EUROPEAN COMMISSION

Directorate-General for Health & Consumers

Scientific Committee on Consumer Safety

SCCS

OPINION on

Picramic acid and sodium picramate

COLIPA n° B28

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

The SCCS adopted this opinion at its 9th plenary meeting on 14 December 2010

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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

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1. BACKGROUND

Submission I for Picramic acid with the chemical name 2-amino-4,6-dinitrophenol was submitted in January 1988 by COLIPA [1, 2].

Submission II for Picramic acid was submitted in May 1993 by COLIPA [2]. The Scientific Committee on Cosmetology (SCC) adopted at its 51st plenary meeting on 7 October 1992 an opinion (SPC/319/91) with the final conclusion that:

"Picramic acid has moderate acute toxicity by the oral route. However, studies suggest that dermal penetration from hair dye formulation is low. There was no evidence of skin irritation with a 2.5% solution. Mild transient conjunctival irritation was seen with a 2.5% solution instilled into the eye and rinsed out after 10 seconds; although no data are available from animals not subjected to very rapid washout. The compound was a mild sensitiser in a maximisation test in guinea pigs. In a 90-day oral study a minimal effect level of 20 mg/kg bw was reported. The compound clearly has mutagenic potential. Positive results were consistently obtained in assays for gene mutation in Salmonella. It is essential to ascertain whether this potential can be expressed in vivo. Studies in the whole animal have been limited to the bone marrow. Negative results were obtained from a micronucleus test but only one harvest time was used. Negative results were also reported in an assay for SCE induction in bone marrow. No conclusions can be drawn regarding the carcinogenicity of picramic acid. No adverse effects were reported in an oral teratogenicity study in rats at up to 15 mg/kg bw. In vivo data are needed from a well conducted micronucleus test, to a current protocol, and also from an in vivo liver UDS assay."

Submission III was submitted in July 2005 by COLIPA and concerned sodium picramate (CAS 831-52-7). According to this submission, sodium picramate and picramic acid are used in hair colouring formulations. As the pKa of picramic acid is around 4, it is always the picramate which is available in typical hair dye formulation (pH 6.5 – pH 10). Therefore the submission discussed mainly sodium picramate.

Sodium picramate, a non-reactive dye, is used as a direct hair colouring agent up to on-head concentration of 0.6% in non-oxidative as well as in oxidative hair dye formulation. For non-oxidative hair dye formulations it is common practice to apply 35 – 50 mL of the undiluted formulation while for oxidative ones 100 mL are applied after mixing with H_2O_2. The application time for both formulation types covers a period of 30 minutes followed by washing off with water and shampoo. It is assumed that application may be repeated weekly.

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

2. TERMS OF REFERENCE

1. *Does the Scientific Committee on Consumer Safety (SCCS) consider sodium picramate and picramic acid safe for use as a non-oxidative hair dye with an on-head concentration of maximum 0.6% taken into account the scientific data provided?*

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

2. *Does the SCCS consider sodium picramate and picramic acid safe for use in oxidative hair dye formulations with an on-head concentration of maximum 0.6% taken into account the scientific data provided?*

3. *Does the SCCS recommend any further restrictions with regard to the use of sodium picramate and picramic in any non-oxidative or 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

Picramic acid (INCI name)
Sodium picramate (INCI name)

3.1.1.2. Chemical names

Acid
2-Amino-4,6-dinitrophenol
2,4-Dinitro-6-aminophenol
Phenol, 2-amino-4,6-dinitro-
1-Amino-3,5-dinitro-2-hydroxybenzene

Sodium salt
2-Amino-4,6-dinitrophenol, sodium salt
Phenol, 2-amino-4,6-dinitro-, sodium salt
Picramic acid, sodium salt
Sodium 2-amino-4,6-dinitrobenzenolate

3.1.1.3. Trade names and abbreviations

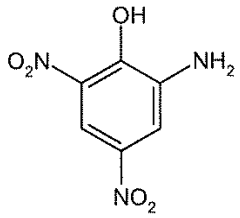
Acid
CI 76540
Oxidation base 21

Sodium salt
Rodol 4R (Lowenstein)

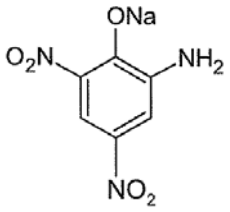
3.1.1.4. CAS / EC number

	Picramic acid	Sodium picramate
CAS:	96-91-3	831-52-7
EC:	202-544-6	212-603-8

3.1.1.5. Structural formula



Picramic acid



Sodium picramate

3.1.1.6. Empirical formula

Formula: $C_6H_5N_3O_5$ (picramic acid)
$C_6H_4N_3NaO_5$ (sodium picramate)

3.1.2. Physical form

Brown orange powder (picramic acid)
Red-brown paste (sodium picramate)

3.1.3. Molecular weight

Molecular weight: 199.12 g/mol (picramic acid)
221.10 g/mol (sodium picramate)

3.1.4. Purity, composition and substance codes

Picramic acid

Purity: > 97.0% (HPLC, relative to standard)
Content: 97.0 – 100% (relative to standard)
> 60%, w/w (non dried material, HPLC, relative to standard)

Sodium picramate

Purity: > 50% (HPLC, relative to standard)

Batch comparison

	Sodium picramate		Picramic acid	
Batch	**145/03**	**50/04**	**DO 422**	**RK 140983**
Purity	**62.6%**	**62.4%**	**99.0%**	**98.9%**
Water	~ 30%	30%	/	/
Crystal water	8%	8%	/	/
2,4-dinitrophenol	n.d. *	n.d.	0.10%	0.014%
2,4-dinitrochlorobenzene	n.d. *	n.d.	n.d. **	n.d. **
Picric acid	n.d.	0.4%	655 mg/kg bw	0.11%

* detection limit = 0.1%
** detection limit = 5 mg/kg bw

The purity of batch 145/03 is characterised by HPLC, NMR, UV, MS and IR. There are no indications of impurities. Water is the only impurity identified.

3.1.5. Impurities / accompanying contaminants

		Picramic acid	Sodium picramate
Picric acid:		< 2000 ppm	< 0.3%
2,4-Dinitrophenol:		< 0.15%	< 0.1%
Dinitrochlorobenzene:			< 0.1%
Sulphated ash:		< 1%	< 1%
Solvent content (loss on drying):		< 40% (non dried material)	< 50%
Heavy metal content:	Ar	< 5 ppm	< 5 ppm
	Sb	< 5 ppm	< 5 ppm
	Pb	< 20 ppm	< 20 ppm
	Cd	< 10 ppm	< 10 ppm
	Hg	<5 ppm	< 5 ppm

These figures are taken from summary submission III 2005. No experimental data were provided.

