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

CONCERNING

2-NITRO-5-GLYCERYL METHYLANILINE

COLIPA n° B60

adopted by the SCCNFP during the 24th plenary meeting
of 24-25 June 2003

1. Terms of Reference

1.1 Context of the question

The adaptation to technical progress of the Annexes to Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products.

1.2 Request to the SCCNFP

The SCCNFP is requested to answer the following questions :

* Is 2-Nitro-5-glyceryl methylaniline safe for use in cosmetic products?

* Does the SCCNFP propose any restrictions or conditions for its use in cosmetic products?

1.3 Statement on the toxicological evaluation

The SCCNFP is the scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food products intended for consumers.

The Commission's general policy regarding research on animals supports the development of alternative methods to replace or to reduce animal testing when possible. In this context, the SCCNFP has a specific working group on alternatives to animal testing which, in co-operation with other Commission services such as ECVAM (European Centre for Validation of Alternative Methods), evaluates these methods.
The extent to which these validated methods are applicable to cosmetic products and its ingredients is a matter of the SCCNFP.

SCCNFP opinions include evaluations of experiments using laboratory animals; such tests are conducted in accordance with all legal provisions and preferably under chemical law regulations. Only in cases where no alternative method is available will such tests be evaluated and the resulting data accepted, in order to meet the fundamental requirements of the protection of consumer health.

2. Toxicological Evaluation and Characterisation

2.1. General

2.1.1. Primary name

2-Nitro-5-glyceryl methylaniline (INCI)

2.1.2. Chemical names

1-methylamino-2-nitro-5-(2,3-dihydroxy-propyloxy)-benzene
3-(3-(Methylamino)-4-nitrophenoxy)-1,2,propanediol
3-(3-(Methylamino)-4-nitrophenoxy)-propane1,2-diol
Methylamino-1-nitro-2(β,γ-dihydroxypropyl),oxy-5-benzene
2-Nitro-5-(2,3-dihydroxypropylether)-methylaniline

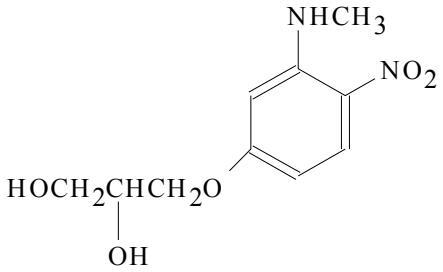
2.1.3. Trade names and abbreviations

IMEXINE FT (Chimex)
COLIPA n° B60

2.1.4. CAS/EINECS no.

CAS n° : 80062-31-3
EINECS n° : 279-383-3

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : $C_{10}H_{14}N_2O_5$
Mol weight : 242

2.1.7. Purity, composition and substance codes

The purity is stated by HPLC > 98% (peak area)

Possible impurities include :	reagents and intermediate reaction products 2,4-dichloro-1-nitro-benzene (less than 100 ppm) (5-chloro-2-nitro-phenyl)-methylamine (about 100 ppm) (5-hydroxy-2-nitrophenyl)-methylamine (75 ppm) (5-(2,2-dimethyl-(1,3)-dioxolan-4-ylmethoxy)-2-nitrophenyl)-methylamine (0.095%)
solvents	methanol (100ppm), isopropanol (330 ppm), toluene (less than 50 ppm), other NaCl (less than 0.25%)

2.1.8. Physical properties

Appearance	:	heterogeneous yellow powder with green coloured highlight, almost odourless
Melting point	:	95-97 °C
Boiling point	:	/
Density	:	/
Rel. vap. dens.	:	/
Vapour Press.	:	/
Log P_{ow}	:	/

2.1.9. Solubility

Water	:	insoluble
Ethanol	:	soluble
Receptor fluid	:	/

General comments on analytical and physico-chemical characterisation

* Purity of 2-nitro-5-glyceryl methylamine in several test batches is not reported.
* No quantitative data on solubility and stability of 2-nitro-5-glyceryl methylamine have been provided. Solubility of the compound in the receptor fluid used for percutaneous absorption study has not been reported.
* Relevant physico-chemical parameters are not given.
* 2-nitro-5-glyceryl methylamine is a secondary amine and thus it is prone to nitrosation. The content of nitrosamine in the dye as well as in the hair dye formulations are required.

