Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

N-(hydroxymethyl)acrylamide; methylolacrylamide; [NMA]

EC Number: 213-103-2

CAS Number: 924-42-5

CLH-O-0000001412-86-211/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

8 June 2018

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

International Chemical Identification:

N-(hydroxymethyl)acrylamide

EC Number: **213-103-2**

CAS Number: **924-42-5**

Index Number: **-**

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON N-(HYDROXYMETHYL)ACRYLAMIDE; METHYLOLACRYLAMIDE; [NMA]

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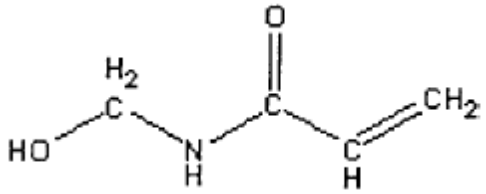
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON N-(HYDROXYMETHYL)ACRYLAMIDE; METHYLOLACRYLAMIDE; [NMA]

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	N-(hydroxymethyl)acrylamide (NMA)
Other names (usual name, trade name, abbreviation)	2-Propenamide,N-(hydroxymethyl) N-(hydroxyl-methyl)acrylamide (NHMA) N-methylolacrylamide N-(hydroxymethyl)-2-propenamide N-(hydroxymethyl)prop-2-enamide N-Metanolacrylamide, Monomethylolacrylamide
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	213-103-2
EC name (if available and appropriate)	N-(hydroxymethyl)acrylamide
CAS number (if available)	924-42-5
Other identity code (if available)	-
Molecular formula	C4H7NO2
Structural formula	
SMILES notation (if available)	OCNC(=O)C=C
Molecular weight or molecular weight range	101.1 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	*[If the substance structure demonstrates stereo-isomerism the ratio of these stereo-isomers should be specified. If the ratio is unknown it should be stated as such. For optical isomers a measure of optical activity (specific rotation) should be specified.]*
Description of the manufacturing process and identity of the source (for UVCB substances only)	*[In the case of UVCB substance a full manufacturing process description should be provided including the identity of the source or starting materials and their ratio. Any relevant process parameters should also be specified.]*
Degree of purity (%) (if relevant for the entry in Annex VI)	>80% (calculated on dry weight) 40 to 85 % in aqueous solution

1.2 Composition of the substance

NMA is essentially marketed as an aqueous solution. According to the substance definition given in REACH, a substance is identified as:

A chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition.

The composition information reported in a number of registration dossiers submitted to ECHA includes a substantial amount of water. Such information is not necessarily correct and does not necessarily reflect the composition that would be appropriately reported following the substance definition given in REACH.

This classification proposal only covers the mono-constituent substance N-(hydroxymethyl)acrylamide.

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
N-(hydroxymethyl)-Acrylamide EC no: 213-103-2	40 to 85 % in aqueous solution >80% (calculated on dry weight)	none	See table below

There are 780 notifications in 15 aggregated notifications on the 17/01/2017. These self-classifications cover the different classes as follows:

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Classification		Number of notifiers
Hazard class and category code	Hazard statement code	
Acute Tox. 3	H301	511
Acute Tox. 4	H312	357
Skin Irrit. 2	H315	126
Eye Irrit. 2	H319	68
Skin Sens. 1	H317	446
Skin Sens. 1B	H317	66
STOT SE 2	H371 (Nervous System)	1
STOT RE 1	H372 (Peripheral nerv...) (oral)	67
STOT RE 1	H372	60
STOT RE 1	H372 (not available)	66
STOT RE 1	H372 (unknown)	62
STOT RE 1	H372 (Damage to organ…)	25
STOT RE 2	H373(Neurotoxicity)	355
STOT RE 2	H373 (Peripheral nerv…)	2
STOT RE 2	H373(Not known)(oral)	1
Muta. 1A	H340 (Oral)	1
Muta. 1B	H340 (Oral)	511
Muta. 2	H341	60
Carc. 1A	H350 (oral)	1
Carc. 1B	H350 (oral)	511
Carc. 2	H350	90
Repr. 2	H361 (oral)	540
Aquatic chronic 4	H413	80
Not classified	Not classified	35

This table shows discrepancies between the self-classifications proposed by notifiers for CMR and STOT RE classifications:

- For mutagenicity, there is one notifier classifying in category 1A, 511 in 1B, 60 in category 2 and 208 not classifying this endpoint.
- For carcinogenicity, there is one notifier classifying in category 1A, 511 in 1B, 90 in category 2 and 178 not classifying this endpoint.
- For reprotoxicity, there are 540 notifiers classifying in category 2 and 240 not classifying this endpoint.
- For STOT RE 2, there are 358 notifiers classifying in category 2 versus 280 in category 1 and 142 not classifying this endpoint.

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Formaldehyde EC no: 200-001-8 CAS: 50-00-0	0.0 - 2.0% (in aqueous solution)	Acute Tox. 3* H301 Acute Tox. 3* H311 Acute Tox. 3* H331 Skin Corr. 1B H314 Skin Sens. 1 H317 Muta.2 H341 Carc. 1B H350 Specific Concentration limits*, Skin Corr. 1B; H314: C ≥ 25% Skin Sens. 1; H317: C ≥ 0,2% Eye Irrit. 2; H319: 5% ≤ C < 25% STOT SE 3; H335: C ≥ 5% Skin Irrit. 2; H315: 5% ≤ C < 25% Nota B and Nota D	Existing harmonized classification	The impact of the classification of these impurities depends on the level of these impurities in the pure, diluted or formulated substance.
Acrylamide EC no: 201-173-7 CAS : 79-06-1	0.0 - 10.0% (in aqueous solution)	Acute Tox. 3* H301 Acute Tox. 4* H312 Acute Tox. 4* H332 Skin Irrit.2. 1B H315 Skin Sens. 1 H317 Eye Irrit. 2 H319 Muta.1B H340 Carc. 1B H350 Repr. 2 H361f*** STOT RE 1H372** Nota D	Existing harmonized classification	The impact of the classification of these impurities depends on the level of these impurities in the pure, diluted or formulated substance.

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
Mequinol EC no.: 205-769-	Stabilizer to prevent polymerization	30 ppm (typical concentration in aqueous solution)	Acute tox 4*, H302 Skin sens 1, H317 Eye Irrit. 2 , H319	Existing harmonized classification	-

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
8					
oxygen CAS no.: 7782-44-7	stabilizer to prevent polymerization	No data	Press. Gas Ox. Gas 1 – H270	Existing harmonized classification	-
Cupric ions CAS no.: 15158-11-9	stabilizer to prevent polymerization	No data	None	-	-

There are different compositions of N-hydroxymethyl-acrylamide (NMA) claimed by the notifier. There are some uncertainties regarding the impact of impurities on the classification of the substance NMA. An impact of both relevant impurities, acrylamide and formaldehyde, present in the different batches used in toxicological studies on the classification of the substance cannot be excluded. So it is important to consider the different batches used in the different toxicological studies to conclude if the effects observed are related to NMA or to the impurities. NMA was tested using different batches with different purities. The different batches used in the toxicological studies are indicated in the following table.

Table 5: Information on the tested batches in toxicity studies performed with NMA

Study	Substance tested	Analytical purity	Batch tested	Impurities (identity and concentrations)
ADME Matthews J.M (2001)	NMA	98% in water	5597-62-03 RTI	not specified
ADME Fennell 2003	NMA	99%(from TCI America Portland, OR) as a powder)	Not specified	not specified 1H- and 13C-NMR analysis appeared consistent with the specified purity and did not indicate the presence of any free formaldehyde or acrylamide.
Oral Repeated Doses studies NTP (1989), Bucher J.R (1990)	NMA	approximately >98% in water	1-45-000	unknown but evidence indicates that 1% may have been a polymer of NMA, which would not have been detected by the analytical methods used
Vitro genotoxicity assays Ames assay Bucher J.R (1990) Chromosomal Aberrations assay in CHO cells Bucher J. (1990)	NMA	approximately >98% in water	1-45-000	unkown but evidence indicates that 1% may have been a polymer of NMA, which would not have been detected by the analytical methods used

Vivo genotoxicity assay Lethal dominant assay Chapin RE. (1995)	NMA	97-99%	not specified	not specified
Vivo genotoxicity assays Bone marrow micronucleus Witt *et al.* (2003) Mouse dominant lethal assay Witt *et al.* (2003)	NMA	98% in deionized distilled water for the drinking water study or hank's balanced salt solution (HBSS) (for the i.p injection studies)	From NTP chemical repository at Research Triangle Institute (RTI log No. 5597-62-10)	Not specified
Rat and mice Carcinogenicity studies NTP (1989)	NMA	98% in water	1-45-000	unknown but evidence indicates that 1% may have been a polymer of NMA
Neurotoxicity assay in rat Tanii H. and Hashimoto K. (1983)	No data			

The composition of the substance tested in the toxicological studies was not fully clarified. Furthermore, with a NMA content above 97%, the toxicological batches are not the most appropriate to cover the substance as placed on the market (40 to 85% as aqueous solution). A carcinogenicity NTP assay (1989)[1] reported that in the batch number 1-45-000 (batch used in most studies) NMA is present at a purity up to 98%, the impurities are unknown but evidence indicates that 1% may have been a polymer of NMA, which would not have been detected by the analytical methods used. Toxicity appears largely restricted to the monomer and acrylamide polymers are thought to pose little hazard to public health or the environment (Kirk-Othmer, 1978). So the polymers of NMA can be considered of not toxicologically concern.

According to the registrants, two impurities classified are present in the composition of NMA substance: formaldehyde and acrylamide (AA)

- Formaldehyde is a toxicological relevant impurity in particular since it is classified for carcinogenicity and genotoxicity endpoints. In a carcinogenicity NTP (1989) study in rodent, subsequent stability studies specifically designed to evaluate possible formaldehyde formation during storage indicated a slow production of formaldehyde, with a maximum concentration of approximately 25 ppm or 0.0025% (higher than generic limit concentration for classification) in the high concentration mixture at the end of 2 weeks.

[1] For information the National Toxicology Program (NTP) regarding the identity of the impurities in the composition of N-(Hydroxymethyl)-acrylamide provided the Chemical Characterization report of N-(Hydroxymethyl)-acrylamide. – revised report chemical characterization and dosage formulation report– June 1998. They reported that at the time of the study, identifying impurities present at >1% would normally have been done, but in this case the two HPLC methods disagreed regarding the number and size of the impurities present with one having an impurity slightly over 1% and the other having no impurities >1%. It is likely they decided not to pursue the identity of the single impurity over 1%. In any case, none of the impurities < 1% would have been identified at that time.

- Acrylamide (AA) is also a toxicological relevant impurity in the composition of the substance since AA is classified for mutagenicity, carcinogenicity, neurotoxicity and reprotoxicity endpoints. No information of the presence of this substance in the tested batched considered for toxicological endpoint.

Overall, among all the toxicity studies, there is no information of the presence of these impurities at a concentration higher than generic limit concentration for classification in the tested batches.

There are some persistent doubts on the fact that the effects (mutagenicity, carcinogenicity and neurotoxicity) observed with NMA could be due to the presence of classified impurities content. Indeed, for most of the compositions of NMA registered under REACH regulation, the levels of formaldehyde and/or acrylamide should lead to a classification of the substance by calculation. Therefore, it can be questioned if proposing a classification entry for NMA is really justified in particular taking into account the classification of AA. However, it remains that some registered compositions do not contain sufficient impurities to impact the classification of NMA (see confidential annex). In addition, it is considered that NMA has intrinsic properties for carcinogenicity, genotoxicity and neurotoxicity to justify its own classification and Annex VI entry (see section 8 toxicokinetics and section 9 evaluation of toxicological hazards).

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No existing Annex VI entry										
Dossier submitters proposal		N-(hydroxymethyl)acrylamide	213-103-2	924-42-5	Carc. 1B Muta. 1B STOT RE1	H350 H340 H372 (peripheral nervous system)	Dgr GHS 08	H350 H340 H372 (peripheral nervous system)			
Resulting Annex VI entry if agreed by RAC and COM		N-(hydroxymethyl)acrylamide	213-103-2	924-42-5	Carc. 1B Muta. 1B STOT RE1	H350 H340 H372 (peripheral nervous system)	Dgr GHS 08	H350 H340 H372 (peripheral nervous system)			

Table 7: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not assessed in this dossier	Yes/No
Flammable gases (including chemically unstable gases)	Hazard class not applicable	Yes/No
Oxidising gases	Hazard class not applicable	Yes/No
Gases under pressure	Hazard class not applicable	Yes/No
Flammable liquids	Hazard class not assessed in this dossier	Yes/No
Flammable solids	Hazard class not applicable	Yes/No
Self-reactive substances	Hazard class not assessed in this dossier	Yes/No
Pyrophoric liquids	Hazard class not assessed in this dossier	Yes/No
Pyrophoric solids	Hazard class not applicable	Yes/No
Self-heating substances	Hazard class not assessed in this dossier	Yes/No
Substances which in contact with water emit flammable gases	Hazard class not assessed in this dossier	Yes/No
Oxidising liquids	Hazard class not assessed in this dossier	Yes/No
Oxidising solids	Hazard class not applicable	Yes/No
Organic peroxides	Hazard class not assessed in this dossier	Yes/No
Corrosive to metals	Hazard class not assessed in this dossier	Yes/No
Acute toxicity via oral route	Hazard class not assessed in this dossier	No
Acute toxicity via dermal route	Hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	Hazard class not assessed in this dossier	No
Skin corrosion/irritation	Hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	Hazard class not assessed in this dossier	No
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation	Hazard class not assessed in this dossier	No
Germ cell mutagenicity		Yes
Carcinogenicity		Yes
Reproductive toxicity	Data lacking	No
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure		Yes
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Hazard class not assessed in this dossier	No
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

N-(hydroxymethyl)acrylamide (NMA) has not previously been assessed for harmonised classification by RAC or TC C&L.

RAC general comment
Composition The Dossier Submitter (DS) reported that two impurities of N-(hydroxymethyl)acrylamide (NMA) may potentially contribute to the results of the studies with NMA. These are acrylamide (AA) and formaldehyde. According to Paulsson *et al*. (2002), NMA is synthesized by a reaction between AA and formaldehyde. An impact of these impurities in the toxicological studies cannot be fully excluded. For most compositions of NMA registered under REACH, the levels of AA and formaldehyde are above the GCL of 0.1% for classification for carcinogenicity and mutagenicity. The composition of the substance tested in the toxicological studies are not fully clarified, and is reported to be either >98% or 97-99%. There was no information regarding impurities in the tested substances, but there are indications that ~1% could be a polymer of NMA (NTP, 1989). The matter is discussed further in the context of the different hazard classes. *Toxicokinetics* Several toxicokinetic studies have been performed with NMA. In a study with intravenous administration of 140 mg/kg bw to male rats, it was reported that the distribution was rapid in total body water with a first-order elimination rate constant of 0.45 h^{-1} from the blood compartment, with a half-life of 1.55 h (Edwards *et al*., 1975). There was evidence of glutathione conjugation, but no evidence of conversion to acrylamide *in vivo*. It is not known if NMA was converted to an epoxide metabolite. In a study with B6CF1 male mice, radioactive NMA at 150 mg/kg bw (0.88-8.1 µCi radiolabel) was given both by single oral gavage and intraperitoneally (i.p.) (Mathews *et al*., 2001). Total excretion at 72 h after oral exposure was estimated to be 79.1 ± 12.3%, with 42.9 ± 34.6% excretion via urine, 11 ± 1.6% in CO_2 and 24.9 ± 22.1% in the faeces. Total excretion after intra-peritoneal administration was estimated to be 86.7 ± 4.7%, with 72.6 ± 7.1% excretion via urine, 9.8 ± 1.1% as CO2 and 4 ± 2.9% via faeces. The highest percentage of radioactivity was found in blood, possibly as a haemoglobin adduct. No epoxide was found. The major urinary metabolite was the glutathione conjugate. No potential for bioaccumulation was found in this study. NMA has structural analogies with acrylamide (AA), and possible metabolic links between these substances have therefore been studied. In the study by Paulsson *et al*. (2002) in male rats (Sprague Dawley), haemoglobin (Hb) adducts were measured as a biomarker of exposure following administration of a single oral dose of 100 mg/kg bw acrylamide (AA) or 142 mg/kg bw NMA (equivalent to 1.4 nmol/kg bw for both compounds). N-(2-carbamoylethyl)valine (AA Val) adduct formation after AA treatment was ~26.2 (21.1-31.4) nmol/g globin per mmol/kg bw, while the AA Val adduct formation after NMA treatment was ~9.8 (6.9-12.8) nmol/g globin per mmol/kg bw. The ratios of GA Val : AA Val were 0.26 for AA treatment and 0.23 for NMA

treatment where GA Val is the epoxymetabolite (N-(2-carbamoyl-2-hydroxyethyl)valine).

