (HPLC, 254 nm)
Vehicle:	dimethyl sulfoxide (DMSO)
Concentration:	2.5, 5 and 10% (w/v)
Positive control:	alpha-hexylcinnamaldehyde dissolved in acetone/olive oil (4/1 v/v)
GLP:	in compliance
Study period:	11 to 23 October 2012

The highest concentration of HC Red No. 18 that could be technically achieved was a 10% (w/v) solution in DMSO. The highest non-irritant test concentration was determined in a pre-test was performed in two animals that were treated with concentrations of 5% and 10% (w/v) in DMSO. No erythema or signs of systemic toxicity were observed at these concentrations.
In the main LLNA study, mice were treated with the test item at concentrations of 2.5, 5 and 10% (w/v) in DMSO by topical application to the dorsum of each ear lobe for three consecutive days. A control group of four female mice was treated with the vehicle DMSO alone. Five days after the first topical application, [^{3}H]-methylthymidine (3HTdR) was intravenously injected into the tail vein. Lymphocyte proliferation was determined by measuring 3HTdR incorporation in the pooled auricular lymph node cells. A test item is regarded as a sensitizer in the LLNA if the exposure to one or more test concentrations resulted in a 3-fold or greater increase in incorporation of 3HTdR compared with concurrent controls, as indicated by the stimulation index (SI).

Results:

Concentration (in % (w/v))	SI
2.5	0.9
5	0.9
10	1.5

Conclusions:
Based on the test results, the test item HC Red No. 18 does not show an allergenic potential when tested up to the concentration of 10%. The EC3 value could not be calculated, since all SI values were below 3.

SCCS comment
In the LLNA, a 1.5-fold increase of lymphocyte proliferation was found in the highest dose tested. HC Red No. 18 is not a skin sensitiser under the conditions of this test.

3.3.4 Dermal / percutaneous absorption

Experiment 1: oxidative conditions

Guideline:	OECD 428 (2004)
Test system:	Frozen dermatomed pig ear skin (430-450 µm)
Membrane integrity:	Electrical resistance barrier integrity
Replicates:	12 (6 donors)
Test substance:	FPK-245
Batch:	WK120919
Purity:	99.85%
Test item:	Cream (batch No. C1R2011003.04) with 3% FPK-245 mixed with hydrogen peroxide lotion 6% (batch No. B1E1995011.2) (mixing ratio:1/1)
Dose applied:	20 µL/cm^2 of the test item (approx. 300 µgFPK-245/cm²)
Exposed area:	1 cm^2
Exposure period:	30 minutes
Sampling period:	24 hours
Receptor fluid:	20% EtOH/PBS
Solubility of test substance in receptor fluid:	Up to 2.20 µg/mL
Mass balance analysis:	Provided
Tape stripping:	Yes (4 pools of 5 strips each)
Method of Analysis:	LC-MS/MS
Positive control:	Benzoic acid
Negative control:	2-Ethylhexyl trans-4-methoxycinnamate
GLP:	In compliance
Study period:	February - March 2013

Two experiments were performed on thawed dermatomed pig skin samples under static and non-occluded conditions. The thickness of the skin used was 430-450 µm. The blank

samples were collected immediately after filling the donor chambers at the maximal flow rate of the pump, but before the application of the test item. FPK-245 was not detected in the blank samples. The conductivity across the skin samples of each chamber was determined before treatment and after the last sampling as a measure of skin integrity. The integrity was given for all skin samples before and after the treatment
In each experiment 6 chambers were analyzed and skin samples from 3 donors per experiment were used for the evaluation of each test preparation. A 20 µL aliquot of the test item, corresponding to approx. 300 µg FPK-245, were applied on each skin sample. The test item was left on the skin for 0.5 hours and was then washed off using 9 times (2x 1 mL H_2O warm + 5x1 mL 10% shampoo + 2x1 mL H_2O warm). The dermal delivery was monitored over 24 hours. The *stratum corneum* was separated by tape stripping from the remaining epidermis and dermis layers. The tape strips (5 strips per sample, 4 samples) were pooled and extracted for analysis. The remaining skin compartments were also extracted for their content of the test substance. The sample solutions from the skin dermal absorption assay were analysed by LC-MS/MS for the presence of FPK-245. The LOD was defined as 0.50 ng/mL in receptor solution and extraction solution. The LLOQ was 1.0 ng/mL in both, receptor solution and extraction solution.
Controls with benzoic acid (positive) and 2-ethylhexyl trans-4-methoxycinnamate (negative) on human skin were used to check the performance of the skin penetration system at least once a year.

Results
The integrity of the skin was demonstrated prior to application and after the last sampling. The conductivity prior to the experiment was in the acceptable range of < 900 µS/cm for all skin samples used. All 12 chambers met the acceptance criteria and could be used to calculate the dermal delivery.
Details of the results are summarised in the following table:

Cream with 3 % FPK-245 under oxidative conditions						
Amount of FPK-245	Expressed as µg/cm² of skin surface mean S.D. (n = 12)#			Expressed as % of dose mean S.D. (n = 12)#		
Applied Dose	220	±	10.8	100	±	4.90
Unabsorbed Dose	231	±	16.2	105	±	7.34
Adsorbed Dose *Stratum corneum* (isolated by stripping)	0.122	±	0.0762	0.0554	±	0.0346
Absorbable Dose Skin extracts (isolated after 24 hours)	0.328	±	0.202	0.148	±	0.0898
Absorbed dose	0.645	±	0.297	0.291	±	0.132
Recovery	233	±	16.3	105	±	4.00
Dermal Delivery (receptor fluid + epidermis + dermis)	0.973	±	0.381	0.439	±	0.168

Conclusion
It can be stated that under the experimental oxidative conditions reported, cream with 3% FPK-245 showed low penetration into the viable skin layers and into the receptor fluid with dermal delivery (receptor fluid, epidermis, dermis) of 0.97 ± 0.38 µg/cm² (0.44 ± 0.17 % of applied dose).

SCCS comment
In accordance with the SCCS Notes of Guidance, the mean + 1 SD i.e. 1.35 µg/cm² will be used for the MoS calculation under oxidative conditions.

Experiment 2: non-oxidative conditions

Guideline:	OECD 428 (2004)
Test system:	Frozen dermatomed pig ear skin (430-450 µm)
Membrane integrity:	Electrical resistance barrier integrity
Replicates:	12 (6 donors)
Test substance:	FPK-245
Batch:	WK120919
Purity:	99.85%
Test item:	Cream (batch No. C1R2011003.07) with 1.5% FPK-245
Dose applied:	20 µL/cm^2 of the test item (approx. 300 µg FPK-245/cm^2)
Exposed skin area:	1 cm^2
Exposure time:	30 minutes
Sampling period:	24 hours
Receptor fluid:	20% EtOH/PBS
Solubility of test substance in receptor fluid:	Up to 2.20 µg/mL
Mass balance analysis:	Provided
Tape stripping:	Yes (4 pools of 5 strips each)
Method of Analysis:	LC-MS/MS
Positive control:	Benzoic acid
Negative control:	2-Ethylhexyl trans-4-methoxycinnamate
GLP:	In compliance
Study period:	February - March 2013

Two experiments were performed on thawed dermatomed pig skin samples under static and non-occluded conditions. The thickness of the skin used was 430 - 450 µm. The blank samples were collected immediately after filling the donor chambers at the maximal flow rate of the pump, but before the application of the test item. FPK-245 was not detected in the blank samples. The conductivity across the skin samples of each chamber was determined before treatment and after the last sampling as a measure of skin integrity. The integrity was given for all skin samples before and after the treatment.
In each experiment, 6 chambers were analysed and skin samples from 3 donors per experiment were used for the evaluation of each test preparation. A 20 µL aliquot of the test item, corresponding to approx. 300 µg FPK-245, was applied on each skin sample. The test item was left on the skin for 0.5 hours and was then washed off 9 times (2x 1 mL H_2O warm + 5x1 mL 10% shampoo + 2x1 mL H_2O warm). The dermal delivery was monitored over 24 hours. The *stratum corneum* was separated by tape stripping from the remaining epidermis and dermis layers. The tape strips (5 strips per sample, 4 samples) were pooled and extracted for analysis. The remaining skin compartments were also extracted for their content of the test substance. The sample solutions from the skin dermal absorption assay were analysed by LC-MS/MS for the presence of FPK-245. The LOD was defined as 0.50 ng/mL in receptor solution and extraction solution. The LLOQ was 1.0 ng/mL in both, receptor solution and extraction solution.
Controls with benzoic acid (positive) and 2-ethylhexyl trans-4-methoxycinnamate (negative) on human skin were used to check the performance of the skin penetration system at least once a year.