2.2. Function and uses

2-Nitro-5-glyceryl methylaniline is used in semi-permanent hair dye formulations at a maximum concentration of 1%.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

Guideline	:	OECD n° 401
Species	:	Sprague Dawley rats, OFA-SD (IOPS)
Group size	:	5 males + 5 females
Substance	:	COLIPA B60 suspended at 1% in methylcellulose
Batch no	:	op 8
Purity	:	99.9 %
Dose	:	1000 and 2000 mg/kg in a volume of 10 ml/kg
Observation period	:	14 days
GLP	:	in compliance

Groups of 5 male and 5 female rats received a single dose of 1000 or 2000 mg/kg bw. The animals were observed twice daily for 14 days for mortality and clinical abnormalities. Body weights and macroscopic observations were recorded, but histological examinations were not performed.

Results
Mortalities (90%) were reported at 2000 mg/kg bw only. Sedation, dyspnea and ptosis were the main clinical signs of toxicity. Recovery of surviving animals was complete by day 3. In surviving animals, there were no treatment-related changes in body weight and no macroscopic abnormalities recorded. There was an orange coloration of tissues of all animals treated with 2000 mg/kg bw and found dead during the study. The LD_{50} of the test substance was reported to be higher than 1000 mg/kg but less than 2000 mg/kg.

Ref. : 1

2.3.2. Acute dermal toxicity

No data

2.3.3. Acute inhalation toxicity

No data

2.3.4. Repeated dose oral toxicity

Guideline	:	OECD n° 407
Species	:	Sprague Dawley rat - Crl CD (SD) BR
Group sizes	:	10 males and 10 females
Substance	:	B60 suspended in 0.5% carboxymethylcellulose
Batch no	:	op 6
Purity	:	99.9 %
Dose levels	:	0, 100 300 and 1000 mg/kg bw/day in a volume of 5 ml/kg

Exposure : 29 or 30 days
GLP : in compliance

The test substance, suspended in 0.5% carboxymethylcellulose, was administered at 100, 300 and 1000 mg/kg bw/day daily for 29 or 30 days by gavage. The control group received the vehicle alone.

All animals were observed twice daily for mortality and once daily for clinical signs. Body weight and food consumption were recorded at weekly intervals. Ophthalmoscopic examinations were performed before the start of treatment and during week 4. Blood samples were taken from all animals during week 4 for haematological and clinical chemistry investigations. Urine samples were collected during week 4 from all animals. At autopsy, organ weights were recorded and the main organs were examined macroscopically and histologically. The eye was not examined histologically.

Results
No mortalities occurred due to the test substance. One mortality in the male high dose group was considered to be due to a gavaging error. Body weights and food consumption of treated animals were comparable to controls. Hypersalivation was noted in 2/10 males and 5/10 females in the group given 1000 mg/kg bw/day. Due to the nature of the compound, the urine of the treated groups was coloured yellow throughout the test period and a yellowish staining of the fur was noted in rats given B60 at 300 or 1000 mg/kg bw/day, from day 2 onwards.

Ophthalmoscopic examination revealed a treatment related bilateral coloration of the fundus in 7/10 males and 4/10 females from the 300 mg/kg bw/day group and 9/9 males and 9/10 females from the 1000 mg/kg bw/day groups. Haematological and urinalysis results did not show any treatment related changes. At termination of the experiment, absolute and relative liver weights were slightly increased at the top dose of 1000 mg/kg bw/day, but there were no related histopathological findings. All other macroscopic and microscopic observations revealed no abnormalities related to the treatment.

The authors considered that the discoloration of the eye was due to the staining properties of the dye and was not of toxicological significance. They therefore concluded that 300 mg/kg bw/day was the “No Toxic Effect Level”.

On the basis of ophthalmoscopic examination 100 mg/kg bw/day should therefore be considered as the NOAEL.

Ref. : 5

2.3.5. Repeated dose dermal toxicity

No data

2.3.6. Repeated dose inhalation toxicity

No data

2.3.7. Sub-chronic oral toxicity

No data

2.3.8. Sub-chronic dermal toxicity

No data

2.3.9. Sub-chronic inhalation toxicity

No data

2.3.10. Chronic toxicity

No data

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

Guideline	:	OECD n° 404
Species	:	rabbit (New Zealand white)
Group size	:	3 males
Substance	:	COLIPA B60 suspended at 1 % in 1,2-propanediol
Batch no	:	op 8
Purity	:	99.9 %
Dose	:	0.5 ml
GLP	:	in compliance

0.5 ml of a 1% suspension of the test substance in 1,2-propanediol was applied to the right clipped dorsal flank of 3 male rabbits. The left flank did not receive any substance and served as a control. The test substance was kept in contact with the skin for 4 hours under a semi-occlusive patch. Any reactions were evaluated 60 minutes and 24, 48 and 72 hours following the removal of the semi-occlusive dressing. At termination, histopathological examination of the treated skin was carried out.

