Scientific Committee on Consumer Products

SCCP

OPINION ON

PHENYL METHYL PYRAZOLONE

COLIPA N° A39

The SCCP adopted this opinion during its 10th plenary of 19 December 2006

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 Products (SCCP), 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 Evaluation Agency (EMEA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCP

Questions concerning the safety of consumer products (non-food products intended for the consumer).

In particular, the Committee addresses questions related to the safety and allergenic properties of cosmetic products and ingredients with respect to their impact on consumer health, toys, textiles, clothing, personal care products, domestic products such as detergents and consumer services such as tattooing.

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1. BACKGROUND

Submission I for phenyl methyl pyrazolone was submitted in July 1993 by COLIPA[1,2].

The Scientific Committee of Cosmetology (SCC) has at its 53rd meeting on 23 June 1993 expressed its opinion (SPC/1272/94 rev.1) on Norantipyrine (former name) or phenyl methyl pyrazolone with the conclusion:

"Norantipyrine is slightly toxic, on the basis of its acute toxicity. Norantipyrine, at a concentration of 1 %, showed no signs of irritation. The sensitization test was carried out inadequately. In the 28-day study with rats, effects were still found in the 1000 mg/kg bw group. The dose level without effect is 200 mg/kg bw. In the carcinogenicity study no neoplastic lesions were observed. No adverse effects were reported in an oral teratogenicity study up to 1000 mg/kg b.w. (the highest concentration tested). With the exception of the mouse lymphoma assay with metabolic activation, Norantipyrine was not genotoxic in all tests. The cutaneous absorption was 0.08 % for skin with and without hair.
For normal use of hair dye, the following calculation can be made: 0.25 g of Norantipyrine comes in contact with the human skin in permanent hair dye condition (based on a usage volume of 100 ml containing maximal 0.25 % Norantipyrine). With a maximal penetration, under normal condition, of 0.08 %, this results in a dermal absorption of 0.2 mg per treatment, which is 0.003 mg/kg bw (assuming a body weight of 60 kg). So a margin of safety of 66670 can be calculated between the figure for human exposure to oxidative hair dye and the no effect level found in rats in the 28-day study. It should be noted that the NOAEL stems from a daily exposure for 28 days, whereas human exposure to permanent hair dye is unlikely to be more than once a month. Need for an adequate sensitization test."

Submission II for this substance was submitted in July 1997 by COLIPA[2].

Submission III for this substance was submitted in November 1998 by COLIPA[2].

The Scientific Committee on Cosmetics on Non-Food Products (SCCNFP) adopted at the plenary meeting on 23 June 1999 the opinion (SCCNFP/0134/99) on phenyl methyl pyrazolone with the opinion*: "The SCCNFP is of the opinion that norantipyrine can be used safely in permanent hair dye formulations at a maximum concentration of 0.5%. Since permanent hair dyes are mixed with hydrogen peroxide before application, the in-use concentration is 0.25%."*

The substance is currently regulated by the Cosmetics Directive (76/786/EC), Annex III, Part 2 under entry 20 on the List of substances provisionally allowed, which cosmetic products must not contain except subject to restrictions and conditions laid down.

Submission IV of phenyl methyl pyrazolone was submitted by COLIPA in July 2005. According to this submission phenyl methyl pyrazolone is used in oxidative hair dye formulations at a maximum concentration of 0.5%, which after mixing typically in 1:1 proportions ratio with hydrogen peroxide prior to use, corresponds to a concentration of 0.25% upon application (final, on-head concentration).

Submission IV 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.

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1 COLIPA - European Cosmetics Toiletry and Perfumery Association
2 According to records of COLIPA

2. TERMS OF REFERENCE

1. *Does the Scientific Committee on Consumer Products (SCCP) consider phenyl methyl pyrazolone safe for use as an oxidative hair dye with a concentration on the head of maximum 0.25 % taken into account the scientific data provided?*

2. *Does the SCCP recommend any restrictions with regard to the use of phenyl methyl pyrazolone in oxidative hair dye formulations?*

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Phenyl methyl pyrazolone (INCI)

3.1.1.2. Chemical names

3H-pyrazol-3-one, 2,4-dihydro-5-methyl-2-phenyl- (CAS)
1-Phenyl-3-methylpyrazol-5-one
2-pyrazolin-5-one, 3-methyl-1-phenyl
3-methyl-1-phenyl-2-pyrazoline-5-one
3-methyl-1-phenyl-5-pyrazolone

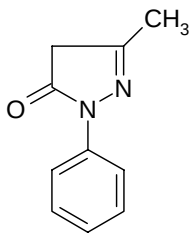
3.1.1.3. Trade names and abbreviations

Phenyl methyl pyrazolone
Norantipyrine
MCI186
Edaravone
COLIPA n° : A039

3.1.1.4. CAS / EINECS number

CAS : 89-25-8
EINECS : 201-891-0

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_{10}H_{10}N_2O$

3.1.2. Physical form

Light beige powder

3.1.3. Molecular weight

Molecular weight: 174.2

3.1.4. Purity, composition and substance codes

Purity and impurities in various batches of phenyl methyl pyrazolone.

Property	**Batch number**		
	91N058	**62**	**500 ref. 8060110***
Identification/characterisation	IR, NMR, MS	UV, IR, NMR, MS, elemental analysis	UV, IR, elemental analysis
Melting point	127-131°C	122°C	129°C
Titre (g/100g)[1]	99.8	98.5	99.8
HPLC purity (% peak area)	>99.5	>99.5	
Impurities (g/100 g) Acetoacetamide Phenylhydrazine	 <0.05 ND <0.01ND	 <0.05 ND <0.01 D	 Not done <0.02 ND
Water content (g/100g)	0.7	0.04	
Loss on drying (g/100g)		<0.1	
Sulphuric ash content (g/100)	<0.05		< 0.1
Solvent residues	Not applicable as water is used as medium for synthesis		

ND Not Detected
D Detected
* also designated as 8060110 as well as pt.8060110, only certificate provided, no supporting data
[1] Neutralisation of amine function by perchloric acid in acetic acid medium

3.1.5. Impurities / accompanying contaminants

See 3.1.4
Metal content in Batch 62
Hg: <0.1 ppm
Ag, Al, As, Ba, Bi, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Pd, Pt, Sb, Se, Sn, V, Zn: each < 1ppm
Ti: 2 ppm
Fe: 9 ppm

3.1.6. Solubility

Water: 2.07±0.07 g/l, 20°C
Ethanol: >1 g/l, <10 g/l
DMSO: ≥20 g/l

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow} : 0.74 (at 24°C, pH 7.2)

3.1.8. Additional physical and chemical specifications

Organoleptic properties:	/
Melting point:	122°C - 131°C
Boiling point:	/
Flash point:	/
Vapour pressure:	/
Density:	/
Viscosity:	/
pKa:	/
Refractive index:	/
UV:	λ_{max} 244.9 nm

3.1.9. Homogeneity and Stability

2 mg/ml and 200 mg/ml solutions of phenyl methyl pyrazolone in 0.5% methyl cellulose (MC), at room temperature up to 6 hours and up to 9 days at 4°C, were stable (maximum deviation from original concentration = 9%) when stored protected from light and under inert gas atmosphere.