Results
The integrity of the skin was demonstrated prior to application and after the last sampling. The conductivity prior to the experiment was in the acceptable range of < 900 µS/cm for all skin samples used. Eleven chambers met the acceptance criteria and could be used to calculate the dermal delivery.
Details of the results are summarised in the following table:

Cream with 1.5 % FPK-245 under non-oxidative conditions						
Amount of FPK-245	Expressed as µg/cm^2 of skin surface mean S.D. (n = 11)#			Expressed as % of dose mean S.D. (n = 11)#		
Applied Dose	189	±	19.9	100	±	10.5
Unabsorbed Dose	193	±	13.7	102	±	7.22
Adsorbed Dose *Stratum corneum* (isolated by stripping)	0.136	±	0.154	0.0717	±	0.0815
Absorbable Dose Skin extracts (isolated after 24 hours)	1.21	±	0.956	0.637	±	0.460
Absorbed dose	1.43	±	0.984	0.793	±	0.580
Recovery	198	±	12.2	105	±	9.17
Dermal Delivery (receptor fluid + epidermis + dermis)	2.65	±	0.776	1.43	±	0.487
# only valid values with a recovery of > 85% were used. samples < LOD were replaced by value of LOD (0.50 ng/mL in matrix A and B) samples < LLOQ were replaced by value of LLOQ (1.00 ng/mL in matrix A and B)						

Conclusion

It can be stated that under the experimental non-oxidative conditions reported, cream with 1.5% FPK-245 showed a low penetration into the viable skin layers and into the receptor fluid with dermal delivery of FPK-245 of 2.65 ± 0.78 µg/cm^2 (1.43 ± 0.49 % of applied dose).

Ref. 11

SCCS comment

FPK-245 is only intended to be used as a hair dye ingredient in oxidative hair colouring products at on-head concentrations of up to 1.5%. Therefore, the reported dermal delivery value under non-oxidative conditions will not be used for MoS calculations.

3.3.5 Repeated dose toxicity

3.3.5.1 Repeated Dose (14 days) oral toxicity

Guideline: OECD TG 407 (2008), with some exceptions
Species/strain: Rats/ Crl:CD(SD)
Group size: 5/sex/group
Test substance: FPK-245
Batch: WK120914
Purity: 99.69%
Vehicle: 0.5 w/v% Methylcellulose solution
Dose levels: 0, 30, 100, 300(200), and 1000(600) mg/kg bw/day.
Dose volume: 10 ml/kg/day
Route: Oral
Administration: Gavage
GLP: In compliance
Study period: 27 September 2012 – 19 October 2012

In a dose-range finding study, the test substance was administered by oral gavage to male and female rats (5/sex/group) at dose levels of 0, 30, 100, 300(200), and 1000(600) mg/kg bw/day once daily for 14 days. Due to severe clinical signs (death and/or remarkable decreases in body weight and food consumption) during the first week of dosing, the dose levels were reduced from 1000 mg/kg bw/day to 600 mg/kg bw/day from day 7 onwards, and from 300 mg/kg bw/day to 200 mg/kg bw/day from day 10 onwards.
The study was performed according to OECD TG 407 with the following exceptions: the dosing period and the organ weight were not conducted in compliance with this TG, and the detailed clinical observations, stability, concentration, homogeneity of the test substance formulations, and histopathology were not conducted.

Results
The stability of the test substance was confirmed with the certificate of analysis provided by the sponsor. The test substance was stable at room temperature for at least 2 years.

Deaths occurred at 1000(600) mg/kg bw/day (2 males on day 8 and all 5 females on days 6-13). The remaining 3 males at 1000(600) mg/kg bw/day and 2 females at 300(200) mg/kg bw/day were subjected to moribund sacrifice on day 10, and days 6-8, respectively. Prior to death / sacrifice, these animals exhibited deterioration of conditions such as a decrease in locomotor activity, abnormal gait, and decreases in body weight and food consumption.
No deaths were reported at the lower dose levels (30 and 100 mg/kg bw/day). Chromaturia (orange urine) and colouring of fur due to the chromaturia were noted in males and females at 30 mg/kg bw/day.

In the surviving animals, a statistically significantly lower mean body weight was observed in males at 1000(600) mg/kg bw/day on days 3 and 6, and a decrease in body weight was noted in one male and in one female at 300(200) mg/kg bw/day. Food consumption was decreased in the same animals.
Several changes related to the test substance or severely affected health conditions were noted in clinical chemistry.
A statistically significant increase in relative liver weights was noted in males at 300(200) mg/kg bw/day and in females from 100 mg/kg bw/day. A statistically significant increase in relative thyroids weights was observed in females at 300(200) mg/kg bw/day.
At necropsy, a small spleen was noted in one surviving male at 300(200) mg/kg bw/day).

Conclusion
The NOAEL was 100 mg/kg bw/day in males and 30 mg/kg bw/day in females under the conditions of this study.

Ref. 14

3.3.5.2 Sub-chronic (90 days) toxicity (oral)

Guideline:	OECD TG 408 (1997)
Species/strain:	Rats/ Crl:CD(SD)
Group size:	15/sex/group (control and high-dose groups), 10/sex/group (other groups)
Test substance:	FPK-254
Batch:	WK120919
Purity:	99.85%
Vehicle:	0.5% Methylcellulose solution
Dose levels:	0, 3, 30, and 150(75/50) mg/kg bw/day
Dose volume:	10 ml/kg bw/day
Route:	Oral

Administration: Gavage
GLP: In compliance
Study period: 30 October 2012 – 07 May 2013

The test substance was administered by oral gavage to male and female rats (10/sex/group) at dose levels of 0, 3, 30, and 150/75/50 mg/kg bw/day once daily for 90 days. The dose levels were based on the findings in the 14-day dose-range finding study described above (Ref.: 14). A further 5 males and 5 females were assigned to the control and the high dose groups to assess the reversibility of the effects after a 4-week recovery period.
Due to one death of a female at 150 mg/kg bw/day on day 15, and a decrease in locomotor activity and abnormal gait in females on the same day, the dose level was reduced from 150 mg/kg bw/day to 75 mg/kg bw/day for females from day 16 onwards. Another 6 females 150/75 mg/kg bw/day died or exhibited moribundity between day 16 and 20, and a decrease in locomotor activity and abnormal gait were noted in several females at the same day; therefore, the dose level was reduced from 75 mg/kg bw/day to 50 mg/kg bw/day for females from day 21 onwards.
In males, suppression of body weight gain and a decrease in food consumption were noted at 150 mg/kg bw/day on day 36 and three males exhibited abnormal gait and decrease in body weight; therefore, the dose level was reduced from 150 mg/kg bw/day to 75 mg/kg bw/day for males from day 37 onwards. Suppression of body weight gain was noted at 150/75 mg/kg bw/day on day 43, and a decrease in locomotor activity was noted in one male, and a decrease in body weight and reduced food consumption were noted in two males; therefore, the dose level was reduced from 75 mg/kg bw/day to 50 mg/kg bw/day for males from day 44 onwards.