Results
No oedema was noted in any of the 3 animals. Reddish coloration of the skin made it impossible to evaluate erythema. Histological examination of samples of treated skin revealed no relevant abnormalities.

The test substance was considered as non-irritant to rabbit skin when applied as a 1% suspension in 1,2-propanediol.

Ref. : 3

2.4.2. Irritation (mucous membranes)

Guideline	:	OECD n° 405
Species	:	rabbit (New Zealand white)
Route	:	eye
Group size	:	3 males
Substance	:	1% B60 suspended in 1,2-propanediol
Batch no	:	op 8
Purity	:	99.9 %
Dose	:	0.1 ml
GLP	:	in compliance

0.1 ml of a 1% suspension of the test substance in 1,2-propanediol was instilled into the left eye of 3 male rabbits, the right eyes served as control. The material was not rinsed out. Eye irritation was scored one hour, 24, 48 and 72 hours following instillation.

Results
One hour after instillation, slight chemosis and redness of the conjunctivae were observed. After 24, 48 and 72 hours, no reactions to the conjunctivae were seen. No irritation of the iris and no corneal opacity were noted.
The test substance was considered as non-irritant to the rabbit eye when applied as a 1 % suspension in 1,2-propanediol.

Ref. : 2

2.5. Sensitisation

Guideline	:	/		
Species	:	guinea pigs (Albino Hartley)		
Group sizes	:	10 males and 10 females		
Substance	:	1% COLIPA B60 suspended in propylene glycol		
Batch no	:	/		
Purity	:	/		
Concentrations used	:	Topical induction	:	0.5 ml
		Challenge	:	0.5 ml
GLP	:	not in compliance		

The method used was in accordance to Brulos et al: J Soc Cosmet Chem, 1977 28 357-365.
Ten male and 10 female guinea pigs received an intradermal injection of 0.1 ml of Freund's complete adjuvant diluted to 50% in sterile isotonic saline, behind the right shank on days 1 and 10.
Following the second intradermal injections, 10 topical applications of the test substance as a 1% suspension in propylene glycol were administered at a dose volume of 0.5 ml per animal. The material was applied under occlusive patch and left for 48 hours at each application. These applications were made to the clipped area of skin just above the injection site within a period of 4 weeks. The actual interval between topical induction and challenge was 12 days (day 24 to 35).

On day 36, 0.5 ml of the test suspension was applied on an untreated area under an occlusive patch for 48 hours.
Readings were made 1 hour, 6, 24 and 48 hours following removal of the occlusive patch (challenge). Histological examination of the test slides showed no abnormalities.

Results
The study does not conform with OECD guidelines.

Ref. : 4

2.6. Teratogenicity

Guideline	:	OECD n° 414
Species	:	Sprague Dawley rat - Crl CD (SD) BR
Group sizes	:	at least 20 females
Substance	:	B60 suspended in 0.5% carboxymethylcellulose
Batch no	:	op 8
Purity	:	99.9 %
Dose levels	:	0, 100 300 and 1000 mg/kg bw/day in a volume of 5 ml/kg
Administration	:	days 6-15 of gestation
GLP	:	in compliance

The test substance, suspended in 0.5% carboxymethylcellulose, was administered at 0, 100, 300 and 1000 mg/kg bw/day (to groups of 22, 23, 23, and 24 pregnant rats, respectively) on days 6 - 15 of gestation, inclusive. The dams were observed daily for clinical signs of toxicity and twice daily (except weekends) for signs of mortality. Body weights and food consumption were recorded on days 0, 6, 9, 12, 15 and 20. On day 20 of gestation, the dams were sacrificed. Ovaries and uteri were examined, foetal sex ratio, foetal body weights, number and position of implantations (live foetuses, early and late intra-uterine deaths) and the number of corpora lutea were determined. Foetuses were examined for external, skeletal and visceral deviations. The dams were examined macroscopically.