0.1 mg/ml and 250 mg/ml solutions of phenylmethyl pyrazolone in DMSO were stable at room temperature up to 4 hours study period (maximum deviation from original concentration = 2%) when stored protected from light and under inert gas atmosphere.

The solutions of phenyl methyl pyrazolone in MC were found to be homogeneous during the 9 days storage period (Coefficient of Variation (CV) maximum 6%), when stored at 4°C, protected from light and under inert gas atmosphere

General comments to physico-chemical characterisation

- Stability of phenyl methyl pyrazolone in marketed products is not reported
- No supporting data is provided for the chemical characterisation of phenyl methyl pyrazolone, batch 500 ref. 8060110.

3.2. Function and uses

Phenyl methyl pyrazolone is used in oxidative hair dye formulations at a maximum concentration of 0.5%, which after mixing typically in 1:1 ratio with hydrogen peroxide prior to use, corresponds to a concentration of 0.25% upon application.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline:	OECD 401
Species/strain:	Rats, Sprague-Dawley
Group size:	Five male and five female, fasted
Test substance:	1-Phenyl-3-methyl-5-pyrazolone
Batch:	pt 8060110
Purity:	Not available
Dose:	2000 mg/kg, 10 ml/kg volume
Vehicle:	1,2-propanediol
Route:	Oral
Exposure:	Single administration and 14-day observation period
GLP:	in compliance

On the day of the exposure the animals (5 male and 5 female) were about 6 weeks old and had a mean weight of 175 ± 6 g for the males and 150 ± 6 g for the females.
The day before treatment, the animals were fasted for a period of about 18 h before administration of the test substance. The test substance was administered suspended, at a dose level of 2000 mg/kg. The administration was performed in a single dose by oral route.
No clinical signs or deaths were seen during the 14 days observation period. The exposure did not have an effect to the body weight gain.
On day 15, the animals were sacrificed and the macroscopic examination of the main organs of the animals sacrificed at the end of the study revealed no apparent abnormality. No samples were taken for histological examinations.

Ref.: 1

Guideline:	OECD 401
Species/strain:	Rats, Sprague-Dawley
Group size:	three groups 10 animals in each (five male and five female), fasted
Test substance:	1-Phenyl-3-methyl-5-pyrazolone
Batch:	91N058
Purity:	99.8%
Dose:	2000 mg/kg, 10 ml/kg volume
Vehicle:	0.5% methylcellulose; 0.5% carboxymethylcellulose; propylene glycol
Route:	Oral
Exposure:	single administration and 14-day observation period
GLP:	in compliance

The animals were fasted for an overnight period, but they had free access to water. Food was given about 4 h after the treatment. The administration was performed in a single dose by oral route using three vehicles. The dose in each case was 2000 mg/kg.

In treated group 1 (vehicle: 0.5% methylcellulose), hypoactivity or sedation, piloerection, ptosis of the eyelids, reddish colouration of the extremites, dyspnoea and rhinorrhea were observed in all animals on day 1. Lateral recumbence was noted in one female prior to death. Tremors were also observed in one male, which was found dead on day 2. Recovery was mentioned to be complete in the other animals.

In treated group 2 (vehicle: 0.5% carboxymethylcellulose), hypoactivity or sedation, piloerection, ptosis of the eyelids, reddish colouration of the extremities, dyspnoea and rhinorrhea were observed in all animals on day 1. One male died 4 hours after treatment,

and 2 females died 4 hours or 6 hours after treatment, respectively. Recovery was mentioned to be complete in the other animals.

In the treated group 3 (vehicle: propylene glycol) hypoactivity or sedation, piloerection, ptosis of the eyelids, reddish colouration of the extremities, dyspnoea and rhinorrhea were observed in all animals on day 1. No death occurred. Recovery was complete in all animals 6 hours after dosing.
Body weight gain of the surviving animals of all groups was not affected by treatment with the test substance.

Mortality was 20% in group 1, 30% in group 2 and 0% in group 3. Mortality occurred on day 1 or 2. At necropsy, no apparent macroscopic abnormalities were observed in the animals which died during the study or in the animals killed at the end of the observation period.

Ref.: 2

3.3.1.2. Acute dermal toxicity

No data submitted

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline:	OECD 404
Species:	New Zealand White Rabbit
Group:	3 male
Substance:	1-phenyl-3-methyl-5-pyrazolone
Batch:	pt 8060110
Purity:	99.8%
Dose:	0.5 ml of 1% test substance in propylene glycol; non-occlusive dressing
GLP:	in compliance

Approximately 24 hours prior to the beginning of the study, the flanks of each animal were partially clipped. A 0.5 ml sample of 1-phenyl-3-methyl-5-pyrazolone at 1% in propylene glycol (99% purity) was applied to the right flank of each animal. It was held in contact with the skin for 4 hours by means of a non-occlusive dressing. The untreated left flank served as control. Subsequently, the dressings were removed, and the treated area was observed 1, 24, 48 and 72 hours after dressing removal.

Results
A slight (1/3 rabbits) to well-defined (2/3 rabbits) erythema was observed one hour after dressing removal. There were no skin reactions at 24, 48, 72 hours. No oedema was observed at any time point.

Conclusion
Under the conditions of this study, 1-phenyl-3-methyl-5-pyrazolone at 1% in propylene glycol caused transient mild irritation to rabbit skin.

Ref.: 3

3.3.2.2. Mucous membrane irritation

Guideline:	OECD 405
Species:	New Zealand White Rabbit
Group:	3 male
Substance:	1-phenyl-3-methyl-5-pyrazolone
Batch:	pt 8060110
Purity:	99.8%
Dose:	0.1 ml of 1% test substance in propylene glycol; non-occlusive dressing
GLP:	in compliance

A 0.1 ml sample of PMP at 1% in propylene glycol was instilled into the conjunctival sac of the left eye of the animals after gently pulling the lower lid away from the eye ball. The upper and lower lids were held together for several seconds to avoid any loss of test substance, and the eyes were not rinsed after administration of the test substance. The untreated right eye served as control, and the ocular reactions were assessed 1, 24, 48 and 72 hours after instillation.

Results
No ocular reactions were observed.