Results
The stability of the test substance was confirmed with the certificate of analysis provided by the sponsor. The test substance is stable at room temperature for at least 2 years.
The homogeneity and stability of the dosing formulations (0.3 and 15 mg/ml) was confirmed for 8 days in 3.8-6.2°C protected from light, followed by 6 hours at room temperature protected from light.
The concentration and homogeneity of the test substance in the dosing formulations were confirmed (0.3, 3, 5, 7.5 and 15 mg/ml).

Deaths occurred at 150/75 mg/kg bw/day (5 females between days 15 and 20), and two females at this dose level were sacrificed in moribundity on day 20. After the dose level was reduced to 50 mg/kg bw/day, one female was sacrificed in moribundity on day 20. A decrease in locomotor activity and/or abnormal gait was observed in most of the animals. In addition, two animals exhibited irregular respiration, and two females exhibited lacrimation and/or soiled perineal region before death or moribund sacrifice.

In the surviving animals at 150/75/50 mg/kg bw/day, abnormal gait was noted in five males and five females from day 35-70 for males and from day 16-38 for females. A decrease in locomotor activity was noted in three of these animals from day 43-58 for males and from day 22-31 for females, and irregular respiration was observed in one male from day 54-61. In the recovery period, no abnormal signs were noted in any animals.

Chromaturia (orange urine) and colouring of fur due to the chromaturia were noted in males at 150/75/50 mg/kg bw/day and females at 30 mg/kg bw/day and higher including animals that died and were sacrificed in moribundity. The colouring of fur remained during the recovery period.

Within the neurofunctional tests, no abnormalities were noted for the sensory reactivity to stimuli or motor activity measurements. The grip strengths were statistically significantly decreased in forelimbs in males and females at 150/75/50 mg/kg bw/day and in hindlimbs

in males and females at 30 mg/kg bw/day and higher in the last week of the dosing period. These changes recovered at the end of the recovery period.

In the surviving animals, mean body weight in males and females at 150/75/50 mg/kg bw/day was statistically significantly reduced from day 29 for males and day 15 for females. In the recovery period, no statistically significant changes were noted in males at 150/75/50 mg/kg bw/day. In females, low mean body weight still remained until the end of the recovery period; the mean body weight gain exceeded that of the control group in the last week of the recovery period.

Statistically significant decreases in food consumption were noted in the surviving males and females at 150/75/50 mg/kg bw/day from day 36-50 for males and from day 22-36 for females. In females of this group, decreased food consumption was also observed on days 64 and 85. In the recovery period, a statistically significant increase was noted in males at 150/75/50 mg/kg bw/day on day 106. In females, statistically significant decreases were recorded on days 99 and 106 at 150/75/50 mg/kg bw/day but not thereafter.

Ophthalmology and urinalysis were not affected in any groups.

In the surviving animals, no treatment-related changes were noted in haematology and clinical chemistry in any groups.
In the moribund sacrificed animals, haematology showed increases in haemoglobin concentration and haematocrit and decreases in reticulocyte ratio and count, white blood cell count, lymphocyte ratio and count, eosinophil ratio and count, white blood cell count, lymphocyte ratio and count, eosinophil ratio and count. Statistical significant changes were also noted in other haematology parameters but were considered by the study report authors to be within physiological range since they were within the background data of the test facility.
In the moribund sacrificed animals, clinical chemistry revealed increases in alkaline phosphatase, glucose, calcium, inorganic phosphorus, potassium and decrease in chloride. Statistical significant changes were also noted in other clinical chemistry parameters but were considered by the study report authors to be within physiological range since they were within the background data of the test facility.

Statistically significant increases were noted in absolute and relative liver weights in males at 150/75/50 mg/kg bw/day and females at 30 mg/kg bw/day and higher at termination, and remained higher (statistically significant) at the end of the recovery period at 150/75/50 mg/kg bw/day in animals of both sexes.
Moribund sacrifice animals showed decreases in absolute and relative spleen weights in two females at 150/75/50 mg/kg bw/day.
Additional weight changes noted in some organs in males or females at 150/75/50 mg/kg bw/day and for the heart weight in females at 30 mg/kg bw/day at the end of the dosing and/or recovery periods were considered by the study report authors to be due to low body weight because their absolute weights were comparable to those in the control group, and related changes were not observed in histopathological examination.

Necropsy at scheduled sacrifice revealed a treatment-related change in the femoral muscle in the form of thinning in two females at 150/75/50 mg/kg bw/day; this was not observed in the recovery period.
In dead or moribund sacrifice animals, treatment-related changes were noted in the stomach, thymus, spleen, and femoral muscle at 150/75/50 mg/kg bw/day in the form of blackish patches in the stomach, small thymus, small spleen and in the thinning of femoral muscle in single females.
Additional findings in other organs were observed but were not considered by the study report authors to be treatment-related but rather common spontaneous changes in normal rats.

In the scheduled sacrifice animals after the dosing period, the primary treatment-related histopathological changes were observed in the liver and femoral muscle. The minimal centrilobular hypertrophy of hepatocytes was observed in males and females at 150/75/50 mg/kg bw/day. In the femoral muscle, atrophy, degeneration and/or regeneration of muscle fibre were observed in males and females with a dose-dependent increased severity at 30 mg/kg bw/day and higher, and was more severe in females that in males. After the 4-week recovery period, atrophy of muscle fibre was still observed in males and females at 150/75/50 mg/kg bw/day, but its grade was minimal. Minimal to mild hypertrophy of muscle fibre was observed in males and females at 150/75/50 mg/kg bw/day, whereas degeneration and regeneration of muscle fibre were not observed in any animals.

In the females that died or were sacrificed in moribundity, atrophy, degeneration and/or regeneration of muscle fibre were observed as in the scheduled sacrifice animals. Their grades were minimal to mild. Other observed histopathological changes were considered by the study report authors to be secondary changes related to poor health conditions or death, or to be common spontaneous changes found in normal rats, or the lack of dose-relation.

Conclusion
The NOAEL of FPK-245 was shown to be 3 mg/kg bw/day in male and female rats after continuous oral administration for 91 days.

Ref. 15

SCCS comment
The SCCS agrees with the NOAEL of 3 mg/kg bw/day; this NOAEL is used for the MOS calculation.

3.3.5.3 Chronic (> 12 months) toxicity

No data

3.3.6 Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial Reverse Mutation Test

Guideline:	OECD 471, Commission Regulation (EC) 440/2008, B.13/14, Japanese Guidelines
Species/strain:	Salmonella typhimurium strains TA 1535, TA 1537, TA 98, TA 100 and the Escherichia coli strain WP2 uvrA
Replicates:	Triplicate plates, 2 independent tests
Test substance:	FPK-245
Batch:	06041404
Purity:	99.88%
Solvent	DMSO
Concentrations:	Experiment I: 3; 10; 33; 100; 333, 1000; 2500 and 5000 µg/plate without and with hamster liver S9-mix
Experiment II:	3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate without and with rat liver S9
Treatment:	Pre-incubation method at 30 °C for 30 minutes and incubation for at least 48 hours at 37 °C in the dark without and with S9-mix

GLP: In compliance
Study period: 21 June 2012 – 27 July 2012

FPK-245 was investigated for its potential to induce gene mutations in Salmonella typhimurium strains TA 1535, TA 1537, TA 98, TA 100 and the Escherichia coli strain WP2 uvrA in the pre-incubation test using Syrian golden hamster liver S9 as metabolic activation (experiment I), and in the plate incorporation assay using Phenobarbital/ β-naphthoflavone induced Wistar rat liver S9 (experiment II). The assays were performed in two independent experiments, with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate. Toxicity was evaluated for 8 concentrations up to the prescribed maximum concentration of 5000 µg/plate on the basis of a reduction in the number of revertant colonies and/or clearing of the bacterial background lawn. Since in the pre-experiment evaluable plates were obtained for five concentrations or more in all strains used, the pre-experiment is reported as the experiment I. The experiment I was performed with pre-incubation test, and the experiment II was performed with direct plate incorporation method. Negative and positive controls were in accordance with the OECD guideline.