Results
Dams : no treatment related mortalities were observed. One mortality in the 300 mg/kg/day group was attributed to gavaging error. All treated animals presented lemon coloured urine from day 7 to day 16. One female (1000 mg/kg/day) was culled following observation of a reddish nasal discharge and piloerection on days 9 and 10 post-coitum. At necropsy, most organs of the culled dam were stained yellow and the stomach showed some ulcerated foci. No abortions were reported.
Maternal body weight gain and food consumption were slightly reduced in the highest dose group compared to the control group. No abnormalities were recorded at necropsy. No changes were noted in the other treated groups.

Foetuses : Litter parameters were comparable between the control and treated groups. External examination revealed no treatment-related foetal malformations. No soft tissue malformations were observed. The only skeletal malformation noted was the number of foetuses from the 1000 mg/kg bw group with a delayed ossification of the fourth metacarpus when compared to controls. This observation was considered by the authors to be related to the slight maternotoxicity seen in this group.

The study authors concluded that, at 300 mg/kg bw/day, there was no evidence of maternal toxicity, embryotoxicity or teratogenicity, whereas the 1000 mg/kg bw/day dose level was slightly maternotoxic but neither embryotoxic nor teratogenic.

Ref. : 6

2.7. Toxicokinetics (incl. Percutaneous Absorption)

Guideline	:	/
Tissue	:	Human breast epidermis
Method	:	Franz diffusion cell (static)
Substance	:	0.915% B60 w/w in a water-based formulation
Batch no	:	op 6
Purity	:	99.9 %
Dose levels	:	40 mg of formulation on 2 cm^2 skin
Duration	:	30 min
GLP	:	not in compliance

The penetration of the test substance was evaluated in Franz diffusion cells using human breast epidermis. Integrity of the epidermal sheets was assessed using 10-fold magnification. Approximately 40 mg of a formulation containing 0.915% of the test compound w/w were applied on 2 cm^2 of skin in the presence and absence of 10 mg of finely cut hair and left for 30 minutes after which time the upper part of the skin was washed and dried. Test material in the receiving chamber was measured by HPLC 4.5 hours after beginning the application. Eight Franz cells were used in determinations with hair and 9 without hair.

Results
After 4 hours 30 minutes the mean quantity of test compound that had passed through the epidermis was reported by the authors to be :

* in presence of hair, a mean of 28 ng/cm^2 corresponding to 0.014% of the applied amount. In 8 of the 9 Franz cells used in this study the concentration of test material in the receiving fluid was below the level of detection (10 ng/ml corresponding to 18.5 ng/cm^2, and 0.01%). In the remaining cell the amount penetrating was 65.13 ng/cm^2.
* in absence of hair, a mean of 68 ng/cm^2 (range 25.6 to 121.9 ng/cm^2) which corresponds to 0.034% of the test compound applied on the skin.

In vitro methods for determining skin penetration require an intact epidermal sheet. This study involved examination under low power microscopy to assess the integrity of the epidermal sheet. This is a relatively crude and unreliable form of assessment. It is therefore highly likely that the single high value reported in the study conducted in the presence of hair is the result of a damaged epidermis in that diffusion cell. Therefore the high value should be considered an artefact and the 8 cells showing penetration to be below detectable limits would be a more reliable reflection of the skin penetration capacity of B60.

The study is considered inadequate (duration of exposure, final recovery (mass balance) not considered).

Ref. : 10

2.8. Mutagenicity/Genotoxicity

2.8.1 Mutagenicity/Genotoxicity *in vitro*

Bacterial Reverse Mutation Test

Guideline	:	/
Species/strain	:	*S. typhimurium,* TA98, TA100, TA1535, TA1537, TA 1538
Replicates	:	Triplicate plates, only 1 test performed
Test substance	:	B 60 dissolved in DMSO
Batch no	:	Batch op26
Purity	:	/
Concentrations	:	9 concentrations – direct plate incorporation assay. With and without metabolic activation 10, 20 , 50, 100, 250, 500, 1000, 2500 & 5000 μg/plate
GLP	:	/

2-Nitro-5-glyceryl methylaniline has been investigated for gene mutation in *S. typhimurium* using the direct plate incorporation method both with or without S9 mix. Liver S9 fraction from Aroclor 1254-induced rats was used as the exogenous metabolic activation system.