Similarly, Fennel *et al*. (2003) measured Hb adducts in male rats (F344) after exposure to a single oral dose of 50 mg/kg bw AA or 71 mg/kg bw NMA. AA Val adduct formation after AA treatment was 26.4 ± 4.9 nmol/g globin per mmol/kg bw, while the AA Val adduct formation after NMA treatment was 56.2 ± 8.1 nmol/g globin per mmol/kg bw. The ratio of GA Val : AA Val were 0.38 for AA treatment and 0.03 for NMA treatment.

AA is known to form glutathione conjugates and it is also transformed by CYP2E1 into glycidylamide (GA) (Sumner *et al.*, 1997, 1999). GA is the epoxide known to be responsible for the genotoxicity and carcinogenicity induced by AA (Segerback, 1995). In studies where rats (Edwards *et al*., 1975) and mice (Mathews *et al*., 2001) were exposed to NMA, no formation of an epoxide was seen, in contrast to what has been shown for AA. Paulsson *et al*. (2002) and Fennell *et al*. (2003) measured the formation of Hb adducts with the aim to investigate if NMA are converted to AA *in vivo* prior to formation of Hb-adducts (see the figures below). These studies suggested that NMA forms glutathione conjugates, but other metabolic pathways are unknown. For AA, the Hb-adducts represent the time concentration integral of AA and GA present in the circulation during the lifetime of the erythrocytes (~125 days), and the adduct concentration is proportional to the internal dose (Bergmark, 1997; Fennell *et al*., 2005).

Figure. Potential metabolic pathways for NMA (abbreviation MAA used by the author in this figure) and AA (from Paulsson et al., 2002)

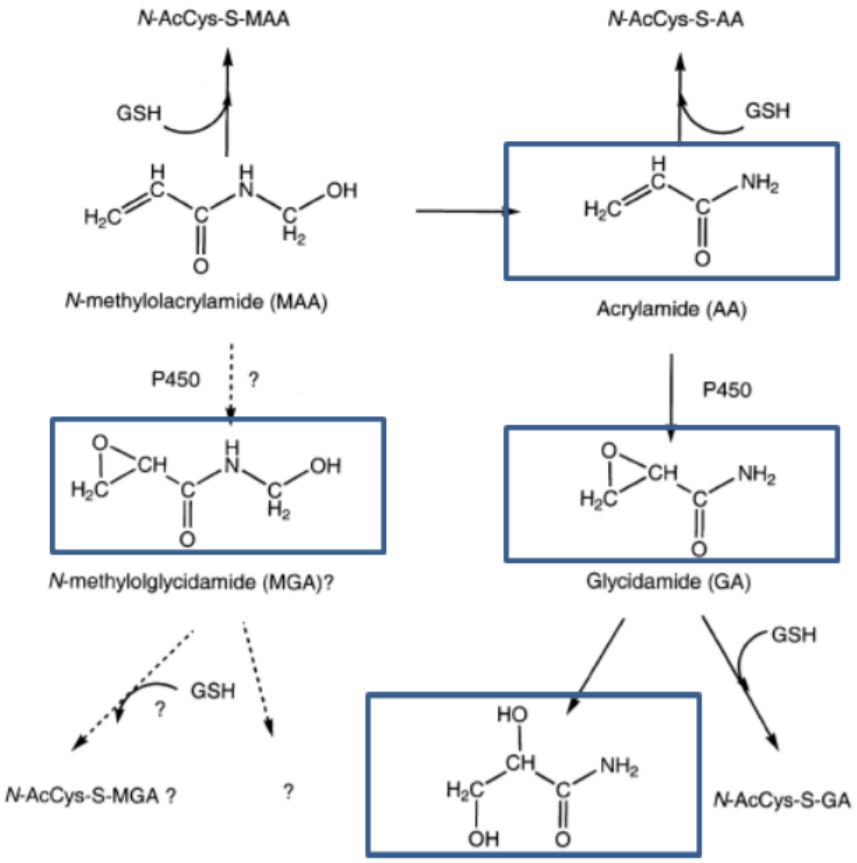
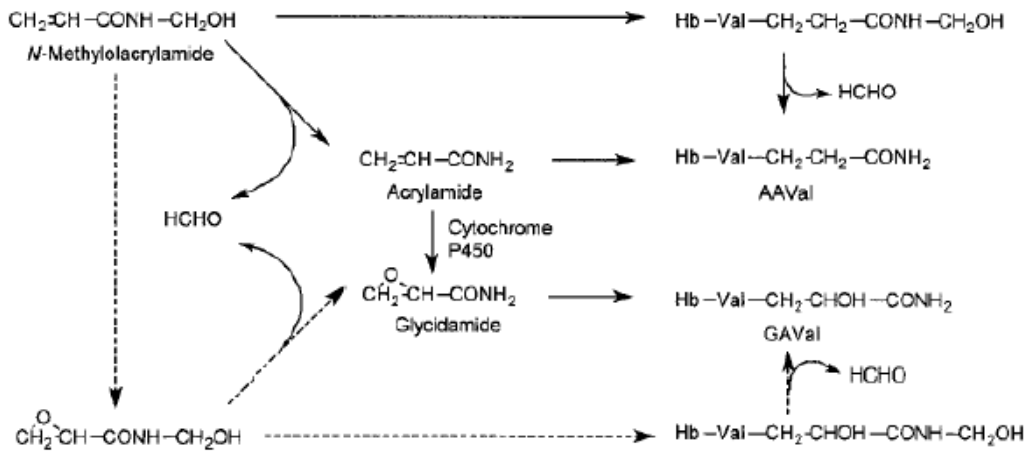


Figure. Possible routes of reaction of NMA to produce AA Val and GA Val (From Fennel et al., 2003)



The DS also performed a QSAR analysis using the OECD QSAR toolbox v3.3.0 which predicted several possible metabolites of NMA, including AA and the epoxide GA. This analysis supported the hypothesis that NMA is converted to AA and also to epoxides, including GA. Based on the available information, there are indications that NMA is converted into AA and epoxides, however no clear evidence for this hypothesis is available.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

A substance with CMR classification is normally subject to harmonised classification (Art. 36 CLP regulation). NMA is currently not classified according to Annex VI of CLP. Available data show that NMA has a CMR property, i.e. carcinogenicity and mutagenicity that justify a harmonised classification and labelling as Muta. 1B and Carc. 1B according to article 36 of CLP.

Furthermore, differences in self classifications for STOT RE justify the need for action at Community level. Based on available animal data in the chronic assay performed in rat and mice with NMA supported by human data, classification as STOT RE1 is warranted.

5 IDENTIFIED USES

The substance is manufactured and used at industrial sites only. This substance is used in the polymer products. This substance has an industrial use resulting in manufacture of another substance (use of intermediates). The sectors of uses are : agriculture, forestry and fishing and formulation of mixtures and/or re-packaging. This substance is used for the manufacture of: chemicals and plastic products.

Other uses are reported in the literature but seems to be not relevant anymore.

6 PHYSICOCHEMICAL PROPERTIES

The physico - chemical properties are reported for a 48% aqueous solution of NMA containing formaldehyde and acrylamide as impurities. Information relative to the physicochemical properties come from the REACH registration dossier.

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Liquid, clear, colourless to light-yellow, odour: formaldehyde	- Material Safety Data Sheet, "Flocryl NMA 48", SNF SAS, 2010 - Registration dossier, (IUCLID 6)	No data provided to evaluate the value.
Melting/freezing point	-10°C at 1 atm	- Material Safety Data Sheet, "Flocryl NMA 48", SNF SAS, 2010 - Registration dossier, (IUCLID 6)	No data provided to evaluate the value.
Boiling point	At temperatures above 50°C, polymerisation could be initiated over time. Substance will polymerise before reaching its boiling point.	- Material Safety Data Sheet, "Flocryl NMA 48", SNF SAS, 2010 - Registration dossier, (IUCLID 6)	Statement
Relative density	1.07-1.10 at 25°C	- Material Safety Data Sheet, "Flocryl NMA 48", SNF SAS, 2010 - Registration dossier, (IUCLID 6)	No data provided to evaluate the value.
Vapour pressure	20-30 mm Hg at 25°C	- Material Safety Data Sheet, "Flocryl NMA 48", SNF SAS, 2010 - Registration dossier, (IUCLID 6)	No data provided to evaluate the value.
Surface tension	Based on the structure of the substance no surface activity is expected.	Registration dossier, (IUCLID 6)	Statement
Water solubility	1000 g/L at 25°C and pH7 Very soluble	Registration dossier, (IUCLID 6)	Calculation based on structure using EPIWIN Suite Software
Partition coefficient n-octanol/water	Log Pow = -1.81 at 20°C and pH 7	- Material Safety Data Sheet, "Flocryl NMA 48", SNF SAS, 2010 - Registration dossier, (IUCLID 6)	Estimated using KOWWIN, version 1.67
Flash point	> 93 °C	MSDS Sigma-Aldrich	No data provided to evaluate the value.
Flammability	At temperatures above 50°C, polymerisation could be initiated over time. Substance polymerises	Registration dossier, (IUCLID 6)	Statement

Property	Value	Reference	Comment (e.g. measured or estimated)
	exothermically above its melting point in the absence of stabilisers and does not combust.		
Explosive properties	The substance is an amide. There is no evidence of amides having explosive properties.	Registration dossier, (IUCLID 6)	Statement
Self-ignition temperature	At temperatures above 50°C, polymerisation could be initiated over time. Substance polymerises exothermically above its melting point in the absence of stabilisers and does not undergo auto-ignition.	Registration dossier, (IUCLID 6)	Statement
Oxidising properties	The substance contains no chemical groups which cause oxidisation.	Registration dossier, (IUCLID 6)	Statement
Granulometry	Not relevant Substance is manufactured and supplied as a liquid.	Registration dossier, (IUCLID 6)	
Stability in organic solvents and identity of relevant degradation products	Soluble in polar solvents (alcohols) and not soluble in nonpolar solvents (hydrocarbon, chloroform, …)	Material Safety Data Sheet, "Flocryl NMA 48", SNF SAS, 2010	No data provided to evaluate the value.
Dissociation constant	Substance is covalent and does not contain dissociating groups	Registration dossier, (IUCLID 6)	Statement
pH	pH = 6 – 7	- Material Safety Data Sheet, "Flocryl NMA 48", SNF SAS, 2010 - Registration dossier, (IUCLID 6)	No data provided to evaluate the value.
Viscosity	1 -7 mPa.s at 25°C (dynamic)	- Material Safety Data Sheet, "Flocryl NMA 48", SNF SAS, 2010 - Registration dossier, (IUCLID 6)	No data provided to evaluate the value.

7 EVALUATION OF PHYSICAL HAZARDS

The substance is not classified for the physico-chemical aspect. See table of summary of physico-chemical properties above. Physical hazards are not further assessed in this dossier.

8 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Some data on acrylamide (AA) have been reported here in order to assess the potential influence of AA on the toxicity of NMA. Indeed, in addition to be a potential impurity of NMA, it has been checked if AA could

be a metabolite of NMA. This is of particular importance to conclude if the mutagenic, carcinogenic and neurotoxic effects reported in studies performed with NMA are related to the substance itself or to the presence of AA as an impurity or as a metabolite.

Table 9: Summary table of key studies for toxicokinetics studies

Method	Results	Remarks	Reference
Porton male rats (3-7 per different time points) Intravenous administration in 0.9% saline Single dose at different points of exposure: 140 mg/kg bw, substance labelled N-hydroxy(14C)methyl acrylamide (NMA) No OECD guideline, no GLP	NMA was distributed rapidly in total body water, half-life < 2 hours, first-order rate of elimination of 0.45/h from the blood compartment. Evidence for glutathione conjugation with NMA in the bile with the substance labelled in the methylene carbon, but no evidence found for conversion to acrylamide *in vivo*. It is not known whether NMA is converted to an epoxide metabolite.	Key study Reliability 2 with restrictions (klimisch score) Substance tested: NMA Purity not specified	Edwards (1975)
Pharmacokinetic study (Absorption, distribution, excretion) B6C3F1 male mouse (4 males per dose) Oral gavage and intraperitoneal administration Exposure regime: Single gavage and single ip exposures Doses/conc.: 150 mg/kg NMA containing 0.88-8.1 µCi radiolabel for both exposure routes Vehicle : water - Tissues and body fluids sampled: urine, faeces, blood, adipose tissue,brain, epidymis, heart, kidney, liver, lung, muscle, fore-stomach, glandular stomach and cage washes and red blood cells - Time and frequency of sampling: after 8, 24, 48 and 72 hours for excreta, tissues	Absorption: well absorbed based on high percentage of NMA and CO_2 recovered in breath and urine Excretion: Total excretion 72 hours after oral administration was 79.1±12.3%: 42.9±34.6% in urine, 11±1.62% in CO_2 and 24.9±22.1% in faeces Total excretion 72 hours after ip administration was 86.7±4.7%: 72.6±7.11% in urine, 9.8±1.12% in CO_2 and 4.06±2.9% in faeces Distribution: blood contained the highest percentage of radioactivity, presumably as the haemoglobin adduct. All organs had a tissue to blood ratio of 0.25-0.56 with adipose and skin on the low end and spleen and kidney on the high end. 25% of the remaining dose was found in the blood. Metabolism: NMA reacts with glutathione to produce the glutathione adduct across the carbonyl group. No epoxide was found in this study. Metabolites identified: yes Details on metabolites: The mercapturate N-acetyl-S-(3-hydroxymethylamino-3-oxopropyl)cysteine, arising from direct conjugation of NMA with glutathione and subsequent metabolism, was the major	Key study Reliability 2 with restriction (klimisch score) Substance tested: NMA Batch number : 5597-62-03 RTI	Mathews (2001)

at sacrifice (72 hours) -Method type(s) for identification: GC/MS/MS, NMR, HPLC, LSS Limits of detection and quantification: not specified No OECD guideline, no GLP	urinary metabolite. It represented about 41 % of the urinary metabolites after intraperitoneal administration and about 45% of the urinary metabolites (not including parent) after oral administration. Evaluation of results: no bioaccumulation potential		
Measures of Hb adducts Male Sprague Dawley rat (6 per group) 8 week old Single oral dose of 100 mg/AA/kg bw or 142 mg NMA/kg bw (1.4 mmol/kg bw for both compounds) Measures by LC/MS/MS of AA Val N-(2-carbamoylethyl)valine (AA val derived from AM) and N-(2-carbamoylethyl)valine (GA Val derived from GA) measured following 3 animals treated with 1 mg/kg bw mitomycin as positive controls No OECD guideline; GLP	<u>Valine adduct formation</u> AAVal adduct formation with AA treatment (26.2 (21.1-31.4) nmol/g globin per mmol/kg body weight) higher than with NMA treatment (9.8 (6.9-12.8) nmol/g globin per mmol/kg body weight in rat. Ratio of GAVal:AAVal after AA= 0.26 and NMA= 0.23.	Supportive study Reliability 2 with restrictions (klimisch score) Substances tested: Acrylamide (AA) and NMA (solution 48 % in water)	Paulsson (2002)
Measures of Hb adducts Male (F344) rats (4 per group); 9-10 weeks old Measures by LC/MS/MS of AA Val N-(2-carbamoylethyl)valine (AA val derived from AM) and N-(2-carbamoylethyl)valine (GA Val derived from GA) measured following Single oral dose of 50 mg/kg AA (equivalent to measured dose of 59.5±8.0 mg AA/kg) or 71 mg/kg NMA (equivalent to measured dose of 73±3.9 mg NMA/kg) ; dose solutions in water solution No OECD guideline; No GLP	<u>Valine adduct formation</u> AAVal adduct formation with NMA treatment (56.2 ± 8.1 nmol/g globin per mmol/kg body weight) AAVal adduct formation AA treatment (26.4 ± 4.9 nmol/g globin per mmol/kg body weight) Ratio of GAVal:AAVal of 0.38 for AA treatment and 0.03 for NMA treatment	Supportive study Reliability 2 with restriction (klimisch score) Substances tested: Acrylamide (AA) and NMA (minimum purity: 99% as a powder)	Fennell (2003)

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON N-(HYDROXYMETHYL)ACRYLAMIDE; METHYLOLACRYLAMIDE; [NMA]

Detailed study summaries are available in Annex I of the CLH report.