Results
Toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), were observed with and without metabolic activation in all strains used in both independent experiments.
No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with FPK-245 at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

Conclusions
Under the experimental conditions reported, the FPK-245 Was not genotoxic (mutagenic) in the Salmonella typhimurium and Escherichia coli reverse mutation assay.

Ref. 13

In vitro Mammalian Cell Gene Mutation Test (*hprt*-locus)

Guideline:	OECD 476, Commission Regulation (EC) 440/2008, B.17, Japanese Guidelines
Cells:	Chinese Hamster V-79 cell line
Replicates:	Duplicate cultures in 2 independent tests
Test substance:	FPK-245
Batch:	WK120919
Purity:	99.85%
Solvent:	deionised water
Concentrations:	Experiment I: 2.5, 5.0, 10.0, 20.0 and 30.0 µg/ml without S9-mixand 20.0, 40.0, 80.0, 120.0 and 160.0 µg/ml with S9-mix Experiment II: 10.0, 20.0, 40.0, 80.0 and 120.0 µg/ml without S9-mix and 5.0, 10.0, 20.0, 40.0 and 80.0 µg/ml with S9-mix
Treatment:	Experiment I: 4 h treatment both without and with S9-mix; expression period 7 days and a selection period of 8 days. Experiment II: 4 h treatment with S9-mix; expression period 7 days and a selection period of 8 days. 24 h treatment without S9-mix; expression period 7 days and a selection period of 8 days.
GLP:	in compliance
Study period:	March 2013

The potential of FPK-245 to induce gene mutations at the *HPRT* locus was examined in V79 cells of the Chinese hamster without and with metabolic activation of Phenobarbital/β-naphthoflavone-induced S9 liver fraction of male Wistar rats. Test concentrations were based on the results of a pre-test on toxicity. Toxicity of FPK-245 is indicated by a reduction of the cloning efficiency. Concentrations were tested with and without metabolic activation with concentrations between 2.5 and 160 µg/ml. The assay was performed in two independent experiments, using two parallel cultures each. The first main experiment was performed with and without liver microsomal activation S9-mix and a treatment of 4 hours. The second experiment was performed with a treatment of 4 hours with and 24 hours without metabolic activation. Toxicity was measured in the main experiments as percentage of cloning efficiency of the treated cultures relative to the cloning efficiency of the solvent control cultures. Negative and positive controls were in accordance with the OECD guideline.

Results
The concentration range of the main experiments was limited by precipitation and cytotoxicity of the test item. Relevant cytotoxic effects in both parallel cultures occurred in experiment I at 160 µg/mL with metabolic activation in the presence of precipitation of the test item. In the second experiment without metabolic activation, cytotoxic effects were noted at 120 µg/mL, again, in the presence of precipitation. In the second experiment with metabolic activation a steep drop of the relative cloning efficiency and the relative cell density was noted at the highest soluble concentrations of 80 µg/mL.
There was no substantial or reproducible concentration-dependent increase in the mutant frequency in both main experiments at any evaluated concentration including those leading to pronounced cytotoxicity and precipitation. The induction factor was far from reaching or exceeding the threshold of three times the corresponding solvent control at any of the data points.

Conclusions
Under the experimental conditions used, FPK-245 was shown to be non-mutagenic in this gene mutation test in mammalian cells when tested up to precipitating concentrations in the presence and absence of metabolic activation.

Ref. 16

Micronucleus Test in Human Lymphocytes

Guideline:	OECD 487, Commission Regulation (EC) 640/2012, B.49
Cells:	Human Lymphocytes from healthy donors
Replicates:	parallel cultures in 2 independent experiments
Test substance:	FPK-245
Batch:	WK120919
Purity:	99.85%
Solvent:	deionised water
Dose levels:	Experiment I: 2.5, 5.0, 10.0, 20.0, 40.0, 50.0, 60.0, 80.0 and 100.0 µg/ml without S9-mix; 25.0, 50.0 and 75.0 µg/ml with S9-mix Experiment II: 5.0, 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0 and 100.0 µg/ml without S9-mix; 25.0, 50.0 and 75.0 µg/ml with S9-mix
Treatment:	Experiment I: 4 h treatment both with and without S9-mix; harvest time 40 h after the beginning of treatment Experiment II: 20 h treatment without S9-mix; harvest time 40 h after the beginning of treatment. 4 h treatment with S9-mix; harvest time 40 h after the beginning of treatment
GLP:	In compliance
Study period:	5 February 2013 - 14 August 2013

The potential of FPK-245 to induce micronuclei in Human lymphocytes *in vitro* was examined with and without metabolic activation of Phenobarbital/β-naphthoflavone-induced S9 liver fraction of rats. Blood samples were obtained from a healthy female (experiment I) and male (experiment II) donors not receiving medication. Human lymphocytes were stimulated for proliferation by the addition of the mitogen PHA to the culture medium for a period of 48 hours. The cell harvest time point was approximately 2 – 2.5 x AGT. Any specific cell cycle time delay induced by the test item was not accounted for directly. The assay was performed in two independent experiments, using two parallel cultures each.
A preliminary cytotoxicity test on the reduction in the cytokinesis block proliferation index (CBPI) in comparison with controls with 4 h treatment and a harvest time of 40 h with and without S9-mix was performed using concentrations up to 300.0 µg/mL of the test item (without S9 mix) and 400.0 µg/mL (with S9 mix) µg/ml in order to determine the toxicity of FPK-245, the solubility during exposure and thus the test concentrations for the main micronucleus test. Since the cultures in this preliminary cytotoxicity test fulfilled the requirements for cytogenetic evaluation and the experimental conditions were identical to those required in the main test, this preliminary test was designated Experiment I.
The treatment period in the main test was either 4 h with or without S9-mix or 20 h without S9-mix. The harvest time was 40 h after the beginning of culture. The final 20 h before harvest was in the presence of cytochalasin B (at a final concentration of 4 µg/ml).
For assessment of cytotoxicity, the relative CBPI was estimated as compared to the respective solvent control. Negative and positive controls were in accordance with the draft guideline.

Results
In the pre-test for toxicity, precipitation of the test item was observed at the end of treatment at 150.0 µg/mL and above without S9 mix and at 100.0 µg/mL and above with S9 mix. In Experiment I, visible precipitation of the test item in the culture medium was observed at 150.0 mg/mL and above in the absence of S9 mix and at 100.0 µg/mL and above in the presence of S9 mix at the end of treatment. In addition, precipitation occurred in Experiment II at 150.0 µg/mL and above in the absence of S9 mix and at 100.0 µg/mL and above in the presence of S9 mix at the end of treatment.
No relevant influence on osmolarity or pH value was observed.
In the absence and presence of S9 mix, pronounced cytotoxicity was observed at the highest evaluated concentration.
In both experiments, in the absence and presence of S9 mix, no biologically relevant increase in the number of cells carrying micronuclei was observed. The micronucleus rates of the cells after treatment with the test item did not exceed the range of the solvent control values and were within the range of the laboratory historical control data.

Conclusions
FPK-245 did not induce micronuclei in Human lymphocytes under the experimental conditions used and thus was shown to be non-mutagenic in this HPRT assay when tested up to cytotoxic and precipitating concentrations in the presence and absence of metabolic activation.

Ref. 2

SCCS comment on *in vitro* genotoxicity:
Three *in vitro* genotoxicity/mutagenicity tests were performed with negative results. In the Ames test and micronucleus assay, FPK-245 was dissolved in DMSO while in the mammalian gene mutation test deionised water was used as solvent and precipitation of FPK-245 already occurred in lower concentrations.