Conclusion
Under the conditions of this study, 1-phenyl-3-methyl-5-pyrazolone at 1% in propylene glycol was non-irritating to rabbit eyes.

Ref.: 4

3.3.3. Skin sensitisation

Murine Local Lymph Node Assay

Guideline:	OECD 429
Species:	CBA/J mice
Group:	28 females in each of 2 independent studies
Substance:	phenyl methyl pyrazolone
Batch:	62
Purity:	98.5%
Dose:	1, 2.5, 5, 10 and 25% v/v in DMSO (first study) 0,1, 0.25, 0.5, 1 and 2.5% v/v in DMSO (second study)
GLP:	in compliance

Fifty-six (56) female CBA/J mice were used in the main study to assess the sensitising potential of phenyl methyl pyrazolone. This assessment was made through two independent experiments using 28 animals each.

In the first experiment, animals were separated in 7 groups (4 mice/group) consisting of:

- 5 treated groups receiving phenyl methyl pyrazolone at 1, 2.5, 5, 10 and 25% (w/v) in dimethylsulfoxide (DMSO). This vehicle was selected on the basis of the results of a previous solubility study showing that 25% (w/v) phenyl methyl pyrazolone in DMSO was the maximal practicable concentration [16]. This concentration was non-irritant in a preliminary test.
- A negative control group receiving the vehicle (DMSO) alone
- A positive control group receiving alpha-hexylcinnamaldehyde (HCA) at 25% (v/v) in DMSO

As positive results were observed at all concentrations in the first experiment, a similar second experiment was conducted at the lower concentrations of 0.1, 0.25, 0.5, 1 and 2.5% (w/v) phenyl methyl pyrazolone.

In each of these experiments, the test substance, DMSO or HCA was applied over the ears (25 µl per ear) of respective animals for three consecutive days designated as days 1, 2 and 3. After 2 days of resting (day 6), mice received a single intravenous injection of tritiated methyl thymidine (^{3}H-TdR). Lymph nodes draining the application sites (auricular nodes) were sampled, pooled per group, and the proliferation of lymphocytes was evaluated by measuring the incorporation of ^{3}H-TdR. The values obtained were used to calculate stimulation indices (SI). The irritant potential of the test item was assessed by measuring ear thickness on days 1, 2, 3 and 6.

Results
SI values of 11.45 and 6.12 were obtained with the positive control HCA in experiment 1 and 2, respectively.

In the first experiment, lymphoproliferative responses were observed at all concentrations tested, which were attributed to delayed contact hypersensitivity in the absence of local irritation.

Concentration (%)	Signs of local sensitisation	Stimulation index (SI)
1	no	3.17
2.5	no	9.15
5	no	15.22
10	no	12.18
25	no	9.81

In the second experiment, the threshold positive value of 3 was approached at the concentration of 2.5%.

Concentration (%)	Signs of local sensitisation	Stimulation index (SI)
0.1	no	1.67
0.25	no	0.72
0.5	no	2.77
1	no	1.70
2.5	no	2.79

Differences in the determination of the EC_3 value were observed (EC_3 1% in first experiment, EC_3 2.5% in second experiment).

Conclusion
Under the conditions of this study, phenyl methyl pyrazolone induced delayed contact hypersensitivity. According to the EC_3 value estimated in these studies, phenyl methyl pyrazolone was considered to have a strong (first experiment) or moderate (second experiment) sensitising potential.

Ref.: 5

3.3.4. Dermal / percutaneous absorption

***In Vitro* Percutaneous Absorption Study using Human Dermatomed Skin**

Guideline: Draft OECD
Species: Human
Group: breast and abdominal skin from 7 females
Substance: phenyl methyl pyrazolone
Batch: 62
Purity: 98.5%
Radiolabelled: 4-[benzene ring-U-^{14}C]-phenyl-3-methylpyrazolone
Batch: CFQ13912 Batch 1

Radio-purity: 98.6%
GLP: in compliance

Human breast and abdominal skin samples were obtained from seven different female donors subjected to plastic surgery. The skin was transferred stored on ice and kept frozen at –20°C until use.

Skin samples were dermatomed (390-400 µm in thickness) and mounted in flow-through diffusion cells, using bovine serum albumin (5%, w/v) in calcium and magnesium free phosphate-buffered saline as the receptor fluid.

The integrity of the skin was checked by determination of the permeability coefficient for tritiated water ($<2.5.10^{-3}$ cm/h for all selected membranes).

Twenty-four diffusion cells were used in two separate experiments, and skin was maintained at approximately 32°C.

In a first experiment (oxidative conditions), a typical oxidative hair dye formulation containing 0.5% phenyl methyl pyrazolone associated with the primary intermediate para-phenylenediamine (PPD, 0.3%) was mixed with the developer (1:1, w/w) to yield a final target concentration of 0.25 % phenyl methyl pyrazolone. About twenty (20) mg/cm² of this mixture (corresponding to exactly 50.5 µg/cm² of phenyl methyl pyrazolone) was applied to the skin surface and left for 30 minutes. After this time period, the remaining formulation on the skin surface was removed using a standardized washing procedure, simulating use conditions. Twenty-four (24) hours after application, the percutaneous absorption of [^{14}C]- phenyl methyl pyrazolone was estimated by measuring its concentration by liquid scintillation counting (following combustion for non-liquid samples) in the following compartments/samples: skin washes (dislodgeable dose), *stratum corneum* (isolated by tape strippings), living epidermis/dermis, unexposed skin and receptor fluid.

In a second experiment, a similar experimental procedure was applied to evaluate the percutaneous absorption of phenyl methyl pyrazolone in non-oxidative conditions, using a formulation without primary intermediate and mixed with water (1:1, w/w) to yield a final concentration of 0.25% phenyl methyl pyrazolone (about 20 mg/cm^2 were applied, corresponding exactly to 50.5 µg/cm^2).

Results
20 samples of human skin yielded data that could be analysed (8 in oxidative conditions and 12 in non-oxidative conditions).

Most of the phenyl methyl pyrazolone applied on the skin surface was removed with the skin washes (about 92% and 93% of the applied dose in oxidative and non-oxidative conditions, respectively), and the total recovery rate was about 95% and 96% in oxidative and non-oxidative conditions, respectively.

The mean amounts of phenyl methyl pyrazolone considered as absorbed (dermal delivery) were estimated as follows (sum of amounts measured in living epidermis/dermis and receptor fluid): 0.31 ± 0.24 µg equiv/cm^2 (0.56 ± 0.44% of the applied dose) and 1.60 ± 0.30 µg equiv/cm^2 (2.92 ± 0.53% of the applied dose) in oxidative and non-oxidative conditions, respectively.