3.1.6. Solubility

	Picramic acid	sodium picramate
Water:	< 10 g/l	10 g/l (some insoluble material)
DMSO:	> 100 g/l	> 100 g/l
Ethanol:	< 60 g/l	< 10 g/l

Taken from the summary of submission III, 2005. No experimental data were provided.

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}:	/	picramic acid
	- 2.97	sodium picramate

The Log Pow of sodium picramate (batch 145/03) was determined according to OECD guideline 107 (flask shaking method).

Ref.: 3

3.1.8. Additional physical and chemical specifications

	Picramic acid	sodium picramate
Melting point:	169-170 °C	98.8 °C (decomposition)
Boiling point:	/	/
Flash point:	/	/
Vapour pressure:	/	/
Density:	/	/
Viscosity:	/	/
pKa:	/	/
Refractive index:	/	/
UV_Vis spectrum (200-800 nm)	/	/

3.1.9. Homogeneity and Stability

Sodium picramate is stable under normal laboratory conditions. Solutions of this chemical in water, DMSO are stable for 48 hours under lab conditions.

Solutions in methanol are stable at 4 °C for 11 weeks. The variation of recovery experiments is <10%

General Comments to physico-chemical characterisation

The data concerning impurities, solubility, identity and other physico chemical parameters of the four batches used cannot be traced back to the original data.

The identity of batch 145/03 as well as its purity is well established. This should also apply to batch 50/04 (but cannot be traced back to the original data). These batches do not contain measurable concentrations of impurities except water (38%) (145/03) and in the case of batch 50/04, additionally 0.4% of picric acid.

The purity of the two batches of picramic acid (DO 422 and RK 140983) is 98.9 and 99% according to submission III, but no experimental data were submitted.

There is inconsistency regarding the water content described in the purity and impurity tables.

Despite the lack of information, the impurities are not expected to be of toxicological concern with respect to the maximum use concentration of 0.6% B28.

The stability of the substance itself and its solutions was sufficient in toxicity testing. The stability of B28 in typical hair dye formulations was not reported. The stability in an oxidative environment has not been demonstrated.

The batches used for toxicity testing were not specified in several cases.

3.2. Function and uses

Sodium picramate and picramic acid are used in hair colouring formulations. As the pKa of Picramic acid is around 4, it is always the picramate which is available in a typical hair dye formulation (pH 6.5- pH 10).

Sodium picramate, a non reactive dye, is used as a direct hair colouring agent up to an on-head concentration of 0.6% in non-oxidative as well as in oxidative hair dye formulation. Sodium picramate is said to be stable under conditions used in oxidative formulations (no data provided) and does not take part on the oxidation colouring forming mechanism.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline:	/
Species/strain:	rat, CFY
Group size:	60 (5 males and 5 females per group)
Test substance:	picramic acid
Batch:	/
Purity:	/
Vehicle:	10% suspension in aqueous gum tragacanth (0.5%) containing 0.05% sodium sulfite
Dose:	0, 100, 160, 250, 400 and 640 mg/kg bw
Dosage volumes:	1.0 to 6.4 ml/kg bw
Route:	oral intubation
GLP statement:	not in compliance
Study period:	August/September 1976

Rats of the CFY strain in the weight range of 95 to 120 g were starved overnight before treatment. Picramic acid was prepared as a 10% suspension in aqueous gum tragacanth (0.5%) and administered by oral intubation at a range of dosage volumes of 1.0 to 6.4 ml/kg bw, corresponding to doses from 100 to 640 mg/kg bw. Rats treated with the vehicle alone (6.4 ml/kg bw) served as controls. During the observation period of 14 days, a record was kept of mortalities and signs of toxicity. All rats that died were examined macroscopically to identify the target organs and surviving animals were similarly examined after the observation period to detect possible damage. From the mortality data the LD_{50} and its 95% confidence limits were calculated.

Results
The results of preliminary range finding tests indicated that the median lethal oral dose (LD_{50}), was in the region of 100 to 400 mg/kg bw. Dosing was then extended to larger groups of rats (five males and five females) in order to set the median lethal dose more precisely. Signs of reactions to treatment were observed shortly after dosing, including lethargy, piloerection and orange staining of external extremities. These signs were accompanied within five hours by gasping in six rats treated at 100 mg/kg bw. Death occurred from within one to 19 hours of treatment. Autopsy revealed discoloration of the liver, pallor of the kidneys and spleen, and orange staining of the inner body wall. Recovery of survivors, as judged by external appearance and behaviour was apparently complete within five days after treatment. Bodyweight gains were within normal limits compared with controls and normal autopsy findings. The acute median lethal oral dose (LD_{50}) and its 95% confidence limits to rats of picramic acid were calculated to be 110 (63-176) mg/kg bw.

Ref.: 15

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: /
Species/strain: rabbit
Group size: 3 animals
Test substance: picramic acid
Batch: /
Purity: /
Vehicle: distilled water containing 0.05% sodium sulfite, buffered to pH 7
Dose level: 2.5% aqueous solution
Dose volume: 0.5 ml
GLP: not in compliance
Study period: September 1976

0.5 ml of 2.5% aqueous solution of the test material was applied on 2.5 cm² under an occlusive patch to intact and abraded skin on dorsal aspect for 24 hr.

Results
No reaction was observed over 72 hr.

Conclusion
Under the condition of the test, a 2.5% aqueous solution of picramic acid was not irritant to rabbit skin.

Ref.: 5

Comment
The test did not conform to a guideline.

3.3.2.2. Mucous membrane irritation

Guideline: /
Species/strain: rabbit
Group size: 3 animals
Test substance: picramic acid
Batch: /
Purity: /
Vehicle: distilled water containing 0.05% sodium sulfite, buffered to pH 7
Dose level: 2.5% aqueous solution
Dosing volume: 0.1 ml
GLP: not in compliance
Study period: October 1976

0.1 ml of 2.5% aqueous solution of the test substance was instilled into the conjunctival sac of one eye of each animal. These eyes were rinsed with 20 ml distilled water after 10 seconds.

Results
In all 3 animals, there was conjunctival redness persisting to 4 days, and up to 7 days in one animal.

Conclusion
Under the condition of the test, a 2.5% aqueous solution of picramic acid was irritant to rabbit eyes.

Ref.: 6

Comment
The test did not conform to a guideline.

3.3.3. Skin sensitisation

Local Lymph Node Assay (LLNA)

Guideline:	OECD 429 (2002)
Species/strain:	mice, CBA/CaOlaHsd (nulliparous and non-pregnant)
Group size:	24 females (4 per group)
Test substance:	sodium picramate
Batch:	145/03
Purity:	62.6%
Vehicle:	DMSO
Concentration:	0, 1, 5, 10, 25 and 50% w/v
Positive control:	α-hexylcinnamaldehyde (85% pure) (in acetone:olive oil; 4:1); October 2003
GLP:	in compliance
Study period:	29 January – 2 February 2004

25 µL test preparation applied to dorsal aspect of ear lobe daily for 3 days. 5 days after the first application, 250 µL of a solution containing 81.3 µCi/mL ^{3}HTdR given by IV injection into a tail vein. 4h later the animals were killed and the draining lymph nodes dissected out. Incorporation of ^{3}HTdR was measure by β-scintillation.