Two key studies were performed in rats and mice.

Rats

Edwards *et al* in 1975 reported that NMA was distributed rapidly in total body water, with a first-order rate of elimination of 0.45/h from the blood compartment following an intravenous administration at 140 mg/kg bw to rat. Evidence for glutathione conjugation with NMA in the bile was found with the substance labelled in the methylene carbon, but no evidence was found for conversion to acrylamide *in vivo*. It is not known from this study whether NMA is converted to an epoxide metabolite (like its structural analogue, acrylamide (AA)). The authors concluded that the distribution and reactivity of the two compounds AA and NMA were very similar. Both compounds distribute very rapidly throughout the total body water and are removed with a half-life of less than 2 hours. No data were available on urinary metabolites.

Mice

In Mathews *et al.* (2001) study, radioactive NMA was administered at 150 mg/kg containing 0.88-8.1 μCi radiolabel by two exposure routes (single oral gavage and intraperitoneal (ip) routes) to B6C3F1male mice. Air, urine and faeces were collected and assayed for radioactivity. Organs were isolated, weighed and radioactivity content determined. Urinary metabolites were assayed and haemoglobin adducts measured. The substance is well absorbed. Total excretion at 72 hours after oral administration was estimated 79.1±12.3% corresponding to 42.9±34.6% in urine, 11±1.62% in CO_2 and 24.9±22.1% in faeces. Total excretion at 72 hours after ip administration was estimated 86.7±4.7%. corresponding to 72.6±7.11% in urine; 9.8±1.12% in CO_2 and 4.06±2.9% in faeces. Blood contained the highest percentage of radioactivity, presumably as haemoglobin adduct. All organs had a tissue to blood ratio of 0.25-0.56 with adipose and skin on the low end and spleen and kidney on the high end. Twenty five percent of the remaining dose was found in the blood. NMA reacts with glutathione to produce the glutathione adduct across the carbonyl group. No epoxide was found in this study. It was concluded that NMA when administered either intraperitoneally or by gavage to mice at 150 mg/kg was excreted primarily in urine and faeces. About 10% of the radioactivity was excreted as $^{14}CO_2$ after either route and between 4 to 25% was excreted in the faeces depending on the route of exposure. The high percent of the dose recovered in urine and as CO_2 indicates that NMA is well absorbed after either route of administration. NMA did not accumulate in any tissue sampled after either route. Less than 5% of the administered radioactivity remained in the tissues sampled 72 h after dosing. Among the metabolites identified, the mercapturate N-acetyl-S-(3-hydroxymethylamino-3-oxopropyl) cysteine, arising from direct conjugation of NMA with glutathione and subsequent metabolism, was the major urinary metabolite. It represented about 41 % of the urinary metabolites after intraperitoneal administration and about 45% of the urinary metabolites (not including parent) after oral administration. No bioaccumulation potential was showed based on study results.

Analogy with Acrylamide (AA)

Due to its structural analogy with AA which is already classified for its mutagenic, carcinogenic and neurotoxic effects, possible metabolic link between AA and NMA has been checked. From both studies, it cannot be concluded if NMA will be metabolized into AA or into an epoxide. To well understand the mechanisms behind the effects reported in studies performed with NMA, we need to

know if NMA could be metabolized into AA and/or an epoxide. AA is mainly metabolized by conjugation to glutathione (GSH) but it is also transformed into glycidamide (GA) by P450 enzymes (CYP2E1) (Sumner *et al.*, 1997, 1999). This epoxide is known to be responsible of AA-induced genotoxicity and carcinogenesis due to its reactivity towards DNA (Segerback, 1995). From Edwards *et al (1975)* and Mathews *et al.* (2001), there is no evidence of transformation of NMA to AA or to an epoxide. Some addition information can be provided from studies carried out by Paulsson *et al*. (2002) and Fennell *et al*. (2003) who measured Hb adduct after AA and NMA exposure. The aim of these reports was to investigate whether NMA is converted to AA *in vivo* prior to adduct formation. The proposed metabolic pathways proposed by Paulsson *et al.*, 2002 and Fennell *et al*., 2003 suggest that NMA is conjugated to GSH, but other potential metabolic pathways, e.g. by the P450 system, are unknown (Figure 1 and 2).

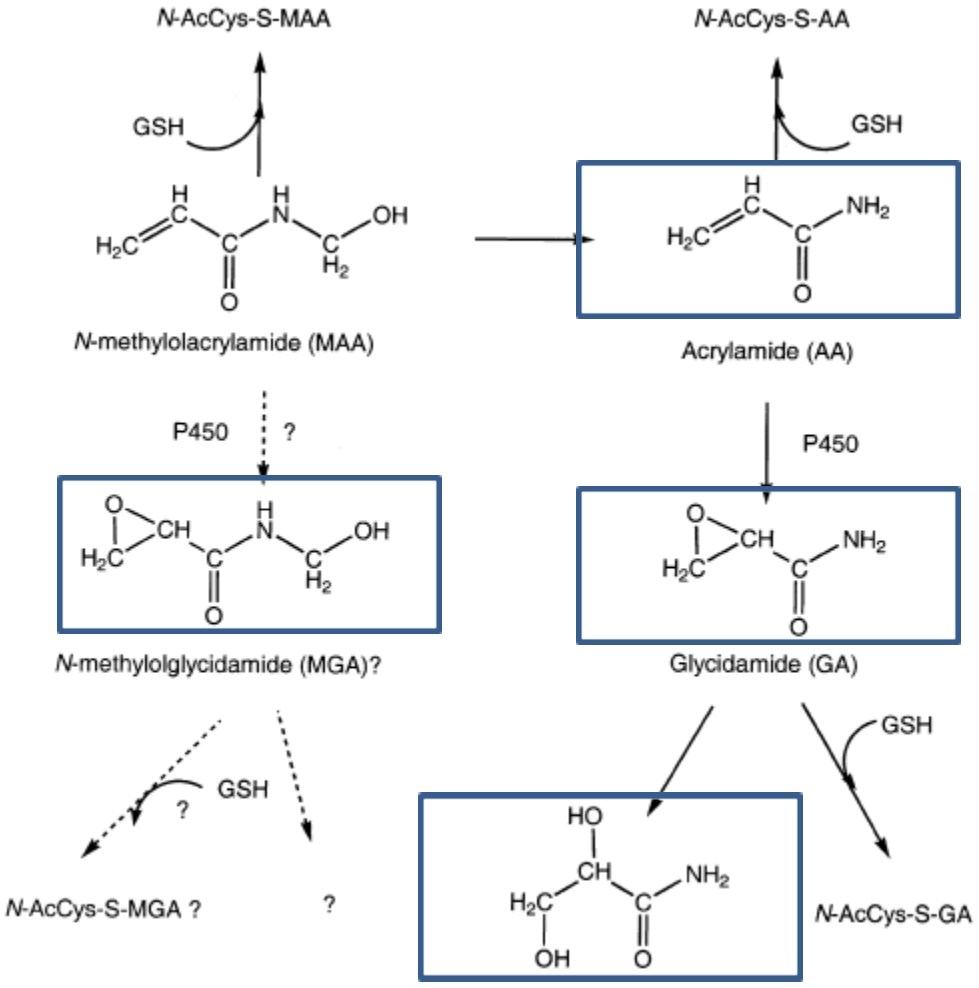


Figure 1: Potential metabolic pathways for AA and NMA (from Paulsson *et al*. (2002) (MAA reported in the figure from Paulsson *et al.* (2002) refers to NMA)

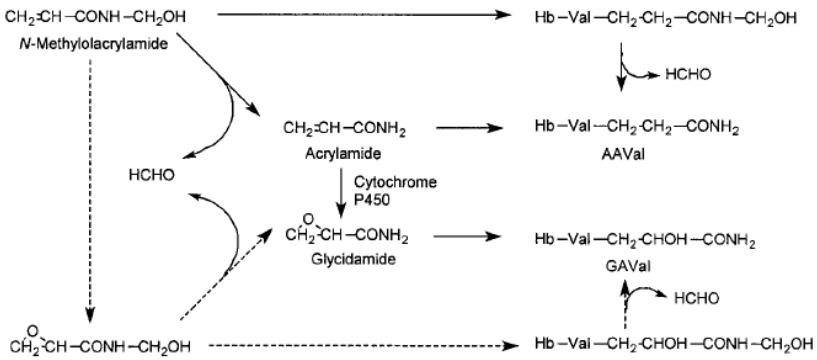
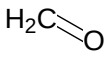
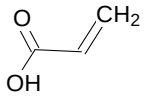
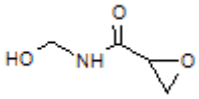
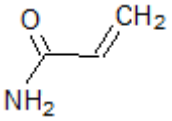
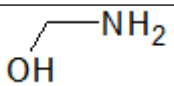
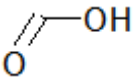
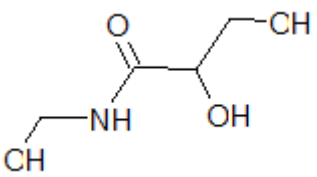
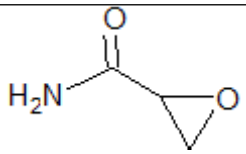
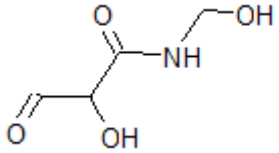
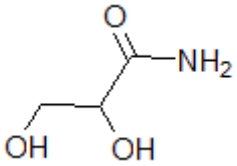


Figure 2: Possible routes of reaction of NMA to produce AA Val and GA Val (from Fennell (2003) (N-methylolacrylamide refers to NMA).

In conclusion, based on the available data, the conversion of NMA into AA and /or into an epoxide is plausible even if not directly confirmed by experimental data. In order to go further into metabolism hypothesis, a QSAR analysis has been realized for NMA with OECD QSAR toolbox v3.3.0.

Table 10: Summary table of predicted metabolites of NMA by OECD QSAR toolbox v3.3.0

Simulated metabolites	**Structure**	**Harmonised classification (focus on CMR and STOT RE)**	**Self-classification (focus on CMR and STOT RE)**
Formaldehyde CAS no 50-00-0		Carc. 1B Muta 2	Carc. 1B Muta 2
Acrylic acid CAS no 79-10-7		No classification for CMR	No self-classification for CMR
N-Methyloglycidamide		-	-
Acrylamide CAS 79-06-1		Muta. 1B Carc. 1B Repr. 2 STOT RE 1	Muta. 1B Carc. 1B Repr. 2 STOT RE 1
unknown		-	-
Formic Acid CAS no 64-18-6		-	-
unknown		-	-

Glycidamide Oxirane-2-carboxamide CAS no 5694-00-8		No harmonized classifcation	Carc 1B
unknown		-	-
unknown		-	-

Ten metabolites (Rat liver S9 metabolism simulator) were identified. QSAR analysis supports the hypothesis of the conversion of NMA to AA and to epoxides, including glycidamide. In addition, four unknown compounds may also be formed. However, further experimental data is needed to confirm the conversion of NMA into acrylamide and into epoxide metabolite. At this time, even it cannot be proven, it cannot be neither excluded that NMA is converted into AA from available *in vivo* studies and by QSAR modelisation. Further experimental data on metabolic conversion of the substance would be needed to reach a firm conclusion.

Overall, one hypothesis to explain the effects reported with NMA is the transformation into AA and then into an epoxide.

8.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

The substance is rapidly absorbed and slowly metabolised. NMA administered to rats intraveinously was distributed rapidly in body; its distribution in tissues and subcellularly is similar to that of acrylamide. It is eliminated via glutathione conjugation and subsequent renal excretion which excludes bioaccumulation.

The mercapturate N-acetyl-S-(3-hydroxymethylamino-3-oxopropyl) cysteine, arising from direct conjugation of NMA with glutathione and subsequent metabolism, is the major urinary metabolite. In addition to conjugation with GSH, other potential metabolic pathways, e.g by the P450 system, are unknown.

QSAR analysis by OECD toolbox supports the hypothesis of the conversion of NMA to AA. However, there is no direct evidence if NMA can be converted to acrylamide or an epoxide from *in vivo* studies in rodents.

DEREK modelisation shows consistent predictions regarding carcinogenicity and neurotoxicity between NMA and AA confirming their structural similarities. For genotoxicity, an additional alert was identified for NMA regarding mutagenicity *in vitro*.

Table 11: Summary table of predicted toxicity of NMA and AA by Derek KB 2015 2.0

	NMA	AA
Carcinogenicity	**Carcinogenicity in mammal is PROBABLE** Alert matched: 744 alpha,beta-Unsaturated amide, nitrile or nitro compound Exact example match: NMA	**Carcinogenicity in mammal is PROBABLE** Alert matched: 744 alpha,beta-Unsaturated amide, nitrile or nitro compound Exact example match: AA
Genotoxicity	**Chromosome damage *in vitro* in mammal is PLAUSIBLE** Alert matched: 311 alpha,beta-Unsaturated amide or thioamide	**Chromosome damage *in vitro* in mammal is PROBABLE** Alert matched: 311 alpha,beta-Unsaturated amide or thioamide Exact example match: AA
	Mutagenicity *in vitro* in bacterium is EQUIVOCAL Alert matched: 307 N-Methylol compound or precursor	**Mutagenicity *in vitro* in bacterium is INACTIVE** No misclassified or unclassified features
Neurotoxicity	**Neurotoxicity in mammal is PLAUSIBLE** Alert matched: 124 Acrylamide or glycidamide	**Neurotoxicity in mammal is PROBABLE** Alert matched: 124 Acrylamide or glycidamide Exact example match: AA
Other endpoints	**Skin sensitisation in mammal is PLAUSIBLE** Alert matched: 426 Formaldehyde donor	-
	HERG channel inhibition *in vitro* in mammal is DOUBTED	

In conclusion, based on the knowledge on the NMA composition in registration dossiers and in batch tested in toxicological studies, on kinetics available with NMA and on OECD QSAR toolbox and DEREK predictions, it is considered that the effects reported in the studies performed with NMA are due to intrinsic properties of this substance.

9 EVALUATION OF HEALTH HAZARDS

9.1 Acute toxicity - oral route

Not evaluated.

9.2 Acute toxicity - dermal route

Not evaluated.

9.3 Acute toxicity - inhalation route

Not evaluated.

9.4 Skin corrosion/irritation

Not evaluated.

9.5 Serious eye damage/eye irritation

Not evaluated.

9.6 Respiratory sensitisation

Not evaluated.

9.7 Skin sensitisation

Not evaluated.