	Oxidative conditions		**Non-oxidative conditions**	
Cutaneous Distribution		**% applied dose**	**µg equiv/cm²**	**% applied dose**
Dislodgeable dose	50.85 ± 1.85	91.88 ± 3.20	51.05 ± 1.20	93.33 ± 2.30
Unabsorbed dose*	52.32 ± 1.65	94.54 ± 2.89	52.44 ± 0.87	95.87 ± 1.57
Receptor fluid	0.07 ± 0.03	0.13 ± 0.05	0.66 ± 0.12	1.21 ± 0.22
Dermal delivery**	**0.31 ± 0.24**	0.56 ± 0.44	1.60 ± 0.30	2.92 ± 0.53

* dislodgeable dose + *stratum corneum* + unexposed skin
** receptor fluid + living epidermis/dermis

Under oxidative conditions, the range for dermal delivery was 0.01–1.01 µg equiv/cm² or 0.18–1.83% of the applied dose. A_{max} was in cell 8.

Under non-oxidative conditions, the range for dermal delivery was 1.01–2.56 µg equiv/cm² or 1.86 – 4.73% of the applied dose. A_{max} was in cell 45.

Conclusion
The amounts of phenyl methyl pyrazolone considered as absorbed from a hair colouring formulation containing phenyl methyl pyrazolone at a final concentration of 0.25% in oxidative conditions were 0.31 ± 0.24 µg/cm^2 (0.56 ± 0.44% of the applied dose), A_{max} = 1.01 µg/cm^2, and in non-oxidative conditions 1.60 ± 0.30 µg/cm^2 (2.92 ± 0.53% of the applied dose), A_{max} = 2.56 µg/cm^2.

Ref.: 15

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity

No data available, except for a 4-week dose finding study.

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

Guideline:	OECD 408
Species/strain:	Sprague-Dawley rat
Group size:	10 per sex and per dose
Test substance:	1-Phenyl-3-methyl-5-pyrazolone (PMP)
Batch:	62
Purity:	98.5
Dose:	0, 20, 100, and 500 mg/kg/day
Route:	Oral
Exposure:	13 weeks in 0.5% aqueous methylcellulose (5ml/kg)
GLP:	in compliance

The subchronic toxicity of PMP by gavage was investigated in a 13-week toxicity study in SD rats. PMP was given at 20, 100, or 500 mg/kg/day. There were no deaths in the 13-week study. Clinical signs included hypoactivity, half-closed eyes, round back and piloerection with a dose related incidence and frequency at 100 and 500 mg/kg/day doses. The signs were observed rapidly and transiently after dosing. The observations can be considered to be related to the mode of administration of PMP, which results in a transiently high plasma concentration.
At 500 mg/kg/day, mean food intake and body weight gain were slightly lower than in the controls.
At 500 mg/kg/day, changes indicative of regenerative haemolytic anaemia we observed. The observations were related to a decrease of erythrocyte count, packed cell volume and haemoglobin concentration and increase in mean cell volume and reticulocyte count, biochemical changes (increased blood total and direct bilirubin concentrations, urinary traces of bilirubin), and pathological changes in spleen weight, microscopic red pulp congestion, and haemosiderosis in the spleen.
Other observations were related to clotting times in males at 500 mg/kg/day, lower blood glucose level in males at 100 and 500 mg/kg/day as well as urinary traces of nitrites and marked discoloration of urine at 500 mg/kg/day.

PMP was detected in the plasma at all dose levels. Plasma levels were comparable after single dosing and at the end of the dosing period. The Cmax was measured 0.5 h after dosing, and systemic exposure increased with dose level.
The NOAEL of the study was 20 mg/kg/day, a dose level associated in week 13 with systemic exposure (AUC 0-24) values of 4.3 µg.h/ml in males and 15.1 µg.h/ml in females.
Ref.: 6

Comment
Clinical signs attributed to phenyl methyl pyrazolone but related to the mode of administration were observed at 100 mg/kg/day and higher. In males, the exposure of 100 mg/kg/day resulted in lower blood levels of glucose. The SCCP considers the NOEL to be 20 mg/kg bw and the NOAEL to be 100 mg/kg bw.

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity/Genotoxicity *in vitro*

Bacterial gene mutation assay

Guideline:	OECD 471 (1997)
Species/strain:	*Salmonella typhimurium*, TA1535, TA1537, TA98, TA100 and TA102
Replicates:	Triplicates per concentration in two independent experiments both in the presence and absence of Aroclor 1254 induced rat liver S9
Assay conditions:	Direct plate incorporation method, apart from the second test with S9 mix, which was performed according to the pre-incubation method
Test substance:	Phenyl methyl pyrazolone (PMP)
Batch:	62
Purity:	98.5%
Concentrations:	313, 625, 1250, 2500 and 5000 µg/ml (µg/plate) in both experiments with and without metabolic activation
Solvent:	DMSO
GLP:	in compliance

PMP was tested in a preliminary toxicity test (TA98, TA100 and TA102) and in two independent experiments in the absence and presence of metabolic activation (S9 mix prepared from the livers of rats given Aroclor 1254). The experiments were conducted according to the direct plating incorporation method, apart from the second test with S9 mix, which was performed according to the pre-incubation method with a preincubation period of 60 minutes at 37°C. Since PMP was freely soluble and non-toxic, it was tested up to the maximum recommended dose, 5000 µg/ml, in experiments 1 and 2, both in the absence and presence of S9 mix. Negative and positive controls were in accordance with OECD guidelines.

Results
There was a moderate precipitation at highest concentration tested and a coloration in plates at concentration at and above 625 µg/plate. No noteworthy toxicity measured as a decrease in revertant colonies and/or a thinning of the background lawn, was observed in any strain tested, either in the presence or absence of S9 mix. No biological relevant increase in revertants was observed compared to controls, in any strain, in both experiments and both test conditions. All solvent controls were within the historical data range, and all positive controls used gave a distinct increase of induced revertant colonies.

Conclusion
Under the experimental conditions used in this study, PMP was not mutagenic in the gene mutation test in bacteria either in the presence or absence of metabolic activation.