Concentration	Stimulation Index
Test item	
1%	1.2
5%	2.7
10%	3.6
25%	7.9
50%	11.4
α-hexylcinnamaldehyde	
5%	0.87
10%	2.26
25%	6.01

Results
The calculated EC3 value for sodium picramate was 6.7% w/v. For the positive control, α-hexylcinnamaldehyde was 12.96%. Therefore, sodium picramate is a skin sensitiser.

Conclusion
In the LLNA, sodium picramate is a moderate skin sensitiser.

Ref.: 12

3.3.4. Dermal / percutaneous absorption

Guideline:	OECD 428 (2004)
Tissue:	dermatomed pig skin (fresh), 300 µm thickness
Group size:	18 (3 x 6) membranes, 18 donors
Skin integrity:	conductivity < 900 µS
Diffusion cell:	glass flow-through diffusion cell, 1.135 cm diameter
Test substance:	B28
Batch:	145/03
Purity:	62.6%
Test item:	standard formulation with 2% B28 (corresponding to 1.25% active ingredient) + hydrogen peroxide, 6% (1:1)

Dose volume: 20 µL/cm² (100 µg B28)
Receptor fluid: saline, 0.9% NaCl
Solubility receptor fluid: /
Stability receptor fluid: 30 days at room temperature
Method of Analysis: HPLC
GLP: in compliance
Study period: 4 April – 27 May 2005

Composition of 'standard formulation'

Ingredient	*Weight (%)*
B28	2.0
Cetearyl Alcohol	8.00
2-Octyldodecanol-1	1.00
Oleyl Alcohol	1.50
Sodium Lauryl Sulfate	1.00
Water	78.2
Ammonium Bicarbonate	0.3
Ammonium Hydroxide	5.20
Water	4.80

20 µL of the test preparation was placed into each diffusion cell. After 30 minutes each cell was rinsed twice with 1mL water, washed twice with 1mL of a 10% shampoo solution and then rinsed 4 times with 1mL of water.

Initially, 2 experiments were envisaged but, as one chamber from each series was excluded, a third experiment was undertaken.

Experiment 1

	Amount recovered (µg/cm²)					
Chamber	**1**	**2**	**3**	**4**	**5**	**6**
Amount applied	62.394	57.291	60.128	53.300	51.352	39.153
Skin extract (absorption)	0.00	0.00	0.00	0.00	0.00	0.00
Penetrated	0.225	0.342	0.336	0.342	0.159	0.058
Bioavailable (µg/cm²)	0.225	0.342	0.336	0.342	0.159	0.058
Bioavailable (%)	0.361	0.597	0.559	0.641	0.309	0.148
Recovery (%)	105.2	95.4	99.0	90.7	92.8	130.9

Experiment 2

	Amount recovered (µg/cm²)					
Chamber	**1**	**2**	**3**	**4**	**5**	**6**
Amount applied	101.38	77.616	62.842	71.189	55.984	53.882
Skin extract (absorption)	0.00	0.00	0.00	0.00	0.00	0.00
Penetrated	0.087	0.062	1.060	0.244	0.005	0.019
Bioavailable (µg/cm²)	0.087	0.062	1.060	0.244	0.005	0.019
Bioavailable (%)	0.086	0.080	1.687	0.342	0.008	0.034
Recovery (%)	93.3	97.1	94.0	76.4	89.5	88.6

Experiment 3

	Amount recovered (µg/cm²)					
Chamber	**1**	**2**	**3**	**4**	**5**	**6**
Amount applied	66.085	57.76	56.671	50.874	50.364	44.633
Skin extract (absorption)	0.00	0.00	0.00	0.00	0.00	0.00
Penetrated	0.082	0.240	0.913	1.014	0.772	0.866

	Amount recovered (µg/cm²)					
Chamber	**1**	**2**	**3**	**4**	**5**	**6**
Bioavailable (µg/cm²)	0.082	0.240	0.913	1.014	0.772	0.866
Bioavailable (%)	0.124	0.416	1.611	1.993	1.533	1.940
Recovery (%)	110.2	106.8	113.8	96.3	89.0	67.9

The values of the shaded diffusion cells were not considered for the calculation of the dermal absorption, since the mass balance analysis showed a recovery outside the range of 100 ± 15%.

Summary table

	µg/cm²		%	
	mean	**SD**	**mean**	**SD**
Receptor fluid	0.377	0.373	0.669	0.684
Stratum corneum (isolated by tape stripping)	0.137	0.063	0.231	0.113
Epidermis + Upper dermis (24 hrs)	0.000	0.000	0.000	0.000
Washing solution (after 30 min.)	59.041	13.644	96.314	7.976
Dermal absorption	**0.377**	**0.373**	**0.669**	**0.684**
Total balance (recovery)	59.688	13.539	97.437	8.000

From an oxidative hair dye formulation containing 1% B28 (62.6% active ingredient), the amount of active B28 absorbed was 0.377 ± 0.373 (range 0.005 - 1.06) µg/cm² or 0.669 ± 0.684 (range 0.008-1.993) % of the applied dose (recalculated to pure dye).

Ref.: 4

Comment
Because of the high variability of the data, the mean + 2 SD (0.38 + 2 x 0.37 = 1.12 µg/cm²) is used for calculating the MOS under oxidative conditions. No study under non-oxidative conditions was provided.

The CIR dermal absorption

The CIR (Cosmetic Ingredient Review; Becker, Bergfeld, Belsito et al, 2003) reports on an unpublished study of Hazelton Laboratories Europe, (picramic acid/percutaneous absorption in the rat; unpublished data submitted by CTFA, 1994) in which [^{14}C]-picramic acid in a hair dye product was tested in pigmented rats of the PVG-strain.

0.1mL of a hair dye formulation containing approximately 15 mg [^{14}C]-picramic acid was applied for 0.5 hours to a clipped area 30 x 30mm of the dorsolumbar skin of each of 3 male and 3 female rats. The dose of picramic acid applied to the skin was 1.667 mg/cm². Within 72h of application to the skin, 0.38% of the picramic acid in the hair dye formulation had been recovered from the urine (0.22%) and faeces (0.16%).

