EUROPEAN COMMISSION

Directorate-General for Health & Consumers

Scientific Committee on Consumer Safety

SCCS

OPINION ON

N-Methyl-2-pyrrolidone (NMP)

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

The SCCS adopted this opinion at its 10th plenary meeting

of 22 March 2011

About the Scientific Committees

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

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

SCCS

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

Scientific Committee members

Jürgen Angerer, Ulrike Bernauer, Claire Chambers, Qasim Chaudhry, Gisela Degen, Thomas Platzek, Suresh Chandra Rastogi, Vera Rogiers, Christophe Rousselle, Tore Sanner, Jacqueline van Engelen, Maria Pilar Vinardell, Rosemary Waring, Ian R. White

Contact
European Commission
Health & Consumers
Directorate C: Public Health and Risk Assessment
Unit C7 - Risk Assessment
Office: B232 B-1049 Brussels
Sanco-Sc6-Secretariat@ec.europa.eu

ISSN 1831-4767 ISBN 978-92-79-12775-5
Doi:10.2772/31663 ND-AQ-09-038-EN-N

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ACKNOWLEDGMENTS

WG members

Prof. J. Angerer
Dr. U. Bernauer
Dr. C. Chambers
Prof. G. Degen
Dr. W. Lilienblum (associate scientific adviser)
Dr. S.C. Rastogi
Prof. V. Rogiers
Prof. T. Sanner (chairman and rapporteur)
Dr. J. van Engelen
Prof. R. Waring
Dr. I.R. White

Keywords: SCCS, scientific opinion, N-methyl-2-pyrrolidone, directive 76/768/ECC, CAS 872-50-4, 51013-18-4, EC 212-828-1

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on N-methyl-2-pyrrolidone (NMP), 22 March 2011

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

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1. BACKGROUND

The Cosmetics Directive as modified by the Council and the European Parliament (2003/15/EC[1]), which is based on an opinion of the SCCNFP of September 2001 (SCCNFP/0474/01, final), stipulates that *"the use in cosmetic products of substances classified as carcinogenic, mutagenic or toxic for reproduction, of category 1, 2 and 3, under Annex I to Directive 67/548/EEC shall be prohibited. To that end the Commission shall adopt the necessary measures in accordance with the procedure referred to in Article 10(2). A substance classified in category 3 may be used in cosmetics if the substance has been evaluated by the SCCNFP and found acceptable for use in cosmetic products."*

In order to implement that provision, the Commission consulted the SCCNFP and, on 25 May 2004, the SCCNFP confirmed its opinion of 25 September 2001 (SCCNFP/0825/04). The Commission adopted Directive 2004/93/EC in order to amend accordingly Annexes II and III of the Cosmetics Directive. Subsequently, the SCCP has been consulted following each adaptation of Annex I to Council Directive 67/548/EEC[2].

On 21 August 2008 and on 15 of January 2009 the Commission adopted respectively Directives 2008/58/EC[3] and 2009/2/EC[4] amending Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances providing new classifications. The classification provided by these two Directives has been taken over by Commission Regulation 790/2009, amending EC Regulation 1272/2008, which deleted Annex I of Council Directive 67/548/EEC.

In order to consider the implementation of these new classifications, the Commission services are consulting the SCCS regarding the safety of the substances concerned by the new classification and which are not yet banned within the Cosmetics Directive.

Separate scientific requests were issued to evaluate the continued safe use of already regulated boron and perborate compounds, which were classified with specific concentration limits by the Regulation 790/2009/EC.

The aim of the present scientific request is to ensure that the same approach - as for the boron and perborate compounds – is taken for other substances classified as CMR 1B or 2 with specific concentration limits. Two other CMR substances have a specific concentration limits above 0.1%.

The 2 substances in question are trisodium nitrilotriacetate EC 225-768-6 and N-methyl-2-pyrrolidone EC 212-828-1, each of which has a specific concentration limit of 5%.

- trisodium nitrilotriacetate, EC 225-768-6, is classified Carc. 2 and with a specific concentration limit of 5%. It is not used directly as ingredient, but it is present as an additive in some ingredients according to Colipa.

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1 OJ L 66, 11.03.2003, p. 26. See recital (12).
(12) "The SCCNFP stated in its opinion of 25 September 2001 that substances classified pursuant to Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances(2) as carcinogenic (except substances only carcinogenic by inhalation), mutagenic or toxic for reproduction, of category 1 or 2, and substances with similar potential, must not be intentionally added to cosmetic products, and that substances classified pursuant to Directive 67/548/EEC as carcinogenic, mutagenic or toxic for reproduction, of category 3, and substances with similar potential, must not be intentionally added to cosmetic products unless it can be demonstrated that their levels do not pose a threat to the health of the consumer."
(2) OJ 196, 16.8.1967, p. 1. Directive as last amended by Commission. Directive 2001/59/EC (OJ L 225, 21.8.2001, p. 1).

2 SCCP/0888/05 and SCCP/0913/05.

3 OJ L 246, 15.09.2008, p. 1.

4 OJ L 11, 16.01.2009, p. 6

- N-methyl-2-pyrrolidone, EC 212-828-1, is classified Repr. 1B and with a specific concentration limit of 5%. As it is used in cosmetic products in a concentration below 5% and Industry awaits the approach taken by the Commission on the boron compounds, no dossier is deemed so far necessary.

2. TERMS OF REFERENCE

1. *Based on the current knowledge on the chemistry, biology, toxicology and taking into account the scientific data used for the classification purposes of trisodium nitrilotriacetate and N-methyl-2-pyrrolidone classified respectively as a carcinogen category 2 and a reprotox 1B substance with a specific concentration limit of 5%, does the SCCS consider safe the continuous use of these two substances in cosmetic products up to the specific concentration limit set out in the Commission Regulation 790/2009?*

As this mandate concerns two unrelated substances, it is addressed in two separate opinions. The present opinion concerns the safety of N-methyl-2-pyrrolidone (NMP) only. Trisodium nitrilotriacetate (NTA) was assessed in opinion SCCS/1391/10, adopted on 14 December 2010.

3. OPINION

3.1 Chemical and Physical Specifications

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

Methyl pyrrolidone (INCI name)
1-methylpyrrolidin-2-one (IUPAC name)

3.1.1.2 Chemical names

1-methyl-2-pyrrolidone (EC name)
N-methyl-2-pyrrolidone
N-methylpyrrolidione
N-methylpyrrolidone

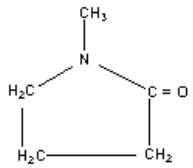
3.1.1.3 Trade names and abbreviations

NMP

3.1.1.4 CAS / EC number

CAS: 872-50-4 / 51013-18-4
EC: 212-828-1/ -

3.1.1.5 Structural formula



3.1.1.6 Empirical formula

Formula: C_5H_9NO

3.1.2 Physical form

Colourless liquid with a mild amine odour

3.1.3 Molecular weight

Molecular weight: 99.1 g/mol

3.1.4 Purity, composition and substance codes

80 – 100%

Ref.: 65

3.1.5 Impurities / accompanying contaminants

Dimethylpyrolidones (mixtures of isomers)	< 0.4 %
Methylamine	< 0.005 %
γ-Butyrolactone	< 0.05 %
Water	< 0.05 %

3.1.6 Solubility

Completely miscible with water
Highly soluble in alcohols, ethers, acetates and aromatics
Moderately soluble in aliphatic hydrocarbons

3.1.7 Partition coefficient (Log Pow)

Log P_{ow}: -0.46 (experimental)

3.1.8 Additional physical and chemical specifications

Melting point:	-23.5 °C
Boiling point:	204.3 °C
Flash point:	/
Rel. vapour density:	3.4 (air = 1)
Density:	1.028 g/cm³
Vapour pressure:	32 Pa (20 °C): 45 Pa (25 °C)
Other properties:	NMP is a basic and polar compound with high stability. It is only slowly oxidized by air and not corrosive. NMP is a very hygroscopic solvent
Conversion factor (20 °C):	1 ppm = 4.12 mg/m³; 1 mg/m³ = 0.24 ppm

3.1.9 Homogeneity and Stability

No data submitted

3.2 Function and uses

NMP is mainly used as a solvent for extraction in the petrochemical industry, as a reactive medium in polymeric and non-polymeric chemical reactions, as a remover of graffiti, as a paint stripper in the occupational setting, and for stripping and cleaning applications in the microelectronics fabrication industry. It is also used as a formulating agent in pigments, dyes and inks, and in insecticides, herbicides and fungicides. NMP is further used as an intermediate in the pharmaceutical industry. NMP is also used to enhance the absorption of topically applied drugs.

NMP is used as a solvent and a surfactant in cosmetic products. There are no known natural sources of NMP.

Comment
The final concentration of NMP in cosmetic products is not known. It is noted that NMP may enhance the dermal absorption of other cosmetic ingredients.

3.3 Toxicological Evaluation

Most of the information in this opinion until about year 2000 is taken from the "Concise International Assessment Document 35 N-Methyl-2-pyrrolidine", WHO (1) and the "Final proposal for classification of 1-Methyl-2-Pyrrolidone", ECB (2). Later information is mainly from published scientific articles.

3.3.1 Acute toxicity

3.3.1.1 Acute oral toxicity

Rats: LD_{50}: 4150 (3100-5560) mg/kg bw (3).
Rats: LD_{50}: 3906 (3100-4400) mg/kg bw (4).
Mice: LD_{50}: 7710 (5900-9600) mg/kg bw (4).

3.3.1.2 Acute dermal toxicity

Rats: LD_{50}: 2500 - 5000 mg/kg bw (5).

3.3.1.3 Acute inhalation toxicity

Rats: LC_{50} > 5.1 mg/l (87% of aerosol particles< 8.5 µm) head only, 4hr. (IUCLID dataset (19.02.00)

General comment
NMP has low acute toxicity by oral, dermal, and inhalation routes.

3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

Skin irritation tests in New Zealand White rabbits (n = 6) exposed to 0.5 ml NMP (100%) were performed according to Draize (6). The test sites were occluded for 24 h and then examined for skin reactions. Only slight erythema was observed. When the examination was repeated 72 h and 7 days after the start of exposure, no effects were observed. The tests showed a low potential for skin irritation and resulted (for both intact and abraded skin and averaged reading from 24 and 72 h) in a primary irritation index of 0.5 (out of a maximum 8).

Ref.: 3

Repeated daily dermal application of 450 mg/kg bw to rabbits caused painful and severe haemorrhage and eschar formation after four doses; the reaction to a dose of 150 mg/kg bw per day was less marked.

Ref.: 1, 7

Aqueous solutions of NMP were tested for primary skin irritation in 10 male albino guinea-pigs. Twenty-four hours after application, slight erythema was observed in two guinea-pigs with the 50% solution and in 0 with the 5% solution. After 48 h, no effects were registered.

Ref.: 1, 8

Three employees were exposed to 99% NMP for a few minutes several times during 3 days. Acute painful swelling and excessive wrinkling of the skin of their hands was observed

without signs of inflammation which probably could be attributed to the powerful hygroscopic effect of NMP on the stratum corneum.

Ref.: 2, 9

Skin irritation was reported in several workers after a few days of working with NMP. They used a paper cloth to wipe surplus of NMP from plastic pieces that had been dipped in the solvent. Analyses by gas chromatography indicated that no compounds other than NMP were present in detectable quantities in the samples of NMP discarded after use.

Ref.: 2, 10

See also Section 3.3.3. Skin sensitization

Comment
In rabbits and guinea-pigs, slight or moderate irritation has been reported. Irritation of the skin has been reported in workers with prolonged or repeated exposure (dermatitis, oedema, redness blister or cracking).