Ref.: 7

Mammalian Cell Mutation Test in Mouse Lymphoma Cells (*tk locus*)

Guideline:	OECD 476 (1997)
Species/strain:	Mouse lymphoma cell line L5178Y *tk*+/-
Replicates:	Duplicate cultures in two independent experiments
Metabolic act.:	Aroclor 1254 induced rat liver S9
Test substance:	Phenyl methyl pyrazolone (PMP)
Batch:	62
Purity:	98.5%
Concentrations:	Experiment 1: (in the presence and absence of S9 mix): 0.313, 0.625, 1.25, 2.5, 5 and 10 mM (equivalent to 54, 109, 218, 435, 870 and 1740 µg/ml). Experiment 2 (in the presence and absence of S9 mix): 0.625, 1.25, 2.5, 5, 7.5 and 10 mM (equivalent to 109, 218, 435, 870, 1305 and 1740 µg/ml)
Treatment:	Pulse (3h) treatment both in the absence and presence of S9. Two days recovery followed by 11-12 days expression period.
Solvent:	DMSO
GLP:	in compliance

PMP (A039) was evaluated in two independent experiments using duplicate cultures each. Both experiments used a pulse (3-hour) treatment and were conducted in the absence and presence of metabolic activation (S9 mix prepared from the liver of rats given Aroclor 1254, and purchased from Moltox™). According to the results in the pre-test PMP was freely soluble and moderately toxic, therefore the top concentration used in each experiment and test condition was 1740 µg/ml (equivalent to 10 mM). Six different concentrations were evaluated.
Cyclophosphamide (CPA), in the presence of S9 mix and methylmethane sulfonate (MMS) in the absence of S9 mix were used as positive controls. Negative controls consisted of cultures treated with the solvent alone (DMSO). Cells were suspended in culture medium and exposed to various concentrations of the test item, to solvent or positive controls. After the treatment period (3 hours), the cells were re-suspended in culture medium. They were transferred to flasks for growth through the expression period (48 hours). At the end of the expression period, acceptable cultures were then plated for viability (7 days) or TFT resistance (11-12 days).
In addition to the numbers of mutant colonies, the size of the colonies was determined, but the number of small versus large colonies was not evaluated.

Results
When tested up to 10 mM, there were no increases in mutant frequency in the absence of S9 mix. In the presence of S9 mix, dose-related increases in mutant frequency were observed at all concentrations tested in experiment 1 and at the three highest concentrations tested in experiment 2 (up to 3.9-fold the mean control value). It should be noted that in experiment 2 no excessive cytotoxicity was observed. Mutation frequencies in solvent negative controls remained within normal ranges for all experiment. However, in experiment 2 in the presence of S9 mix, the mutant frequency observed for control cultures was high (179), and above the upper limit of the laboratory historical negative control range (56 – 146). Treatment with positive controls CPA and MMS yielded distinct increases in mutant frequency.

Conclusion
Under the conditions of this study, PMP was considered to be genotoxic (mutagenic or clastogenic) in the mouse lymphoma assay (*tk* locus) in the presence of metabolic activation

Ref.: 8

Remark
It was not possible to distinguish between the induction of point mutations versus chromosomal aberrations because the number of small versus large colonies was not evaluated either for positive and negative controls or for any of the tested concentrations.

Mammalian Cell Gene Mutation Test in Mouse Lymphoma Cells (*hprt locus*)

Guideline:	OECD 476
Species/strain:	Mouse lymphoma cell line L5178Y [*hprt* locus for 6-thioguanine (6-TG) resistance]
Replicates:	Duplicate cultures in two independent experiments (single cultures for positive controls)
Metabolic act.:	Aroclor 1254 rat liver S9
Test substance:	Phenyl methyl pyrazolone (PMP)
Batch:	62
Purity:	98.5%
Concentrations:	Experiment 1: (in the presence and absence of S9 mix): 300, 600, 900, 1200, 1500 and 1740 µg/ml (equivalent to 10 mM) Experiment 2: (in the presence and absence of S9 mix): 200, 500, 800, 1100 (not evaluated in the presence of S9 mix due to contamination), 1400 and 1740 µg/ml (equivalent to 10 mM)
Treatment:	Pulse (3h) treatment both in the absence and presence of S9. Expression period was 7 days.
Solvent:	DMSO
GLP:	in compliance

PMP was evaluated for its ability to induce point mutations in the *hprt* locus in the mouse lymphoma cell line L5178Y. Two independent experiments using duplicate cultures each (single cultures for positive controls). Both experiments used a pulse (3-hour) treatment and were conducted in the absence and presence of metabolic activation (S9 mix prepared from the liver of rats given Aroclor 1254). According to the results in the pre-test PMP was freely soluble and moderately toxic, therefore the top concentration used in each experiment and test condition was 1740 µg/ml (equivalent to 10 mM). Five - six different concentrations were evaluated. Cells were treated for 3 hours followed by an expression period of 7 days to fix the DNA damage to stable *hprt*-mutations. At the end of the expression period, acceptable cultures were then plated for viability (2 plates per culture, 7 days) or 6-TG resistance (4 plates per culture, 11-12 days). Benzo(a)pyrene, BP, in the presence of S9 mix and 4-nitroquinoline 1-oxide, NQO, in the absence of S9 mix were used as positive controls. Negative controls consisted of cultures treated with the solvent alone (DMSO).

Results
When tested up to 10 mM, there were no increases in mutant frequency either in the presence or absence of S9 mix, in both experiments. A slight linear trend was observed in the absence of S9 mix in experiment 1. However, as there were no statistically significant increases in mutant frequency for any concentration tested, and as all mutant frequencies were similar to the laboratory historical negative control mean value, this isolated linear trend was considered to have no biological significance. It should be noted that a number of individual cultures were not evaluated for mutation in the presence of S9 in experiment 2, due to non-addition of 6-TG to mutant plates. However, there was clearly no evidence of induction of mutations in either experiment, and the loss of data from individual cultures in a single experiment was therefore considered not to invalidate the study.
Mutation frequencies in solvent negative controls remained within normal ranges, and treatment with positive controls NQO and BP yielded distinct increases in mutant frequency.

Conclusion

Under the conditions of this study, PMP was considered not to be mutagenic in mouse lymphoma cells (*hprt* locus) either in the absence or presence of metabolic activation.

Ref.: 9

In Vitro Micronucleus Test in cultured Human Lymphocytes

Guideline:	OECD draft guideline 487 (2004)
Species/strain:	Cultured human peripheral blood lymphocytes from two healthy, non-smoking male volunteers
Metabolic act.:	Aroclor 1254 induced rat liver S9
Test substance:	Phenyl methyl pyrazolone (PMP)
Batch:	62
Purity:	98.5%
Concentrations:	Experiment 1 in the absence of S9: 234, 365 and 891 µg/ml (up to 60% reduction in RI)
Experiment 1 in the presence of S9: 187, 713 and 1740 µg/ml (equivalent to 10 mM)	
	Experiment 2 in the absence of S9: 365, 570, 1114 and 1392 µg/ml (up to 75% reduction in RI)
	Experiment 2 in the presence of S9: 1114, 1392 and 1740 µg/ml (equivalent to 10 mM)
Treatment:	Experiment 1: 24 hours mitogen (PHA) stimulation before treatment
	Experiment 2: 48 hours mitogen (PHA) stimulation before treatment
	Both experiments:
	With S9: 3 h treatment followed by 45 h recovery period
	Without S9: 20 h treatment follower by 28 h recovery period
	The last 27 hours of incubation in the presence of cytochalasin B
Solvent:	DMSO
GLP:	In compliance

PMP was evaluated for its ability to induce micronuclei (clastogenic and aneugenic potential) using duplicate cultures of human lymphocytes in two independent experiments in the absence and presence of metabolic activation. The top concentration in each experiment and test condition was either 1740 µg/ml (equivalent to 10 mM) or was selected on the basis of cytotoxicity criteria; it was in the latter case selected to yield approximately 60% cytotoxicity (reduction in replication index, RI).