The above study was under non-oxidative conditions. Although the original data has not been made available, an absorption of 0.38% (worst case) of the applied dose of picramic acid can be derived.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (14 days) oral / dermal / inhalation toxicity

Guideline:	OECD 407 (1995)
Species/strain:	rat, HanBrl:WIST (SPF)
Group size:	40 (5 males and females per group)
Test substance:	sodium picramate
Batch:	50/04
Purity:	62.4%
Vehicle:	bi-distilled water
Dose levels:	0, 20, 100 and 250 mg/kg bw/day
Dose volume:	10 ml/kg bw
Route:	oral gavage
Administration:	daily for 14 days
GLP:	not mentioned
Study period:	26 July – 16 August 2004

In this 14-Day Oral Range-Finding Toxicity Study in the Wistar Rat, sodium picramate was administered by daily gavage to SPF-bred Wistar rats of both sexes at dose levels of 20, 100, and 250 mg/kg bw/d for a period of 14 days. A control group was treated similarly with the vehicle (H_20 bidest.) only. A total of 40 rats was used in this study. The groups comprised 5 animals per sex which were sacrificed after 14 days of treatment. Clinical signs, food consumption and body weights were recorded periodically during acclimatization and the treatment period. At the end of the treatment period, all animals were killed, necropsied and examined *post mortem*. Histological examinations were performed on organs and tissues from all control and high dose animals, and all gross lesions from all animals were recorded.

Results
Deaths were observed at the high dose [two males: Days 5 and 7; three females: Days 1, 3 and 7].
Control animals, animals treated at the dose of 20 mg/kg bw/day or 100 mg/kg bw/day showed no treatment related clinical effects.
In rats treated at the dose of 250 mg/kg bw/day several clinical signs were observed: during the first treatment week, slightly ruffled fur was observed in one female on treatment days 4 and 5 and in an other one on treatment day 3; slight emaciation was observed in four females and two males in the first treatment week and moderate emaciation in a further male on treatment day 6. Decreased spontaneous activity was observed in one female on treatment day 4 and 5 and in one male on treatment day 6. Slightly brown urine was seen in two females and three males on the last day of treatment.
The mean absolute food intake was slightly decreased in males and females treated at the dose of 250 mg/kg bw/day and the mean relative food intake was also decreased in males and females treated at this dose when compared to control rats.
The mean absolute body weights were decreased in males treated at the dose of 250 mg/kg bw/day when compared to the control group. In females of this dose group a not statistically significant decrease was observed. The mean body weight gain was decreased in males and females from the high dose group.
Increased dose-related mean spleen weights and spleen to body ratios were observed in males and females rats treated at 250 mg/kg bw/day. The mean liver to body weight ratio was increased in males treated at the dose of 100 mg/kg bw/day or 250 mg/kg bw/day and in females at the dose of 250 mg/kg bw/day and 100 mg/kg bw/day but at this dose the increase was not statistically significant. An increase of the mean brain to body weight ratio was also observed in male rats at the dose of 250 mg/kg bw/day.
The macroscopic lesions observed and possibly related to treatment consisted of enlarged spleen observed in 3 male rats and 1 female rat of the 250 mg/kg bw group. They could be

correlated to the increase of the weight of the spleen and were considered related to the treatment. Reduced size of testes, epididymes, prostate and seminal vesicles was seen in three males treated at the dose of 250 mg/kg bw/day. Changes in colon as foci, nodules or thickened organ were observed in three male rats and one female rats treated at the dose of 250 mg/kg bw/day and in one female rat treated at the dose of 100 mg/kg bw/day. Thickened caecum was observed in three male rats and foci were seen on the caecum of two males and two females treated at the dose of 250 mg/kg bw/day.
Other signs observed were discoloration of lung, lung not collapsed and thickened thymus, but they were not considered to be related to the treatment with sodium picramate.

Conclusion
Based on the results of this study, dose levels of 5, 15 and 80 mg/kg bw/day of sodium picramate were proposed for the 90-day study in Wistar rats.

Ref.: 14

Comments
In this study, the No Observed Adverse Effect level (NOAEL) was 20 mg/kg bw/day, corresponding to 12.5 mg/kg bw/day active ingredient.
The SCCS noticed that in this study rats treated at the dose of 100 mg/kg bw/day did not show any clinical effect whereas the LD50 calculated from the acute toxicity study was 110 mg/kg bw/day. This discrepancy in the toxicity of B28 may be related to the different batches of B28 tested with different levels of impurity.

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

Guideline:	OECD 408 (1998)
Species/strain:	rat, HanBrl:WIST (SPF)
Group size:	100 (10 males and 10 females per group) (group 1 and 4: + 5 males and 5 females for 4 weeks recovery)
Test substance:	sodium picramate (paste with 30% water)
Batch:	50/04
Purity:	62.4%
Vehicle:	bi-distilled water
Dose levels:	0, 5, 15 and 80 mg/kg bw/day
Stability:	7 days in vehicle under storage conditions
Dose volume:	10 ml/kg bw
Route:	oral gavage
Administration:	daily for 13 weeks
GLP:	in compliance
Study period:	8 November 2004 – 24 May 2005

Twenty rats (10 per sex) of the Wistar strain were used per dose and control group. Additional 10 rats (5 per sex) in both the control and high dose groups were assessed for recovery, four weeks after the last administration. The test procedure followed the OECD guideline and was conducted in compliance with the principles of GLP. Aliquots of 10 ml/kg bw of sodium picramate (purity 62.4%) were administered in a single dose by gavage. The test substance was given as an aqueous solution for 91 consecutive days in daily doses of 5, 15 and 80 mg/kg bw/day based on the results of a dose range finding study (ref. 14). The control animals received the vehicle alone (bi-distilled water). During the study the mortality, signs of intoxication, body weight and food consumption were recorded. The animals of the recovery groups were additionally examined during the 4-week treatment-free period. Ophthalmoscopic examinations were performed in all rats at acclimatisation, at the end of the treatment period in control and high-dose rats, and in low- and mid-dose groups if sodium picramate related changes were found in the high dose group. If sodium picramate related changes were observed in week 13, ophthalmoscopic examinations of both eyes of all animals were performed after the application of a mydriatic solution.

Blood samples were withdrawn for haematology and blood chemistry analysis. Urine samples were collected for urinalysis. All animals were killed, necropsied and examined *post mortem*. Histological examinations were performed on organs and tissues from all control and high dose animals, in animals which died spontaneously and in all gross lesions.