3.3.2.2 Mucous membrane irritation

Primary eye irritation tests according to Draize (6) were performed in New Zealand White rabbits (n = 9). Intraocular applications of 0.1 ml NMP into one eye (the other eye served as untreated control) caused conjunctival effects, such as corneal opacity, iritis, and conjunctivitis. The effects faded within 21 days after the application. When the exposed eye was washed out 30 s after the application (performed in three of the nine exposed rabbits), the effects faded within 14 days. The primary irritation index scores for unwashed/washed eye were 41/35, 40/26, 34/18, 8/1, 4/0, and 0/- after 1, 2, 3, 7, 14, and 21 days post-exposure, respectively. The tests in the rabbits indicated a moderate potential for eye irritation.

Ref.: 3

Six volunteers (human males) were exposed by inhalation to 0, 10, 25, and 50 mg NMP/m³ for 8 hr on one single day. No discomfort of the eyes was reported by the subjects. The authors do not exclude that extremely high peak exposures or warm NMP vapours may condense to an aerosol vapour that may be irritating to the eyes.

Ref.: 11

Workers exposed to NMP in electronic industries reported severe eye irritation and headache. Levels of NMP vapours ranging from 49 to 83 ppm (i.e. 202 to 342 mg/m³) were qualified "unbearable". Concentrations of 15-17 ppm (i.e. 62-70 mg/m³) were described as "immediately uncomfortable". However these reports present several limitations regarding the methods used to assess the exposure levels and the responses (e.g. informal interviews, subjective judgements).

Ref.: 12

See also Section 3.3.11. Human data

Comment
NMP causes eye irritation.

3.3.3 Skin sensitisation

Sensitization potential tests, defined as the increase of response at challenge after a series of four intradermal injections (0.1 ml of 1% NMP in 0.9% saline solution; one injection per week), were performed in 10 male albino guinea-pigs. Two weeks after the intradermal injections, the animals were exposed to aqueous solutions of NMP. About 0.05 ml each of a 5% and a 50% (v/v) solution were applied and lightly rubbed in to the shaved intact

shoulder skin. Nine guinea-pigs that did not have intradermal injections of NMP were used as control animals. No sensitization was found when the animals were examined after 24 and 48 h.

After 24 h, there was slight erythema at the 50% solution test sites in 6 out of 10 challenged guinea-pigs and in 4 out of 9 controls. No effects were observed when animals were examined after 48 h. The 5% NMP solution caused no irritation.

Ref.: 1, 8

Comment
No evidence of skin sensitisation has been found in the assays reported.

3.3.4 Dermal / percutaneous absorption

In rats, NMP is rapidly absorbed upon inhalation, ingestion, and dermal application and widely distributed throughout the body. The peak plasma concentration in rats after administration of a mixture of [2-^{14}C]-NMP and [5-^{14}C]-2-pyrrolidone by gastric intubation (112/75 mg/kg bw in 0.6 ml distilled water) occurred after 2 h; after application to the skin (2.5/1.67 mg/cm² skin on 9 cm² in 150 µl isopropanol), the peak plasma concentration occurred after 1 h for males and 2 h for females. Following dermal application of the two compounds, the plasma concentrations showed little variation 1–6 h after administration, indicating that the absorption through the skin during this period was relatively constant. The percutaneous absorption, expressed as the total excretion in urine, faeces, and expired air, was 69% in males and 78% in females. The levels of total radioactivity in plasma were markedly higher in female rats than in male rats for 12 h after the application, reflecting a greater percutaneous absorption in females.

Ref.: 13, 14

The percutaneous absorption of NMP may differ when NMP is applied as pure NMP or as an NMP solution. In a dermal absorption study in the rat, the absorbed amounts of applications of pure NMP, 30% NMP in water, and 30% NMP in (R)-(+)-limonene were 31%, 3.5%, and 72%, respectively.

Ref.: 15

In rats exposed whole body by inhalation to 618 mg NMP/m³ for 6 h, the NMP concentration in the blood increased from 0 to 4 h after termination of the exposure. Such an increase is due to a percutaneous uptake of adsorbed NMP on fur and skin when the animals are whole-body exposed to aerosol NMP. When a solution of 10% NMP as a penetration enhancer was studied for 24 h in vitro, the skin permeability of NMP was 4 times higher in rats than in humans.

Ref.: 16, 17

The *in vivo* percutaneous absorption of [^{14}C]-NMP in rats was determined by sacrificing the animals at different times after topical application of neat [^{14}C]NMP (20 and 40 µl/cm²). After topical application of neat NMP, the percentage of the absorbed dose increased rapidly with exposure time and accounted for about 60% of the dose after 2 h of exposure. For a 24-h dermal exposure to neat [^{14}C]-NMP (20 µl/cm²), the percentage of the absorbed dose calculated from the content of radioactivity in excreta and carcass was 85 ± 4% of the applied dose.

Ref.: 18

Following topical administration of NMP radiolabelled with ^{14}C on the C2 atom to rat skin at doses of 0.2, 2 and 20 mg/cm², applied to an area of 12 cm², there was 50% absorption of the 2 lower doses while 75% of the 20 mg/cm² dose was absorbed, suggesting that NMP promotes its own absorption. Maximum blood levels were observed approximately 8 hours after application.

Ref.: 19

Six female and six male volunteers (groups 1 and 2) were topically exposed for 6 hours to 300 mg of NMP. An additional group of six male volunteers (group 3) was exposed to 300 mg of NMP in a 50% water solution. None of participants reported any kind of irritation. All of them displayed a slight redness at the site of application, which faded within 4 (range 2–6) hours. Shortly after the redness disappeared, a slight dryness occurred which disappeared within 4 (range 2–12) days. For groups 1 and 2, 16% and 18% of the applied dose were recovered in the urine as the sum of NMP and its metabolites. For group 3, 4% was recovered. The absorbed NMP dose of the volunteers exposed to pure NMP was approximately 4–5 times higher than that of the group exposed to a 50% water mixture although the applied dose was the same and the surface area of the patch used for the 50% mixture was twice as big. The differences between the male and female volunteers were small also for the other parameters studied.

Ref.: 20

Four male volunteers were exposed to liquid NMP under occlusive conditions on the back of one hand with varying exposure times and solvent concentrations. Urine was collected before, during and after the exposure and analysed for the main NMP metabolites 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP) and 2-hydroxy-N-methylsuccinimide (2-HMSI) (see also section 3.3.9. toxicokinetics). The average pad surface area was ± 10 cm². After the exposure the pads were removed and residual NMP was washed off the skin. The six experimental designs comprised the application of 100% NMP for 2 h (D1), 50% NMP for 2 h (D2), 10% NMP for 2 h (D3), 100% NMP for 30 min (D4), 50% NMP for 30 min (D5) and 10% NMP for 30 min (D6). Of six exposure conditions, only three designs (D1, D2, D4) could be used for the study calculations as the two 10% NMP dilution experiments (D3, D6) yielded a large number of samples with metabolite concentrations below the limits of detection. Also, the 50% solution only had viable results when applied to the skin over a period of 2 h (D2), but not in the 30 min experiment (D5). The 120 min exposure to 100% NMP resulted in the elimination of 115±18 mg NMP, while only 18±6 mg (16% of 115 mg) were found in the 50% NMP approach of D2. The 30 min exposure to 100% NMP (D4) yielded an average amount of 31±8 mg NMP (27% of 115 mg), as would be expected for a linear proportion between dermal absorption and exposure time. The urinary concentration of the metabolites upon exposure to undiluted NMP for 2 h increased rapidly with 5-HNMP reaching a maximum at 4–5 h and 2-HMSI after 26–29 h. The application of aqueous NMP solutions resulted in a delay of the peak time for 5-HNMP of approximately 6 h as compared with the undiluted solvent. With the calculated total amount of NMP in urine, the duration of exposure and the pad size as a measure for the exposed skin surface, the average dermal absorption in the D1 experiment (100% NMP, 120 min) was 5.4±1.5 mg NMP cm^2/h. This value is in good accordance with the 6.5±2.0 mg NMP/cm²/h from the 30 min exposure to 100% NMP (D4) while the absorption was reduced to 0.9±0.5 mg NMP/cm²/h in the 50% NMP experiment accounting for only 16% of the D1 results with undiluted NMP.

Ref.: 21

In a dermal application experiment, 12 humans volunteers were exposed to 300 mg NMP through a dermal patch (filter paper, diameter 5 cm, protected with aluminium foil and attached by Dermalock) applied on the anterior face of the left forearm for 6 h. Five urine fractions were collected during 48 h following the onset of application. The mean dermal absorption of NMP was 67.9% (60.8 – 77.4%).

Ref.: 61

Comment
NMP is readily absorbed by all routes of exposure. Due to its low vapour pressure, absorption through the skin represents the most likely and potentially the most significant route of exposure to NMP under most known consumer use conditions.

In vivo rat studies indicate that 70 – 85% is absorbed when high concentrations of NMP are tested. The absorption at lower concentrations was less. It is noted that the absorption of

NMP depend on the solvent. In a rat experiment only 3.5% NMP in a 30% aqueous solution was absorbed, but 72% of NMP in a 30% solution in limonene was absorbed. In the case of human studies 16 – 68% was absorbed. Also in this case, the absorption was higher for neat NMP than for diluted NMP.

In the absence of adequate experimental data with concentrations of NMP relevant for this assessment, the SCCS will use the default value of 100% dermal absorption in the calculation of the MOS. This value is also used by ECHA (65).

3.3.5 Repeated dose toxicity

3.3.5.1 Repeated Dose (30 days) oral toxicity

Oral

Rats

Rats (10 per sex) were intubated 5 days/week for 4 weeks with 0, 257, 514, 1028, or 2060 mg NMP/kg bw/day. In males, a dose-dependent decrease was observed in body weight at 1028 and 2060 mg/kg bw/day (11% and 16%, respectively), and a decrease in relative and absolute testes weight was observed in nine animals at 2060 mg/kg bw/day. The histological examination showed adverse effects on seminiferous tubule epithelium and formation of multinucleate giant cells and clumping of sloughed-off cells. In both sexes, a dose-dependent increase in relative liver and kidney weights and a decrease in body weight gain were observed at 1028 and 2060 mg/kg bw/day, and lymphocyte count decreased following exposure to 1028 and 2060 mg/kg bw/day. At 2060 mg/kg bw/day, symptoms of general toxicity, such as tremor, restlessness, ruffled fur, and defensive reactions, were registered. The NOAEL was 514 mg NMP/kg bw.

Ref.: 22

In a repeated-dose toxicity study, rats (Orl:CD.BR) (five per sex) were given 0, 2000, 6000, 18 000, or 30 000 mg NMP/kg diet for 28 days. The mean daily NMP doses were 0, 149, 429, 1234, and 2019 mg/kg bw/day in males and 0, 161, 493, 1548, and 2268 mg/kg bw/day in females. The purity of NMP was 99.9%.

No mortality occurred. Lower body weights and body weight gains were noted at ≥ 1234 mg/kg bw/day in males and at 2268 mg/kg bw/day in females. In males, the body weight gains over test days 0-28 were by 40 and 72 % less than controls at 1234 and 2019 mg/kg bw/day, respectively. In females, it was reduced by 52 % at the high dose. These decreases were correlated with lower food consumption. Slight, but non-significant lower cumulative weight gain was observed in males at 429 mg/kg bw/day and in females at 1548 mg/kg bw/day.

Several serum parameters were mildly affected: Decreases in glucose (≥ 1234 mg/kg bw/day) and alkaline phosphatase activity (ALP) (2019 mg/kg bw/day) in males; minimal decreases in total proteins and albumin and mild increase in cholesterol in males and females at the highest dose.