9.8 Germ cell mutagenicity

Table 12: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Bacterial reverse mutation assay (e.g. Ames test) (gene mutation) Similar to guideline OECD, no GLP As reported by Ames *et al* (1975) with modifications described in Zeiger *et al* (1988) and Harworth *et al* (1983) Limitations: - 4 strains instead of 5 recommanded - limited data on test system and conditions - dose rationale not specified Key study Reliability 2 with restrictions (klimisch score)	NMA batch number 1-45-000 (purity > 98% in water) No analytical purity	*S.typhimurium* strains TA97, TA 98, TA 100 and TA 1535 (met. Act. : with and without 0, 100, 333, 1000, 3333, 10000 µg/plate	Negative Toxicity was observed at 10000 mg/plate and since the test article is infinitely soluble this must have been due to cytotoxicity and not to precipitation	NTP (1989)
Sister chromatid exchanges (SCEs) No OECD guideline, no GLP As reported by Galloway *et al*. (1985, 1987) Supportive study Reliability 2 with restrictions (klimisch score)	NMA batch number 1-45-000 (purity > 98% in water)	Chinese hamster ovary (CHO) (met. Act.: with and without) Test concentrations : 16.7, 50, 125, 166.7, 250 µg/mL (-S9) (26-28h exposure) and 166.7, 500, 1700 µg/mL (+S9) (26-28h exposure) Solvent : DMSO Positive controls : - S9: Mitomycin C +S9: Cyclophophamide	Weakly increased frequency of sister chromatid exchange with and without metabolic activation. No information if this increase is dose-related and statistically significant.	NTP (1989)
Chromosome aberration Similar to OECD guideline 473, no GLP As reported by Galloway *et al.* (1985,	NMA batch number 1-45-000 (purity > 98% in water)	Chinese hamster ovary (CHO) (met. Act.: with and without) Test concentrations : 16.7, 50, 125, 166.7, 250 µg/mL (-S9) (10-13h exposure) and 166.7, 500, 1700	Positive Dose-related increase in chromosomal aberrations both with and without activation using rat liver S9	NTP (1989)

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
1987) Because of significant chemical-induced cell cycle delay, the incubation period was extended to approximately 5 hours. Key study Reliability 2 with restrictions (klimisch score)		µg/mL (+S9) (10-13h exposure) Solvent : DMSO Positive controls : - S9: Mitomycin C +S9: Cyclophophamide		

Detailed study summaries are available in Annex I of the CLH report.

Table 13: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Dominant lethal assay including in a RABC study No OECD Guideline No GLP Key study Reliability : 2 with restrictions (Klimisch score)	NMA (97-99% purity) (batch not specified)	Mouse (Swiss) male oral: drinking water 0 ppm; 60 ppm; 180 ppm; 360 ppm (nominal in water) Number of animals : 20, 20, 19 and 20 for each group respectively 13 week treatment Positive control: none Negative controls: concurrent vehicle	<u>At 360 ppm :</u> Early fetal resorptions: 2.98 vs 0.79 in controls ($p<0.05$) Total implantation losses: 3.18 vs 1.06 in controls with a dose-related trend ($p> 0.05$) Live fetuses :10.5 vs 13.6 in controls ($p<0.05$). <u>At 180 ppm:</u> Early fetal deaths not statistically significantly: 1.31 vs 0.79 in controls Total implantation losses significant: 1.52 vs 1.06 in controls <u>At 60 ppm:</u> No significant change ➔ NMA induced dominant lethal mutations after almost 13 weeks of treatment in the dominant lethal phase of a continuous breeding (RABC) study	Chapin (1995)

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			Toxicity: yes (neurotoxicity - Grip strength)	
Dominant lethal assay in acute and subchronic studies No OECD guideline. GLP Key study Relability 2 with restrictions (klimisch score)	NMA (purity 97-99%) Batch number : RTI log No. 5597-62-10	Male B6C3F1 mice (8-week-old) Hank's Balanced Salt Solution (HBSS) as negative control intraperitoneal (i.p.) injection **Acute studies** 1st study 30 males were administered NMA as a single i.p. injection of 150 mg/kg NMA, 20 males as controls 13 matings for controls and for treated groups 2nd study a second male dominant lethal experiment was conducted using 5 daily injections of 50 mg/kg per day; 36 males were administered NMA and 20 males were given HBSS as the negative control. The mating interval covered 2.5–6.5 days after the final i.p. treatment. 3 matings for control and treated group **13-Week Drinking Water Study** tested concentrations: 180, 360, 540, 720 ppm (approximately equivalent to doses of 37, 68, 90–95 and120–125 mg/kg/day) 30 males each in the 180 and 360 ppm dosed group, 10 males each in the 540 and 720 ppm dosed groups and 20	**Acute studies**: 1st study: non significant increase in the first two mating intervals (post-treatment days 0.5–3.5 and 4.5–7.5). 2nd study: negative **13-Week Drinking Water Study** First mating interval (days 7–11 of treatment): increased dead implants (4.7%, 10.3%, 11.4%, 22.6% for each dose respectively compared to a control value of 4.9 %) After only 1 week of treatment a dose-related increase in the dominant lethal response was observed, with the highest dose of 720 ppm showing a statistically significant elevation in mutations compared to the control value and with marginal increases seen at concentrations of 360 and 540 ppm. The response at the lowest dose of 180 ppm was not different from control values. Second mating interval (days 49–53 of treatment): increased dead implants (8.8%, 32.7%, 27.1%, 63.8% for each dose respectively compared to a control value of 4.9 %). After 8 weeks of treatment a dose-related increase in dominant lethal mutations observed, with responses at the 3 highest doses significantly elevated over the control value (although the two middle doses were not significantly different from each other) and with the low dose elevated, but not significantly. Third and final mating interval (days 84–88 of treatment): increased dead implants (13.9%, 20.7%, 32.5%, and 63.6% for each dose respectively compared to a control value of 5.0).	Witt (2003)

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		males in the control group. 4 matings for control and treated group No positive control Collection of urine at 8, 24, 48, and 72 hr.	After 13 weeks of treatment significant dose-related increases in dominant lethal responses at all four dose levels. These germ cell effects appear to have reached a plateau by 8 weeks of treatment, as the frequency of dominant lethal mutation in the highest three dose levels did not change appreciably from the second to the third mating interval.	
Micronucleus assay Assessment of micronucleus (MN) induction with Hb adduct measurement. Similar to OECD Guideline GLP negative controls: concurrent vehicle saline under sterile conditions Positive control : Mitomycine C (MMC) Key study Reliability 2 with restrictions (klimisch score)	NMA (48 % in water) purity > 98% in water AA was also tested in this study	**Male CBA mice (8-week-old)** 6 animals per dose group intraperitoneal (i.p.) injection Tested concentrations : 35, 71 and 142 mg NMA/kg body weight for 13 weeks Mice were sacrificed after 48h and blood was collected for MN test and Hb adduct measurement. **Male Sprague–Dawley rats (8-week-old)** 4 animals per dose group Tested concentrations : 142 mg/kg body weight for 13 weeks intraperitoneal (i.p.) injection One group of rats was sacrificed after 24 h and one group after 48 h and bone marrow was collected for the MN test and blood for the Hb adduct (N-(2-carbamoyl-2-hydroxyethyl)valine (GAVal derived from Glycidamide) analysis.	**Mice:** Positive Dose-dependent increases in both Hb adduct level and MN frequency in peripheral erythrocytes. The ratio between incremental MN frequency and the metabolite adduct level (MN-increase/GA–Val adduct), was three times higher after NMA treatment than after AA treatment. **Rat:** Negative No increase in micronuclei frequency in bone marrow erythrocytes.	Paulsson (2002)

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Micronucleus assay on bone marrow of mice in acute and subchronic study Similar to OECD guideline, GLP. Key study Reliabiliy 2 (with restrictions (Klimisch score)	NMA (purity 97-99%) Batch number : RTI log No. 5597-62-10	Male B6C3F1 mice (8-week-old) **Acute study:** NMA administered twice by i.p. injection or gavage at doses ranging from 37.5–150 mg/kg 10 animals per group positive control : DMBA (dimethylbenzantracene) **Subchronic 31-day study:** 10 male mice per treatment group, gavage 7 days per week for 31 days with NMA (dissolved in tap water). Doses : 42, 84, and 168 mg/kg Positive control: urethane **Subchronic 13-Week Drinking Water Study*:*** At the end of the 13-week dosing period of the drinking water dominant lethal study (see Witt, 2003 above), blood samples were obtained by tail snip from six randomly selected male mice from each treatment group.	**Acute Study** No significant increases in the frequencies of MN-PCE. **Subchronic 31-day Gavage Study** No significant increases in the frequencies of MN-PCE or MN-NCE in the bone marrow or peripheral blood. **Subchronic 13-Week Drinking Water Study** No significant increases in the frequencies of MN-PCE or MN-NCE noted in the bone marrow or peripheral blood.	Witt (2003)

Detailed study summaries are available in Annex I of the CLH report.

9.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

***In vitro* studies:**

In *in vitro* studies, NMA was not mutagenic in S. *typhimurium* strains TA97, TA98, TA100, or TA1535 when tested with or without exogenous metabolic activation. In studies with CHO cells,

NMA induced both sister chromatid exchanges (SCEs) with and without metabolic activation. NMA caused a dose-related increase in chromosomal aberrations both with and without activation using rat liver S9 in CHO cells.

***In vivo* studies:**

In micronucleus assays, micronucleus induction was observed only in mouse peripheral erythrocytes in one study (Paulsson *et al*., 2002). This result was not reproductible in another study performed in 2003 by Witt *et al.*, probably due to differences in protocol, route of administration, duration of exposure, strain of species used,..).

Two dominant lethal studies were reported and show that NMA induce heritable mutations in mice. Chapin *et al.* in 1995 reported some early fetal resorptions, total implantation losses (with a dose-related trend) in the high dose group of 360 ppm ($p<0.05$). At the mid dose of 180 ppm, an increase of total implantation losses was statistically significant but early fetal deaths were not statistically significant. NMA induced dominant lethal mutations after almost 13 weeks of treatment in the dominant lethal assay including in a NTP continuous breeding study. In a second study by Witt *et al.* in 2003, NMA clearly induces genetic damage in the germ cells of male mice. The observed germ cell response was dependent on the route of administration and exposure duration, with positive effects seen following subchronic oral administration in drinking water but not after single or 5 fractionated doses i.p. injections.

Furthermore, NMA is structurally close to AA which is a known mutagen. In particular, AA shows preferential binding to protamine in mouse sperm (Sega *et al.*, 1989; Sega *et al.*, 1991); this is consistent with the observed greater sensitivity of germ cells to acrylamide-induced genetic damage. Therefore, these data support the fact that NMA may cause genetic defect.

No reliable study of mutagenicity in human is available.

9.8.2 Comparison with the CLP criteria

Table 14: Results of genotoxicity studies in comparison to the CLP criteria

Toxicological results	**CLP criteria**
No clear positive evidence was observed from human data. Thus, a classification category 1A is not appropriate for NMA	The classification in Category 1A is based on positive evidence from human epidemiological studies.

Testing *in vitro*: Bacterial mutation assays: Negative Tests involving mammalian cells: Positive (Sister Chromosomal Exchange and chromosomal aberration test in CHO cells) Testing *in vivo* (experiments in mammals): On somatic cells (MN assays): - Contradictory results after ip administration in mice - Negative after oral administration in mice - Negative after ip administration in rats On germ cells (lethal dominant tests): - Positive in 2 assays in mice In conclusion, results are positive in at least two valid *in vivo* mammalian germ cell mutagenicity test. There are also positive results from one valid *in vivo* mammalian somatic cell, but this result was not reproductible in one other micronucleus assay. Finally, the fact that NMA is a structural analogue of AA which is already classified as Muta. Cat. 1B and that a metabolisation to AA cannot be excluded supports the need to classify NMA as a mutagen agent. Thus, based on these results, a classification mutagen category 1B is considered appropriate for NMA.	The classification in Category 1B is based on: — positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or — positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells *in vivo*, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or — positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.
Clear genetic damages on germ cells reported with NMA are sufficient to propose a classification mutagen category 1B.	The classification in Category 2 is based on: — positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from: — somatic cell mutagenicity tests *in vivo*, in mammals; or — other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays. Note: Substances which are positive in *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

9.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Positive results on germ cell in mice were reported in two independent dominant lethal studies. It can be discussed if these results are due to NMA itself or due to AA as an impurity or a metabolite since AA is already classified as Muta. Cat. 1B. Indeed, there are some existing NMA composition which contains AA as an impurity at levels higher than the generic concentration limit for mixture classification (0.1%). In this context, purity of NMA tested in genotoxic studies has been checked

but no adequate information can be found on the level of AA in the batches tested. Therefore, the influence of AA on the results of the available studies performed with NMA is uncertain. In addition, there were some investigations on the biotransformation of NMA to AA. At this time, although some data suggest that NMA could be metabolized into AA, there is no clear evidence of this transformation. Moreover, using DEREK modelisation, it has been found that NMA may possesses additional intrinsic genotoxic properties compared to AA. In conclusion, it has been considered that the effects found in the genotoxic studies are related to an intrinsic property of NMA or of its metabolites and thus NMA is proposed to be classified Muta. 1B, H340.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

For the assessment of germ cell mutagenicity, the DS evaluated three *in vitro* and four *in vivo* studies. The *in vitro* studies included one Ames test (Bacterial reverse mutation assays), one Sister Chromatid Exchange (SCE) test in Chinese hamster ovary (CHO) cells, and one Chromosome Aberration (CA) test in CHO cells. None of the *in vitro* tests were performed according to OECD test guidelines (TG) or GLP. However, the Ames and CA tests were performed in accordance with TGs similar to the OECD TGs. The *in vivo* studies included two dominant lethal assays in male mice and two micronucleus assays, one in male rats and mice and one in male mice. None of the assays were performed according to an OECD TG, but one dominant lethal assay was performed according to GLP. The two micronucleus assays were performed according to GLP and were similar to OECD TG.

In vitro studies

NMA was not mutagenic in S. *typhimurium* strains TA97, TA98, TA100 and TA1535 when tested with or without metabolic activation. In CHO cells, a weak increase in SCE was reported without a dose-response relationship both with and without metabolic activation. A dose-related increase in CA with and without metabolic activation using rat liver S9 was reported. The NMA tested had a purity > 98%.

In vivo studies

In the first dominant lethal assay in Swiss male mice (NMA purity 97-99%, Chapin *et al.*, 1995), NMA induced heritable mutations evident as a statistically significant increase in early foetal resorptions and total implantation losses with a dose-related trend. The treatment was for 13 weeks with doses of 0, 60, 180 and 360 ppm in drinking water.

In the second dominant lethal assay in B6C3F1 male mice (NMA purity 97-99%, Witt *et al.*, 2003) an acute i.p. study and a 13-week drinking water study was included. In the acute study, a single i.p. dose of 150 mg/kg bw or five i.p. doses of 50 mg/kg bw/day of NMA induced no effects on implantations, live embryos, dead implants or resorptions. In the 13-week drinking water study NMA induced genetic damage in the germ cells of male mice which was shown to reach a plateau after 8 weeks of treatment.

In the first micronucleus assay (NMA purity 98%, Paulsson *et al.*, 2002), NMA was given via i.p. for 13 weeks at doses of 0, 35, 71 and 142 mg/kg bw/d to male CB mice and male Sprague-Dawley rats. In mice, NMA induced a dose-depended increase in both Hb-adducts and micronucleus (MN) frequency in peripheral lymphocytes. In the rats no

increase in MN was reported.

In the second micronucleus assay (NMA purity 97-99%, Witt *et al*., 2003), NMA was given twice with a 24 hour interval by i.p. or by gavage at doses ranging from 37.5 to 150 mg/kg bw. In the subchronic study NMA was given by gavage at doses of 42, 84 and 168 mg/kg bw for 31 days. Further, a subchronic 13-week study was performed with 0, 180, 360, 540 and 720 ppm NMA in drinking water (the corresponding doses in mg/kg bw are given in the table below). In the acute study, no increase in MN frequencies in Poly Chromatic Erythrocytes (PCE) was reported. In the subchronic 31 day gavage and in the 13-week drinking water study no increase in MN in PCE or NCE (normochromatic erythrocytes) in bone marrow or peripheral blood was reported.

Based on the available data, the DS proposed a classification for NMA as Muta. 1B based on the following; results were positive in at least two valid *in vivo* mammalian germ cell mutagenicity tests. There were also positive results from one valid *in vivo* mammalian somatic cell test, but this result was not reproducible in one other MN assay.

Furthermore, the DS considered that NMA is a structural analogue of AA which is already classified as Muta. 1B. and as metabolisation of NMA to AA cannot be excluded, this supports the need to classify NMA as a mutagenic agent.