Results
No rats died during the study. No clinical signs were observed in the low dose group (5 mg/kg bw/day). Deep yellow urine was observed in almost all rats from the medium dose group (15 mg/kg bw/day) or the high dose group (80 mg/kg bw/day) during the 13 weeks of treatment and first week of recovery period. Rats from the high dose group also showed slight tan fur during the treatment period. Other clinical signs as hair loss, scabbed wound or necrosis on the neck were observed in males or females rats from the high and control dose groups. These signs were not considered related to the treatment. Ophthalmoscopic investigations revealed no evidence of eye toxicity.
Food intake was not significantly affected except in the high dose group. In males treated with 80 mg/kg bw/day the mean absolute and relative food consumption was slightly increased from week 3 until the end of the treatment when compared with controls. In females from the high dose group, this increase was only observed at the last week of the treatment.
Body weight and body weight gain were not affected during the treatment.
Haematological changes were observed after 13 weeks in females from the medium groups and in both sexes from the high dose group: slight increase in the mean corpuscular volume and mean corpuscular haemoglobin and a decrease in the mean haemoglobin concentration (high dose group only); increase in the mean relative and absolute reticulocyte counts and in the mean reticulocyte maturity index in the males and females from the high dose group and the females from the medium dose group; moderate increase in the leukocyte count and in the mean relative and absolute values of neutrophils in the high dose group; at this high dose group effects on lymphocytes and monocyte count were observed. These effects were considered test item related and were reversible at the end of the recovery period. Non dose related platelet effects were also observed in the males from the medium and high dose group.
Clinical biochemistry changes were observed in males and females from the high dose group only at the end of the treatment. These effects may reflect metabolic changes. Effects on the electrolyte parameters Na^+, Cl^- (males and females) were observed and related to the nature of the test item itself (sodium salt) and most of these effects regressed during recovery.
Some minor changes in parameters of urinalysis were observed in males and females rats from the high dose group and urine discoloration in males rats from the medium dose group. Increase in urine turbidity was also observed in male rats from the high dose group. All these effects were reversible after the recovery period.
Increase in liver, kidney and spleen weights and in their ratios to body and brain weights were observed at the end of treatment in rats from the high dose group. These effects were considered related to the treatment. They were reversible after the recovery period.
Decreases in mean testes weights, testes to body and brain weights ratios were observed in male rats from the high dose group at the end of treatment. These effects were not reversible after the recovery period.
Decreases in mean epididymidis weights, epididymidis to body and brain weights ratios were observed in male rats from the high dose group at the end of the recovery period but not during the treatment.
Macroscopic and microscopic examination after terminal necropsy showed lesions in the testes and epididymides of male rats from the high dose group: size reduction of testes (in nine rats) and epididymides (in six rats) and concerning epididymides thickening (in one rat), nodules (in one rat), foci (in two rats) or cysts (in two rats). Severe tubular degeneration, sperm granuloma, azoospermia or oligospermia were reported on the testis of nine rats from the high dose group; these lesions were also observed after the recovery period. As prostate and seminal vesicles were not affected, the authors considered that a

testosterone effect is unlikely and the lesions recorded are considered to be of primary cytotoxic nature to sperm.
Macroscopic lesions (foci or thickening) were also observed on stomach, caecum or mesenteric lymph nodes of some male or female rats. Microscopic findings on gastrointestinal tracts as ulceration or inflammation, fibrosis were reported on some male or female rats from the medium or high dose groups, some of them were always observed after the recovery period.
Microscopic findings were reported in the spleen and bone marrow in rats from the high dose group of both sexes (extramedullary and medullary hemopoiesis), in the liver in male or female rats from the medium or high dose groups (hepatocellular hypertrophy, in the kidneys in male or female rats from the medium or high dose groups (tubular cell swelling) and in the adrenals in male rats from the high dose group (cortical vacuolation). These findings were not longer present after the recovery period.
Extramedullary and medullary hemopoiesis were considered by the authors of minor severity degrees and secondary to intestinal perforation, inflammation and haemorrhage. Hepatocellular hypertrophy was considered to be of adaptative metabolic nature. The increased cortical vacuolation in adrenals was considered related to stress hormone production (fatty change) and the nature of the renal lesions was considered unclear.

Conclusion
Under the conditions of this experiment, sodium picramate revealed primary findings of toxicity in the testes/epididymides and gastrointestinal tract.
Due to haemototoxicity and microscopic findings in the gastro-intestinal tract, liver and kidney at 15 mg/kg bw/d the No Observed Adverse Effect Level (NOAEL) in rats after daily oral treatment is determined to be 5 mg/kg bw/day, corresponding to 3.1 mg/kg bw/day active ingredient.

Ref.: 13

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

Summary on toxicity

Study	Species	Sex	Effects	Critical doses	Ref
acute, oral	rat SFY	m and f	non specific	LD50 =110 mg/kg bw	15
14-day, oral	rat HanBrL: WIST	m and f	**250 mg/kg bw:** 2m and 3 f †, clinical effect ↓ bw, ↓ food consumption, ↑ spleen, liver w, brain; ↓ size reproductive organs (m); histological changes in colon **100 mg/kg bw**: ↑bw liver; histological changes in colon (one f)	NOAEL = 20 mg/kg bw/d	14
90-day, oral	rat HanBrL: WIST	m and f	**80 mg/kg bw**: ↑ food consumption (m and f); haematological changes (m and f); clinical biochemistry changes (metabolic changes);↑ liver (reversible increase), kidney and spleen weight and ↓ in testes weights (irreversible increase) and tubular degeneration; ulceration or inflammation of the caecum (m and f), hemopoiesis extra or intra medullary, vacuolation in the adrenals (m) **15 mg/kg bw**: haematological changes (f); ulceration or inflammation of the caecum (f)	NOAEL = 5 (a.i.: 3.1 mg/kg bw/d)	13
Teratogenicity, oral	Rat, Wistar	f	**60 mg/kg bw**: ↑ in foetal body weight and uterine weights	NOAEL = 30 mg/kg bw/d	16

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial Reverse Mutation Assay

Guideline:	OECD 471 (1997)
Species/Strain:	*Salmonella typhimurium* TA 98, TA 100, TA 102, TA1535, TA 1537
Replicates:	triplicate
Test substance:	sodium picramate
Batch:	145/03
Purity:	62.6%
Vehicle:	de-ionised water
Concentration:	3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate
Treatment:	experiment I: plate incorporate test, without and with S9-mix
	experiment II: pre-incubation test, without and with S9-mix
	experiment IIA: pre-incubation test, TA 98 with S9-mix
Control:	without S9-mix: sodium azide, 4-nitro-o-phenylenediamine, methyl methane sulfonate
	with S9-mix: 2-aminoanthracene
GLP:	in compliance
Study period:	14 May – 7 June 2004

The potential of sodium picramate to induce gene mutations according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the *Salmonella typhimurium* strains TA 1535. TA 1537, TA 98, TA 100 and TA 102 were investigated.
The assay was performed with and without liver microsomal activation except experiment IIA was performed with metabolic activation, only. Each concentration and the controls were tested in triplicate.