In experiment 1, cultures were incubated in the presence of the mitogen phytohaemagglutinin (PHA) for 24 hours and then received a 20- or 3-hour treatment in the absence or presence of S9 mix, respectively. Cells were harvested 72 hours after the beginning of incubation (the last 27 hours of incubation being in the presence of cytochalasin B). In experiment 2, a similar test procedure was used except that cultures were incubated in the presence of PHA for 48 hours prior to treatment (harvesting took place 96 hours after the beginning of incubation).
Lymphocyte preparations were stained and examined microscopically for determining the RI and the proportion of micronucleated binucleate (MNBN) cells when selected. Two thousand binucleate cells per concentration were analysed blindly. Known clastogens in the presence (cyclophosphamide, CPA) or absence of S9 (4-nitroquinoline-1-oxide, NQO and vinblastine, VIN). Solvent-treated cultures (DMSO, four replicates) were used as negative controls.

Results
The highest concentrations tested in the absence of S9 (890.9 µg/ml and 1392 µg/ml) induced 60% and 75% reduction of RI in the two independent experiments, respectively. At the highest concentration tested in the presence of S9 (1740 µg/ml = 10 mM), induced reduction in RI of 56% and 15%, respectively in the 2 experiments.
Treatment of cultures with PMP in the presence of S9 was not associated with any increase in MNBN cell frequencies in either experiment. In the absence of S9, statistically significant

increases in MNBN cell frequencies were observed at most PMP concentrations tested. However, there was no dose-relationship, the statistically significant increases observed were set against low values for concurrent solvent controls, and all MNBN frequencies obtained in PMP-treated cultures remained within the laboratory historical negative solvent control range. Accordingly, these increases observed only in the absence of S9 were considered to be chance events devoid of biological significance. Treatment of cultures with positive controls NQO, CPA and VIN resulted in consistent significant increases in MNBN frequencies, compared to concurrent solvent controls, thus showing the sensitivity of the test system and procedure used.

Conclusion
Under the conditions of the study, PMP was not genotoxic (clastogenic and/or aneugenic) in cultured human lymphocytes either in the presence or absence of metabolic activation.

Ref.: 10

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Rat bone marrow micronucleus test

Guideline:	OECD 474 (1997)
Species/strain:	Sprague-Dawley (SD) rats
Group size:	Five/sex/dose level
Test substance:	Phenyl methyl pyrazolone (PMP)
Batch:	62
Purity:	98.5%
Dose level:	0, 500, 1000 or 2000 mg/kg bw administrated as single dose
Route:	Oral gavage
Vehicle:	0.5% aqueous carboxymethylcellulose (10 ml/kg)
Sacrifice times:	24 hours after dosing. Additional negative control (0.5% aqueous carboxymethylcellulose) and high dose (2000 mg/kg) PMP groups (5 rats/sex) were killed 48 hours after dosing.
GLP:	In compliance

PMP has been investigated for its clastogenic and aneugenic activity in the *in vivo* micronucleus assay in rat bone marrow cells. Groups of SD rats (5/sex/dose level) received a single oral (gavage) dose of PMP at 0, 500, 1000 or 2000 mg/kg in 0.5% aqueous carboxymethylcellulose (10 ml/kg). These dose levels were selected on the basis of the results of dose range finding test where clinical signs but no deaths were observed at 2000 mg/kg.
In the main test, an additional positive control group of 5 rats/sex was given a single oral dose of cyclophosphamide (CPA) at 60 mg/kg. Animals were sacrificed 24 hours after dosing. Additional negative control (0.5% aqueous carboxymethylcellulose) and high dose (2000 mg/kg) PMP groups (5 rats/sex) were sacrificed 48 hours after dosing.
For each animal, smears were prepared from the tibial bone marrow, stained with acridine orange and scored blind for the incidence of micro-nucleated polychromatic erythrocytes (MN-PCE, 2000 PCE counted) and for the polychromatic/normochromatic erythrocyte ratio (PCE/NCE ratio, 500 erythrocytes counted).

Results
There were no deaths following treatment with PMP up to 2000 mg/kg. Clinical signs were observed at all dose levels: squinted eyes, hypoactivity and upright posture at 500 mg/kg and higher, and oral/ocular discharge and irregular breathing at 2000 mg/kg.
There were no biologically or statistically significant increases in the incidence of MN-PCE in any group treated with PMP. There was no indication of bone marrow toxicity since PCE/NCE ratios were similar for PMP and control groups. However, the oral bioavailability of PMP was suggested by the clinical signs observed in the present study. Moreover, in a recent 13-week oral toxicity study (CIT Study No. 26900 TCR [6]), systemic exposure to PMP was

demonstrated in rats given a single gavage at lower dose levels (Area Under the Curve values of approximately 5, 80 and 300 µg.h/mL at 20, 100 and 500 mg/kg, respectively).

When compared to controls, the incidence of MN-PCE was statistically significantly increased (approximately 70- and 26-fold for males and females, respectively) in animals given the positive control CPA, showing the adequate sensitivity of the test system and procedure used.

Conclusion
Under the conditions of the study, PMP did not produce cytogenetic damage leading to micronucleus formation in the bone marrow of rats treated orally up to 2000 mg/kg, the assay limit dose. Bone marrow toxicity was not demonstrated, but clinical signs of toxicity indicate systemic exposure.