Thus, based on these results, classification as Muta. 1B was considered appropriate by the DS for NMA.

Comments received during public consultation

Comments were received from three MSCAs. Two MSCAs supported the DS proposal to classify NMA as Muta. 1B since the two dominant lethal tests in mice with exposure to NMA showed positive results. One of these MSCA questioned the possibility that the observed mutagenicity could be due to the presence of AA as an impurity in NMA and suggested to check the dose of AA required to induce such effects and compare this to the maximum theoretical amount of AA in the dominant lethal studies with NMA. The DS compared the carcinogenicity data and the doses which induced tumours for AA and NMA and concluded that AA induced lung and ovary tumours at doses which were lower than for NMA. The similarity in target organs and the observation that AA seems more potent than NMA were considered to be consistent with NMA being metabolised to AA.

The third MSCA did not support a classification of NMA as Muta. 1B, but considered that the data were more in favour of a classification as Muta. 2. This was based on the following;

- No *in vivo* mutagenicity tests were performed according to OECD TG, and the results from the two MN *in vivo* tests were contradictory. Further, no positive controls were included in the dominant lethal tests, which is considered a requirement according to OECD TG 478.
- The MSCA also questioned the DS statement that NMA is a structural analogue of AA which is classified as Muta. 1B, supporting classification of NMA as Muta. 1B. The issue was raised that since according to the CLP regulation (table 3.5.1), substances which are positive in *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Muta. 2. As a reliable positive *in vitro* mammalian mutagenicity assay is available for NMA (NTP, 1989) and the DS

postulated structural similarity to AA, the classification criteria for Muta. 2 were considered to be met.

Assessment and comparison with the classification criteria

For the assessment of germ cell mutagenicity the DS included three *in vitro* studies and four *in vivo* studies. The *in vitro* studies were one Ames test as well as one SCE test and one CA test, both of which were performed in Chinese hamster ovary (CHO) cells. The *in vivo* studies included two dominant lethal assays in male mice and two micronucleus assays, one in male rats and mice and one in male mice. None of the tests were performed according to OECD TG.

In vitro studies

NMA was not mutagenic in *S. typhimurium* strains TA97, TA98, TA100 and TA1535 when tested with or without metabolic activation in a study similar to OECD TG. The doses tested were from 100 to 10000 µg/plate. A weak increase in SCE, however, not reaching statistical significance, was reported in CHO cells with and without metabolic activation (non OECD TG). The doses tested were: 16.7, 50, 125, 166.7, 250 µg/mL (-S9) and 166.7, 500, 1700 µg/mL (+S9). In the CA assay in CHO cells (similar to OECD TG), a dose-related increase in CA with and without metabolic activation using rat liver S9 was reported (see the table below).

Table: Induction of CA in CHO cells

	Dose **µg/mL**	Total cells –S9 / +S9	# of CA –S9 / +S9	CA / cell –S9 / +S9	% cells with CA –S9 / +S9
Negative control*	-	200/200	2/3	0.01/0.02	0.5/0.5
NMA	250	200/200	16/95	0.08/0.48	7.0/11.5
NMA	375	200/25	48/95	0.24/3.80	18.5/56.0
NMA	500	50/25	57/149	1.14/5.96	52.0/92.0
Positive control*	0.05	200/200	67/34	0.34/0.17	24.0/14.0
Positive control**	0.08	25/25	15/42	0.60/1.68	40.0/72.0

*Dimethyl sulfoxide; **Mitomycin C

In vivo studies

Positive results were reported in both dominant lethal assays in male mice (non OECD TG). In the first dominant lethal assay in male Swiss mice (Chapin *et al.*, 1995) the treatment was for 13 weeks with doses of 0, 60, 180 and 360 ppm NMA in drinking water (19-20 male mice/group). No positive control was included. NMA induced heritable mutations, evident as a statistically significant increase in early foetal resorptions and total implantation losses, both with a dose-related trend (see table below).

Table: Dominant lethal data in male Swiss mice following exposure to NMA

Dose (ppm)/mg/kg bw/day	0	(60)/13	(180)/37	(360)/68
Number of litters/male	20	20	19	20
Early resorptions	0.79 ± 0.19	1.04 ± 0.20	1.31 ± 0.20*	2.98 ± 0.26*/**
Dead foetuses	0.23 ± 0.09	0.08 ± 0.05	0.10 ± 0.06	0.11 ± 0.06
Total implantation losses	1.06 ± 0.21	1.15 ± 0.19	1.52 ± 0.22*	3.18 ± 0.25*/**
Live foetuses	13.6 ± 0.4	12.9 ± 0.40	13.2 ± 0.30	10.5 ± 0.5*/**

*Statistically significant different from controls ($p < 0.05$); **Dose-related trend ($p < 0.05$)

In the second dominant lethal assay (non OECD TG) in B6C3F1 male mice (Witt *et al*., 2003), an acute i.p. study (30 male mice/group) and a 13-week drinking water study (30 male mice/group) was included. In the acute study, a single i.p. dose of 150 mg/kg bw or five i.p. doses of 50 mg/kg bw/day of NMA induced no effects on implantations, live embryos, dead implants or resorptions. In the 13-week drinking water study NMA induced genetic damage in the germ cells of male mice following exposure to NMA (0, 37, 68, 90 and 120 mg/kg bw/day), which was shown to reach a plateau after 8 weeks of treatment (see table below). No positive control was included in the study.

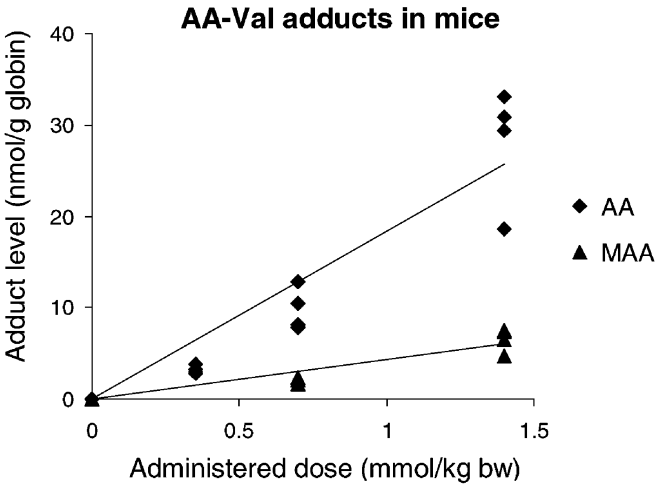
Table: Number of dead implants for three different mating intervals (Witt et al., 2003)

Dead implants	Control	180 ppm 37 mg/kg bw/day	360 ppm 68 mg/kg bw/day	540 ppm 90 mg/kg bw/day	720 ppm 120 mg/kg bw/day
1. mating (7-11 days)	4.9%	4.7%	10.3%	11.4%	22.6%*
2. mating (49-53 days)	4.9%	8.8%	32.7%*	27.1%*	63.8%*
3. mating (84-88 days)	5.0%	13.9%*	20.7%*	32.5%*	63.6%*

*Statistically significant $p \leq 0.001$ (Dunnett's test for pairwise comparisons)

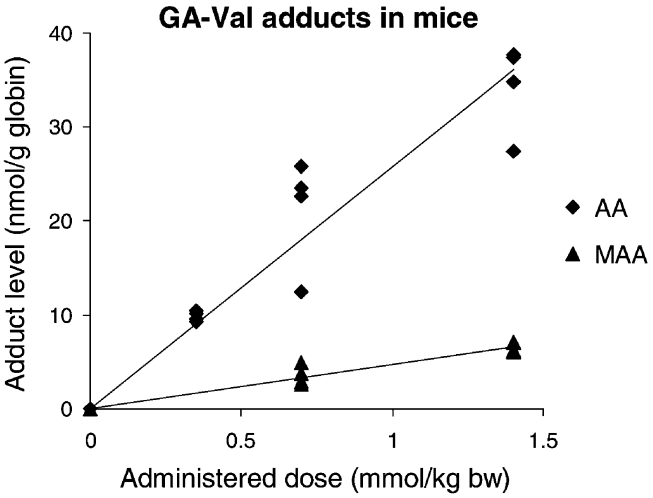
In the first micronucleus assay (Paulsson *et al*., 2002, similar to OECD TG) NMA and AA was given to male CBA mice and male Sprague-Dawley rats via i.p. for 13 weeks at doses of 0.35, 0.7 and 1.4 mmol/kg bw/day corresponding to 0, 35, 71 and 142 mg/kg bw/day NMA and 25, 50 and 100 mg/kg bw/day AA. The rats received only the highest dose of NMA and AA. MMC (1 mg/kg bw/day) was included as a positive control in rats. In male mice exposed to NMA a dose-depended increase in Hb-adducts from NMA or AA was measured as N-(2-carbamoylethyl)valine adducts (AA Val) by the *N*-alkyl Edman method. Using this method analysis of Hb-adducts following *in vitro* or *in vivo* MAA exposure is shown to result in an Edman derivative that is similar to the AA Val adduct formed following exposure to AA (Tareke, 1998). In the study by Paulsson *et al*. (2002), the adducts formed from NMA or AA and its epoxymetabolites (N-(2-carbamoyl-2-hydroxyethyl)valine) were analysed as AA Val adducts and GA Val adducts, respectively (see the figures below).

Figure: AA Val adducts in male mice following exposure to NMA and AA (from Paulsson et al., 2002):



Val: Valine, AA: Acrylamide, MAA the same as NMA

Figure: GA Val adducts in male mice following exposure to NMA and AA (from Paulsson et al., 2002):

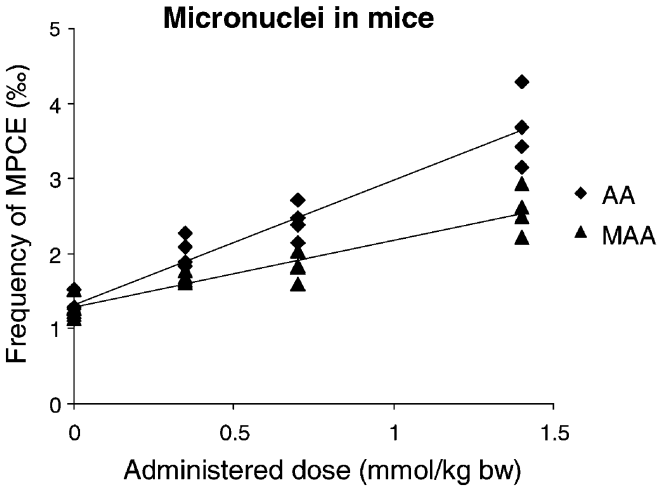


GA: Glycidamide, AA: Acrylamide, MAA the same as NMA

Data from the lowest dose was excluded for NMA due to a suspected mistake at injection (adduct level approximately the same as for controls).

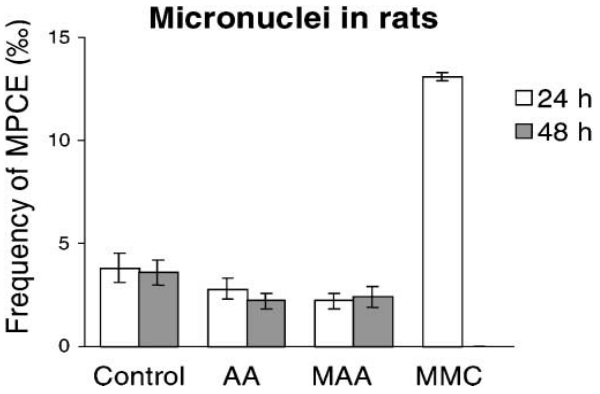
An increase in MN frequency in peripheral blood erythrocytes was also reported in male mice (see figure below).

Figure: MN in male mice following exposure to NMA and AA (from Paulsson et al., 2002):



In rats, no increase in Hb-adducts or MN in bone marrow were reported following exposure to NMA and AA, however, a statistically significant increase in MN following exposure to the positive control MMC was reported (see figure below).

Figure: MN in male rats following exposure to NMA, AA and the positive control MMC.



The Hb-adduct levels in mice and rats were shown to be three to six times higher in AA-treated mice and rats compared to NMA-treated mice and rats which may be due to a slower reaction of NMA compared with AA (see table below).

Table: Hb-Val adduct levels in mice and rats following exposure to NMA and AA (1.4 mmol/kg bw).

Hb-Val adduct levels	Adduct	Mouse	Rat	Mouse/Rat
AA	AA-Val	20.0 (16.4–23.6)	26.2 (21.1–31.4)	0.76 (0.59–1.1)
	GA-Val	24.5 (21.8–27.2)	6.8 (5.9–7.7)	3.6 (2.7–5.4)
NMA	AA-Val	4.7 (3.9–5.4)	9.8 (6.9–12.8)	0.47 (0.31–0.97)

	GA-Val	4.6 (4.3–4.9)	2.2 (1.9–2.5)	2.1 (1.8–2.5)

As regards the induction of MN, an increased frequency of MN in peripheral blood erythrocytes was reported following exposure to both NMA and AA in male mice, however, the potency of NMA was only half that of AA (see figure showing MN in male mice, above). The study indicated a more efficient metabolic conversion of NMA or AA to the epoxy metabolites in the mouse compared to rats. Since GA is considered to be the genotoxic species after uptake of AA, this may at least in part explain the reported lack of effect of both AA and NMA in the rat MN assay.

In the second micronucleus assay (Witt *et al*., 2003, similar to OECD TG), NMA was given by different NMA administration and by different exposure duration. In the acute study NMA was given twice with 24 h intervals by i.p. or by gavage at doses ranging from 37.5 to 150 mg/kg. The positive control was dimethylbenzanthracene (DMBA). In a sub-chronic study NMA was given by gavage at doses of 42, 84 and 168 mg/kg bw/day for 31 days. The positive control was urethane (466.7 mg/kg bw/day). Further, a subchronic 13-week study was performed with 0, 180, 360, 540 and 720 ppm NMA in drinking water (equivalent to 0, 37, 68, 90 and 120 mg/kg bw/day). In the acute study, no increase in MN frequencies in PCE was reported in the bone marrow following exposure to NMA, however, a clear increase was reported for DMBA. In the sub-chronic 31 day gavage and in the 13-week drinking water study no increase in MN in PCE or NCE in the bone marrow or the peripheral blood, respectively, was reported. In the positive control urethane showed a clear increase in MN-PCE and MN-NCE in the 31-day gavage study.

The difference in the methods used in two MN studies included: 1) the NMA used was from different suppliers (in Paulsson *et al*. (2002) it was from Fluka-Chemika where the NMA was 48% in water, and in Witt *et al*. (2003) from the NTP chemical repository at Research Triangle Institute); 2) the strain of mice was different (Paulsson *et al*., 2002 used CBA mice and Witt *et al*., 2003 used B6C3F1 mice); 3) the method of data collection (Paulsson *et al*. (2002) employed the more sensitive flow cytometric measurements of 160000 PCE in each of four animals per treatment group than in the Witt *et al*. (2003) study), indicating that the scoring technique used by Paulsson *et al*. (2002) provided a greater power to detect an increase in MN over the background level.

In summary:

No reliable study of mutagenicity in humans is available. Thus, RAC considers that classification as Muta. 1A is not justified.

Experimental data following exposure to NMA was available from germ cells, somatic cells and from *in vitro* studies.

Germ cells: Two independent dominant lethal assays, in which male mice were exposed to NMA, reported positive results, however, the studies were not performed according to OECD TG and no positive control was included, both of which could be seen as limitations of the studies. One of the studies reported a statistically significant increase in early foetal resorptions and total implantation losses with a dose-related trend, and the second study reported an increase in dead implants in three matings. However, it is in principle possible that the positive results are related either to exposure to NMA or due to AA as an impurity in NMA (the purity of NMA was 97-99% without any further information regarding the impurity), since AA is classified as Muta. 1B. RAC concludes that it is not reasonable that the positive results would be caused by AA alone, although a contribution

from AA cannot be excluded. As regards the absence of a positive control in the studies, according to the OECD TG 478 a positive control shall be included in the dominant lethal assay unless the laboratory has demonstrated proficiency in the conduct of the test and has used the test routinely in the recent past (e.g. within the last 5 years). The DS informed that the two studies were performed according to the RACB (Reproductive Assessment by Continuous Breeding) protocol from NTP, and the design has been used by NTP for 15 years.