Reduced background growth was observed with and without metabolic activation at 5000 µg/plate in strain TA 100 in experiment I and at 1000 - 5000 µg/plate in strain TA 102 in experiment I and II.
Toxic effects, evident as a reduction in the number of revertants were observed at higher concentrations in all strains with and without metabolic activation in experiment I and II.
A dose dependent increase in revertant colony numbers was observed in strain TA 98 in experiment II in the presence of metabolic activation. The number of revertant colonies reached or exceeded the threshold of twice the number of the corresponding solvent control at concentrations of 100, 333 and 1000 µg/plate. A third experiment using the pre-incubation procedure was performed with strain TA 98 with metabolic activation to verify the results of the second experiment. This additional experiment showed a concentration dependent mutagenic response exceeding the threshold of 2.0 at 333 and 1000 µg/plate. The additional experiment is reported as experiment II A.
Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revenant colonies.

Conclusion
Under the experimental conditions reported, the test item induced gene mutations by frame shifts in the tester strain TA 98 in the presence of metabolic activation.

Ref.: 7

In vitro Mammalian Cell Gene Mutation Test

Guideline:	OECD 476 (1997)	
Species/strain:	L5178Y $tk^{+/-}$ mouse lymphoma cells	
Replicates:	two parallel cultures in two independent experiments, with and without S9-mix	
Test substance:	sodium picramate	
Batch:	145/03	
Purity:	62.6%	
Vehicle:	de-ionised water	
Concentrations:	experiment 1:	112.5, 225, 450, 900, 1350 and 1800 µg/ml with and without S9-mix
	experiment 2:	28.1, 56.3, 112.5, 225, 337.5 and 450 µg/ml without S9-mix 112.5, 225, 450, 750, 900, 1050 and 1200 µg/ml with S9-mix
Treatment	experiment 1:	4h treatment with and without S9-mix
	experiment 2:	4h treatment with and 24h without S9-mix
Control:	without S9-mix:	methyl methane sulfonate
	with S9-mix:	cyclophosphamide
GLP:	in compliance	
Study period:	10 February – 13 April 2004	

The potential of sodium picramate to induce mutations at the mouse lymphoma thymidine kinase locus using the cell line L5178Y was investigated.
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 and a treatment period of 4 h. The duration of treatment in the second experiment was 4 h with - and 24 h without metabolic activation.
The highest applied concentration in the pre-test on toxicity (3600 µg/mL corresponding to approximately 10 mM) was chosen with regard to the molecular mass and the purity of the test item.
No substantial and reproducible concentration-dependent increase in mutant colony numbers was observed in both main experiments. No relevant shift of the ratio of small versus large colonies was observed up to the maximal concentration of the test item.
Appropriate reference mutagens were used as positive controls and showed a distinct in crease in induced mutant colonies, indicating that the tests were sensitive and valid.
The concentration range of the main experiments was adjusted to toxicity data and the occurrence of precipitation.

Conclusion
Under the experimental conditions reported, the test item did not induce mutations in the mouse lymphoma thymidine kinase locus assay using the cell line L5178Y in the absence and presence of metabolic activation.

Ref.: 10

In vitro Mammalian Chromosome Aberration Test

Guideline:	OECD 473
Species/strain:	K1-BH(4) Chinese hamster ovary cells
Replicates:	
Test item:	picramic acid
Batch:	DO 422
Purity:	> 99%
Vehicle:	DMSO

Concentrations:	0, 0.57, 1.71, 5.14, 15.43, 46.29, 138.88, 416.66 and 1250 µg/ml without and with S9-mix
Performance:	2h treatment followed 22h recovery
Positive controls:	without S9-mix: methylmethanesulfonate with S9-mix: cyclophosphamide
GLP:	in compliance
Study period:	1 October – 24 November 1992

The test substance was assayed in an *in vitro* cytogenetic assay using cultures of Chinese hamster ovary (CHO) cells both in the absence and presence of metabolic activation by a rat liver post-mitochondrial fraction (S9-mix) from Aroclor-1254 induced animals.

Cells were exposed to concentrations of 0.57, 1.71, 5.14, 15.43, 46.29, 138.88, 416.66 and 1250 µg/ml in the absence and presence of S9-mix for two hours followed by a recovery period of 22 hours. Cells were harvested at 24 hours after start of treatment. Methylmethanesulfonate (25 ug/ml, without S9-mix) and cyclophosphamide (12.5 µg/ml, S9-mix activated) were used as positive control agents.

Results
With and without S9-mix, at 24 hours sampling time after treatment of cells even at the highest testable concentration, no significant toxicity was observed and at concentrations of 138.88, 416.66 and 1250 µg/ml. No statistically significant differences in the number of cells with aberrations were found between treated and control cultures.
The positive controls induced statistically significant increases in cells with chromosomal aberrations.

Conclusion
It was concluded that the test substance did not induce chromosomal aberrations in Chinese hamster ovary cells in vitro when tested under the experimental conditions reported.

Ref.: 8

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

In vivo Mammalian Erythrocytes Micronucleus Test

Guideline:	OECD 474
Species/strain:	mice, Crl:NMRI BR
Group size:	5 males and 5 females
Test substance:	picramic acid
Batch:	RK140983
Purity:	> 99%
Vehicle:	carboxymethylcellulose, 2%
Dose level:	50 mg/kg bw (24h treatment test and positive control groups; 48h treatment negative control)
Route:	oral
Control:	cyclophosphamide
GLP:	in compliance
Study period:	22 July – 3 September 1992

Based on data from data of a preliminary toxicity assay the test article was administered orally in a single dose of 50 mg/kg bw to 2 groups of NMRI mice each comprising 5 males and 5 females. Concurrent control groups, each containing 5 male and 5 female mice, were run: the negative control group received only the vehicle (2% carboxymethylcellulose), whilst the positive control group was treated with cyclophosphamide at a dose of 40 mg/kg bw. The test groups and the negative control groups were sacrificed 24 and 48 h after treatment, respectively. Samples of bone marrow were taken and subsequently analysed. Positive control animals were sacrificed at 24 h p.a. and treated accordingly.

Results
An increase in the number of normochromatic erythrocytes and decrease in the number of polychromatic erythrocytes respectively could not be observed in the treated groups. This indicates that B 28 exerted no toxic influence in the bone marrow.
A single oral administration of picramic acid at a dose of 50 mg/kg bw to male and female mice did not produce a significant increase in the frequency of micronuclei in the polychromatic erythrocytes. According to historical data from NMRI mice, the mean values of all parameters measured were within the respective normal range.
The positive control group, treated with cyclophosphamide, revealed a significant increase in the number of micronucleated polychromatic erythrocytes.

Conclusion
Under the experimental conditions used picramic acid did not induce an increase in the number of polychromatic erythrocytes with micronuclei in treated mice and, consequently, was not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice.