Ref.: 11

Rat liver *in vivo/in vitro* UDS assay

Guidelines:	OECD 486 (draft, 1996)
Species/Strain:	Male Sprague-Dawley (SD) rats
Replicates:	Two independent experiments. Three animals per group were evaluated
Animals per dose:	4 male rats
Assay conditions:	The rats were sacrificed 14 and 2 hours after exposure in the first and second experiment respectively. Three rats per dose groups were evaluated. Two slides with 50 cells were scored for each animal. For some animals 3 slides were scored in order to obtain 100 cells per animal.
Test Substance:	Phenyl methyl pyrazolone (PMP)
Batch:	91N058
Purity:	99.8%
Dose level:	0, 250, 500, 1000 and 2000 mg/kg bw in the first experiment 0, 125, 250, 500 and 1000 mg/kg bw in the second experiment
Route:	Oral gavage
Vehicle:	0.5% aqueous carboxymethylcellulose (10 ml/kg)
GLP:	In compliance

The ability of PMP to induce DNA damage was assessed in the *in vivo* unscheduled DNA synthesis (UDS) assay in rat hepatocytes. The test substances and control substances were administered as a single dose by oral gavage. The dose levels were selected on the basis of the results from a previous acute oral toxicity study in rats where no deaths were observed at 2000 mg/kg [1]. Animals were sacrificed and sampled at 14 and 2 hours after treatment in the first and second experiment respectively.
For each animal, hepatocytes were isolated from the liver, and primary cultures from three animals per group were evaluated for Unscheduled DNA Synthesis (UDS). After attachment of the cells, cultures were labelled with tritiated thymidine for 4 hours and then incubated overnight with unlabelled thymidine. Autoradiographic slides were prepared and were evaluated blindly for UDS in 100 total cells from two different slides per animal [mean net nuclear grain count, mean percentage of nuclei with five or more net nuclear grains (cells in repair)]. 2-acetylaminofluorene (2-AAF) and methylnitrosourea (MNU) were used as positive controls in the first and second experiment respectively.

Results
Following treatment in experiment 1, clinical signs were observed at 500 mg/kg and higher, and all animals given 2000 mg/kg were found dead within 14 hours of dosing. Lower dose levels were therefore used in the second experiment where piloerection was observed at 250 mg/kg and higher.

In the first experiment, reduced levels of radio-labelling and reduced cell numbers were observed at 1000 mg/kg, indicating cytotoxicity at this dose level. These cytotoxic effects were even more severe in experiment 2 at 1000 mg/kg, and this dose level was therefore not selected for scoring in this experiment.

No changes from controls in mean nuclear labelling and percentage of cells in repair were observed in PMP-treated rats at any dose level and killing time. Net nuclear grain values remained negative for all PMP-treated animals.
When compared to controls, the administration of 2-AAF and MNU produced significant increases in nuclear labelling (both net nuclear grain count and percentage of cells in repair), showing the sensitivity of the test system and procedure used.

Conclusion
Under the conditions of the study, there was no DNA damage leading to unscheduled DNA synthesis in hepatocytes derived from rats treated orally with PMP up to the maximum tolerated dose of 1000 mg/kg. There was an indication of cytotoxic effect in the liver after exposure to the test substance.

Ref.: 12

3.3.7.	Carcinogenicity

Oral administration, mice

Guideline:	/
Species/strain:	B6C3F_1 mice
Group size:	49-50 animals per sex and dose, matched controls 20 animals per sex
Test substance:	l-Phenyl-3-methyl-5-pyrazolone in the diet
Batch:	Aldrich Chemical Company
Purity:	Melting point 128-130 °C; HPLC: one homogenous peak; TLC: 5 (chloroform:methanol as solvent) and 2 (ethylacetate as solvent) impurities in batch 1 (used for the first 5 months), 2 (chloroform: methanol as solvent) and 1 (ethylacetate as solvent) impurities in batch 2 (purchased 5 months later than batch 1); UV analysis: λ_{max} = 246, with ε =1.3 x10^4 (Batch 1) and 1.22 x 10^4 (Batch 2) observed in 0.l N NaOH
Dose level:	0, 7,500, 15,000 ppm for 102 weeks. The animals were then observed for 2 additional weeks.
Route:	Oral, in diet
Exposure:	102 weeks
GLP:	GLP not mentioned

A bioassay of l-phenyl-3-methyl-5-pyrazolone for possible carcinogenicity was conducted using
B6C3F1 mice. l-Phenyl-3-methyl-5-pyrazolone was administered in the feed, at either of two concentrations, to groups of 49 or 50 male and 50 female animals. The high and low concentrations of l-phenyl-3-methyl-5-pyrazolone utilized were, respectively, 15,000 and 7,500 ppm. Twenty animals of each sex were placed on test as controls. A 102-week period of chemical administration was followed by an additional 2-week observation period. An adequate numbers of animals survived sufficiently long to be at risk from late-developing tumours. Compound-related mean body weight depression was observed. There were no tumours in either sex of mice for which a significant positive association could be established between chemical administration and incidence. Under the conditions of this bioassay, there was no evidence for the carcinogenicity of l-phenyl-3-methyl-5-pyrazolone to B6C3F1 mice.

It is concluded that under the conditions of this bioassay, l-phenyl-3-methyl-5-pyrazolone was not carcinogenic for B6C3F_1 mice.

Ref.: 13

Oral administration, rats

Guideline:	/
Species/strain:	Fischer 344 rats
Group size:	49-50 animals per sex and dose, matched controls 20 animals per sex
Test substance:	l-phenyl-3-methyl-5-pyrazolone in the diet
Batch:	Aldrich Chemical Company
Purity:	Melting point 128-130 °C; HPLC: one homogenous peak; TLC: 5 (chloroform:methanol as solvent) and 2 (ethylacetate as solvent) impurities in batch 1 (used for the first 5 months), 2 chloroform: methanol as solvent) and 1 (ethylacetate as solvent) impurities in batch 2 (purchased 5 months later than batch 1); UV analysis: λ_{max} = 246 with ε=1.3 x10^4 (Batch 1) and 1.22 x 10^4 (Batch 2) observed in 0.l N NaOH
Dose level:	0, 2,500, and 5,000 ppm in feed. The animals were then observed for 2 additional weeks.
Route:	Oral, in diet
Exposure:	103 weeks
GLP:	GLP not mentioned

A bioassay of l-phenyl-3-methyl-5-pyrazolone for possible carcinogenicity was conducted using Fischer 344 rats. l-Phenyl-3-methyl-5-pyrazolone was administered in the feed, at either of two concentrations, to groups of 49 or 50 male and 50 female animals. The high and low concentrations of l-phenyl-3-methyl-5-pyrazolone utilized were 5000 and 2500 ppm. Twenty animals of each sex were placed on test as controls. After a 103-week period of chemical administration, there was an additional observation period of 2 weeks. Adequate numbers of animals survived sufficiently long to be at risk from late-developing tumours. Compound-related mean body weight depression was not observed. In addition, no significant accelerated mortality or other signs of toxicity were associated with the dietary administration of l-phenyl-3-methyl-5-pyrazolone to rats; therefore, it is possible that the compound was not administered to rats at the maximum tolerated concentration. There were no tumours in either sex of rats for which a significant positive association could be established between chemical administration and incidence. Under the conditions of this bioassay, there was no evidence for the carcinogenicity of l-phenyl-3-methyl-5-pyrazolone to Fischer 344 rats.