Moderate leucopoenia and histopathological alterations (hypo-cellular bone marrow in males and females, testicular degeneration and atrophy in males, and thymus atrophy in females) were observed at the highest dose in males and females. They were judged secondary to nutritional and body weight effects. Centrilobular hepatocellular hypertrophy occurred in males and females at the two highest doses (0/5 males or females in the control and the two lowest doses; 5/5 and 4/5 males at 1234 and 2019 mg/kg bw/day, respectively; 3/5 and 5/5 females at 1548 and 2268 mg/kg bw/day, respectively). It was considered an adaptative response.

Males showed degeneration and/or atrophy of the testicular seminiferous tubules (0/5, 0/5, 0/5, 1/5, and 5/5, at 0, 149, 429, 1234, and 2019 mg/kg bw/day, respectively). The weight of testes was also altered (not detailed).

The authors concluded that, rather than specific organ toxicity, a general systemic response seemed to occur. The NOAEL was 429 mg/kg bw/day (6000 ppm) for males and (1548 mg/kg bw/day (18000 ppm) for females.

Ref.: 23

Mice

In a repeated-dose toxicity study, mice (B6C3F1/CrlBR) (five per sex) were given 0, 500, 2500, 7500, or 10 000 mg NMP/kg diet for 28 days. The mean daily NMP dose was 0, 130, 720, 2130, and 2670 mg/kg bw/day in males and 0, 180, 920, 2970, and 4060 mg/kg bw/day in females. The purity of NMP was 99.9%.

Experimental evaluations included food consumption, body weight, haematology, clinical chemistry, and gross and microscopic examination of a standard set of tissues.

The death of one male in the 2670 mg/kg bw/day group was considered treatment-related. Morphological examination revealed pathological changes, consistent with the renal effects observed in the surviving animals. There was no effect on body weight or food consumption, or changes in haematological parameters at any doses, in either males or females. Except from a lower serum ALP in females at 4060 mg/kg bw/day, there were no changes in the clinical chemistry parameters.

Cloudy swelling of the epithelia of the distal parts of the renal tubuli was observed in 4 males and 3 females at 10 000 mg/kg and in 2 males at 7500 mg/kg. The NOAEL was 720 mg/kg bw/day (2500 mg/kg) in males and 2970 mg/kg bw/day (7500 mg/kg) in females, based on the kidney histopathology.

Ref.: 23

Inhalation

Rats

Rats (Crl:CD) (15/sex/dose) were exposed to 100, 500, and 1000 mg/m³ NMP (aerosol-vapour mixture) with an additional control (air) (> 95 % of the droplets below 10 μm in diameter) 6 hr/day, 5 days/week for 4 weeks. The purity of NMP was 100%. Experimental evaluations included body weight, haematology, clinical chemistry, and gross and microscopic examination of a standard set of tissues.

At all levels, all rats showed signs of lethargy and irregular respiration after approximately 3-4 hr of exposure. These signs persisted until the end of exposure. Rats at 100 and 500 mg/m³ recovered within 30-45 minutes post-exposure, but only a few rats recovered by 18 hr post-exposure at the high dose.

At 1000 mg/m³: Out of 30 rats, 8 rats died and 5 rats were euthanised within the first 9 days of exposure. Because of the respiratory difficulty and of the mortality, exposure was discontinued after 10 days and the surviving rats were allowed to recover for 2 weeks. A decrease in weight gain was found after 5 days of exposure. After 10 days of exposure, the relative and absolute number of neutrophils was increased, while the number of lymphocytes was decreased. They returned to normal values after 2 weeks of recovery. Histopathological examination of dead animals revealed focal pneumonia, hypoplasia and

haemorrhage in the bone marrow, and atrophy of the lymphoid tissue in the spleen and thymus.

At 100 and 500 mg/m³: There were no changes in body weight, blood and urinary analysis, and no pathological lesions after 4 weeks of exposure or 2 weeks post-exposure.

Ref.: 24

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

Oral

Rats

Rats (Crl:CD) (10 per sex) were administered 0, 3000, 7500, or 18 000 mg NMP/kg diet for 90 days. The mean daily NMP dose was 0, 169, 433, and 1057 mg/kg bw/day in males and 0, 217, 565, and 1344 mg/kg bw/day in females. The purity of NMP was 99.9%. In the subchronic assay, evaluations included clinical signs, food consumption, body weight, haematology and clinical chemistry, ophthalmologic evaluation and complete gross and histopathological examinations. In the neurobehavioral assay, evaluations included clinical signs, food consumption, body weight, neurobehavioral tests, and detailed histopathological examination of muscle and central and peripheral systems.

NMP had no effect on survival, haematology, clinical chemistry or urinary parameters in males or females, at any concentration. Body weight, body weight gain and food consumption were lower over the 90-day feeding period at 18000 mg/kg, and during the first half of treatment at 7500 mg/kg. (The body weight gains over the 90-day test period were 28 and 25 % lower than control in the 18000 mg/kg males and females, respectively).

Neurobehavioral parameters were not affected, except from an increase in foot splay in males at 7500 and 18000 ppm, and a higher incidence of low arousal and slight palpebral closure in the open field evaluation in the 18000 ppm males. No morphological changes were evident in either the peripheral or central nervous system. The picture of effects was considered to be treatment-related and suggestive of a sedative effect.

Females had increased absolute and relative liver weights that were associated with an increased incidence of centrilobular hepatocellular hypertrophy (0/10 at 0, 3000 and 7500 mg/kg, and 6/10 at 18000 mg/kg). Relative and absolute kidney weights were increased at 18000 mg/kg in both sexes, but no pathological changes were found. Changes in the relative weight of lungs, brain (males and females), and testes (13% increase) occurred at 18000 mg/kg. They were not associated with morphological changes.

The NOAEL was 169 mg/kg bw/day in males and 217 mg/kg bw/day in females (3000 mg/kg for both sexes) based on body weight effects and changes in three neurobehavioral parameters (males only) at higher doses.

Ref.: 25

Mice

Mice (B6C3F1) (20 per sex) were administered 0, 1000, 2500, or 7500 mg NMP/kg diet for 90 days. The mean daily NMP dose was 0, 277, 619, and 1931 mg/kg bw/day. The purity of NMP was 99.9%. Experimental evaluations included clinical signs, food consumption, body weight, haematology and clinical chemistry, ophthalmologic evaluation, and complete gross and histopathological examinations.

There was no adverse effect on survival, body weight, food consumption or haematological parameters at any dose, in either males or females. At 2500 and/or 7500 mg/kg bw/day, increase in serum cholesterol (females), and decreases in serum triglycerides, calcium, and

ALP (males) occurred at 28 days, but not at 90 days. Liver weight (absolute and relative) was increased in males at 2500 and 7500 mg/kg. Relative liver weight was also slightly higher at all doses in females, although a dose-response relationship was not evident.

Centrilobular hepatocellular hypertrophy was observed at 7500 mg/kg (1/10, 0/10, 2/10, and 9/10 in males at 0, 1000, 2500, and 7500 mg/kg, respectively; and 1/10, 0/10, 3/10, and 10/10 in females at 0, 1000, 2500, and 7500 mg/kg, respectively). These findings were regarded as an adaptation process, but were clearly attributed to NMP exposure. No other histopathological changes were detected.

The NOAEL was set at 277 mg/kg bw/day (1000 mg/kg) based on the liver responses at higher doses. (Transient changes in biochemical parameters were also observed at > 1000 mg/kg).

Ref.: 25

Dogs

Dogs (Beagle) (six per sex) that were administered NMP (purity: 99.9%) at doses of 0, 25, 79, or 250 mg/kg bw/day in the diet for 90 days showed no statistically significant adverse effects. A dose-dependent decrease in body weight gain and an increase in platelet count and megakaryocytes within a normal range were observed. At exposure termination, no significant differences between high-dose and control groups were reported. However, the authors mentioned several findings considered incidental or of doubtful significance: trend towards a decrease in body weight gains with increasing doses (body weights showed no significant differences), slight increase in platelet count with increased megakaryocytes and decrease in male serum cholesterol with increasing doses.

The NOAEL for dietary exposure in dogs in this study is 250 mg/kg bw/day.

Ref.: 26

Inhalation

Rats

In a medium-term exposure study, rats (10 per sex per dose level) were exposed (head only) to 0, 500, 1000, or 3000 mg NMP/m³ for 6 h/day, 5 days/week, for 13 weeks. These groups were sacrificed and examined at the end of exposure. An additional two satellite groups (10 rats per sex per dose level) were identically exposed to 0 or 3000 mg/m³ and sacrificed after 13 weeks of exposure and a 4-week post-exposure period to obtain information on the reversibility of possible effects. The generated NMP atmospheres consisted of a large proportion (82–92%) of respirable aerosol particles (MMAD 2.1–3.5 µm; relative humidity 52–61%).

Dark yellow discoloration of the urine was found at all levels, and nasal irritation as shown by crust formation on nasal edges at 1000 mg/m³ was observed at the end of the exposure period.

At 3000 mg/m³, non-specific clinical symptoms and irritation of the respiratory tract were registered. In male rats, body weight was significantly decreased (34%) and absolute testes weight was decreased. Cell loss in germinal epithelium of testes in 4 out of 10 male rats was noted. Slight increases in erythrocytes, haemoglobin, haematocrit, and mean corpuscular volume were observed. In female rats, the number of polymorphonuclear neutrophils increased and the number of lymphocytes decreased. Examination of the satellite group at the end of the 4-week post-exposure observation period showed a significant lower body weight gain in males compared with the controls. The testes effects

registered in the 3000 mg/m³ group sacrificed at the end of exposure were also registered in the satellite group at the end of the 4-week post-exposure observation period.
The NOAEL was 500 mg NMP/m³ for both male and female rats.

Ref.: 27

Comment
No dermal exposure data were found. A dose-dependant yellow coloration of urine was generally reported in rodents, whatever the route of administration. Although the urine discolouration was compound-related, it was not associated to changes in kidneys. It was probably due to the presence of a metabolite of NMP and reflected body impregnation.

A NOAEL of 169 mg/kg bw/day in male rats was found in a 90-day study based on body weight effects and changes in three neurobehavioral parameters. The NOAEL was 277 mg/kg bw/day in mice based on the liver responses in a 90-day study and 89 mg/kg bw/day in male mice in a 2-year study based on increased liver weighs and 173 mg/kg bw/day based on liver tumours (see Section 3.3.7. Carcinogenicity) A NOAEL of 250 mg/kg bw/day (highest dose tested) was found in a 90-day study with dogs.

After inhalation, the NOAEL was 500 mg NMP/m³ for both male and female rats.

3.3.5.3 Chronic (> 12 months) toxicity

See section 3.3.7 Carcinogenicity

3.3.6 Mutagenicity / Genotoxicity

In vitro

NMP has been tested in bacterial mutagenicity assays in the dose range of 0.01–1000 µmol/plate (0.99 µg/plate to 99 mg/plate) with and without metabolic activation by Aroclor-induced rat liver S9. In the direct plate incorporation in *Salmonella typhimurium* strains TA97, TA98, TA100, TA102, and TA104 at highest dose, signs of cytotoxicity (decreased number of revertants or bacterial lawn thinning) were observed. In strains TA102 and TA104 without activation, a minor and not dose-related increase in the number of revertants was observed. When using a preincubation method in strains TA98 and TA104, no effects were registered (Ref. 28). Also, in another preincubation test in strains TA98, TA100, TA1535, and TA1537 (NMP dose levels up to 10 mg/plate) with and without Aroclor-induced rat or hamster liver S9, no mutagenic activity was observed (Ref. 29). Other studies, also using Salmonella typhimurium strains for testing the mutagenicity of NMP, reported no mutagenic activity (Refs. 30, 31).