Results
Toxicity : no data.
With or without S9 mix : no dose related or biologically relevant increase in revertant numbers was observed, in any of the 5 *S. typhimurium* tester strains.

Conclusions
Based on the reversion rate, and under the conditions of the assays performed, it is concluded that the test agent B 60 is negative in the *S. typhimurium* tester strains in the absence or in the presence of S9 mix. However, the test is unsuitable for genotoxicity evaluation for the following reasons : purity is not known, no guidelines were followed, no quality insurance is given and no independent repeat-experiment has been performed

Ref. : 7

***In Vitro* Mammalian Chromosomal Aberration Test, study 1**

Guideline	:	/
Species/strain	:	Chinese Hamster Ovary Cells
Replicates	:	Duplicate cultures but no independent experiment
Test substance	:	2-Nitro-5-glyceryl methylaniline in DMSO solution
Batch no	:	/
Purity	:	/
Concentrations	:	Preliminary dose range finding study : No raw data given Test without S9 : 0, 0.5, 1, 2 and 4 mg/ml Test with S9 : 0, 0.5, 1, 2 and 4 mg/ml
GLP	:	/

2-Nitro-5-glyceryl methylaniline has been investigated for induction of chromosomal aberrations in CHO cells. The test concentrations were established from a preliminary toxicity study.
Liver S9 fraction from Aroclor 1254-induced rats was used as the exogenous metabolic activation system.

Samples were exposed during 1 hours with or without S9. Cultures were kept for 19 hours before harvest.

Results
Toxicity : no raw data given

Structural chromosome aberrations

With or Without S9 mix :
While a slight dose dependent trend of aberrations was found, no statistics have been used to evaluate the incidence of aberrant cells. Significant increase in the aberration rate was observed as compared to the corresponding solvent control, mainly in the absence of activation.

Polyploidy : not taken into account

Conclusions
There are clear indication of clastogenicity while the treatment time was short (1 h). No statistics have been performed.
However, according to the modern standard strategies and guidelines, the assay is unsuitable for evaluation (there is no independent repeat study, the exposure and expression period are inadequately selected, test substances is not characterised, batch and purity is not given.).

Ref. : 8

***In Vitro* Mammalian Chromosomal Aberration Test, study 2**

Guideline	:	OECD 473, EC B10.
Species/strain	:	Chinese Hamster Ovary Cells (CHO)
Replicates	:	Duplicate cultures, single experiment.
Test substance	:	2-nitro-5-glyceryl methylaniline in DMSO
	:	IMEXINE FT
Batch no	:	Batch No 0503126
Purity	:	/
Concentrations	:	25 - 250 µg/ml with and without metabolic activation.
GLP	:	in compliance

2-nitro-5-glyceryl methylaniline has been investigated for induction of chromosomal aberrations in Chinese hamster CHO cells Liver S9 fraction from Aroclor 1254-induced rats was used as the exogenous metabolic activation system.
The test concentrations were established from a preliminary toxicity study. With respect to the molecular weight of 2-nitro-5-glyceryl methylaniline, the maximum concentration tested was 2420 µg/ml (that correspond to 10 mM) has been selected.

Exposure period	Recovery	Preparation interval	Doses µg/ml
Experiment without S9 mix			
3 hours	17 hours	20 hours	407 169 2420
Experiment with S9 mix			
3 hours	17 hours	20 hours	1186 1694 2420

Results
pH and Osmolarity
On the post-treatment medium, no marked influence of the pH or osmolarity was noted as compared to the concurrent vehicle controls.

Toxicity
A 68 % reduction of the mitotic index was noted in the absence of activation at the top dose level (2420 µg/ml). With S9, at the top dose the mitotic index reduction was 31% of the control.

Structural chromosome aberrations
* without activation system.
- A significant and biologically relevant increase in the number of cells with structural chromosomal aberrations was noted at the top dose of 2420 µg/ml.
- 2420 µg/ml14 %.

* with activation system.
- A statistically and/or biologically significant dose-dependent relevant increase in the number of aberrant cells was observed as compared to the corresponding solvent control at the top dose of 2420 µg/ml.
- 2420 µg/ml 13.5 %

Polyploidy
Taken into account that no specific positive control agent has been used in this assay, and that only metaphases with 19 -23 chromosomes were considered for scoring, polyploidy means a number of chromosome > to 23 or endoreduplication.