Somatic cells: NMA induced PCE-MN in one study in mice following NMA i.p. exposure for 13 weeks. However, another independent study in mice with i.p., gavage and drinking water administration of NMA, was negative, but there were differences in the methodology used in the two studies. The differences were related to the supply of NMA, the mouse strain used and the sensitivity of the scoring technique for MN. These differences may have had an influence on the different results from the two mouse studies. In rats no induction of MN in bone-marrow was reported.

In vitro studies: The bacterial mutation assay was negative. However, in mammalian cells NMA induced SCE and CA in CHO cells.

A classification as Muta. 1B is based on positive results(s) from *in vivo* heritable mutagenicity tests in mammals, or positive results from *in vivo* somatic cell mutagenicity tests in mammals in combination with some evidence that the substance has the potential to cause mutations in germ cells.

Based on the data available for NMA with positive results in two independent Dominant Lethal Assays, one positive results from a test for the induction of MN and in the absence of information regarding the presence of AA as an impurity in NMA in these studies, RAC considers that classification of NMA as Muta. 1B is justified.

9.9 Carcinogenicity

Table 15: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Carcinogenicity study in Fischer rat 50 males and 50 females per dose group Equivalent or similar to OECD Guideline 451 GLP Key study Reliability 2 with	NMA Batch number 1-45-000 (purity > 98% in water) oral: gavage 0, 6 and 12 mg/kg in water (nominal conc.) Vehicle: water Exposure: 103 weeks (5 days a	Mortality: The survival of low dose female rats was significantly lower than that of vehicle controls after day 550. No significant differences in survival were observed between any other groups of either sex. No treatment- related neoplasms. Incidence of keratoacanthomas of the skin in low dose male rats was significantly greater than that in the controls (1/50 control, 6/50 low dose, 3/50 high dose). Incidence of all skin tumors combined (basal-cell papillomas, basosquamous tumors, keratoacanthomas, squamous-cell papillomas, or sebaceous adenomas; 5/50 control, 8/50 low dose, and 5/50 high dose) were not increased in dosed male rats.	NTP (1989), Bucher (1990), IARC (1994)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
restriction (klimisch score)	week)	Cystic degeneration of the liver at a marginally increased incidence in high dose male rats (10/50 control, 8/50 low dose, 19/50 high dose). No other non neoplastic lesions related to NMA.	
Carcinogenicity study in mouse (B6C3F1) 50 male/50 female per dose group Equivalent or similar to OECD Guideline 451 (Carcinogenicity Studies) GLP Key study Reliability 2 with restriction (klimisch score)	NMA Batch number 1-45-000 (purity > 98% in water) oral: gavage 0, 25, 50 mg/kg in water; Vehicle: water Exposure: 105 weeks (5 days a week)	Mortality: Deaths of eight low dose male mice between week 8 and week 32 were considered to be due to an urinary infection; all other early deaths of low dose males and the majority of early deaths of high dose male mice were attributed to the presence of tumors. No significant differences in survival were observed between any groups of either sex. **Treatment-related neoplastic effects: yes** <u>Hardarian gland:</u> Adenomas: increase in males: 1/48; 14/49; 29/50 ($p < 0.001$ at all doses tested), respectively and in females: 5/47; 8/45; 20/48 ($p < 0.001$, at 50 mg/kg/d), respectively. Increase exceeded the historical data. Carcinomas: not significantly increased. The incidence of adenomas and carcinomas (combined) was increased in both sexes and exceeded the historical control data (HCD). <u>Liver :</u> Hepatocellular adenomas: increase (male: 8/50; 4/50; 19/50; $p < 0.05$, at 50 mg/kg bw; female: 3/50; 4/50; 17/49; $p < 0.001$, at 50 mg/kg bw). The incidences also exceeded HCD. Hepatocellular carcinomas: marginally increase in male: 6/50; 13/50; 12/50 ($p = 0.023$ at both doses), respectively. Adjusted rates of carcinomas in males were outside of the HCD. The incidence of hepatocellular adenomas and carcinomas (combined) showed a positive trend, and the incidences were higher than those in the vehicle controls: males (12/50; 17/50; 26/50 ($p < 0.001$ at 50 mg/kg/d); females (6/50; 7/50; 17/49 ($p = 0.002$ at 50 mg/kg/d). <u>Lung:</u> Alveolar bronchiolar adenomas: increase (3/49; 6/50; 11/50; $p < 0.05$, at 50 mg/kg/d) Alveolar bronchiolar carcinomas: increase (2/49; 4/50; 10/50; $p < 0.05$, at 50 mg/kg/d). The incidence of alveolar-bronchiolar adenomas and carcinomas (combined) showed a positive trend in male (5/49; 10/50; 18/50; $p < 0.001$, at 50 mg/kg/d)	NTP (1989), Bucher (1990), IARC (1994)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		and in female (6/50; 8/50; 13/49; $p < 0.05$, at 50 mg/kg/d). All these incidences were outside the ranges of HCD. Ovary: Benign granulosa-cell tumours: increase (0/50; 5/45; 5/47; $p < 0.05$, at all doses). Forestomach: Squamous papillomas in few animals (male: 0/50; 1/49; 2/48; female: 0/46; 0/16; 2/44) – not statistically significant. Anterior Pituitary gland: Adenomas of the pars distalis: significantly decrease in female at both tested doses (13/49; 5/14; 4/43). **Non-neoplastic lesions: yes** Lung: chronic inflammation (male: 8/49; 12/50; 20/50; female: 12/50; 28/50; 14/49) and alveolar epithelial hyperplasia were observed at slightly increased incidences (male: 10/49; 17/50; 19/50; female: 8/50; 26/50; 17/49). These two lesions generally occurred together and appeared to be part of the same lesion. Ovary: Ovarian atrophy was observed at increased incidences in female mice receiving NMA (3/50; 39/45; 38/47). Atrophy was characterized by a complete absence of follicular and luteal activity, often accompanied by a decrease in ovarian size. Spleen: Hematopoietic cell proliferation in the spleen was increased at the highest dose (male: 11/50; 13/26; 38/50; female: 15/50; 10/19; 40/48). The proliferation was considered a secondary response to neoplastic and inflammatory lesions in various organs. Kidney: Chronic nephropathy was increased in the high dose female mice (10/50; 3/11; 23/48). The nephropathy was generally of minimal to mild severity and was consistent with changes in the kidney of aging B6C3F1 mice.	

Detailed study summaries are available in Annex I of the CLH report.

9.9.1 Short summary and overall relevance of the provided information on carcinogenicity

The substance was tested for carcinogenicity by gavage in a chronic bioassay in rats and mice.

No carcinogenic effect was found in rats. In mice, increased tumours in Harderian gland, liver, lung and ovary were reported.

The incidences of Harderian gland adenomas were increased in males given both doses tested (low and high): control, 1/48; low-dose, 14/49; and high dose 29/50 ($p < 0.001$) and in females given the high-dose: control, 5/47; low-dose, 8/45; and high-dose, 20/48 ($p < 0.001$). The values of incidences of adenomas exceeded the historical data. The incidences of carcinomas of the Harderian gland were not significantly increased by NMA administration (male: 1/48; 0/49; 2/50; female: 0/47; 3/45; 2/48). The incidence of adenomas and carcinomas (combined) was increased in both sexes and exceeded the historical control values.

The incidences of hepatocellular adenomas were increased in male and female mice given 50 mg/kg bw NMA (male: 8/50; 4/50; 19/50; $p < 0.05$; female: 3/50; 4/50; 17/49; $p < 0.001$). The incidences also exceeded historical control values. The incidences of hepatocellular carcinomas were marginally increased only in treated male mice: control, 6/50; low-dose, 13/50; and high-dose, 12/50 ($p = 0.023$, incidental tumor test for comparison between low-dose and control). Adjusted rates of carcinomas in males were outside of the historical control data. The incidence of hepatocellular adenomas and carcinomas (combined) showed a positive trend, and the incidences in high-dose males and females were higher than those in the vehicle controls: males -control, 12/50; low-dose, 17/50; and high-dose, 26/50 ($p < 0.001$); females-control, 6/50; low-dose, 7/50; and high-dose, 17/49 ($p = 0.002$).

In high-dose males only, the incidences of alveolar bronchiolar adenomas (control, 3/49; low-dose, 6/50; and high-dose, 11/50; $p < 0.05$) and carcinomas were increased (control, 2/49; low-dose, 4/50; and high-dose, 10/50; $p < 0.05$). The incidence of alveolar-bronchiolar adenomas and carcinomas (combined) showed a positive trend in male mice and was statistically significant at the highest dose (control, 5/49; low-dose, 10/50; and high-dose, 18/50; $p < 0.001$). The incidence of alveolar-bronchiolar adenomas and carcinomas (combined) was increased in high-dose females (control, 6/50; low-dose, 8/50; and high-dose, 13/49; $p < 0.05$). All these incidences were outside the ranges of historical control values.

The incidences of benign granulosa-cell tumours of the ovary were increased in treated groups (control, 0/50; low-dose, 5/45; and high-dose, 5/47; $p < 0.05$).

Non-neoplasic effects were found in the lung, ovaries, spleen and kidneys (see table above).

Based on these results, the NTP (1989) concluded that there is no evidence of carcinogenicity activity in rats but a clear evidence of carcinogenic activity in mice. In 1994, IARC concluded that NMA is not classifiable as to its carcinogenicity to humans (Group 3) based on inadequate evidence in humans and limited evidence in experimental animals for the carcinogenicity. No further explanations are provided by the IARC to justify this conclusion.

Based on the present evaluation, beside the two types of tumours that can be of questionable relevance to humans (Harderian gland tumour with no human equivalent and liver tumour frequently observed in B6C3F1 mice (NTP, 2007b, Haseman *et al*., 1998, Battershill J.M and Fielder R.J 1998), the lung and ovary tumours observed in the study in mice coupled with the mutagenicity profile of NMA are judged to be sufficient evidence for classification. Furthermore, AA, a structural analoguous which is a known carcinogen, supports the fact that NMA is a presumed human carcinogen.

9.9.2 Comparison with the CLP criteria

Table 16**:** Results of carcinogenicity studies in comparison to the CLP criteria

Toxicological results	CLP criteria
No data human data is available regarding carcinogenicity of NMA. Thus a classification as Carc. 1A is not appropriate for NMA.	Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence
Strenght of evidence: A clear carcinogenic effect (with both malignant and benign tumours) was reported by the NTP in mice. No evidence of carcinogenic effect was observed in rats. Thus, carcinogenic effect, including malignant tumours, was reported in both sexes in one species in a well-performed study. Tumour type and background incidence: Adenomas of the Harderian gland, hepatocellular adenomas/carcinomas, alveolar/bronchiolar adenomas/carcinomas and benign granulosa cell tumours of the ovary were reported. These tumours were statistically significantly increased from control values and exceeded the historical control data. Among these tumours, two types of tumours are of questionable relevance for classification. Adenomas of the Harderian gland is a tumour of questionable human relevance because this tissue has no human equivalent. According to CLP guidance (2015), tumours occurring in such tissues indicate that the substance has the potential to induce carcinogenic effects. A classification on this basis cannot be automatically ruled out in particular in a context of tumour response in other sites. Furthermore, liver tumour is particularly prevalent in B6C3F1 mice. However, it can be noted that the incidence (as adjusted rate) for adenomas/carcinomas of the liver found with NMA exceeded the historical control values. Therefore, the biological significance of these liver tumours are confirmed. Alveolar/bronchiolar adenomas/carcinomas and benign granulosa cell tumours of the ovary are considered relevant to humans. Multi-site responses: NMA induces tumours in various tissues (Harderian gland, liver, lung and ovary) Progression of lesions to malignancy: Malignant tumours were already found in liver	Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence. The classification is based on strength of evidence together with additional considerations: — animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen). Carcinogenicity in experimental animals — sufficient evidence of carcinogenicity: a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites. The placing of a substance in Category 2 (suspected human carcinogens) is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies. Carcinogenicity in experimental animals — limited evidence of carcinogenicity: the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the

and lung. Reduced tumour latency : Yes the first observation of tumor occurrence was about 700 days for control group and between 300 and 600 days for tested groups in mice. Whether responses are in single or both sexes: Tumours were reported in both sexes in mice. Whether responses are in a single species or several species: Tumours occurred in mice but not in rats. Structural similarity to a substance(s) for which there is good evidence of carcinogenicity: AA, which is classified Carc. 1B, is a structural analogue of NMA. Moreover a metabolism to AA cannot be excluded. Routes of exposure: The available carcinogenicity studies were performed by oral route. There is no data for other routes. Comparison of absorption, distribution, metabolism and excretion between test animals and humans: No information The possibility of a confounding effect of excessive toxicity at test doses: No significant differences in survival were observed between any groups of either sex in the mice study. Some non-neoplasic effects were reported in mice. Mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity: At this time, the mode of action is not fully clarified. A link between genotoxicity and carcinogenicity is expected considering the results obtained in genotoxicity studies. In addition, it can be hypothesized that, as for AA, an epoxide metabolite may be involved in the carcinogenicity of NMA. However, at this time, there is no clear evidence of the formation of this metabolite after NMA administration. Consideration of mutagenicity: There is clear evidence of *in vivo* mutagenicity with NMA.	agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs. Additional considerations: (a) tumour type and background incidence; (b) multi-site responses; (c) progression of lesions to malignancy; (d) reduced tumour latency; (e) whether responses are in single or both sexes; (f) whether responses are in a single species or several species; (g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity; (h) routes of exposure; (i) comparison of absorption, distribution, metabolism and excretion between test animals and humans; (j) the possibility of a confounding effect of excessive toxicity at test doses; (k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity. Mutagenicity: it is recognised that genetic events are central in the overall process of cancer development. Therefore evidence of mutagenic activity *in vivo* may indicate that a substance has a potential for carcinogenic effects.

Based on these results, there is sufficiently convincing evidence to propose a classification for NMA as Category 1B and not as Category 2.	

9.9.3 Conclusion on classification and labelling for carcinogenicity

Based on the results of the carcinogenicity studies available with NMA, there is no evidence of carcinogenicity in rats but a clear evidence in mice (both sexes). Hardarian gland tumours is of questionable relevance for classification purpose since there is no human equivalent tissue. However, it cannot automatically be ruled out that the substance could induce similar tumours of comparable cell/tissue origin (e.g. squamous cell tumours at other epithelial tissues) in humans (CLP guidance, 2015). Liver tumours are associated with a high spontaneous tumour incidence in B6C3F1 mice, however, since the increase exceeded the historical control data, the biological significance of these tumours are confirmed. Anyway, adenomas of the Harderian gland, alveolar/bronchiolar adenomas/carcinomas and benign granulosa cell tumours of the ovary were considered relevant for classification. In addition, the evidence from *in vivo* mutagenicity with NMA supports the carcinogenicity potential of NMA.

It can be discussed if these results are due to NMA itself or due to AA as an impurity or a metabolite since AA is already classified as Carc. Cat. 1B. Indeed, there are some existing NMA composition which contains AA as an impurity at levels higher than the generic concentration limit for mixture classification (0.1%). In this context, purity of NMA tested in carcinogenicity studies has been checked but no adequate information can be found on the level of AA in the batch tested. Therefore, the influence of AA on the results of the available studies performed with NMA is uncertain. In addition, there were some investigations on the biotransformation of NMA to AA. At this time, although some data suggest that NMA could be metabolized into AA, there is no clear evidence of this transformation. Anyway, when using DEREK modelisation, it has been found that NMA possesses intrinsic carcinogenic properties. In conclusion, it has been considered that the effects found in the carcinogenicity study in mice are related to an intrinsic property of NMA or of its metabolites and thus NMA is proposed to be classified Carc. 1B, H340.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

NMA was tested in two carcinogenicity bioassays by gavage, one in rats and one in mice. Both studies were performed according to GLP and to a test guideline equivalent or similar to OECD TG 451. The purity of NMA was > 98% in both studies.