Ref.: 9

Comment
The bioavailability of the test compound in the bone marrow was not demonstrated.

Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *In Vivo*

Guideline:	OECD 486 (1997)
Species/strain:	rat, Wistar HanlBm: WIST (SPF)
Group size:	32 (4 per group and preparation interval)
Test substance:	sodium picramate
Batch:	145/03
Purity:	62.6%
Vehicle:	de-ionised water
Dose level:	55 and 110 mg/kg bw (2 and 16h preparation interval)
Dosing volume:	10 ml/kg bw
Route:	oral
Control:	2h preparation interval: N,N'-dimethylhydrazinedihydrochloride (sym.) 16h preparation interval: 2-acetylaminofluorene
GLP:	in compliance
Study period:	12 October – 7 December 2004

The test item was assessed in the *in vivo* UDS assay for its potential to induce DNA repair (UDS) in the hepatocytes of rats.
The test item was formulated in deionised water, which was used as vehicle control. The volume administered orally was 10 ml/kg bw. After a treatment period of 2 and 16 hours, respectively, the animals were anaesthetised and sacrificed by liver perfusion. Primary hepatocyte cultures were established and exposed for 4 hours to 3HTdR (methyl-3H-thymidine) which is incorporated if UDS occurs (2).
The test item was tested at the following dose levels: 2 and 16 hours preparation intervals: 55 and 110 mg/kg bw.
The highest dose was estimated in a pre-experiment to be the maximum applicable dose, at which clinical signs of toxicity occurred without affecting the survival rates.
The urine of the treated animals was orange indicating the systemic distribution of the test item and thus, its bioavailability.
For each experimental group including the controls, hepatocytes from three treated animals were assessed for the occurrence of UDS.

Results
The viability of the hepatocytes was not substantially affected by the in vivo treatment with the test item.
None of the tested dose levels revealed UDS induction in the hepatocytes of the treated animals as compared to the corresponding vehicle controls.
Appropriate reference mutagens [DMH 2h treatment (10), 40 mg/kg bw and 2-AAF, 16 h treatment 100 mg/kg bw] were used as positive controls. Treatment with the positive control substances revealed distinct increases in the number of nuclear and net grain counts.

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

Ref.: 11

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

Guideline:	OECD 414
Species/strain:	rat, Wistar derived SPF-Albino Crl:Wi/Br
Group size:	80 (20 females per group)
Test substance:	picramic acid
Batch:	/
Purity:	100%
Vehicle:	0.5% sodium carboxymethylcellulose
Dose levels:	0, 10, 30 and 60 mg/kg bw/d
Dose volume:	10 ml/kg bw
Route:	oral gavage
Administration:	once daily during day 5 to day 15 of gestation
GLP statement:	in compliance
Study period:	24 August – 22 September 1988

80 pregnant rats (10 per sex) of the Wistar strain were treated once daily by oral gavage of picramic acid in 0.5% sodium carboxymethylcellulose during day 5 to day 15 of gestation at doses of 0, 10, 30 and 60 mg/kg bw/d. The animals received a constant volume of 10 ml/kg bw/d. The test procedure followed the OECD guideline and was conducted in compliance with the principles of GLP.
During the study the mortality, signs of intoxication, body weight and food consumption were recorded. All mated females were sacrified on day 20 of gestation. In the pregnant female, a complete autopsy and a macroscopic examination of the organs were carried out. Uterus were weighed and examined. For each ovary, corpora lutea were counted and foetuses were individually weighed and sexed. A gross examination of all foetuses was performed and one-third of the foetuses were examined for visceral anomalies. The other foetuses were evaluated for skeletal defects.

Results
No rats died during the treatment period. No toxic effects were reported during the study. Females of all dose groups had orange-brown discoloured urine throughout the application period at dose related intensity.
Mean maternal bodyweight gains and mean food consumptions over the gestation period were normal when compared to the control group.
Gross necropsy did not reveal any organ alterations related to treatment.
No significant differences in the number of viable foetuses, the male to female sex ratio, birth- position, number of runts, post-implantation losses, implantations, resorptions and corpora lutea between dosage groups and the control group were observed.
The highest dose group showed an increase in foetal body weight and uteri weights with a tendency towards dose-relation. Examination of the foetuses yielded minor variations (wavy ribs) at comparable inter-group frequencies and incidences within the historical control animals of this strain.
There were no biologically significant differences in the number of litters with malformations or developmental variations between any of the dose groups and the control group.

Conclusion
Due to increase in foetal body weight and uteri weights at 60 mg/kg bw/d, the No Observed Adverse Effect Level (NOAEL) of picramic acid in female rats after daily oral treatment is determined to be 30 mg/kg bw/day for the maternal and foetal organisms.

Ref.: 16

Comment
It is questionable if the increase of the foetal weights observed at 60 mg/kg bw/d is an adverse effect. However, as a conservative approach, the conclusion by the study authors is taken over.

3.3.9. Toxicokinetics

No data submitted

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

No data submitted

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

No data submitted

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Picramic acid

(oxidative conditions)

Absorption through the skin	**A (mean + 2 SD)**	**=**	**1.12 µg/cm²**
Skin Area surface	**SAS**	**=**	**580 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**0.65 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.01 mg/kg bw/d**
No observed adverse effect level (90-day, oral, rat)	**NOAEL**	**=**	**3.1 mg/kg bw/d**
Margin of Safety	**NOAEL / SED**	**=**	**310**

3.3.14. Discussion

Physico-chemical properties
Sodium picramate, a non reactive dye, is used as a direct hair colouring agent up to an on-head concentration of 0.6% in non-oxidative as well as in oxidative hair dye formulation. The information concerning impurities, solubility, identity and other physico chemical parameters of the four batches used cannot be traced back to the original data. The identity of batch 145/03 as well as its purity is well established. This seems also to apply to batch 50/04 (information cannot be traced back). These batches do not contain measurable concentrations of impurities except water (38%) (145/03) and in the case of 50/04 additionally 0.4% of picric acid. The purity of the two batches of picramic acid (DO 422 and RK 140983) is given as 98.9 and 99%, but no studies were submitted. The water content given in the purity and impurities tables are not in agreement.
Despite the lack of information, the impurities are not expected to be of toxicological concern with respect to a maximum use concentration of 0.6% sodium picramate.
The stability of the substance itself and their solutions was sufficient in toxicity testing. The stability of sodium picramate in typical hair dye formulations was not reported. The stability in an oxidative environment has not been demonstrated.

The batches used for toxicity testing were not specified in several cases.