* without activation system
A relevant increase in the number of polyploid metaphases was recorded at the top dose. The number of cells displaying numerical aberrations was of 3.8 % in the control and 7.8 % at the top dose.

* with activation system
A relevant increase in the number of polyploid metaphases was recorded at the top dose. The number of cells displaying numerical aberrations was of 4.8 % in the control and 10.3 % at the top dose. Moreover, in one replicate of the intemediate dose, 8 % of polyploid cells were noted. In addition, many endoreduplicated cells were observed in the presence of the activation system.

Conclusions
The assay is acceptable for evaluation.

IMEXINE FT is considered positive for clastogenic activity in Chinese hamster ovary cell line in the presence of activation under the conditions of this test. In addition indication of aneugencity has been noted under both conditions at the top dose levels.

Ref :11

2.8.2 Mutagenicity/Genotoxicity *in vivo*

Mammalian Erythrocyte Micronucleus Test, study 1

Guideline	:	/
Species	:	Albino Swiss mouse
Group sizes	:	10 males dosed, 5 control and 6 test animals evaluated
Substance	:	B60 dissolved in DMSO
Batch no	:	DG1
Purity	:	/
Dose levels	:	0, 350, 450 and 550 mg/kg in a volume of 10 ml/kg
Route	:	intraperitoneal injection
Administration	:	2 intraperitoneal injections, 24 hours apart.
Sacrifice times	:	after 24, 48 and 72 hours.
GLP	:	/

2-Nitro-5-glyceryl methylaniline has been investigated for induction of micronuclei in the bone marrow cells of male or female mice. Dose levels were determined on the basis of the results of a preliminary dose-range finding study having showed that LD_{50} is 650 mg/kg bw. Negative and positive controls were in accordance with the OECD guideline.

Results

Test doses
2-Nitro-5-glyceryl methylaniline dissolved in DMSO was administrated by 2 single intraperitoneal injections with 24 h interval. Male mice : 0, 350, 450 and 550 mg/kg .
1 sacrifice times was selected : 6 hours after last dosing
Bone marrow smears were obtained from the positive control group 24 after dosing.

Number of cells scored
A total of at least 2000 erythrocytes were examined from each animal ; the incidence of micronucleated erythrocytes and the ratio of polychromatic erythrocytes to normochromatic erythrocytes were calculated.

Results
Reactions to treatment
Toxic effects such as passivity, dyspnea and ataxia were observed in all dosage groups.

Mean values of micronucleated PCE
No statistically significant or biologically relevant increase in the incidence of micronucleated polychromatic cells over the concurrent vehicle control values were observed at any sampling times

PCE/NCE ratio
No significant variations in the ratio of normochromatic to polychromatic erythrocytes, which would have indicated that the bone marrow was reached by the test material and or toxicity of the latter, was noted.

Conclusions
The test does not conform to OECD guidelines. Only male were dosed. The number of animals per dose was insufficient. In addition the test was not conducted under GLP conditions. The test is not acceptable for genotoxicity evaluation.

Ref. : 9

Mammalian Erythrocyte Micronucleus Test, study 2

Guideline	:	OECD 474
Species	:	Swiss Ico: OF1(IOPS caw) mice
Group sizes	:	5 males and 5 females
Test substance	:	2-nitro-5-glyceryl methylaniline in 1 % methylcellulose
	:	IMEXINE FT
Batch no	:	Batch No 0504494
Purity	:	/
Dose levels	:	0, 500, 1000 & 2000 mg/kg bw
Administration	:	2 intragastric gavages on 2 consecutive days (24-hours interval)
Sacrifice times	:	24 hours after the last dosing.
GLP	:	in compliance

2-Nitro-5-glyceryl methylaniline has been investigated for induction of micronuclei in the bone marrow cells of male or female mice. Dose levels were determined by a preliminary range finding study in which no toxic effects were seen. The substance was administered by two single intragastric gavages at 24-hours interval and the groups of animals sacrificed 24 hours after the last administration. Negative and positive controls were in accordance with the OECD guideline.

Results
Test doses
2-nitro-5-glyceryl methylaniline in 1 % methylcellulose, batch No 0504494 (purity not stated was administered by 2 single oral doses at 24-hours intervals.