It is concluded that under the conditions of this bioassay, l-phenyl-3-methyl-5-pyrazolone was not carcinogenic for Fischer 344 rats.

Ref.: 13

Comment
It is concluded that under the conditions of this bioassay, l-phenyl-3-methyl-5-pyrazolone given in diet was not carcinogenic for B6C3F_1 mice or Fischer 344 rats. However, as there were no signs of toxicity in the rat study, it is possible that the compound was not administered to rats at the maximum tolerated concentration.

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

Guideline:	OECD 414
Species/strain:	Sprague-Dawley mated females
Group size:	three groups of 25 pregnant animals
Test substance:	1-Phenyl-3-methyl-5-pyrazolone
Batch:	8060110
Purity:	99.8%
Dose:	0, 40, 200 and 1000 mg/kg, 5 ml/kg/day
Vehicle:	0.5% carboxymethylcellulose
Route:	Oral
Exposure:	Female rats from day 6 to day 15 of pregnancy
GLP:	in compliance

The embryo/foetal toxicity of PMP was evaluated through daily oral gavage exposure (0, 40, 200 and 1000 mg/kg) of the mated SD female rats from day 6 through day 15 of gestation. The study was initiated on 25 mated females/group, but as pregnancy rate was low, additional females were included in the study to yield the aforementioned number of animals in each group.
No clinical signs were observed in the control, 40 and 200 mg/kg/day groups. In the 1000 mg/kg/day group, treated animals presented orange coloured bedding from day 7 to day 16.
The only case of death occurred in one non-pregnant female of the 1000 mg/kg/day group, without identifying the cause of the death.
There were no abortions.
The maternal body weight gain and food consumption of the dams were similar in the control, 40 and 200 mg/kg/day groups pregnancy. In the 1000 mg/kg/day, the body weight gain of the dams during the treatment period was 8% less than in the control group.
No parental macroscopic changes were seen.
There were no treatment-related foetal external or skeletal abnormalities. No foetal soft tissue anomalies were observed in any group.
At 1000 mg/kg/day, maternal toxic effects were observed and associated with lower foetal weight and increased incidence of foetuses with reduced ossification. There were no teratogenic effects, and the NOAEL was 200 mg/kg/day for both maternal and developmental toxicity.

Ref.: 14

3.3.9. Toxicokinetics

No data submitted

3.3.10. Photo-induced toxicity

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

(phenyl methyl pyrazolone)
(Oxidative/permanent)

Maximum absorption through the skin	**A**	**=**	**1.01 µg/cm²**
Skin Area surface	**SAS**	**=**	**700**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**0.707 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dosage (SED)	**SAS x A x 0.001/60**	**=**	**0.012**
No observed adverse effect level (13-week, oral, rat)	**NOAEL**	**=**	**100 mg/kg bw**
Margin of Safety	**NOAEL / SED**	**=**	**8333**

3.3.14. Discussion

Physico-chemical specification
Phenyl methyl pyrazolone is used in oxidative hair dye formulations at a maximum concentration of 0.5%, which after mixing typically in 1:1 ratio with hydrogen peroxide prior to use, corresponds to a concentration of 0.25% upon application. Stability of phenyl methyl pyrazolone in marketed products is not reported

General toxicity
PMP is not acutely toxic. The median lethal dose (LD50) of PMP was consistently above 2000 mg/kg. 4 different vehicles were used.
The repeated daily oral administration of PMP to rats for 13 weeks at 20, 100, and 500 mg/kg/day was mainly associated with changes indicative of regenerative haemolytic anaemia at 500 mg/kg/day. Clinical signs attributed to PMP but related to the mode of administration were observed at 100 mg/kg/day and higher. In males, the exposure of 100 mg/kg/day resulted in lower blood levels of glucose. The NOAEL was determined to be 100 mg/kg/day. The NOEL was set at 20 mg/kg bw.
PMP had no teratogenic potential. Maternal toxicity was seen at 1000 mg/kg/day. The NOAEL of maternal and developmental toxicity was 200 mg/kg/day.

Irritation / sensitisation
Under the conditions of the tests study, 1-phenyl-3-methyl-5-pyrazolone at 1% in propylene glycol caused transient mild irritation to rabbit skin. It was non-irritating to rabbit eyes.
Phenylmethyl pyrazolone induced delayed contact hypersensitivity. It is considered to have a strong sensitising potential.

Dermal absorption
The amounts of phenylmethyl pyrazolone considered as absorbed from a hair colouring formulation containing phenylmethyl pyrazolone at a final concentration of 0.25% in oxidative conditions were 0.31 ± 0.24 µg/cm^2 (0.56 ± 0.44% of the applied dose), A_{max} = 1.01 µg/cm^2 and in non-oxidative conditions 1.60 ± 0.30 µg/cm^2 (2.92 ± 0.53% of the applied dose), A_{max} = 2.56 µg/cm^2.

Mutagenicity
Phenyl methyl pyrazolone did not induce gene mutations in bacteria. It was also not mutagenic in an *in vitro* gene mutation test with mammalian cells (*hprt* locus). A mutagenic

response was observed in the Mouse Lymphoma assay (*tk* locus) but only in the presence of S9. In an *in vitro* micronucleus assay no genotoxic effect (structural and/or numerical chromosomal aberrations) was observed. An *in vivo* bone marrow micronucleus assay in mice or in an *in vivo* UDS assay in rats were negative.
The results of the tests performed indicate that phenyl methyl pyrazolone itself is not mutagenic *in vivo*.
To reach a definitive conclusion, appropriate tests with m-aminophenol in combination with hydrogen peroxide have to be provided.

Carcinogenicity
Under the conditions of the tests, l-phenyl-3-methyl-5-pyrazolone was not carcinogenic for $B6C3F_1$ mice and Fischer 344 rats. However, as there were no signs of toxicity in the rat study, it is possible that the compound was not administered to rats at the maximum tolerated concentration.

4. CONCLUSION

Based on the information provided, the SCCP is of the opinion that the use of phenyl methyl pyrazolone itself as an oxidative hair dye substance at a maximum concentration of 0.25% in the finished cosmetic product (after mixing with hydrogen peroxide) does not pose a risk to the health of the consumer, apart from its sensitising potential.

Phenyl methyl pyrazolone itself is not mutagenic *in vivo*.
However, studies on genotoxicity/mutagenicity in finished hair dye formulations should be undertaken following the relevant SCCNFP/SCCP opinions and in accordance with its Notes of Guidance.

5. MINORITY OPINION

Not applicable

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