Toxicity
In an acute oral toxicity study, the calculated LD_{50} of picramic acid was 110 mg/kg bw.
In an oral 14-day study in rats, the No Observed Adverse Effect Level (NOAEL) was 20 mg/kg bw/day, corresponding to 12.5 mg/kg bw/day active ingredient.
In an oral 90-day study in rats, the No Observed Adverse Effect Level (NOAEL) was 5 mg/kg bw/day corresponding to 3.1 mg/kg bw/day of the active ingredient. Adverse effects observed in this study were mostly related to intestinal perforation and adaptive metabolic reactions in the liver.
No data on two-generation reproductive toxicity was submitted. In a teratogenicity study, the No Observed Adverse Effect Level (NOAEL) for the maternal and foetal organisms was 30 mg/kg bw/day. The only effects considered adverse (questionable) observed at 60 mg/kg bw/day were increases in uterine weights and foetal body weights.

Skin/eye irritation and sensitisation
Under the condition of the test, a 2.5% aqueous solution of picramic acid was not irritant to rabbit skin. A 2.5% aqueous solution of picramic acid was irritant to rabbit eyes.
In the LLNA, sodium picramate is a moderate skin sensitiser.

Percutaneous absorption
Because of the high variability of the data, the mean + 2 SD (0.38 + 2 x 0.37 =1.12 µg/cm²) is used for calculating the MOS under oxidative conditions. No study under non-oxidative conditions was provided by the applicant. However, in a CIR review, a report on an in vivo dermal absorption study in rats was included, resulting in a dermal absorption rate on 0.38% under non-oxidative conditions. Although the conditions of the two experiments are not directly comparable, this value is similar to the absorbed percentage in the in vitro dermal absorption experiment under oxidative conditions (0.67%) and supports the assumption that picramic acid, as a non-reactive hair dye, would have similar absorption rates under oxidative and non-oxidative conditions.

Mutagenicity/genotoxicity
Picramic acid was tested for all three genetic endpoints: gene mutations, structural and numerical chromosomal aberrations. The test compound induced frameshift mutations in bacteria in the presence of metabolic activation. It did not induce gene mutations or chromosomal aberrations in mammalian cells *in vitro*. Picramic acid was not clastogenic and/or aneugenic in an *in vivo* micronucleus assay, and did not cause DNA damage leading to Unscheduled DNA Synthesis (UDS) in hepatocytes derived from rats treated orally up to the maximum applicable dose.
As the genotoxic effects found *in vitro* were not confirmed in *in vivo* tests, picramic acid can be considered to have no *in vivo* genotoxic potential and additional tests are unnecessary.

Carcinogenicity
No data submitted

4. CONCLUSION

Based on the data provided, the SCCS is of the opinion that the use of picramic acid/sodium picramate with a maximum on-head concentration of 0.6% in non-oxidative hair dye formulations does not pose a risk to the health of the consumer. For a final assessment of the use of picramic acid/sodium picramate in oxidative hair dye formulations, data on the stability in an oxidative environment should be provided

5. MINORITY OPINION

Not applicable

6. REFERENCES

1. Röder A. Implementation of an Analytical Method for a Skin Penetration Test. RCC-CCR, Roßdorf, study nr 872901, 25 July 2005
2. Tognucci A. Determination of the NMR-, IR-, UV/VIS Absorption, Mass Spectra and a HPLC Chromatogram RCC Ltd., Study nr 853020, 26 July 2004
3. Weissenfeld M. Determination of the Partition Coefficient (n-Octanol/Water) of Sodium Picramate. RCC Ltd., study nr A12442, 5 July 2005
4. Honarvar N. In Vitro Dermal Absorption through Porcine Ear Skin with Standard Formulation with 2% B028 & 1st Amendment. RCC-CCR, Roßdorf. Study nr 872902, 25 July 2005
5. Kynoch SR, Liggett MP. Irritant effects of Picramic acid on rabbit skin Huntington Research Centre, Cambridgeshire, Study nr 6756/3D/76, 5 November 1976
6. Kynoch SR, Liggett MP. Irritant effects of Picramic acid on rabbit eye mucosa. Huntington Research Centre, Cambridgeshire, Study nr 6749/2D/76, 8 November 1976
7. Sokołowski A, Wollny HE. Salmonella Typhimurium Reverse Mutation Assay with Sodium Picramate RCC-CCR, Roßdorf, Study nr 818502, 6 July 2004
8. Grötsch W. In vitro Chromosome Aberration Assay with B028 in CHO cells. Labor L+S GmbH, Bad Bocklet, Study nr 918542-2, 10 December 1992
9. Grötsch W. In vivo micronucleus test of B028 in mice. Labor L+S GmbH, Bad Boocklet, Study nr 711663, 24 October 1992
10. Wollny HE, Sokołowski A. Cell Mutation Assay at the Thymidine Kinase Locus (TK+/-) in Mouse Lymphoma L5178Y Cells with Sodium Picramate. RCC-CCR, Roßdorf, Study nr 818501, 6 July 2004
11. Honarvar N., Schulz M. In vivo/in vitro Unscheduled DNA Synthesis in Rat Hepatocytes with Sodium Picramate RCC-CCR, Roßdorf, Study nr 854500, 23 February 2005
12. Honarvar N, Schulz M. LLNA in mice with Sodium Picramate RCC-CCR, Roßdorf, Study nr 818100, 11 May 2005
13. Damme B., Flade D., Aluma J. 13-Week Oral Toxicity (Gavage) Study in the Rat. RCC Ltd., Study nr 855195, 13 June 2005
14. Damme B., Flade D. 14-Day Oral Toxicity (Gavage) Study in the Rat RCC Ltd., Study nr 855194, 24 January 2005
15. Kynoch SR, Lloyd GK. Acute oral toxicity to rats of picramic acid. Huntington Research Centre, UK-Cambridgeshire, Study nr 6781-01-76, 22 November 1976
16. Korn W-D, Pfenning M. Teratogenicity study with "Picramic Acid" in Wistar rats. IBR Forschungs GmbH, Hannover, Study No.: 4-4-767-88, 16 December 1988

Literature

- Final Report on the Safety Assessment on Sodium Picramate CIR-Report, Journal of the American College of Toxicology, 11 (4), 1992,447-464
- Becker, LC, Bergfeld WF, Belsito DV, Klaassen CD, Marks JG. Jr, Shank RC., Slaga TJ, Snyder PW, Andersen FA (2009). Amended Safety Assessment of Sodium Picramate and Picramic Acid. International Journal of Toxicology 28 (Suppl 3) 205S-216S
- Contact Sensitisation: Classification according to Potency ECETOC, Technical Report No. 87, April 2003
- Die Aldosylamin- und Ketosylamin-Umlagerung schwach basischer N-Glycoside Heyns K, Beilfuß W, Chem. Ber., 106: 2693-2709, 1973
- A study of the conductivity and dissociation of certain organic acids in aqueous solution at different temperatures. Springer A Jr., Jones HC, Am. Chem. J., 48: 411-453, 1912