Two assays in yeast show that NMP may induce aneuploidy. Incubation of Saccharomyces cerevisiae strain D61.M with NMP in the dose range of 77–230 mmol/litre (7.6–23 g/litre) caused a dose-related effect. Concentrations of 179 mmol/litre (18 g/litre; 1.8%) and higher were toxic and decreased the level of survival by more than 50% (32). The decrease in survival was shown to be the same when NMP was used at a concentration of 2.44% for incubation of the same yeast strain (Ref. 33).

Negative results were obtained in a study of the ability of NMP to induce unscheduled DNA synthesis in rat primary hepatocyte cultures (Ref. 34) and in a study of the mutagenic activity of NMP in L5178Y mouse lymphoma cells (Ref. 35).

In vivo

In a micronucleus test, NMRI mice (both sexes) were orally administered a single dose of 950, 1900, or 3800 mg NMP/kg bw. Irregular respiration, coloured urine, and general poor

health were observed. No clastogenic effects or aneuploidy were observed when mice were examined at 24, 48, and 72 h after dose administration. Positive controls displayed clastogenic and aneugenic activity. Thus, no mutagenic activity with NMP was found.

Ref.: 36

In a bone marrow chromosomal aberration study, Chinese hamsters (both sexes) were exposed to a single oral dose of 1900 or 3800 mg NMP/kg bw. Irregular respiration, coloured urine, and general poor health were observed. At 16 (only high dose level) and 24 h after administration, bone marrow samples were taken. Structural and numerical chromosomal alterations were found in positive control animals but not in NMP-exposed animals, indicating no mutagenic activity of NMP.

Ref.: 36

Signs of toxicity were reported in two older studies (1976): a micronucleus test in Chinese hamsters (both sexes) exposed for 6 weeks (6 h/day, 5 days/week) to 3300 mg NMP/m³ and a germ cell genotoxic activity test (a dominant lethal test) in male NMRI mice with intraperitoneal administration of 391 mg NMP/kg bw (once per week for 8 consecutive weeks). The inhalation study displayed a slight but non-significant increase in structural chromosomal aberrations in the bone marrow. In the intraperitoneal study, a significantly increased post-implantation loss was observed (relative to the control animals).

Ref.: 37

Comment
The studies were not performed to current regulatory standards and could not be fully evaluated for NMP mutagenic activity.

General comment
The results available indicate that NMP can cause aneuploidy in a fungal test *in vitro*. However, NMP does not express a genotoxic effect in a standard bacterial assay and in two well-conducted *in vivo* assays (bone marrow chromosome aberration assay and micronucleus assay). NMP is not considered to have *in vivo* genotoxic potential.

3.3.7 Carcinogenicity

Oral

Rats

Rats (Crl:CD) (62/sex/dose) were administered 0, 1600, 5000, or 15 000 mg NMP/kg diet for 2 years. The mean daily NMP dose was 0, 66.4, 207, and 678 mg/kg bw/day in males and 0, 87.8, 283, and 939 mg/kg bw/day in females. The purity of NMP was 99.8%. Evaluations: Body weight, food consumption, clinical signs, ophthalmology evaluation, haematology at 12, 18 and 24 months (high dose), and microscopic evaluation of a standard set of tissues and organs.

Decrements in body weight, body weight gain, and food intake were noted at 15000 mg/kg, in males and females. At the end of the 2-year feeding period, body weights of 15000 mg/kg males and females were 25 and 35% lower than control, respectively. A substance–related decrease in survival, associated with a chronic progressive nephrotoxicity/uremia occurred in males at 15000 mg/kg (32% survival in controls/24% in treated males). There was a lower survival in males at 5000 mg/kg and in females at 1600 and 15 000 mg/kg, due to combination of single instances of a variety of lesions (e.g. pituitary tumours), which were considered not treatment-related (no dose-response relationship).

No toxicologically significant clinical signs of toxicity, ophthalmic changes or leucocyte differential counts were detected in either males or females, at any doses. Gross

morphological changes were seen in males at 15 000 mg/kg and consisted in increased incidence of small testes (weights not reported, 6/62, 6/62, 8/62, and 14/62 at 0, 1600, 5000, and 15 000 mg/kg, respectively), of fluid within the pleural cavity, and of kidneys with chronic progressive nephropathy.

Microscopic morphological changes: Increased severity of pigment accumulation in macrophages in the spleen was seen in both 15 000 mg/kg males and females. The number of males with severe nephropathy was also increased (7/62, 10/62, 12/62, 25/62 for 0, 1600, 5000 and 15 000 mg/kg, respectively). Several other lesions were reported in the males at the high dose, which may be secondary to the renal failure and/or to the debilited conditions of the animals: i.e. centrilobular fatty change in the liver, thrombus in the renal vein of the kidney, lymphoid depletion of the mesenteric lymph node, hypertrophy/cystic degeneration in the adrenal cortex associated with bilateral degeneration/atrophy of seminiferous tubules in the testes (15/62, 17/62, 15/62, and 35/62 at 0 1600, 5000 and 15 000 mg/kg, respectively), bilateral oligospermia/germ cell debris in the epididymides (11/62, 14/62, 12/62, and 35/62 at 0 1600, 5000 and 15 000 mg/kg, respectively), and fibrous osteodystrophy in the femur/knee joint and sternum. In addition, polytarteris in the cecum, mesenteric lymph node and in the testis were observed. The toxicological significance of these findings was not clear.

NMP was not oncogenic in males and females up to doses of 15 000 mg/kg. The primary NMP-related effect was an increase in chronic progressive nephropathy in males.

The NOAEL was 207 mg/kg bw/day in males and 283 mg/kg bw/day in females (5000 mg/kg in both sexes).

Ref.: 38

Mice

Mice (B6C3F1) (50 /sex/dose) were administered 0, 600, 1200, or 7200 mg NMP/kg diet for 2 years. The mean daily NMP dose was 0, 89, 173, and 1089 mg/kg bw/day for males and 115, 221, and 1399 mg/kg bw/day in females. The purity of NMP was 99.8%. Evaluations: Body weight, food consumption, clinical signs, haematology at 12, and 18 months (high dose), and microscopic evaluation of a standard set of tissues and organs.

There was no adverse effect on the incidences of clinical observations, food consumption, survival, and haematology, in either males or females, at any dose. The body weight of the high-dose males was slightly lower at the end of the treatment (5%).

The absolute and/or relative liver weights were significantly increased in the males and females at 7200 mg/kg, and in the 1200 mg/kg males. They were considered to be the result of liver tumours and/or centrilobular hepatocellular hypertrophy (0/50, 0/50, 3/50, 43/50 in males, at 0, 600, 1200, and 7200 mg/kg, respectively). Changes in the weight of kidney, testes, adrenal, ovarian and brain were noted, without histological correlates and/or dose-response relationship. Therefore, they were not considered to be test substance-related. There was an increased incidence of foci with cellular alterations in the liver at 7200 mg/kg: clear cell foci (males), eosinophilic foci (males and females), and basophilic foci (females).

At 7200 mg/kg, there was an increase in the occurrence of hepatocellular adenomas [5/50 (10%), 2/50 (4%), 4/50 (8%), and 12/50 (24%) at 0, 600, 1200, and 7200 mg/kg, respectively] and carcinomas [4/50 (8%), 1/50 (2%), 3/50 (6%), 13/50 (26%) at 0, 600, 1200, and 7200 mg/kg, respectively] in male mice, and an increased incidence of adenomas in females [2/50 (4%), 2/50 (4%), 1/50 (2%), 7/50 (14%) at 0, 600, 1200, and 7200 mg/kg, respectively]. No evidence of an increase incidence of malignant tumours was seen

at lower doses. There were no NMP-related neoplastic or non-neoplastic changes in other organs.

Ref.: 38

Comment
The authors state that the relative liver weights were significantly increased in the 1200 mg/kg males. It is noted that the relative liver weights (% of body weight) were 3.8, 3.6, 4.0, and 5.0 in the control, 89 mg/kg bw/d, 173 mg/kg bw/d, and 1089 mg/kg bw/d, respectively. Moreover, non-neoplastic and neoplastic lesions in mice were only observed at the highest dose. SCCS considers that the NOAELs are 173 mg/kg bw/d in males and 221 mg/kg bw/d in females.

Inhalation

Rats

In a 2-year inhalation study, Charles River CD rats (Crl:CD) (120/ sex/ dose level) were exposed (whole body) to NMP vapour concentrations of 0, 40, or 400 mg/m³ for 6 h/day, 5 days/week. Ten rats/ sex were subjected to haematology and blood and urine chemistry analysis after 1, 3, 6, 12, and 18 months of exposure. Ten rats/ sex were sacrificed after 3, 12, and 18 months. All surviving rats were killed at the end of 24 months of exposure and subjected to a gross examination. All vital organs and tissues were subjected to microscopic examination. Respiratory tract toxicity was observed at 400 mg/m³ as a minimal inflammation in the lung. Male rats exposed to 400 mg/m³ for 18 months showed higher haematocrit and higher alkaline phosphatase levels in serum than were observed in the control group. There was no such difference after 24 months of exposure. At the 400 mg/m³ dose level, male rats excreted larger urine volumes, and both males and females excreted dark yellow urine. The 2-year study showed a 6% reduction in the mean body weight in male rats at the 400 mg NMP/m³ dose level (statistical significance not reported).

Clinical chemistry, haematology, and gross and microscopic examination were conducted after 1, 3, 6, 12, 18 months (10 animals/sex/dose) and two years (all surviving animals). There were no treatment-related effects on mortality or morbidity.

At 400 mg/m³, males and females discharged dark yellow urine and the males had a greater urine volume. After 2 years, male rats had gained slightly less body weight at 400 mg/m³ (6%). Males at 400 mg/m³ for 18 months had high haematocrits and serum ALP. No other significant changes were found in the haematology, clinical chemistry and urine analysis. Histopathological evaluation revealed a wide variety of spontaneous neoplastic and non-neoplastic lesions that were considered to be mostly age-related and occurred with the same incidence and severity in control and treated animals. A slightly higher incidence of progressive nephropathy was found at 12 months in males exposed to 40 mg/m³, and in rats exposed to 400 mg/m³ that died or were euthanized before 18 months. No difference was observed at the 18 and 24 months. Chronic nephropathy was considered a commonly occurring lesion in the kidneys of aging rats (especially males).

NMP was reported to have no oncogenic potential.

Ref.: 24

Comment
The potential carcinogenicity of NMP has been investigated in two long-term studies with rats and one with mice. No oncogenic potential of NMP in rats was found after oral administration (highest dose 678 mg/kg bw/day) or inhalation exposure (highest dose 400 mg/m³; about 100 mg/kg bw/day). Chronic nephropathy, especially in males, was the main toxic effect recorded. In the oral mice study at the highest dose, an increased frequency of liver adenomas (males and females) and carcinomas (males) was found. No evidence of an

increase incidence of malignant tumours was seen at lower doses and there were no NMP-related neoplastic or non-neoplastic changes in other organs.

NMP is not considered to have genotoxic potential *in vivo*. Thus, the mice liver tumour at the highest dose tested may be induced by a non-genotoxic mechanism and is not relevant in relation to the low exposure from NMP in cosmetics.

3.3.8 Reproductive toxicity

3.3.8.1 Two generation reproduction toxicity

Oral

Rats

Wistar rats, groups of 25 males and 25 females were exposed to NMP at in the diet doses of 0, 50, 160, or 500 mg/kg bw/day. The first parental generation (P1) was exposed during a period prior to mating, gestation, lactation, and weaning of the litter (F1a) and during a period prior to a second mating, gestation, lactation, and weaning of the litter (F1b). The second parental generation (P2 = F1b) was exposed from day 21 postpartum as the P1 generation until the first litter (F2a) and the second litter (F2b) were delivered. The highest dose level caused decreased parental body weight and food consumption and a concomitant reduction in survival and growth rates in the offspring. The highest dose was reduced to 350 mg/kg bw/day from day 126 because of pup toxicity.