In male and female rats, no evidence of carcinogenicity was reported, however, in male and female mice clear evidence of carcinogenicity was reported. These included: Harderian gland adenomas and carcinomas, hepatocellular adenomas and carcinomas, alveolar bronchiolar adenomas and carcinomas, and in female mice benign granulosa-cell tumours in the ovary.

Although the relevance to humans of tumours of the Harderian gland may be questioned, since there is no equivalent human tissue, these were considered relevant for classification by the DS. The DS also noted that although the relevance of liver tumours could also be questionable as the mouse strain used, B6C3F1, is associated with a high spontaneous liver tumour incidence. Since the increase exceeded the historical control data (HCD), the biological significance of these tumours was still considered relevant. Taken together, adenomas of the Harderian gland, alveolar/bronchiolar adenomas/carcinomas, liver tumours and benign granulosa cell tumours of the ovary were all considered relevant for classification by the DS. In addition, the evidence from *in vivo* mutagenicity with NMA supported the carcinogenicity potential of NMA.

The DS discussed in the CLH report whether these results are due to NMA itself or due to AA as an impurity or a metabolite, since AA is already classified as Carc. Cat. 1B (purity of NMA > 98%). In this context, the DS checked the purity of NMA tested in the carcinogenicity studies but no adequate information was found regarding the level of AA in the batches of NMA tested. Therefore, the influence of AA on the results of the available studies performed with NMA was considered uncertain. The DS indicated that there were some investigations on the biotransformation of NMA to AA. Although some data suggested that NMA could be metabolised into AA, there was no clear evidence of this transformation. Structure-based modelling using DEREK (KB 2015 2.0) predicted that NMA potentially possesses intrinsic carcinogenic properties. The DS concluded that the effects found in the carcinogenicity study in mice are related to an intrinsic property of NMA or of its metabolites and therefore the DS proposed that NMA be classified as Carc. 1B, H340.

Comments received during public consultation

Two MSCA commented; one supported the DS proposal to classify NMA as Carc. 1B, whereas the other MSCA questioned the proposed classification of NMA as Carc. 1B. This was related to the fact that there was a clear difference in the carcinogenic response in rats and mice, as tumours did not develop in rats. Furthermore, the only available mutagenicity study in rats and mice (Paulsson *et al.,* 2002) also reported positive results in mice and negative results in rats. The cause of this difference was considered as unclear, and which species was more relevant for humans was considered unknown. The MSCA also mentioned that according to the EU Risk Assessment Report of AA from 2002, AA induced tumours in rats in the thyroid, adrenals, mammary glands and testis. As both the tumour type and species differed, the role of AA in the carcinogenicity of NMA was considered as questionable. The second MSCA

also informed about a carcinogenicity study with exposure to NMA for 26 weeks in rasH2 transgenic mice (Tg mice) and non-Tg mice from 2015. The study was, however, not a carcinogenicity study but the performing laboratory tested NMA for the establishment of a reliable short-term carcinogenicity screening method. In this study, NMA treatment induced the formation of adenomas and adenocarcinomas at week 26 in the Tg mice but not in the non-Tg mice, and no expression of specific genes were apparent in either genotype of mice. The study was considered by the DS to not replace a good quality carcinogenicity study, such as the NTP mouse study.

Assessment and comparison with the classification criteria

The DS included two carcinogenicity studies with exposure to NMA (purity > 98%), one in mice and one in rats. The DS looked into the purity of NMA tested in the carcinogenicity studies but no adequate information was found regarding the level of AA in the batches of NMA tested.

In the rat study F344/N male and female rats (50/dose group) were exposed by gavage for 103 weeks 5 days/week to 0, 6 and 12 mg/kg bw/day of NMA (NTP, 1989).

The survival of the low dose females were statistically significant lower than the control group (see table below).

Table: Survival of male and female rats in the carcinogenicity study

Survival	0	6 mg/kg bw/day	12 mg/kg bw/day
Male	28	22	27
Female	35	22*	33

*p value < 0.007

The mean body weight of high dosed males were 6-7% lower than in the control group and for females 5-6% lower than in the control group.

In the skin, the incidence of keratoacanthomas in low dose male rats was statistically significantly greater than that in the controls (1/50 control, 6/50 low dose, 3/50 high dose). However, the incidence of all skin tumours combined (basal-cell papillomas, basosquamous tumours, keratoacanthomas, squamous-cell papillomas, or sebaceous adenomas were not increased in male rats (5/50 in controls, 8/50 at the low dose, and 5/50 at the high dose).

In the liver, cystic degeneration was increased marginally in high dose male rats (10/50 in control, 8/50 at the low dose, and 19/50 at the high dose).

No other non-neoplastic lesions related to NMA were reported in male or female rats. RAC therefore agrees with the DS that there were no evidence of carcinogenicity in male and female rats.

In the mouse study, B6C3F1 male and female mice (50/dose group) were exposed by gavage for 103 weeks, 5 days/week to 0, 25 and 50 mg/kg bw/day of NMA (NTP, 1989).

Deaths of eight low dose male mice between week 8 and week 32 was reported. However, the deaths were considered to be due to an urinary infection. The other early deaths of low dose males and the majority of the early deaths of high dose male mice were attributed to the presence of tumours. No statistically significant differences in survival were observed between any groups of either sex.

The mean body weights of dosed males were 13% higher than those in the control group and 25% higher for females.

Harderian gland adenomas and carcinomas

The incidence of adenomas and carcinomas in male and female mice is shown in the table below. The incidence of adenomas were statistically significantly increased in both males and females, however, the incidence of carcinomas was not increased. The incidence of adenomas and carcinomas (combined) was increased in both sexes and was outside the historical control data (HCD) from NTP studies.

Table: Harderian gland adenomas and carcinomas in mice.

Males	Control	25 mg/kg bw/day	50 mg/kg bw/day	HCD
Adenomas	1/48 (2%)	14/49 (29%)*	29/50 (58%)*	
Carcinomas	1/48 (2%)	0/49 (0%)	2/50 (4%)	20/350 (6% ± 4%)[a] 73/2040 (4% ± 3%)[b]
Adenomas & carcinomas	2/48 (2%)	14/49 (29%)*	30/50 (60%)*	22/350 (6% ± 4%)[a] 79/2040 (6% ± 4%)[b]
Females	Control	25 mg/kg bw/day	50 mg/kg bw/day	HCD
Adenomas	5/47 (11%)	8/45 (18%)	20/48 (42%)*	9/350 (3% ± 4%)[a] 41/2040 (2% ± 2%)[b]
Carcinomas	0/47 (0%)	3/45 (7%)	2/48 (4%)	
Adenomas & carcinomas	5/47 (11%)	11/45 (24%)	22/48 (46%)*	12/350 (3% ± 4%)[a] 48/2040 (2% ± 2%)[b]

[a]In water gavage vehicle controls
[b]In untreated controls in NTP studies
*$p < 0.001$

The reported adenomas and carcinomas in the Harderian gland in male and female mice, is, however, of questionable relevance for classification purposes since there is no human equivalent tissue. On the other hand, according to the CLP guidance, paragraph 3.6.2.3.2, 2017 it cannot automatically be ruled out that the substance could induce similar tumours of comparable cell/tissue origin in humans (e.g. squamous cell tumours at other epithelial tissues).

Liver: Hepatocellular adenomas and carcinomas

The incidences of hepatocellular adenomas were increased in male and female mice given 50 mg/kg bw/day NMA (see the table below). The incidences also exceeded HCD from NTP studies. The incidences of hepatocellular carcinomas were marginally increased only in

treated male mice. The incidence of hepatocellular adenomas and carcinomas (combined) showed a positive trend, and the incidences in high-dose males and females were higher than those in the vehicle controls.

Table: Hepatocellular adenoma and carcinomas mice

Males	Control	25 mg/kg bw/day	50 mg/kg bw/day	HCD
Adenomas	8/50 (16%)	4/50 (8%)	19/50 (38%)**	54/347 (16% ± 4%)[a] 259/2032 (13% ± 7%)[b]
Carcinomas	6/50 (12%)	13/30 (26%)	12/50 (24%)***	56/347 (16% ± 8%)[a] 379/2032 (19% ± 7%)[b]
Adenomas & carcinomas	12/50 (24%)	17/50 (34%)	26/50 (52%)*	106/347 (31% ± 6%)[a] 609/2032 (30% ± 8%)[b]
Females	Control	25 mg/kg bw/day	50 mg/kg bw/day	HCD
Adenomas	3/50 (6%)	4/50 (8%)	17/49 (35%)*	22/348 (6% ± 5%)[a] 107/2032 (5% ± 4%)[b]
Carcinomas	3/50 (6%)	3/50 (6%)	2/49 (4%)	9/348 (3% ± 2%)[a] 81/2032 (4% ± 2%)[b]
Adenomas & carcinomas	6/50 (12%)	7/50 (14%)	17/49 (35%)****	29/348 (8% ± 5%)[a] 184/2032 (9% ± 5%)[b]

[a]In water gavage vehicle controls
[b]In untreated controls in NTP studies
*p < 0.001, ** p < 0.05, *** p = 0.023, incidental tumour test for comparison between low-dose and control, **** p < 0.002

Lung: Alveolar bronchiolar adenomas and carcinomas

The incidence of alveolar bronchiolar adenomas and carcinomas was increased in the high dose males. The incidence of alveolar-bronchiolar adenomas and carcinomas (combined) showed a positive trend in male mice and was statistically significant at the highest dose. The incidence of alveolar-bronchiolar adenomas and carcinomas (combined) was increased in high-dose females. All the incidences in the high dose males and females were outside the ranges of the HCD from NTP studies (see the table below).

Table: Alveolar bronchiolar adenomas and carcinomas in mice

Males	Control	25 mg/kg bw/day	50 mg/kg bw/day	HCD
Adenomas	3/49 (6%)	6/50 (12%)	11/50 (22%)*	46/347 (13% ± 8%)[a] 255/2034 (13% ± 6%)[b]
Carcinomas	2/49 (4%)	4/50 (8%)	10/50 (20%)*	22/347 (6% ± 5%)[a] 102/2032 (5% ± 3%)[b]
Adenomas & carcinomas	5/49 (10%)	10/50 (20%)	18/50 (36%)*	65/347 (19% ± 8%)[a] 348/2034 (17% ± 7%)[b]
Females	Control	25 mg/kg bw/day	50 mg/kg bw/day	HCD
Adenomas	4/50 (8%)	4/50 (8%)	7/49 (14%)*	25/349 (7% ± 3%)[a] 101/2026 (5% ± 4%)[b]
Carcinomas	2/50 (4%)	5/50 (10%)	7/49 (14%)	8/349 (2% ± 2%)[a] 45/2026 (2% ± 2%)[b]
Adenomas & carcinomas	6/50 (12%)	8/50 (16%)	13/49 (27%)**	33/349 (9% ± 4%)[a] 145/2026 (7% ± 4%)[b]

[a]In water gavage vehicle controls
[b]In untreated controls in NTP studies
*p < 0.05, ** p < 0.001

Ovary: granulosa-cell tumours

The incidences of benign granulosa-cell tumours of the ovary were statistically significantly increased at 25 mg/kg bw/day and 50 mg/kg bw/day in the exposed female mice and were outside the HCD from NTP studies (see the table below).

Table: Benign ovary granulosa cell tumours in female mice

Females	Control	25 mg/kg bw/day	50 mg/kg bw/day	HCD
Benign granulosa-cell tumours	0/50	5/45 (11%)*	5/47 (11%)*	2/339 (0.6% ± 1.0%)[a] 13/1867 (0.7% ± 2.0%)[b]

[a]In water gavage vehicle controls
[b]In untreated controls in NTP studies
*p < 0.05

Non-neoplastic effects were found in the lung as chronic inflammation, in ovaries as ovarian atrophy, in the spleen as haematopoietic cell proliferation and in kidneys as chronic nephropathy in the mice carcinogenicity study (see the table below).

Table: Non-neoplastic lesions in mice

Non-neoplastic lesions	Control	25 mg/kg bw/day	50 mg/kg bw/day	Remarks
Lung (M)	8/49	12/50	20/50	Chronic inflammation*
Lung (M)	10/49	17/50	19/50	Alveolar hyperplasia*
Lung (F)	12/50	28/50	14/49	Chronic inflammation*
Lung (F)	8/50	26/50	17/49	Alveolar hyperplasia*
Spleen (M)	11/50	13/26	38/50	Proliferation secondary to neoplastic and inflammatory lesions in various organs
Spleen (F)	15/50	10/19	40/48	
Kidney (F)	10/50	3/11	23/48	Minimal to mild severity, consistent with changes in aging mice
Ovary	8/49	12/50	20/50	

*occurred together and appeared to be part of the same lesion

In summary, the results from the rat carcinogenicity study showed no carcinogenic potential of NMA. However, in the mouse carcinogenicity study clear evidence of carcinogenic activity of NMA was reported. The lung tumours reported in male and female mice as well as the ovary tumours reported in female mice seen together with the mutagenicity profile of NMA are considered to be sufficient evidence for classification for carcinogenicity.

However, there were two types of tumours reported in the mice study that can be of questionable relevance to humans, the Harderian gland tumour with no human equivalent tissue and the liver tumour that are frequently observed in B6C3F1 mice.

It should be noted that IARC in 1994 concluded that NMA is not classifiable as to its carcinogenicity to humans (Group 3) based on inadequate evidence in humans and limited evidence in experimental animals for the carcinogenicity. No further explanations were provided by the IARC to justify this conclusion. However the two dominant lethal assays as well as the two *in vivo* micronucleus assays were performed after IARC concluded on a classification in group 3.

According to the CLP regulation (Annex I: 3.6.2.2.4), additional considerations as part of a weight of evidence approach has to be taken into account for a classification for carcinogenicity. These factors are assessed below:

a) Tumour type and background incidence:

Adenomas of the Harderian gland, hepatocellular adenomas/carcinomas, alveolar/bronchiolar adenomas/carcinomas and benign granulosa cell tumours of the ovary were reported. These tumours were statistically significantly increased from control values and exceeded the HCD.

Alveolar/bronchiolar adenomas/carcinomas and benign granulosa cell tumours of the ovary

are considered to be relevant to humans.

The Harderian gland and hepatocellular tumours were also considered relevant to humans.

b) Multi-site responses:

NMA induces tumours in various tissues (lung, ovary, Harderian gland, and liver)

c) Progression of lesions to malignancy:

Malignant tumours were found in the lung and liver.

d) Reduced tumour latency:

In mice, the first observation of tumour occurrence in the control group was at about 700 days, but between 300 and 600 days for the exposed groups.

e) Whether responses are in single or both sexes:

Tumours were reported in mice in both sexes.

f) Whether responses are in a single species or several species:

Tumours occurred in mice but not in rats.

g) Structural similarity to a substance(s) for which there is good evidence of carcinogenicity:

AA, which is classified Carc. 1B, is a structural analogue of NMA. Moreover, metabolism to AA cannot be excluded. AA can also have been present as an impurity in the NMA tested, however, no data on this was included by the DS

h) Routes of exposure:

The available carcinogenicity studies were performed by oral route. There is no data for other routes.

i) Comparison of absorption, distribution, metabolism and excretion between test animals and humans:

No information

j) The possibility of a confounding effect of excessive toxicity at test doses:

No significant differences on survival were observed between any groups of either sex in the mice study. Some non-neoplastic effects were reported in mice (see the table above).

k) Mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity:

The mode of action is not fully clarified. A link between genotoxicity and carcinogenicity is expected considering the results obtained in genotoxicity studies. In addition, it can be hypothesized that, as for AA, an epoxide metabolite may be involved in the carcinogenicity of NMA. However, there was no clear evidence of the formation of this metabolite after NMA administration.

Comparison with the CLP criteria

No human data was available. Thus, RAC considers that classification as Carc. 1A is not justified.

Experimental animal data results following exposure to NMA was available from two carcinogenicity studies, one in rats and one in mice. According to the CLP criteria,

classification as Carc. 1B is based on the following:

"*A causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in*

(a) two or more species of animals or

(b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence."