3.3.6.2 Mutagenicity / Genotoxicity in vivo

Mammalian Erythrocyte Micronucleus Test integrated in a 14-day Toxicity Study

Guideline: According to an accepted method (Kawabata et al., 2007)
Species: rats [Crl:CD(SD)
Group sizes: 10 (5 males and 5 females)
Test substance: FPK-245
Batch: WK120914
Purity: 99.69 % (HPLC)
Vehicle: 0.5% methylcellulose solution
Dose levels: 0, 30, 100, 200 (300), and 600 (1000) mg/kg bw/day
Route: once daily oral gavage for 14 days
Sacrifice times: /
GLP: In compliance
Study period: 14 September 2012- 19 October 2012

FPK-245 was investigated for the induction of micronuclei in bone marrow cells of rats. The micronucleus study was integrated in a 14-day oral repeated dose toxicity study in rats.
FPK-245 was administered by oral gavage to male and female rats (5 males and 5 females/group] at dose levels of 0, 30, 100, 200 (300), and 600 (1000) mg/kg bw/day once daily for 14 consecutive days. Due to severe clinical signs including mortalities and/or remarkable decreases in body weight and food consumption during the initial dosing period, the dose levels were reduced from 1000 mg/kg bw/day to 600 mg/kg bw/day from day 7 onwards and from 300 mg/kg bw/day to 200 mg/kg bw/day from day 10 onwards. During the dosing period, the rats were observed 3 times per day for clinical signs; before dosing, just after dosing and 3-4 h after dosing. Outside the dosing period the animals were observed once a day in the morning. All common parameters from a repeated dose toxicity study were measured, e.g. body and organ weights, food consumption, haematology, blood chemistry etc.
A micronucleus test was carried out using a portion of surplus femur obtained at the scheduled sacrifice. Toxicity and thus exposure of the target cells was determined by estimating the ratio between immature and total erythrocytes (IE/TE). Bone marrow preparations were stained with acridine orange and examined microscopically for the IE/TE ratio and micronuclei.

Results
Death occurred in 2 male rats and all females at 1000 (600) mg/kg/day died or lost weight at doses of 600 mg/kg/bw/day or higher. Between day 6-13 and moribundity occurred in 2 females at 300(200) mg/kg/day and 3 males at 1000 (600) mg/kg/day between day 6-10.
Body weight and food consumption decreased on the last day in these animals. Changes in urinalysis, haematology, blood chemistry, organ weight and necropsy related to the test substance were noted.
In the bone marrow cells there was no change in the incidence of micronucleated immature erythrocytes or in the percentage of immature erythrocytes in male or female rats. However, based on the toxicokinetics data, the substance is widely distributed in the body and was shown to also reach the bone as target organ.

Conclusions
Under the experimental conditions used, FPK-245 is not genotoxic (clastogenic and/or aneugenic) in this micronucleus test in bone marrow cells of rats.

Ref. 14

3.3.7 Carcinogenicity

/

3.3.8 Reproductive toxicity

3.3.8.1 Two generation reproduction toxicity

/

3.3.8.2 Other data on fertility and reproduction toxicity

/

3.3.8.3 Developmental Toxicity

Prenatal developmental toxicity range-finding study in rats

Guideline:	/
Species/strain:	Rat, RccHan^{TM}: WIST(SPF)
Group size:	5 females/group
Test substance:	FPK-245
Batch:	WK120919
Purity:	99.85 %
Vehicle:	0.5% Carboxymethylcellulose (CMC) aqueous solution
Dose levels:	0, 50, 100 and 200 mg/kg bw/day
Dose volume:	10 ml/kg bw
Route:	Oral
Administration:	Gavage
GLP:	/
Study period:	2 October 2012- 1 November 2012

FPK-245 was administered by oral gavage to mated female rats (5 females/group) at dose levels of 0, 50, 100 and 200 mg/kg bw/day from gestation day 6 to 20. Control animals were dosed with the vehicle alone.

Results:
The stability of the test substance was confirmed with the certificate of analysis provided by the sponsor. The test substance was stable at room temperature for at least 2 years.
According to the study report authors based upon the results of stability analyses performed within the Harlan study no. D59271, dose formulations were stable for at least 4 hours at room temperature (15 - 25 °C) and for 8 days in refrigerator (2 - 8 °C).

At 100 and 200 mg/kg bw/day severe clinical symptoms starting on gestation day 12 were observed such as uncoordinated movement, prostrate, ruffled fur, chromodachryorrhea, reddish nasal secretion, visible weight loss in some animals. Therefore, the animals were killed in extremis. At 50 mg/kg bw/day, all females survived until the scheduled necropsy.
Clinical findings in the form of reddish discoloured urine due to the colour of the test item were observed starting from a few days after treatment until the end of study in all test item-treated groups.
Food consumption and body weight / body weight gain was dose-dependently affected at 100 and 200 mg/kg bw/day; no effects were noted at 50 mg/kg bw/day.
The macroscopic examination showed no treatment-related changes in any dose group.

All animals were pregnant. Reproduction data of animals treated with 100 and 200 mg/kg bw/day could not be evaluated due to premature termination. At 50 mg/kg bw/day, relevant reproduction parameter (pre- and post-implantation loss and number of foetuses per dam) were not affected by treatment with the test substance.

Due to premature termination of the dams at 100 and 200 mg/kg bw/day, no foetus was available for examination. At 50 mg/kg bw/day, no treatment-related effects were observed for the foetal body weight and sex ratio of the foetuses, or external abnormalities and variations.

Conclusions:
The NOAEL for under the conditions of this prenatal developmental toxicity range-finding study was 50 mg/kg bw/day.

Ref. 9

SCCS comment
The study report (Harlan study no D59271) demonstrating the stability of the test substance has not been submitted.

Prenatal developmental toxicity study in rats

Guideline:	OECD TG 414 (2001)
Species/strain:	Rat, RccHan^{TM}: WIST(SPF)
Group size:	22 females/group
Test substance:	FPK-245
Batch:	WK120919
Purity:	99.85 %
Vehicle:	0.5% carboxymethylcellulose (CMC) aqueous solution
Dose levels:	0, 3, 15 and 50 mg/kg bw/day
Dose volume:	10 ml/kg bw
Route:	Oral
Administration:	Gavage
GLP:	In compliance
Study period:	6 November 2012- 2 May 2013

FPK-245 was administered by oral gavage to mated female rats (22 females/group) at dose levels of 0, 3, 15 and 50 mg/kg bw/day from gestation day 6 to 20. Control animals were dosed with the vehicle alone. The dose levels were based on the findings in the 14-day dose-range finding study described above (Ref.: 9). All females were sacrificed on day 21 post coitum and the foetuses were removed by Caesarean section.

Results:
The stability of the test substance was confirmed with the certificate of analysis provided by the sponsor. The test substance was stable at room temperature for at least 2 years.
According to the study report authors based upon the results of stability analyses performed within the Harlan study no. D59271, dose formulations were stable for at least 4 hours at room temperature (15 - 25 °C) and for 8 days in refrigerator (2 - 8 °C).

The FPK-245 concentrations in the dose formulations ranged from 96.6% to 106.0% with reference to the nominal and were within the accepted range of ±20%. The homogeneous distribution of FPK-245 in the preparations was approved as single results did not deviate

more than 4.7% from the corresponding mean and met the specified acceptance range of ≤15%.

There was no mortality in any dose group.
With the exception of discoloured urine due to the colour of the test item, no further treatment-related clinical finding was noted in any dose group.
At 50 mg/kg bw/day, food consumption was slightly reduced during the first week of treatment attaining statistical significance between days 9 and 12 *post coitum*. At the same dose level, slight test item-related effects were seen on body weight gains and corrected body weight gains; no effect on mean body weight, body weight gains and corrected body weight gains were observed in the other treated groups.
The macroscopic examination showed no treatment-related changes in any dose group.

There were one, three, three and two non-pregnant animals in the control, 3, 15 and 50 mg/kg bw/day groups, respectively.
The relevant reproduction data (pre- and post-implantation loss and number of foetuses per dam) were not affected by treatment with the test substance in any group.

No treatment-related effects were observed for any foetal parameters including of foetal body weight, sex ratio, external, visceral, skeletal and cartilage abnormalities, and variations or ossification and supernumerary ribs in any group.