At 500 mg/kg bw/day, in the P1 generation:
- Decreases in body weights and feed consumption at the end of gestation (day 20) and the beginning of lactation (day 1). (It cannot be excluded that the reduction in the offspring weight may have contributed to the decrease in maternal weight at the end of gestation).
- No change in body weight for male.
- Statistically significantly increased number of stillborn F1a pups and kidney weights in P1 males.
- Statistically significantly decreased in body weight and in number of liveborn.
- Significant decreased in mean litter size at day 4.

At 500 mg/kg bw/day, in the F1 generation:
- Decrease in mean litter size, pup survival and pup body weights during lactation.

At 350 mg/kg bw/day:
- No maternal toxicity or reduced pup survival, no adverse effects in the P1 and F1 (only F1b was exposed at 350 mg/kg) generation including mortality, body weights, feed consumption, clinical observations, reproductive performance or fertility. No effects on histological male and female in the P1.

At 350mg/kg bw/day, in the F2 generation:
- Decreases in the number of pups surviving lactation, and in pup body weights.

No changes in microscopic evaluations of reproductive tissues as well in sperm assessments (P1-F1).

Post weaning developmental landmarks (day of preputial separation and vaginal opening) determined in F1 generation were not affected.

NOAEL for reproductive performance and fertility was 350 mg/kg bw/day for the F0 and F1 parental rats.

NOAEL for developmental toxicity was 160 mg/kg bw/day for F1 and F2 progeny.

Ref.: 39

Rats (30/sex/group) received 0, 50, 160, and 500 mg/kg bw/day by oral diet 10 days prior to mating and continuing throughout mating, gestation and lactation for both generations. Maternal toxicity was reported as reduced food intake, body weight, and/or body weight gain in the F0 and F1 generations at 500 mg/kg bw/day.

There was evidence of developmental toxicity in both generations at 500 mg/kg bw/day, as evidenced by reduced litter size, reduced postnatal survival and pup weight. Significant reductions in the male fertility index and the female fecundity index of the F1 generation were also reported, without a clear NOAEL. Clear adverse effects were found at 500 mg/kg bw/day (Fertility index of males mated twice: 93-83, 72-69, 72-60, and 47-35 in the control, low-, mid- and high-dose groups, respectively. Fecundity index of females mated twice: 96-93, 82-74, 75-64, 61-50 in the control, low-, mid-, and high-dose groups, respectively).

There was also an increased incidence of F1 females with decreased corpora lutea at the high-dose. Histological changes were noted at the high dose. There was a reduced incidence of females with pigmented macrophages, which usually occur at the implantation sites (24, 18, 19, and 11 dams at 0, 50, 160, and 500 mg/kg bw/day, respectively). This correlates well with the findings that 1, 6, 5, and 13 animals at 0, 50, 160, and 500 mg/kg bw/day, respectively, never became pregnant. There was also an increased incidence of females with reduced corpora lutea in the treated groups (1, 3, 5, and 17 dams at 0, 50, 160, and 500 mg/kg bw/day, respectively). Hypospermia was observed in 3 males at 500 mg/kg. Diffuse bilateral testicular atrophy was observed in 1 male at the mid-dose and in 3 males at the high-dose (0 in control). Diffuse unilateral atrophy occurred in 1 male at 50 mg/kg bw/day and in 1 male at 160 mg/kg bw/day.

No other information is available.

Ref.: 40

Inhalation

Rats

In a two-generation reproduction study, rats (Crl:CD) (10 males and 20 females per dose level) were exposed whole body to 0, 41, 206, or 478 mg/m³ of NMP vapour (relative humidity 40–60%) for 6 h/day, 7 days/week, for a minimum of 14 weeks (P0 generation). The P0 generation was 34 days old at exposure onset. At 119 days of age, one male and two females from the same exposure group were allowed to mate. The P0 males were exposed for >100 days (pre-mating and mating periods), and the females were exposed for >106 days (pre-mating, mating, gestation, and lactation periods). At the end of the mating period, 50% of the P0 males were sacrificed and examined for adverse reproductive effects. The other 50% of the P0 males were examined 21 days later (recovery period). From the delivered offspring, exposed from day 4 postpartum, one male and one female per litter were examined for adverse reproductive effects on day 21 postpartum. The remaining offspring were designated as the F1 generation. At the end of the weaning period, the P0 dams were sacrificed and examined for adverse effects on reproduction. In parallel, the sex-specific effects of exposure to 0 and 478 mg/m³ vapour for 6 h/day, 7 days/week, for a minimum of 14 weeks were studied by cross-mating of exposed and unexposed males and females from the F1 generation for production of an F2 generation. No effects on body, testes, or ovarian weights or on reproductive ability were recorded. A 4–11% decrease in pup weight of the F1 offspring whose parents both inhaled NMP was observed from day 1 to day 21 postpartum, but not at day 28 postpartum. This effect was not clearly dose related

and reached statistical significance for the low and high, but not for the intermediate, exposure groups.

Ref.: 41

In a reproduction study, male rats (12 per dose level) were exposed whole body to 0 or 618 mg NMP/m³ (vapour; <50% relative humidity) for 6 h/day, 7 days/week, for 90 days. There were no abnormal histopathological changes or differences in testis weights when rats were examined at the termination of exposure and 90 days later. Nor were there any abnormalities of the semen, sperm cell morphology, or cell concentration.

Ref.: 42

3.3.8.2 Developmental toxicity

Dermal

Rats

In a range-finding study of developmental toxicity, pregnant Sprague-Dawley rats (3–5 per exposure level) were exposed to daily dermal doses (not occlusive, 25 cm², 8 hr/day) of 0, 500, 1100, or 2500 mg NMP/kg bw during days 6 through 15 of gestation. At 2500 mg/kg: all dams died or aborted prior to caesarean. At 1100 mg/kg bw/day: Depressed maternal weight gain during gestation, 4/5 litters completely resorbed. At 500 mg/kg bw/day: No evidence of adverse effects on the mother and the conceptus.

Ref.: 43

In a developmental toxicity study, pregnant Sprague-Dawley rats (about 22 per dose level) were administered daily dermal NMP doses (not occlusive, 25 cm², 8 hr/day) of 0, 75, 237, or 750 mg/kg bw/day during days 6 through 15 of gestation.

Maternal toxicity:
- Patches of dry skin at the application site, the severity of which increased with the dose.
- At the high dose, decrease in the body weight gain during gestation. No information available on maternal weight gain minus uterine weight on GD 21.
- No maternal effects at 75 and 237 mg/kg bw/day.

Developmental toxicity:
- At 750 mg/kg bw/day: Increase in the incidence of resorptions, decreases in the number of viable foetuses and in the foetal body weight (20 %).

Delayed ossification of several bones (i.e. skull, hyoid, sternebrae, vertebrae) and increase in the incidence of extra ribs. Skeletal malformations including fused/split ribs (8 foetuses from 5 litters), and fusion of the exoccipital and atlas bones (4 foetuses from 4 litters). No increase in the incidence of soft tissue variations or malformations.
- No treatment-related effects at 75 and 237 mg/kg bw/day.

NOAEL for developmental toxicity: 237 mg/kg bw/day. NOAEL for maternal toxicity: 237 mg/kg bw/day. The lower maternal weight may be due, at least partly, to the increased resorption rate and the lower foetal body weight.

Ref.: 43

Rabbits

The maternal toxicity in rabbits after dermal application was studied in a range-finding study. Pregnant rabbits (15 per dose level) were exposed daily to dermal doses of 0, 400,

600, or 800 mg/kg bw/day (as 40% aqueous solution). There was maternal toxicity, expressed as prolonged clotting time at 800 mg/kg bw (BASF, 1993a).

Ref.: 44

In a developmental toxicity study, 15 Himalayan pregnant rabbits per dose level were exposed daily by dermal application (semi-occlusive dressing 6hr/day) to 0, 100, 300, or 1000 mg NMP/kg bw for 6 h/day on days 7–19 post-insemination. The application doses were made as 40% aqueous solution. There were no signs of maternal toxicity (death, food consumption, body weight, uterus weight), nor local effects at the application site. There was a significant increase in the incidence of foetuses with skeletal alterations, due to the occurrence of accessory 13^{th} ribs. At 1000 mg/m³, their foetal and litter incidences were 15% and 60%, respectively (historical value 8.4 and 40 %, respectively). There was no effect on foetal body weight, or on the incidence of external, soft tissue and skeletal malformations. The NOAEL was set at 300 mg/kg bw/day.

Ref.: 44

Inhalation

Rats

In a developmental toxicity study, pregnant rats (25 per dose level) were exposed whole body to 0, 100, or 360 mg NMP/m³ (100% pure) for 6 h/day on days 6–15 of gestation. The exposure consisted of a mixture of aerosol/vapour of unknown particle size distribution. No effects of the NMP exposure on the outcome of pregnancy, embryonal growth rate, or development in vital organs and skeletons of the foetuses were found. Nor were there abnormal clinical signs or pathological lesions in the maternal rats. During the first 3 days, lethargy and irregular respiration were observed in the dams exposed to 100 mg/m³.

Ref.: 24

In a developmental study, pregnant rats (Mol:WIST) (27 in the control group and 28 in the exposed group) were exposed whole body to 0 or 680 mg NMP/m³ (≥ 99.5% pure, vapour; <50% relative humidity) for 6 h/day on days 4–20 of gestation. The dose was chosen to correspond to the "worst-case" level of human exposure.

No maternal toxicity was reported (mortality, clinical signs, no reduction in food consumption and in body weight changes, including weight gain corrected from uterus weight). There were significantly more dams with pre-implantation loss (11/20 and 20/23 at 0 and 680 mg NMP/m³, respectively). However, there were no significant differences in the incidence of pre-implantation loss/litter (13.4 and 20.5% at 0 and 680 mg NMP/m³) and in the number of implantations. No effects on corpora lutea, live foetuses and resorptions were found apart from a slight decrease in foetal body weight (significant difference only when adjusted for litter size). The incidence of bones showing delayed ossification tended to increase and was significantly higher for digits and cervical vertebrae. No treatment-related malformations were observed.

Ref.: 45

In a neuro-behavioural teratology study, pregnant rats (Mol:WIST) were exposed whole body to 0 or 622 mg NMP/m³ (≥ 99.5% pure, vapour; <50% relative humidity) for 6 h/day on days 7–20 of gestation. The dose was chosen to minimize maternal toxicity and offspring mortality, based on earlier experience in the laboratory. Maternal weight development during days 7–20 was 15% slower among the exposed dams (no statistical analysis reported). In the exposed group, a lower body weight of the pups and slight delay in achieving some developmental milestones in the pre-weaning period were observed. While most of the behavioural tests gave similar results for the exposed and control animals, an occasionally increased latency in Morris swimming maze and a statistically borderline

impairment in operant behaviour with delayed spatial alternation were noted among the exposed offspring.

Ref.: 46

In a developmental study, pregnant rats (Sprague-Dawley) (25-26 pregnant female/dose) were exposed whole body to 0, 124, 247, or 494 mg NMP/m³ (≥ 99.5% pure, vapour; <45.5% relative humidity) for 6 h/day on days 6–20 of gestation. All the animals survived the exposure.

Maternal body weight gain was significantly decreased at 247 and 494 mg/m³ on GD 6–13 and maternal food consumption was reduced at 494 mg/m³ on GD 13–21. No significant difference in the gestational weight change corrected for the weight of the gravid uterus was observed, whatever NMP concentration. There were no adverse effects on embryo/foetal viability or evidence of teratogenicity at any concentration tested. Foetal toxicity indicated by reduced foetal weight was observed at 494 mg/m³.