1 sacrifice time was selected : 24 h after the last oral administration.
Bone marrow smears were obtained from the positive control group 24 hours after dosing.

Number of cells scored
A total of at least 1000 erythrocytes were examined from each animal ; the incidence of micronucleated erythrocytes and the ratio of polychromatic erythrocytes to normochromatic erythrocytes were calculated.

Results
Mean values of micronucleated PCE

No statistically significant or biologically relevant increase in the incidence of micronucleated polychromatic cells over the concurrent vehicle control values were observed for any dosage groups.

PCE/NCE ratio
No significant reduction in the PCE/NCE ratio was observed in any of the dosage groups of mice treated with Arianor Straw Yellow.

Conclusions

Under the conditions of the test it can be concluded that IMEXINE FT in doses at which no significant variation in the PCE/NCE ratio was observed, does not induce statistically significant increase in the frequency of PCE. The negative and positive controls gave the expected results. However, it should be noted that a trend to a dose response was observed and that there is large inter-individual variations in the individual values.
In the light of the polyploid properties observed *in vitro* on CHO, this should also have been checked in this study.

Ref. : 12

2.11. Safety evaluation

NOT APPLICABLE

2.12. Conclusions

Purity of 2-nitro-5-glyceryl methylamine in several test batches was not reported. Relevant physico-chemical parameters were not given No quantitative data on solubility and stability of 2-nitro-5-glyceryl methylamine have been provided. Solubility of the compound in the receptor fluid used for percutaneous absorption study has not been reported.
2-nitro-5-glyceryl methylamine is a secondary alkanolamine, that may give rise to nitrosamine formation. Therefore, analytical data on the nitrosamine content on more than one sample as well as in hair dye formulations is considered essential.

Acute toxicity was in a range between 1000 and 2000 mg/kg bw whereas the NOAEL in a repeated dose oral toxicity study (30 d) was 100 mg/kg bw.

The test substance was considered as non-irritant to the intact rabbit skin and eye at a 1% suspension in 1,2-propanediol.

The macroscopic and histological results indicated that the test substance did not produce any cutaneous sensitisation reaction in guinea pigs. However, the study does not conform with OECD guidelines.

Teratogenicity studies lead to the result that 300 mg/kg bw showed no evidence of maternal toxicity, embryotoxicity or teratogenicity, whereas the 1000 mg/kg bw dose level was slightly maternotoxic, but neither embryotoxic nor teratogenic.

The percutaneous absorption study is considered inadequate : duration of exposure, final recovery (mass balance) were not considered.

2-Nitro-5-glyceryl methylaniline was tested in procaryotic cells for gene mutation in several tester strains of S. typhimurium . The results are negative. However, the test is unsuitable for genotoxicity evaluation.
The in vitro mammalian chromosomal aberration test is negative. However, this test is unsuitable for genotoxicity evaluation. Another in vitro mammalian chromosomal aberration test has been provided with Imexine FT. This test is suitable for genotoxicity evaluation. It is positive for clastogenicity and equivocal for aneugenicity at the top doses tested both in the presence or absence of an activation system.
2-Nitro-5-glyceryl methylaniline gave negative results in the mammalian erythrocyte micronucleus test. However, the study did not demonstrate that bone marrow was reached by the test agent. In addition, the test is unsuitable for genotoxicity evaluation. Another mammalian erythrocyte micronucleus test has been provided with Imexine FT. This test gave negative results. However, it should be noted that a trend to a dose response was observed and that there is large inter-individual variations in the individual values; in addition no special investigation has been made regarding the aneugenic potential of the test agent.

2.13. References

1. Acute Oral Toxicity in Rats. IMEXINE FT (batch Op 8).Centre International de Toxicologie, (CIT), Evreux, France. Study No 8797 TAR, 25 May 1992.
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3. Opinion of the SCCNFP

The SCCNFP is of the opinion that the information submitted is inadequate to assess the safe use of the substance.

Before any further consideration, the following information is required :

* complete data on physico-chemical and chemical characterisation of the test material.

* nitrosamine content in various batches of 2-nitro-5-glyceryl methylamine and in hair dye formulations containing this chemical.

* percutaneous absorption study in accordance with the Notes of Guidance.

* data on genotoxicity/mutagenicity following the relevant SCCNFP opinions and in accordance with the Notes of Guidance.

4. Other considerations

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5. Minority opinions

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