Conclusions:
The NOAEL for maternal, as well as for developmental toxicity, was 50 mg/kg bw/day.
Ref. 10

SCCS comment
The study report (Harlan study no D59271) demonstrating the stability of the test substance has not been submitted.

3.3.9 Toxicokinetics

3.3.9.1 Toxicokinetics in laboratory animals

Absorption, Distribution, Metabolism and Excretion (ADME) Following Single Percutaneous and Oral Administration in rats

Guideline:	OECD 417 (2010), OECD 427 (2004)
Species/strain:	Rats, (Crl:CD SD)
Group size:	4 Male rats
Test substance:	FPK-245
Batch:	WK120919
Purity:	>99%
Test item:	^{14}C-FPK-245
Batch:	K0166-10
Radiochemical purity:	99.06%; specific activity: 83.29 mCi/mmol
Vehicle:	Oral administration - 0.5% w/v methylcellulose (MC) solution Dermal administration - 4% monoethanolamine/ethanol (50/50, v/v)
Dose levels:	Oral administration: 3, 30, 150 mg/kg bw (for determination of plasma radioactivity); 3 mg/kg bw for other measurements

	Dermal administration: 25 mg/kg bw
Route:	Oral (gavage), dermal
Administration:	Single administration
GLP:	In compliance
Study period:	19 November 2012 – 17 July 2013

Radiolabelled test substance (radiolabel: carbon in benzene ring, not further specified) was administered to male rats at a single percutaneous (25 mg/kg bw; normal and damaged skin for determination of plasma radioactivity, normal skin for other measurements) or oral dose (3, 30, 150 mg/kg bw for determination of plasma radioactivity; 3 mg/kg bw for other measurements) to investigate the plasma concentration, distribution in tissues, and excretion in urine, faeces and bile (in bile-duct cannulated animals), as well as metabolite profiles in plasma, urine, faeces and bile.

Results:
Radioactivity in each lot of the percutaneous dosing formulation and oral dosing formulation met the criteria (95% or higher, actual radiochemical purity: 99.1% and 98.5%).

Absorption:
Results for the determination of parameters in plasma are presented in the table:

	Radioactivity concentration (ng eq. of FPK-245/mL)				
	Percutaneous administration		Oral administration		
	Normal skin (25 mg/kg)	Damaged skin (25 mg/kg)	3 mg/kg	30 mg/kg	150 mg/kg
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
C_{max} (ng eq. of FPK-245/mL)	126.1 ± 154.3	2002 ± 652	1073 ± 220	8611 ± 1122	31340 ± 370
t_{max} (h)	8.7 ± 13.3	0.8 ± 0.3	0.5 ± 0.0	2.5 ± 1.0	5.0 ± 2.6
$t_{1/2}$ (h)	18.7 ± 5.9 [a]	10.5 ± 0.8 [b]	5.6 ± 0.6 [c]	6.0 ± 1.5 [c]	4.5 ± 0.2 [c]
	206.4 [d]	65.8 ± 12.6 [d]	70.0 ± 7.5 [d]	67.3 ± 21.8 [d]	68.3 ± 22.9 [d]
AUC_{0-t} (ng eq.·h/mL)	2965 ± 1458	21400 ± 3860	8179 ± 734	102200 ± 19000	480800 ± 31800
$AUC_{0-\infty}$ (ng eq.·h/mL)	N.C.	22440 ± 3950	8500 ± 858	105200 ± 20800	489000 ± 34900

N.C.: not calculated

In non-fasting male rats receiving ^{14}C-FPK-245 administered as a single oral dose of 3,30 and 150 mg/kg bw, a dose dependency (linear correlation) between the C_{max} and dose (R^2=0.9954) and also between the AUC_{0-t} and dose (R^2=0.9997) was observed. This indicates that absorption did not saturate with dose in the oral administration.

Distribution in tissue:
The results indicated that no significant persistency of radioactivity was detected in any tissue in oral or percutaneous administration.

Excretion in urine and faeces – normal rats:
Following percutaneous application to normal rats, up to 2.97 ± 1.77 and 1.89 ± 1.24 % of the dose was excreted into urine and faeces, respectively, by 168 hours post-dose; the total recovery of radioactivity was 102.35 ± 1.93 % of the dose.
Following oral administration of a single dose of 3 mg/kg bw to normal rats, up to 65.49 ± 3.07 and 30.60 ± 3.71 % of the dose was excreted into urine and faeces, respectively, by 168 hours post-dose; the total recovery of radioactivity was 98.22 ± 1.14 % of the dose.

These results indicated that the dosed radioactivity was always completely excreted by 168 hours post-dose, regardless of the dosing route.

Excretion in bile, urine and faeces – bile-duct cannulated rats:
Following oral administration of a single dose of 3 mg/kg bw to bile-duct cannulated rats, up to 47.61 ± 9.88, 28.45 ± 6.18 and 23.59 ± 2.85 % of the dose was excreted into bile, urine and faeces, respectively, by 48 hours post-dose; the total recovery of radioactivity was 102.06 ± 1.33 % of the dose.
Since animals with bile-duct cannulation showed lower faecal excretion of radioactivity than those without bile-duct cannulation, a portion of the radioactivity excreted into the faeces of rats without bile-duct cannulation was considered to have been excreted via bile after oral administration.
The amount excreted into faeces after oral administration in animals with cannulation was considered to be excreted directly into the faeces and therefore not to be absorbed by the digestive tract.

Metabolic Profile

Plasma:
In total eight radioactive peaks were detected in plasma after a single percutaneous and oral administration. One peak was identified as the parent compound; the other peaks were unknown metabolites.
After a single percutaneous application of 25 mg/kg bw, five radioactive peaks were detected. At 4 hours post-dose, only the parent compound was detected.
After a single oral administration of 3 mg/kg bw/day, seven radioactive peaks were detected 4-hours post-dose. The parent compound accounted for 9.2%. A major metabolite accounted for about 25%.

Urine:
In total, twelve radioactive peaks were detected in urine after a single percutaneous and oral administration. One peak was identified as the parent compound and another peak was identified as 4-amino-2-chlorophenol; the other peaks were unknown metabolites.
After a single percutaneous application of 25 mg/kg bw twelve radioactive peaks were detected at 0-48 hours post-dose; the parent compound accounted for about 0.43% of the dose. The two major metabolites accounted for 0.49 and 0.28 % of the dose, respectively.
After a single oral administration of 3 mg/kg bw, eight radioactive peaks were detected at 0-48 hours post-dose; the parent compound accounted for about 8.2% of the dose. The major metabolite (unknown) accounted for about 11% of the dose.

Faeces:
In total three radioactive peaks were detected in faeces after a single percutaneous and oral administration. One peak was identified as the parent compound; the other peaks were unknown metabolites.
After a single percutaneous application of 25 mg/kg bw, three radioactive peaks were detected at 0-48 hours post-dose; the parent compound accounted for about 0.1 % of the dose. The major metabolites accounted for about 1% and 0.22% of the dose, respectively.
After a single oral administration of 3 mg/kg bw, three radioactive peaks were detected at 0-48 hours post-dose; the parent compound accounted for about 4.2 % of the dose. The major metabolites (unknown) accounted for about 13% and 7% of the dose, respectively.

Bile:
After a single oral administration of 3 mg/kg bw three radioactive peaks were detected. One peak was identified as the parent compound; the other peaks were unknown metabolites. At 0-24 hours post-dose; the parent compound accounted for about 2.4% of the dose. The major metabolite (unknown) accounted for about 1.2% of the dose.

Conclusion
After oral administration, [^{14}C]-FPK-245 was rapidly and completely absorbed, fast and widely distributed and excreted. The bioavailability after oral administration can be considered as very high and the value of 98.14% will be used for risk assessment including MoS calculation [applicant].
After dermal administration to rats, absorption through the intact skin was, as expected, significantly lower than after oral administration. After dermal administration, [^{14}C]-FPK-245 was widely distributed and rapidly excreted via the urine and faeces. The total excretion after 168 hours including cage washings was 5.21% of the dermally administered dose (2.97% in urine, 1.89% in faeces).
Irrespectively of the route of administration, [^{14}C]-FPK-245 was almost completely metabolised and only minor amounts of the unchanged compound were detected. There was no indication of any persistency in any organ or tissue.