The authors concluded that inhalation exposure of pregnant rats to NMP (vapours) during the entire post-implantation phase of gestation is neither teratogenic nor embryo-lethal. Evidence of developmental toxicity was limited to intrauterine growth retardation that occurred in the presence of maternal toxicity.

The NOAEL for maternal and developmental toxicity was 124 and 247 mg/m³, respectively.

Ref.: 54

Rabbits

In a pre-test of developmental toxicity, five pregnant rabbits per dose level were exposed to 0, 300, 1000, or 2000 mg NMP/m³ (vapour/aerosol; MMAD 3.8–4.0 µm) for 6 h/day on days 7–19 post-insemination. Maternal toxicity was expressed as prolonged clotting time, decreased plasma protein content, and increased liver weight at both 1000 and 2000 mg/m³. Small but dose-related decrease was observed in gravid uterine weight (99, 90, 82 and 71 g at 0, 300, 1000, and 2000 mg/m³. Concomitant findings included a dose-related decrease in the number of foetuses (which attained statistical significance at the high dose), and a statistical increase in post-implantation loss at 2000 mg/m³.

In the main study, pregnant rabbits (15 per dose level) exposed head only for 6 h/day to 0, 200, 500, or 1000 mg NMP/m³ (vapour/aerosol; MMAD 2.7–3.5 µm) on days 7–19 post-insemination. No effects on maternal body weight (corrected and uncorrected for uterus weight), food consumption, uterus weight were found. Likewise, no effects on implantations, number of resorptions and live foetuses or foetal body weight were found. At 1000 mg/m³, a slight foetal toxicity was seen as increased occurrence of skeletal variations (accessory 13th ribs) (BASF, 1993b). The two studies show NOAELs for developmental and maternal toxicity of 500 mg/m³.

Ref.: 47, 48

Oral

Rats

In a developmental study, pregnant rats (Crl:CD) (25 per dose level) were given daily NMP (100% pure) doses of 0, 40, 125, or 400 mg/kg bw by oral gavage (5 ml/kg bw) on days 6–15 of gestation. Maternal and foetal toxicity were observed at the highest dose level compared with controls.

- Maternal toxicity: There were no treatment-related clinical observations.

Body weight gain was depressed during treatment at 400 mg/kg bw (GD 6-9, GD 9-12, GD 6-15) (14, 18, and 53 g, respectively at 0 mg/kg bw compared to 7, 15 and 42 g, respectively at 400 mg/kg bw). However, there was no statistical difference in weight gain during the overall gestation period (GD 0-21) and after correction for gravid uterine weight. No changes in food consumption were found.
- Developmental toxicity:
At 400 mg/kg bw/day: Reduced foetal body weight (10-11 %) and an increased incidence of stunted foetuses (foetuses: 1/340, 1/393, 2/395, and 12/397; litters: 1/21, 1/25, 2/24, and 6/25; at 0, 40, 125 and 400 mg/kg bw, respectively). No teratogenic effects.

NOAEL for maternal and developmental toxicity: 125 mg/kg bw/day.

Ref.: 49

In another developmental toxicity study, orally doses of 55, 175, or 540 mg NMP/kg bw/day were administered to pregnant rabbits (15 - 20 per dose level) on days 6–18 of gestation.
- Maternal toxicity:
Decreased food intake and weight gain during dosing at 175 and 540 mg/kg bw/day.
- Developmental toxicity:
At 540 mg/kg bw/day: Increased incidences of resorptions. Cardiovascular malformations and malformed skull bones. Increased incidence of misshapen skull bones and of 27 presacral vertebrae.

NOAEL for maternal toxicity: 55 mg/kg bw/day. NOAEL for developmental toxicity: 175 mg/kg bw/day.

Ref.: 50

Daily doses of 0, 332 or 997 mg NMP/kg bw/day were administered to Sprague-Dawley rats by gavage on days 6–15 of gestation.
At 997 mg/kg bw/day: Marked reductions in maternal body weight and placental weight were observed. There was a large number of resorptions (24/29 dams showed complete resorption) and only 15 live and 1 dead foetus were present at term. Observations in the live foetuses included reduction in foetal weight (37%), malformations considered as indicative of foetal retardation in 8 out of 15 foetuses), and 14 runts.
At 332 mg/kg bw/day: Maternal body weights were not reported. Placental and foetal weight was lower than control (14-20% and 10% respectively).
There was no difference in implantation rate, litter size or resorptions.

LOAEL of maternal and foetal toxicity 332 mg/kg bw/day.

Ref.: 51

The developmental toxicity of NMP was studied in Sprague–Dawley rats after oral administration. Daily doses of 0, 125, 250, 500, and 750 mg/kg bw/day were administered by gavage, on gestational days (GD) 6 through 20.

Significant decreases in maternal body weight gain and food consumption during treatment, and a reduction in absolute weight gain were observed at 500 and 750 mg/kg bw/day. The incidence of resorptions per litter was significantly higher than in controls at 500 mg/kg bw/day, and rose to 91% at 750 mg/kg bw/day. Examination of the foetuses revealed treatment-related malformations, including imperforate anus and absence of tail, anasarca, and malformations of the great vessels and of the cervical arches. The incidence of malformed foetuses per litter and of litters with malformed foetuses was significantly increased at 500 and 750 mg/kg bw/day.

At 250 mg/kg bw/day, one foetus showed malformations similar to those recorded at higher dosages. There was a dose-related decrease in foetal body weights (male, female, and total) that reached statistical significance at 250 mg/kg. A significant increase in incomplete

ossification of skull bones and of sternebrae was also present at 500 and 750 mg/kg bw/day.

In summary, NOAEL for maternal and developmental toxicity was 250 and 125 mg/kg bw/day, respectively. Thus, oral administration of NMP produced developmental toxicity below maternally toxic levels. The authors state that the study was conducted according to the current OECD and EU guidelines.

Ref.: 53

The developmental toxicity of the three main metabolites of NMP was studied in Sprague-Dawley rats. Pregnant rats (groups of 18-24 rats) were given 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP; 0, 250, 500, 750, or 1000 mg/kg bw/day), N-methylsuccinimide (MSI; 0, 500, 750, 1000, or 1250 mg kg bw/day), or 2-hydroxy-N-methylsuccinimide (2-HMSI; 0, 250, 500, 1000, or 1500 mg kg bw/day), by gavage, on gestational days (GD) 6–20.

No evidence of maternal toxicity was observed in dams given 5-HNMP. Administration of 2-HMSI resulted in overt maternal toxicity at 500 mg kg bw/day and higher doses, as indicated by a significant reduction in weight gain and food consumption at the beginning of treatment. There was no evidence of embryo/foetal toxicity in any of the groups treated with 5-HNMP or 2-HMSI. MSI produced marked developmental toxicity in the presence of maternal effects. Maternal body weight gain and food consumption were affected at 750 mg kg bw/day MSI, and above. A significant increase in post-implantation loss occurred at 1250 mg kg bw/day MSI, and the incidence of foetuses with external or with visceral malformations was significantly increased at 1000 and 1250 mg kg bw/day MSI. Malformations mainly consisted of anasarca, cardiovascular defects and diaphragmatic hernia. Foetal weight was significantly reduced at 1000 and 1250 mg kg bw/day. The incidence of skeletal variations (predominantly cervical ribs, and delayed ossification of skull bones and sternebrae) was significantly elevated at 750 mg kg bw/day and higher doses. However, MSI was much less potent than the parent compound.

The authors concluded that the results indicate that the embryotoxic and teratogenic effects of NMP are not attributable to the above metabolites.

Ref.: 55

Mice

Oral daily doses of 0, 1055, or 2637 mg/kg bw/day on days 11–15 of gestation in mice caused an increase in resorption rate, increased incidence of runts, diminished foetal weight and length, and an increased rate of malformations such as cleft palate at the higher dose level. The lower dose level caused no observable embryotoxicity. Both developmental and maternal toxicity are insufficiently reported, and the exposure covers only a part of organogenesis.

Ref.: 51

<u>Intraperitoneal injection</u>

Mice

An intraperitoneal daily dose to mice of 0, 630, or 1570 mg/kg bw on days 11–15 of gestation caused increased resorption rate, increased incidence of runts, diminished foetal weight and length, and an increased rate of malformations such as cleft palate at the high level. No maternal toxicity was observed. The low dose level caused no observable embryotoxicity. No information on maternal toxicity is given in this study; thus, evaluation of the results is difficult (US EPA, 1988).

Ref.: 51

NMP doses of 14–166 mg/kg bw singly or repeatedly intra-peritoneally administered to mice during various phases of pregnancy caused increased post-implantation loss and a reduced body weight of the foetuses. Morphological defects such as exencephaly, open eyelids, microphthalmia, cleft palate, oligodactyly, shortened or kinked tails, fusions and curvature of neck and chest vertebrae, and fusion of sternebrae and ribs were observed. The LOAEL for repeated doses was 74 mg/kg bw administered on days 7–11 of gestation. No information on maternal toxicity is given in this study; thus, evaluation of the results is difficult

Ref.: 52

The results from the reproductive studies of NMP in animals are summarized in Table 1.

Table 1: Summary of reproductive toxicity of NMP in animals

Species; type of study	Exposure (mg/kg bw/d)	Toxicity		NOAEL/LOAEL (mg/kg bw/day)	Reference
		Foetal	Maternal		
Dermal					
Rat; range-finding developmental toxicity study; dermal; days 6–15	0 500 1100 2500		None None Massive resorption; decreased bw gain Lethal	Maternal toxicity: NOAEL = 500 mg/kg bw/day	Becci et al., 1982 (43)
Rat; developmental toxicity study; dermal; days 6–15	0 75 237 750	None None None Increased resorption, delayed ossification	None None None Decreased body weight gain	Developmental toxicity: NOAEL = 237 mg/kg bw/day; Maternal toxicity: NOAEL = 237 mg/kg bw/day	Becci et al., 1982 (43)
Rabbit; developmental toxicity study; dermal; days 7-19; 40% aqueous solution	0 100 300 1000	None None None Increased number of foetuses with skeletal alterations	None None None None	Developmental toxicity: NOAEL = 300 mg/kg bw/day	BASF, 1993a (44)
Rabbit; maternal toxicity; dermal; 40% aqueous solution	0 400 600 800		None None None Increased clotting time	Maternal toxicity NOAEL = 600 mg/kg bw/day	BASF, 1993 a (44)
Oral					
Rat; two-generation	0 50 160 500-350	None None None Decreased number of pups	None None None None	NOAEL reproductive performance and fertility: 350 mg/kg bw/day Developmental toxicity: 160 mg/kg bw/day	BASF, 1999 (39)
Rat; two generation. Staring 10 days prior to mating.	0 50 160 500	None None None Decreased number of pups	None None None Reduced body weight	NOAEL reproductive performance and developmental toxicity: 160 mg/kg bw/day	OEHHA, 1999 (40)
Rat; developmental study, days 6-15, gavage 5 ml/kg	0 40 125 400	None None None Reduced foetal weight and stunted foetuses	None None None Bodyweight gain depressed	Maternal and developmental toxicity: NOAEL = 125 mg/kg bw/day	EXXON, 1992 (49)