Ref. 12

SCCS comment
Following oral administration of a single dose to normal rats, up to approximately 65 % and 31 % of the dose was excreted in the urine and faeces, respectively, indicating an oral absorption of approximately 65 % of the dose from the GI tract. In bile-duct cannulated rats, approximately 48 %, 29 % and 24 % of the dose was excreted in the bile, urine and faeces, respectively, indicating an oral absorption of up to approximately 77 % of the dose from the GI tract (sum of dose excreted in the bile and the urine). Under the conditions of this toxicokinetic study, an oral absorption of 77% is considered based on the results from the bile-duct cannulated rats. The oral absorption of 77% is used for the correction of the NOAEL for the MoS calculation.

3.3.9.2 Toxicokinetics in humans

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3.3.10 Photo-induced toxicity

3.3.10.1 Phototoxicity / photo-irritation and photosensitisation

No data

3.3.10.2 Photomutagenicity / photoclastogenicity

No data

3.3.11 Human data

No data

3.3.12 Special investigations

Cell Transformation Assay in Syrian Hamster Embryo Cells (SHE Assay)

Guideline: OECD Draft Proposal (2012), Commission Regulation (EC) 440/2008 B.21
Species/strain: Syrian golden hamster, LAK:LVG (SYR)
Group size: at least 1.000 colonies (40 dishes: 25-45 colonies/dish)

Test substance: FPK-245
Batch: 06041404
Purity: 99.88%
Solvent: Test item and Benzo[a]pyrene: Dimethylsulfoxide (DMSO); Complete medium containing 0.2% or 10% DMSO
Concentrations: 0.25, 0.5, 1.0, 2.0, 3.0, 4.0 µg/mL
Treatment: Day 0: feeder cell seeding, Day 1: target cell seeding, Day 2: test item treatment, Day 9: fixing and staining of colonies; 24 hours after seeding of the target cells incubation for 7 days at 37 °C in 10% CO_2 atmosphere
Positive control: Benzo[a]pyrene (B[a]P), 5.0 – 10.0 µg/mL culture medium
Negative controls: Complete culture medium (Dulbeccos's Modified Eagle's Medium – LeBoeuf's modification(DMEM-L) supplemented with 20.0% fetal calf serum (FCS) and 2.0% L-glutamin, pH: 6.65 and 6.75).
GLP: in compliance
Study period: 13 June 2012 – 05 September 2012

The potential of FPK-245 to induce colonies with morphologically transformed cells *in vitro* as an indication for a possible carcinogenic potential was examined in SHE cells with a continuous treatment time of at least 7 days.

Method:
After the incubation period, the complete medium was poured off and the cells were fixed. After removal of the methanol, Giemsa solution was added to each dish for about 10 minutes. Thereafter, the dishes were rinsed with purified water, dried and the stained colonies were scored under a stereomicroscope for plating efficiency (PE) and morphological transformation (MT).
For each concentration ≥ 1000 colonies were evaluated and normal (non-transformed) colonies and transformed colonies were enumerated to evaluate the plating efficiency (PE) and the relative plating efficiency (RPE) and morphological transformation frequency (MTF). In addition to the RPE, the colony size and density were recorded as parameters of cytotoxicity.

Results:
Cytotoxicity indicated as reduced relative plating efficiency of about 50 % of control was observed at the two highest concentrations of 3.0 and 4.0 µg/mL.
There were no statistically significant increases in the frequency of morphologically transformed colonies at any of the concentrations tested including those leading to cytotoxicity.
The sensitivity and reliability of the test system was demonstrated as B[a]P) as positive control and induced a statistically significant increase in morphologically transformed colonies.

Conclusions:
FPK-245 did not induce morphological transformation of cell colonies as determined by the *in vitro* cell transformation assay in SHE cells after 7 days of exposure, when tested up to cytotoxic concentrations.

Ref. 5

Medium-term liver carcinogenesis assay in rats

Guideline: /
Species/strain: Male rats, Crl:CD (SD)
Group size: 10/group
Test substance: FPK-245
Batch: WK120919

Purity:	99.85%
Vehicle:	0.5% aqueous methylcellulose solution
Dose levels:	0, 150 and 250 mg/kg bw/day
Dose volume:	/
Route:	Oral
Administration:	Gavage
Positive control:	FPK-132, 600 mg/kg bw/day
Negative control:	Deionized water
GLP:	/
Study period:	5 December 2012 – 25 February 2013

FPK-245 was investigated for its initiating potential using a modified medium-term liver carcinogenesis bioassay. Male Crl:CD(SD) rats were treated by repeated oral application (gavage) with dose levels of 0, 150 and 250 mg/kg bw/day and for comparison with the positive control FPK-132 with a dose level of 600 mg/kg bw/day during a 2-week initiation period. The dose levels for FPK-245 were selected with regard to the results of the previous repeated dose toxicity studies.
All animals were fed a laboratory powder diet during the 2-week initiation period, followed by application of sodium phenobarbital (S.PB), an established liver tumour promoter, at a dietary level of 500 ppm for 6 weeks, from week 3 to week 8. All animals were subjected to partial hepatectomy after week 3 (one week after starting the S.PB treatment) and killed after week 8.
At necropsy, three liver lobes of all surviving animals were cut, fixed, embedded in paraffin wax, sectioned and stained immunohistochemically for glutathione S-transferase placental form (GST-P). The total areas of the liver sections and numbers and areas of GST-P positive foci larger than 0.2 mm in diameter were measured and the results were assessed by comparing the numbers and areas of GST-P positive foci per 1 cm^2 liver section.

Results:
There were no deaths related to FPK-245 administration and no effects on food and water consumption, gross pathology or absolute and relative liver weights.
The immunohistochemical examination showed clearly that no GST-P positive hepatocyte foci were induced by FPK-245.
The only clinical finding, colouring of the urine, was noted in rats at 150 and 250 mg/kg bw/day during the treatment period. Statistically significantly lower body weights were noted only transiently in the 250 mg/kg bw/day group from week 2 onwards. However, no significant changes were found at weeks 6 and 8, indicating recovery.
The positive control, FPK-132, had no effects on water consumption, gross pathology or absolute and relative liver weights but also led to a colouring of urine. Significantly lower body weights were noted transiently from week 1 to week 7, but not at week 8. The sensitivity and suitability of this study design was demonstrated as the positive control FPK-132 clearly increased the numbers and areas of GST-P positive hepatocyte foci per unit area compared to the controls.

Conclusion:
FPK-245 showed no liver carcinogenesis initiating potential in male Crl:CD(SD) rats, when treated orally by gavage up to a dose level of 250 mg/kg bw/day, which caused overt signs of toxicity during the 2-week application period.

Ref.: 3

Sections of the liver were made from a paraffin block prepared in the 90-day study (Ref. 15) and stained with anti-rat liver glutathione S-transferase placental form (GST-P) in the immunohistochemistry.

No statistically significant differences were noted in the GST-P positive areas, number of the GST-P positive areas, number of GST-P positive hepatocytes, or relative GST-P positive area in any group at the end of the dosing or recovery period.

Ref.: 17

An expert report has been performed to review the toxicity data related to FPK-245 and to assess the toxicological relevance of treatment-associated changes. The conclusions of the expert in relation to hepatocarcinogenicity are as follows:
"The lack of a significant increase in GST-P foci in the livers of the toxicity study rats as well as a negative GST-P medium term carcinogenicity assay provide further support for lack of hepatocarcinogenic potential of FPK-245. Based on the results from the 90-day repeated dose toxicity study, the NOAEL for FPK-245 is 3 mg/kg/day."