Species; type of study	Exposure (mg/kg bw/d)	Toxicity		NOAEL/LOAEL (mg/kg bw/day)	Reference
		Foetal	Maternal		
Rat, developmental study, days 6-15,	0 332 997	None Foetal weight reduced Increased resorptions	None Placental weight reduced Maternal weight reduced	Maternal and foetal toxicity: LOAEL = 332 mg/kg bw/day	EPA, 1988 (51)
Rat, developmental study, days 6-20	0 125 250 500 750	None None Decreased foetal weight Malformation Malformation	None None None Reduced weight gain Reduced weight gain	Maternal toxicity: NOAEL = 250 mg/kg bw/day Developmental toxicity: NOAEL = 125 mg/kg bw/day	Saillenfait et al., 2002 (53)
Mouse, developmental study, days 11-15	0 1055 2637	None Increased resorption, malformations	-	Both developmental and maternal toxicity are insufficiently reported.	EPA, 1988 (51)
Rabbit; developmental study, days 6-18.	0 55 175 540	None None None Increased resorptions, malformations	None None Decreased weight gain Decreased weight gain	Maternal toxicity: NOAEL = 55 mg/kg bw/day Developmental toxicity: NOAEL = 175 mg/kg bw/day	GAF, 1992 (50)
Inhalation					
Rat; two-generation; inhalation (whole body), 6 h/day, 7 days/week	0 mg/m^3 41 mg/m^3 206 mg/m^3 478 mg/m^3	None None None Pup body weight decrease (4–11%)	None None None Decrease in response to sound	Reproductive toxicity: NOAEL = 206 mg/m^3; Maternal toxicity: NOAEL = 206 mg/m^3;	Solomon et al., 1995 (41)
Rat; testes and semen toxicity study; inhalation (whole body); 6 h/day, 7 days/week; <90 days	0 mg/m^3 618 mg/m^3	None None	None None	Reproductive toxicity: NOAEL = 618 mg/m^3	Fries et al., 1992 (42)
Rat; developmental toxicity; inhalation (whole body); days 4–20, 6 h/day	0 mg/m^3 680 mg/m^3	None Increased pre-implantation loss but no effect on number of implantations per dam or number of live foetuses; delayed ossification	None None	Developmental toxicity: LOAEL = 680 mg/m^3 Maternal toxicity: NOAEL = 680 mg/m^3	Hass et al., 1995 (45)
Rat; developmental toxicity; inhalation (whole body); days 7–20, 6 h/day	0 mg/m^3 622 mg/m^3	None Decreased body weight; neuro-behavioural effects	None None	Developmental toxicity: LOAEL = 622 mg/m^3 Maternal toxicity: NOAEL = 622 mg/m^3	Hass et al., 1994 (46)
Rat; developmental toxicity; inhalation (whole body); days 6–15, 6 h/day	0 mg/m^3 100 mg/m^3 360 mg/m^3	None None None	None None Lethargy and irregular respiration during the first 3 days of exposure	Developmental toxicity: NOAEL = 360 mg/m^3 Maternal toxicity: NOAEL = 100 mg/m^3	Lee et al., 1987 (24)
Rat; developmental toxicity; inhalation (whole body); days 6-20, 6 h/day	0 mg/m^3 124 mg/m^3 247 mg/m^3 494 mg/m^3	None None None Reduced foetal weight	None None Reduced weight gain Reduced weight gain	Developmental toxicity: NOAEL = 247 mg/m^3 Maternal toxicity: NOAEL = 124 mg/m^3	Saillenfait et al., 2003 (54)

Species; type of study	Exposure (mg/kg bw/d)	Toxicity		NOAEL/LOAEL (mg/kg bw/day)	Reference
		Foetal	Maternal		
Rabbit, developmental toxicity; inhalation (whole body); days 7-19, 6h/day	0 mg/m^3 200 mg/m^3 500 mg/m^3 1000 mg/m^3	None None None Slight foetal toxicity	None None None Small decrease in gravid uterine weight	Developmental and maternal toxicity: NOAEL = 500 mg/m^3	BASF, 1991, 1993b. (47, 48)
Intraperitoneal injection					
Mouse, developmental toxicity; days 11-15	0 630 1570	None Increased resorption rate and malformation	-	Developmental toxicity: LOAEL = 630 mg/kg bw/day	EPA, 1988 (51)
Mouse, developmental toxicity, days 7-11	14-166	Reduced foetal weight and malformation	-	LOAEL for repeated dose = 74 mg/kg bw/day	Schmidt, 1976 (52)

Comments
The developmental effects consisted in embryolethal, teratogenic, and foetotoxic effects after oral (gavage) and dermal administration in rats, after oral (gavage) treatment in rabbits, and *i.p.* injection of mice. Teratogenic effects were not observed after inhalation exposure of rats and rabbits.

In addition, foetal toxicity, expressed as reduced foetal body weight, occurred in the absence of significant maternal toxicity in rats treated by gavage. In one experiment, a foetus showing the characteristic malformations elicited by NMP was also observed at a dose level with no maternal toxicity.

It is considered unlikely that the embryolethality and the foetal malformations could have been secondary to the general toxicity of NMP.

The developmental effects were specific and severe and are considered the most important reprotoxic effect in relation to NMP. As a consequence the NOAEL should be based on developmental toxicity. Table 2 list the lowest NOAEL from the different experiments.

NMP has been classified due to its reprotoxic effect as category 1b; H360: May damage fertility or the unborn child (Previously, Reprotox. Cat.2; R 61: May cause harm to the unborn child).

Table 2: NOAELs for developmental toxicity

Exposure	NOAEL (mg/kg bw/day)	Reference
Dermal		Becci et al., 1982 (43) BASF, 1993a (44)
rat	237	
rabbit	300	
Oral		Sailenfait et al., 2002 (53) GAF, 1992 (50)
rat	125	
rabbit	175	
Inhalation		
rat	60 (247 mg/m^3 6h)	Sailenfait et al., 2003 (54)
rabbit	48 (500 mg/m^3 6h)	BASF, 1991, 1993b (47, 48)

The lowest NOAEL after oral administration of NMP in rats (125 mg/kg bw/day) and will be used in calculation of MOS. This NOAEL was reported for developmental toxicity in two independant studies (49, 53). It is noted that teratogenic effects were not observed after inhalation exposure.

The NOAEL of 48 mg/kg bw/day from the inhalation study of rabbits was used for setting specific concentration limit for NMP (see section 3.3.12 Special investigations). SCCS consider this value uncertain. The experiment was performed prior to 1991, it is not up to modern standards and the description of the experiment is unsatisfactory.

3.3.9 Toxicokinetics

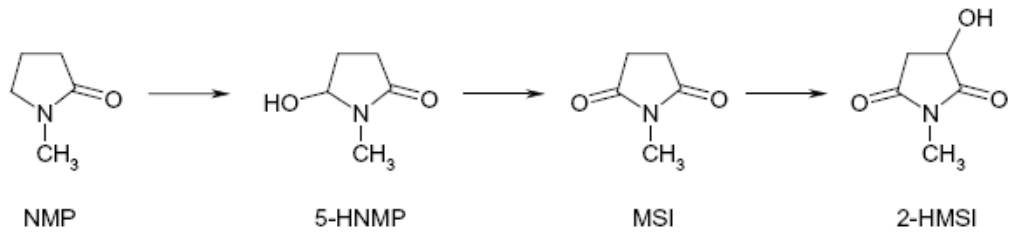


Fig. 1: Proposed metabolism of NMP (Taken from ref. 59)

A metabolic pathway has been suggested for humans: NMP is first hydroxylated to 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP), and then oxidised to N-methylsuccinimide (MSI), which in turn is hydroxylated to 2-hydroxy-N-methylsuccinimide (2-HMSI) (Fig. 1).

In humans, as in rats, NMP is rapidly absorbed via inhalation (56), ingestion (56), and dermal administration (57). An uptake of about 90% by the inhalation route was found when the difference between inhaled and exhaled NMP concentrations was calculated. The peak plasma concentrations after an 8-h exposure to NMP occurred at the termination of exposure for NMP, at 2 h post-exposure for 5-HNMP, at 4 h post-exposure for MSI, and at 16 h post-exposure for 2-HMSI. The half-lives in plasma after a short period of distribution were 4 h, 6 h, 8 h, and 16 h, respectively. The detected amounts in urine after inhalation were as follows: NMP (2%), 5-HNMP (60%), MSI (0.1%), and 2-HMSI (37%). The recovery was about 100%.

After oral administration, the amounts detected in urine were as follows: NMP (1%), 5-HNMP (67%), MSI (0.1%), and 2-HMSI (31%), corresponding to 65% of the administered dose. There was no tendency for coloration in any of the urine samples collected, and none of the synthesized metabolites was coloured (56, 57, 58).

In a 6-h topical single-application study with administration of 300 mg NMP in volunteers (six per sex), the NMP concentration in plasma reached a maximum 3 h after application in both males and females. Twenty-four per cent and 22% of the dose in males and females, respectively, were recovered in urine as NMP and NMP metabolites (58).

Carnerup and co-workers studied the metabolism of NMP in rats in order to determine the biological levels after one oral developmentally toxic dose of NMP. Non-pregnant female Sprague-Dawley rats were given an oral single dose of either a non-toxic dose of 125 mg NMP/kg bw (group 1) by gavage or a developmentally toxic dose of 500 mg/kg bw (group 2). Blood plasma (7 rats per time point) and urine (10 rats per time point) were sampled up to 72 h after administration and analyzed using mass spectrometry. In both plasma and urine NMP, 5-HNMP, MSI, and 2-HMSI and 2-pyrrolidone (2-P) were identified. In urine 48% of the administered dose was recovered as 5-HNMP and 2–5% as 2-HMSI. The total recovery in urine was 53–59%. The peak concentrations for NMP in plasma were 1.2 and 6.9 mmol/l, 0.42 and 0.76 mmol/l for 5-HNMP, 0.07 and 0.31 mmol/l for MSI and for 2-HMSI the concentrations were 0.02 and 0.05 mmol/l for groups 1 and 2, respectively. The authors concluded that, the same metabolites were found in rats as in humans. However, there were some notable differences. The mean urinary excretion of 5-HNMP and 2-HMSI in volunteers orally exposed to NMP corresponded to 44% and 20%, respectively, of the

administered dose. The mean total recovery was 65% In rats, the relative excreted amount of 5-HNMP was higher than in humans while it was 4–9 times lower for 2-HMSI.

Ref.: 59

The relative embryotoxicity of the NMP and its metabolites was evaluated using rat whole embryo culture (WEC) and the BALB/c 3T3 cytotoxicity test. The resulting data were evaluated using two strategies; namely, one based on using all endpoints determined in the WEC and the other including endpoints from both the WEC and the cytotoxicity test. On basis of the first analysis, the substance with the highest embryotoxic potential is NMP, followed by 5-HNMP, 2-HMSI and MSI. Specific dysmorphogeneses induced by NMP and 5-HNMP were aberrations in the head region of the embryos, abnormal development of the second visceral arches and open neural pores. The second evaluation strategy used only two endpoints of the WEC, i.e. the no observed adverse effect concentration (NOAECWEC) and the lowest concentration leading to dysmorphogenesis in 100% of the cultured embryos (ICMax WEC). In addition to these WEC endpoints the IC_{50} 3T3 from the cytotoxicity test (BALB/c 3T3 fibroblasts) was included in the evaluation scheme. These three endpoints were applied to a prediction model developed during a validation study of the ECVAM allowing the classification of the embryotoxic potential of each compound into three classes (non-, weakly- and strongly embryotoxic). Consistent results from both evaluation strategies were observed, whereby NMP and its metabolites revealed a direct embryotoxic potential. Hereby, only NMP and 5-HNMP induced specific embryotoxic effects and were classified as weakly embryotoxic, whereas the other two metabolites, 2-HMSI and MSI, were determined to be non-embryotoxic.

The authors concluded that the results of this *in vitro* study support the hypothesis that NMP, the parent substance, is responsible for the embryotoxic effects observed in vivo whereby its main metabolite, 5-HNMP, may be involved to some extent. The latter metabolites (MSI, 2-HMSI) may be considered as detoxification steps. The authors pointed out that according to the general principles of allometry this would mean that in case of a non-inhalation route of exposure, e.g., after dermal exposure, humans should be about four times more sensitive than rats, unless comparative metabolism studies in rats and humans suggest something different.

Ref.: 60

The involvement of cytochrome P450 2E1 (CYP2E1) in the metabolism of NMP was studied with three experimental approaches: in the rat, in vitro in human microsomes, and in human volunteers. NMP was administered dermally (40 mg/kg bw) to OFA rats to examine the influence of CYP2E1 inhibition. CYP2E1 inhibition led to a statistically significant retardation of 5-HNMP excretion in urinary fractions collected during the first 12 h. In the group of fasted rats, a two-fold increase of CYP2E1activity was observed in comparison with the control group. During the first 6 h after dermal administration of NMP to fasted rats, about 33% of the dose was excreted in urine versus 22% in controls. In vitro, NMP (15 mM) was incubated (up to 120 min) with human liver microsomes and the formation of 5-HNMP followed Michaelis-Menten kinetics with V_{max} of 1.1 nmol/min per mg protein and Km of 2.4 mM. The formation of 5-HNMP was inhibited by 35% in the presence of a monoclonal antibody against CYP2E1, but not by CYP1A2 antibody. In a dermal application experiment, 12 human volunteers were exposed by means of a dermal patch to 300 mg NMP; five urine fractions were collected during the 48 h following the onset of application in order to measure the major metabolites 5-HNMP and 2-HMSI. Before NMP application, a blood sample was collected for the quantification of CYP2E1 mRNA in peripheral blood lymphocytes (PBLs). The mean dermal absorption of NMP was 67.9%. The highest amount of 5-HNMP was excreted in urine in the fraction collected between 6–12 h (12.6% of dose), while 2-HMSI peaked in fractions 12–24 h and 36–48 h (3.3 and 3.2% of dose, respectively). A significant relationship was found between CYP2E1 mRNA content in PBLs and the amount of both the metabolites excreted in urine within 24 h ($r2=0.54$, $P<0.01$). It

is concluded that CYP2E1 is involved in the first steps of NMP metabolism in the rat and, to a lesser extent, in humans.

Ref.: 61

3.3.10 Photo-induced toxicity

No data submitted

3.3.11 Human data

A 23-year-old laboratory technician was occupationally exposed to NMP during her first 20 weeks of pregnancy. The uptake via the lungs was probably of minor importance, as the NMP was handled at room temperature. Hand rinsing of glassware with NMP and cleaning up of an NMP spill in week 16 of pregnancy may have brought about a much larger uptake through the skin. During the 4 days following the spill, malaise, headache, and nausea were experienced. Examination of the pregnancy at week 14 showed no signs of delayed development; however, at week 25, signs of delayed foetal development were observed, and at week 31, a stillborn foetus was delivered. Stillbirth in this period of pregnancy is unusual. However, as the level of exposure is unknown, it is impossible to establish if exposure to NMP is the causative factor.

Ref.: 62, 63

A total of 15 24-h exposures in a repeated-insult patch test in human subjects (n = 50) caused minor to moderate transient irritations. No signs of contact sensitization were observed. Direct contact of skin with NMP caused redness, swelling, thickening, and painful vesicles when NMP was used as a cleaner (10) or as a paint stripper (64).

Workers exposed to NMP in working areas with air concentrations up to 280 mg/m³ reported severe eye irritation and headache. With the methods of assessing the exposure level (sampling on charcoal and tracer gas method) and the response (observation and informal interview), it is impossible to develop a concentration–response relationship (12). Six volunteers exposed to 10, 25, or 50 mg/m³ during 8 h in a chamber study registered their symptoms, before the start of exposure and then every 2 h for 16 h, in a questionnaire on a scale from 0 to 10 (0 = no symptoms and 10 = not tolerated). The volunteers displayed none of the following symptoms: eye or respiratory tract irritation; hacking cough, nose secretion, or blockage, sneezing, itching, or dryness in the mouth and throat, or other symptoms in upper airways; itching, secretion, smarting pain, visual disturbances, or other symptoms such as headache, dizziness, and nausea; and other symptoms. Two volunteers reported detecting an odour at 50 mg/m³. There were no significant differences in the spirometric data displayed by the forced expiratory volume in 1 s, vital capacity, and the highest forced expiratory capacity measured before or after any level of exposure. There were no acute changes in the nasal cavity assessed by continuous acoustic rhinometry. Even though the effects observed in this study were not very pronounced, the possibility of undetected effects still remains (the number of volunteers was only six) (56).

3.3.12 Special investigations

Setting specific concentration limits for classified substances

Under the chemical legislation, the specific concentration limit (SCL) concept allows a fine tuning of the contribution of a certain hazardous substances to the classification of mixtures based on the potency of the substances. There is no detailed and accepted guidance yet developed for the setting of specific concentration limits (SCLs) for reproductive toxicity, as is the case for e.g. carcinogenic substances.

A method named "the German method" has been used for a few substances toxic for reproduction, including NMP. The method is not validated. An EU expert group (linked to ECHA) is currently working to develop "Guidance for setting specific concentration limits for substances classified for reproductive toxicity according to the CLP regulation (EC/1272/2008)". This will probably be finalised in 2011.

The specific concentration limit according to the German method is calculated from the formula:

SCL = NOAEL x 100/1000

In the case of NMP the lowest NOAEL was found for developmental toxicity in rabbits after inhalation (NOAEL = 48 mg/kg bw/day, see Table 2).

SCL = 48 mg/kg bw/day x 100/1000 mg/kg bw/day = 4.8% ~ 5%.

Ref.: 66

3.3.13 Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

N-Methyl-2-pyrrolidone (NMP)

The safety calculation is only considering dermal exposure

Exposure 17.8 g/day, 5% NMP (17.8 x 0.05x1000)		=	890 mg/day
Maximum absorption through the skin (100%)	890 x 1	=	890 mg/day
Typical body weight of human		=	60 kg
Systemic exposure dose (SED)	890/60	=	14.8 mg/kg bw/day
NOAEL, for developmental effect, rat, oral exposure		=	125 mg/kg bw/day
MOS	**NOAEL / SED**	**=**	**8.4**

The MOS is too low to be accepted.

3.3.14 Discussion

The safety has only been evaluated for dermal exposure.
The final concentration of NMP in cosmetic products is not known. It is noted that NMP may enhance the dermal absorption of other cosmetic ingredients.

Physico-chemical properties
NMP is colourless liquid with mild amine odour. It is completely miscible with water. It is only slowly oxidized by air. No information on the stability of NMP in cosmetic products is available.

Acute toxicity
NMP has low acute toxicity by oral, dermal, and inhalation routes.

Irritation /sensitization
Slight or moderate skin irritations have been reported in rabbits and guinea-pigs. Irritation of the skin has also been reported in workers with prolonged or repeated exposure (Dermatitis, oedema, redness blister or cracking). NMP has moderate potential for eye irritation. No evidence for skin sensitisation was observed.

Dermal absorption
NMP is readily absorbed across all body surfaces. Due to its low vapour pressure, absorption through the skin represents the most likely and potentially the most significant route of exposure to NMP under most known consumer use conditions.

In vivo rat studies indicate that 70 – 85% is absorbed when high concentrations of NMP are applied. The absorption at lower concentrations was less. It is noted that the absorption of NMP depends on the solvent. Thus, while in a rat experiment only 3.5% of NMP from a 30% aqueous solution was absorbed, 72% of NMP from a 30% solution in limonene was absorbed. In human studies, 16 – 68% of NMP was absorbed. Also in this case, the absorption was higher for neat NMP than for diluted NMP.

In the absence of adequate experimental data with concentrations of NMP relevant for this assessment, SCCS will use the default value of 100% dermal absorption in the calculation of the MOS. 100% dermal absorption was also used in ECHA's recent proposal for Identification of NMP as a Substance of Very High Concern (SVHC) (Ref. 65).

Repeated dose toxicity
No dermal repeated dose toxicity data were found. A NOAEL of 169 mg/kg bw/day based oral administration in male rats was found in a 90-day study based on body weight effects and changes in three neurobehavioral parameters. The NOAEL was 277 mg/kg bw/day in mice based on the liver responses in a 90-day study and 173 mg/kg bw/day based on liver tumours in male mice. A NOAEL of 250 mg/kg bw/day (highest dose tested) was found in a 90-day study with dogs. The NOAEL was 500 mg NMP/m³ for both male and female rats after inhalation.

Mutagenicity/Genotoxicity
The results available indicate that NMP can cause aneuploidy in a fungal test *in vitro*. However, NMP does not express a genotoxic effect in a standard bacterial assay and in two well-conducted *in vivo* assays (bone marrow chromosome aberration assay and micronucleus assay). NMP is not considered to have *in vivo* genotoxic potential.

Carcinogenicity
The potential carcinogenicity of NMP has been investigated in two long-term studies with rats and one with mice. No oncogenic potential of NMP in rats was found after oral administration (highest dose 678 mg/kg bw/day) or inhalation exposure (highest dose 400 mg/m³; about 100 mg/kg bw/day). Chronic nephropathy, especially in males, was the main toxic effect recorded. In the oral mice study at the highest dose, an increased frequency of liver adenomas (males and females) and carcinomas (males) was found. No evidence of an increase incidence of malignant tumours was seen at lower doses and there were no NMP-related neoplastic or non-neoplastic changes in other organs.

NMP is not considered to have genotoxic potential *in vivo*. Thus, the mice liver tumour at the highest dose tested may be induced by a non-genotoxic mechanism and is not relevant in relation to the low exposure from NMP in cosmetics.

Reproduction toxicity
The developmental effects in animals consisted in embryolethal, teratogenic, and fetotoxic effects after oral (gavage) and dermal administration in rats, after oral (gavage) treatment in rabbits, and *i.p.* injection of mice. Teratogenic effects were not observed after inhalation exposure of rats and rabbits.

In addition, foetal toxicity, expressed as reduced foetal body weight, occurred in the absence of significant maternal toxicity in rats treated by gavage. In one experiment, a foetus showing the characteristic malformations elicited by NMP was also observed at a dose level with no maternal toxicity. It is considered unlikely that the embryolethality and the foetal malformations could have been secondary to the general toxicity of NMP.

The developmental effects were specific and severe and are considered the most important reprotoxic effect in relation to NMP. As a consequence the NOAEL should be based on developmental toxicity. The lowest NOAEL after oral administration of NMP in rats (125 mg/kg bw/day) is considered the most relevant observation and will be used in the calculation of MOS.

Toxicokinetics and metabolism
NMP is readily absorbed by all three routes of exposure (inhalation, oral, and skin administration). Once absorbed, NMP is widely distributed throughout the body, metabolized, and primarily eliminated in the urine (the majority within 24 hours after treatment), with negligible tissue residues remaining after 4-5 days post-dose. NMP was shown to reach the foetus after exposure of pregnant rats and comparable maternal and foetal blood levels of NMP has been found. A metabolic pathway has been suggested for humans: NMP is first hydroxylated to 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP), and then oxidised to N-methylsuccinimide (MSI), which in turn is hydroxylated to 2-hydroxy-N-methylsuccinimide (2-HMSI). In vitro studies suggest that NMP, the parent substance, is responsible for the embryotoxic effects observed in vivo whereby its main metabolite, 5-HNMP, may be involved to some extent. The latter metabolites may be considered as detoxification steps.

4. CONCLUSION

Based on a worst case assessment with a maximum use concentration of 5% NMP in cosmetic products and a dermal absorption of 100%, the Margin of Safety is considered to be too low. There is an absence of specific information on the actual possible maximum concentrations of NMP present in cosmetic products and specific measurement of dermal absorption of it through skin at relevant concentrations.

With the information available at the time of assessment, the SCCS is of the opinion that the presence of NMP with a maximum use concentration of 5% in cosmetic products is not safe for the consumer. A re-evaluation may be possible should relevant data that addresses the above be provided.

5. MINORITY OPINION

Not applicable

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