3.3.13 Safety evaluation (including calculation of the MoS)

FPK-245 is only intended as a hair dye ingredient in oxidative hair colouring products at on-head concentrations of up to 1.5%. Therefore, the value of 0.973 µg/cm² obtained under oxidative conditions will be used for risk assessment and Margin of Safety (MoS) calculation.

CALCULATION OF THE MARGIN OF SAFETY

(oxidative conditions)
(3 % formulation, on-head concentration 1.5 %)

Absorption through the skin	**A**	**=**	**1.35 µg/cm²**
Skin Area surface	**SAS**	**=**	**580 cm²**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**0.783 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.013 mg/kg bw**
No observed adverse effect level (90-day, oral, rat)	**NOAEL**	**=**	**3 mg/kg bw/d**
Bioavailability 77 %*		**=**	**2.31 mg/kg bw/d**
Margin of Safety	**adjusted NOAEL/SED**	**=**	**180**

* based on toxicokinetic study ref 12.

3.3.14 Discussion

Physicochemical properties
HC Red 18 is intended to be used as a hair dye ingredient in oxidative hair colouring products at on-head concentrations of up to 1.5%.
The reported purity and impurity data on HC Red 18 cannot be accepted in the absence of the study report describing analytical methodology, validation of the method, reference standard used for the determination, etc. In addition, further identification data regarding mass spectrometry, ^{13}C-NMR, ^{1}H-NMR for all the batches used in the studies, should be submitted. The impurities of HC Red 18 were not chemically characterised. The synthetic route was provided in additional data. In addition, the impurities should be characterised and any impurity belonging to CMR (carcinogenic, mutagenic and reproduction toxic) must be quantified.

- The SCCS has no information on the batch used for the stability study of HC Red 18 under alkaline peroxide conditions. Information on the batch number, purity and impurity of this batch must be provided.
Methodology used for the determination of water content, ash content and heavy metal content was not described and the study reports were not submitted.
The study reports on water solubility and Log Pow determination of HC Red 18 were not submitted. It is not known whether water solubility and Log Pow were determined by respective EU methods. Physicochemical properties such as melting point and density of HC Red 18 are not reported. Stability of HC Red 18 in typical hair dye formulations is not shown.

General toxicity

No acute oral toxicity study with HC Red 18 was submitted. However, based on the mortalities in the 14-day dose range-finding rat study from 300(200) mg/kg bw/day, HC Red 18 is considered to possess a moderate acute toxic potential following oral administration.
Daily administration of HC Red 18 by oral gavage to Sprague Dawley rats at a dose level of 30 mg/kg bw/day and higher for 90 days resulted in atrophy, degeneration and/or regeneration of muscle fibre in the femoral muscle. At 150/75/50 mg/kg bw/day, severe signs of systemic toxicity, including mortality/moribundity were observed. The NOAEL of 3 mg/kg bw/day is used for the MOS calculation.
In the developmental toxicity study in rats, daily administration by oral gavage to pregnant female Wistar rats at a dose level of 50 mg/kg bw/day during gestation days 6 to 20 resulted in minimal maternal toxicity. The NOAEL for maternal and developmental toxicity is considered to be 50 mg/kg bw/day. Neither malformations nor variations occurred at the highest dose level of 50 mg/kg bw/day.

Irritation/sensitisation

The skin irritation potential of HC Red No. 18 as neat substance has been tested using an *in vitro* skin irritation test method according to OECD TG 439 (July 2010). As red colouring of the tissues by the test item might have occurred, interference with the MTT cell viability endpoint is expected. leading to false estimates of the skin irritation potential. Therefore, the SCCS considers the results obtained from this test as inconclusive.

HC Red No. 18 has been tested as 20% (w/v) suspension and neat substance in state-of-the-art *in vitro* test methods for eye corrosion (BCOP assay, OECD 437 (September 2009) and eye irritation (EpiOcular^{TM}), respectively. Due to red colouring of the tissues by the test item, interference with the endpoints may occur, leading to false estimates of the eye irritation potential. Therefore, the SCCS does not consider the results obtained from these tests as valid.

The skin sensitisation potential of HC Red No. 18 was assessed in one LLNA study performed according to OECD TG429. The test item was tested at concentrations of 2.5, 5 and 10% in DMSO. The highest dose tested was the maximum concentration that could be technically achieved in this vehicle. In the high dose group a slight increase of lymphocyte proliferation was induced, but the SI was below 3. Based on these results, SCCS concludes that HC Red No. 18 is not a skin sensitiser.

Dermal absorption

Two *in vitro* experiments according to OECD 428 (2004), one under oxidative and one under non-oxidative conditions, were performed to measure the dermal absorption of HC Red No. 18. As HC Red No. 18 is only intended to be used as a hair dye ingredient in oxidative hair colouring products, only the dermal delivery of HC Red No. 18 measured under oxidative

conditions i.e. 0.97 ± 0.38 µg/cm² (0.44 ± 0.17 % of applied dose) will be considered. For the calculation of the MoS, a dermal absorption of the mean + 1 SD i.e. 1.35 µg/cm² will be used.

Mutagenicity

Overall, the genotoxicity of HC Red No. 18 was investigated for the three endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy. HC Red No. 18 did not induce gene mutations in bacteria nor at the *HPRT* locus in a mammalian gene mutation test in V79 cells. An *in vitro* micronucleus test in human lymphocytes was also negative. The absence of clastogenicity *in vitro* was confirmed also *in vivo*. HC Red No. 18 was studied in an *in vivo* micronucleus test which was integrated in a 14-day repeated dose toxicity study. Although 14-day treatment resulted in toxicity, no increase in the number of cells with micronuclei was observed.

Consequently, HC Red No. 18 can be considered to have no genotoxic potential and additional tests are not required.

Carcinogenicity

HC Red No. 18 did not induce morphological transformation of cell colonies as determined by the *in vitro* cell transformation assay in SHE cells after 7 days of exposure, when tested up to cytotoxic concentrations.

HC Red No. 18 showed no liver carcinogenesis initiating potential in male Crl:CD(SD) rats, when treated orally by gavage up to a dose level of 250 mg/kg bw/day, which caused overt signs of toxicity during the 2-week application period.

Sections of the liver were made from a paraffin block prepared in the 90-day study and stained with anti-rat liver glutathione S-transferase placental form (GST-P) in the immunohistochemistry. No statistically significant difference was noted in the GST-P positive areas, number of the GST-P positive areas, number of GST-P positive hepatocytes, or relative GST-P positive area in any group at the end of the dosing or recovery period.

An expert report has been performed to review the toxicity data related to HC Red No. 18 and to assess the toxicological relevance of treatment-associated changes. The conclusions of the expert in relation to hepatocarcinogenicity are as follows:

"The lack of a significant increase in GST-P foci in the livers of the toxicity study rats as well as a negative GST-P medium term carcinogenicity assay provide further support for lack of hepatocarcinogenic potential of FPK-245. Based on the results from the 90-day repeated dose toxicity study, the NOAEL for FPK-245 is 3 mg/kg/day."

Toxicokinetics

Under the conditions of the *in vivo* toxicokinetic study, an oral absorption of 77% is considered based on the results from the bile-duct cannulated rats showing an excretion of about 48% and 29% of the dose in bile and urine, respectively. An oral absorption of 77% is used for the correction of the NOAEL for the MoS calculation.

Human data

No data were submitted.

4. CONCLUSION

(1) In light of the data provided, does the SCCS consider HC Red No. 18 (BÍ 24) (FPK 245) safe when used as a hair dye ingredient in oxidative hair colouring products at on-head concentrations of up to 1.5 % under oxidative conditions?

In the light of the data provided, the SCCS considers that the use of HC Red No. 18 (B124) as an ingredient at 1.5% in oxidative hair dye formulations is safe.

(2) Does the SCCS have any further scientific concerns regarding the use of HC Red No. 18 (B124) (FPK 245) in other cosmetic products?

Neither the purity of HC Red No. 18 nor its impurities are quantified adequately.

5. MINORITY OPINION

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6. REFERENCES

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