In the mouse carcinogenicity study performed according to GLP, clear evidence of carcinogenic activity of NMA (purity > 98%) was reported. No adequate information was available regarding the level of AA in the batches of NMA used in the study. However, RAC concludes that it is not reasonable that the positive results would be caused by the influence of AA alone, although a contribution of AA cannot be excluded. In a weight of evidence assessment, the lung tumours reported in male and female mice as well as the ovary tumours in female mice seen together with the mutagenicity profile of NMA are considered to be sufficient evidence for classification for carcinogenicity. Further, in the absence of data indicating that results related to carcinogenicity in mice are not relevant for humans, the effects reported in the mice study is considered relevant for a classification. RAC therefore considers that classification of NMA as Carc. 1B is justified.

9.10 Reproductive toxicity

Not evaluated.

9.10.1 Adverse effects on sexual function and fertility

No data available.

9.10.2 Adverse effects on development

No data available

9.10.3 Adverse effects on or via lactation

No data available.

9.11 Specific target organ toxicity-single exposure

Not evaluated.

9.12 Specific target organ toxicity-repeated exposure

Table 17: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
16-day oral toxicity study by gavage in rats (Fischer 344) and mice B6C3F1 (7 week old) 5 male and 5 female per dose group No Guideline OECD GLP Supportive study Reliability 2 with restriction (klimisch score)	NMA Batch number 1-45-000 (purity > 98% in water) 0, 25, 50, 100, 200, or 400 mg/kg bw/d Vehicle: water Exposure: 16 days (5 times/week)	NOAEL = 50 mg/kg bw/d (male rats) or 100 mg/kg bw/d (female rats and mice both sexes) 200 < LD_{50} < 400 mg/kg bw (male/female) <u>At 100 mg/kg bw/d</u> Rats : 10% decreased body weight in males at the end of the study. Ataxia developed after 7 d in males. Mice: body weight gains were variable and not clearly related to treatment. <u>At 200 mg/kg bw/d</u> Rats : 3/5 male and 2/5 female died. Compound-related clinical signs : ataxia, muscle tremors and hyperirritability. Final mean body weight of male rats: 27% lower than vehicle controls and of female rats: 20% lower than vehicle controls. Compound related lesions in rats included hyperplasia of the bronchiolar and tracheal epithelium, dysplasia of the nasal and tracheal epithelium, centrilobular hepatocellular necrosis, lymphoid depletion of the spleen, and myelin degeneration of the lumbar ventral spinal nerve. Mice : ataxia <u>At 400 mg/kg bw/d</u> Rats: All rats died within 4 days. Mice: All males and 4/5 female died on the 2nd day. Ataxia observed in the surviving female mouse. Weight changes inconsistent among dose groups. Bronchial epithelial hyperplasia (mild) appeared to be dose related in males and females mice. Sinusoidal congestion of the liver and vacuolar degeneration of myocardial fibers in males and females given 400 mg/kg bw/d.	Bucher (1990), NTP (1989)

90-day study by gavage in rats (Fischer 344) and mice (B6C3F1) 10 male and 10 female per dose group 7 week old No Guideline OECD GLP Key study Reliability 2 with restriction (klimisch score)	NMA Batch number 1-45-000 (purity > 98% in water) 12.5, 25, 50, 100 and 200 mg/kg bw /d (nominal in water) by gavage Vehicle: water Exposure: 90 days (5 days/week)	**RAT:** No NOAEL can be derived from this study. LOAEL rat: 12.5 mg/kg bw/d (nominal) (male/female) based on the decreased forelimb and hind limb grip strength in male rats. <u>Mortality :</u> All rats ≥ 100 mg/kg bw/d died. 100% mortality M/F at 200 mg/kg bw/d before the 6th study week. <u>Clinical signs:</u> ≥ 50 mg/kg bw/d: hindlimb ataxia progressing to paralysis. At 50 mg/kg bw/d, all rats appeared to be ataxic in the hind limbs during the 8th week which progressed to hind limbs paresis during the 11th week of the study. At 100 mg/kg bw/d, hind limb ataxia, beginning in the third week of dosing, did not progress to hind limb paralysis in the males until the 6th or 7th week. All male rats exhibited burrowing behaviour after gavage beginning the 4th study week. In many animals of this dose group, a weakened condition, thin appearance and rough hair coats were observed. At 200 mg/kg bw/d, generalised irritability to handling during the first study week (M/F). Only those animals which survived until the third week of dosing had a hind limb ataxia which then progressed to a hind limb paralysis. Weak appearance and rough hair coats were additional observations recorded from many of these animals until death. <u>Body weight/ body weight gain:</u> Lower body weight than controls at all doses (> 10%), with dose-response relationship in both sexes of rats.	Bucher (1990), NTP (1989)

		Neurobehavioral assessments: Decreased forelimb and hindlimb grip strength at doses from 12.5 mg/kg bw/d in male rats. Behavioural tests performed after 6 and 13 weeks of treatment showed dose-related (one-way analysis of variance) decreases in forelimb and hind limb. Grip strength in week 13 was significantly different from controls (Dunnett's test) at doses from 25 mg/kg bw/d in males and from 50 mg/kg bw/d in females. Motor activity was not significantly different at any dose group. At 13 weeks, female animals of the 50 mg/kg bw/d dose group exhibited significantly lower startle responses score as compared with control animals. Landing foot spread was increased at 6 weeks only for female rats receiving 50 mg/kg/d (not tested at 13 weeks since hind limb paresis was present in both sexes). No other group showed effects on landing foot spread. Histopathological lesions: Focal or multifocal necrosis of small neurons in the granular cell layer of the cerebellum at 200 mg/kg/d. Axon filament and myelin sheath degeneration of the brain stem, spinal cord, and/or peripheral nerves was seen in rats at increased incidences at 25 mg/kg/d and higher doses. Inflammation and/or hemorrhage and edema of the urinary bladder mucosa were seen with doses of 25 mg/kg bw/d or more in a few rats that had distended bladders at gross examination. It is unknown if the bladder lesions and /or submucosal hemorrhage, edema, and inflammation bladder lesions are primary effects of the chemical or secondary to peripheral nerve injury leading to difficulty in voiding urine. ➔ **These findings are consistent with the development of peripheral neuropathy.**	

		MICE: No NOAEL can be derived from this study. LOAEL mice: 12.5 mg/kg bw/d (nominal) (male/female) based on on the decreased relative testis weight in male mice. Peripheral neuropathy from 25 mg/kg bw/day. <u>Mortality :</u> All mice that received 200 mg/kg/d NMA died before the end of the study. <u>Body weight:</u> Final mean body weights of dosed and vehicle control mice were similar. <u>Organ weight:</u> A decreased relative testis weight was observed for mice that received 12.5 mg/kg/d or more. The relative kidney weights for male mice receiving 50 or 100 mg/kg/d were greater than that for vehicle controls. However, this difference could be explained by the lower body weights in this dose group. <u>Neurobehavioral assessments:</u> Decreased forelimb grip strength in male and female mice at doses from 25 mg/kg/d. No lesions were apparent in the brainstem, spina cord, or peripheral nerves. However, dose-related decreases in forelimb grip strength were seen in male and female mice at week 6 and 13, and decreases in hindlimb grip strength were noted at week 13 at doses from 25 mg/kg/d. An exaggerated startle response was seen for female mice given 100 mg/kg/d. A reduction in rotarod performance was seen at week 6 for male and female mice receiving 100 mg/kg/d and for male mice receiving 25 mg/kg bw/d performance at 13 week was significantly reduced for mice receiving 100 mg/kg bw/d compared with that for vehicle controls. Motor activity was not affected in animals given NMA. <u>Histopathological lesions:</u> Hepatocellular necrosis and thymic lymphocytic necrosis were compound-related effects in mice given 200 mg/kg/d NMA. Hemorrhage, necrosis, and mineralization of the zona reticularis of the adrenal gland were present in 3/10 female mice given 200 mg/kg/d, and cytoplasmic vacuolization of the adrenal cortex was seen with lower doses. ➔ **These findings in mice are consistent with the development of peripheral neuropathy observed at lower doses in rats.**	

Neurotoxicity study (only summary report available) in Wistar male rats Age not specified 4 male per dose group No OECD guideline, no GLP Supportive study Reliability 3 (summary) (klimisch score)	NMA Purity of substance not specified 0, 3.36, 5.41, 8.65, 13.8 mM (nominal in water) (equivalent to approximately 0, 33.9, 54.6, 87.4, and 139.4 mg/ml, respectively) Vehicle: water Exposure: 90 days (Animals were dosed for 60-90 days in drinking water at 4 different concentrations (4 rats per dose level) chosen by preliminary experiments.)	NOAEL : 11 mg/kg bw/d (nominal) (male) based on: Depression of the [3H]colchicine-binding to neurotubulin (the soluble protein) of sciatic nerves and in the spinal cord of both the cervical and the lumbar regions, but neither in the brain nor the cerebellum.	Tanii H. and Hashimoto (1983)

Detailed study summaries are available in Annex I of the CLH report.

Other studies reporting neurotoxicity not described in the table above are available but of lower quality (Barnes *et al*., 1970; Edwards *et al.*, 1974, 1975b, Godin *et al*., 2002; Tanii *et al*., 1991). Since they do not bring further information compared to the studies summarized in the table, it is not judged necessary to detail these studies.

Table 18: Summary table of human data on STOT RE

Type of data/report	**Test substance**	**Route of exposure** **Relevant information about the study (as applicable)**	**Observations**	**Reference**

Human data on workers Assessment of the health effects of occupational AA exposure using hemoglobin (Hb) adducts as biomarkers of internal dose. *Collection of blood samples* for the workers with recent peripheral nervous symptoms (PNS). Blood samples were drawn for the analysis of gamma-glutamyl transferase and carbohydrate-deficient transferrin in serum and fasting blood glucose according to routine techniques. *Analysis of hemoglobin adducts by GC MS/MS* *Initial health examination and self-reported exposure categorization :* self-administered questionnaire. *Standardized neurophysiological examination:* motor and sensory neurography of the right extremities. Measurement of sensory perception thresholds in the left foot. *Dermatological examination* Supportive study; not used for weight of evidence approach performed with OHAT because this study did not assess a relationship between exposure and effects.	Coexposure AA and NMA Grouting agent, Rhoca Gil® containing both AA and NMA was ready-mixed in the tunnel from two solutions and water According to the declaration of content, Rhoca Gil® contained up to 1.5% AA, about 37% NMA, and about 0.9% formaldehyde Purity not specified	Final study population: 210 men and 3 women workers (median age 44 (range 20 - 62) years). None of the professionals had used appropriate personal protective equipment during construction. 18 non-professionally exposed non-smokers. Adduct concentrations were measured 1 to 2 weeks after discontinuation; effects were studied 2 to 4 weeks after exposure. The full health examination was performed from 14 October to 17 November 1997	Air quality measurements reported close levels of AA and NMA of 0.27 $mg.m^{-3}$ and 0.34 $mg.m^{-3}$. Many of the workers developed health problems (alterations of the peripheral nervous system), The adducts measured can be as much formed by exposure to AA as to NMA. However, it has been shown that for a comparable exposure dose, the production of adducts from NMA is three times less than that resulting from exposure to AA.	Hagmar (2001)

Observational study in 24 exposed worker (average age 43.1 SD 8.6 years old range 31-62) 50 unexposed tunnel workers (average age 43.9 SD 8.6 years old (range 23-60)) served as referents No OECD Guideline, no GLP Study limitations: many outcomes, random associations due to multiple comparisons may have occurred and may have complicated the interpretation of possible effects Key study Weight of evidence approach by OHAT: Study quality level : probably high risk of bias	Coexposure AA and NMA (Rhoca-Gil solution) Purity not specified	Among 73 participants in a health survey of tunnel workers during autumn 1997, selection of 25 tunnel workers which were the most heavily exposed workers; 24 exposed subjects were included in the analyses of symptoms and neurophysiologic measurements (one worker was excluded owing diabetes) Workers exposed to NMA in mixture with AA during grouting operations (Rhoca-Gil solution): grout mixing, injection, equipment disassembly and clean up. Exposure assessment based on qualitative exposure information: exposure assessed by questionnaires and qualitative exposure indices. No measurements of AA or NMA in the working environment during the injection work. Examination of symptoms and nerve conduction properties 4 and 16 months after the cessation of exposure. Visual evoked response (VEP) and electroretinography (ERG) performed 16 months post exposure. Analysis of AA-hemoglobin adducts by GC MS/MS Examination of chromosome aberrations and distribution of Glutathion S transferase (GST) genotypes (M and T) compared to 25 age and smoking matched referents.	The main exposure occurred when mixing and pumping the grouting solution, and the following drilling of holes in the wall when the grouting solution was injected. Slight effects on the peripheral nervous system in tunnel workers. Apart from a possible delayed axonal effect on sensory fibres in the sural nerve, the effects seemed largely to be reversible, with normalisation 16 months post-exposure. Some subclinical effects on photoreceptors (cones) in the central part of the retina were observed. Subjects lacking GST-M1 and GST-T1 seemed to have the highest number of chromatid gaps, indicating that individual susceptibility related to detoxification of AA and NMA may have played a role in the observed effect.	Kjuus (2002, 2004)

Observational study Subjects with known persistent neurological disease, diabetes mellitus, or known alcohol or drug abuse not eligible. Further restriction criteria: impaired colour vision and known eye diseases Key study Weight of evidence approach by OHAT: - Study quality level : definitively or probably low risk of bias	Coexposure AA and NMA Purity not specified	Study of possible persisting visual system effects in tunnel workers previously exposed to AA and NMA during grouting work. 88 participants constituted the study base : 44 exposed tunnel workers (mean age 48.4 SD 9.5 years) 2-10 years after exposure to AA and NMA containing grouting agents, identified from a registry made by the association of employers in the Norwegian construction industry (information on amounts and time periods of use of AA-containing grouts in tunnel projects during 1982-1997 from 4 companies). 44 tunnel workers not involved in grouting operations served as control group (mean age 44.5 SD 10.1 years) randomly recruited from one single construction company No measurements of AA or NMA performed during injection work.	A significantly higher threshold for detecting single stimuli in all parts of the inner 30 degrees of the visual field in the exposed group compared to the control group. On the test of the visual light sensitivity threshold using Humphrey Visual Field Static Perimeter 740, the foveal threshold group difference was 1.4 dB (p=0.002) (mean value, both eyes). On the Lanthony 15 Hue Desaturated test, the exposed subjects made more errors in sorting blue colours, and a statistically significant increase in C-index was observed. Surrogate measures for duration and intensity of exposure gave no further improvement of the model. Slightly reduced light sensitivity and reduced colour discrimination among the exposed subjects compared to the controls.	Goffeng (2008a)

Observational study Study limitations: To limit the possibility of outcome selective recruitment into the study, the exposure assessment was based on described work tasks and length of grouting work, not on subjective exposure assessment by the workers themselves. Subjects with known persistent neurological disease, diabetes mellitus, or known alcohol or drug abuse not eligible for the study. Further restriction criteria were impaired colour vision and known eye diseases. Key study Weight of evidence approach by OHAT: - Study quality level : definitively or probably low risk of bias	Coexposure AA and NMA Purity not specified	Evaluation of nerve conduction, visual evoked responses (VER) and electroretinography (ERG) in tunnel workers previously exposed to AA and NMA containing grouting agents. 44 eligible tunnel workers previously exposed to AA and NMA during grouting operations (2-10 years post exposure) (mean age 47.9 years) identified from a registry of tunnel construction companies using AA containing grouts, established in 1997 by the Norwegian association for Building and Construction industry for surveillance purposes 49 tunnel workers (mean age 44.6 years) with no history of exposure to acrylamide who served as controls, randomonly recruited from one of the four construction companies 24 recently exposed workers to AA and NMA (Kjuus (2004) (mean age 43.7 years) selected from 73 workers who had taken part in railway tunnel construction during a grouting period terminating 16 months prior to a health examination. No measurement of AA or NMA in the working environement performed during injection work in companies represented in the registry.	Neurographic measurements: A statistically significant reduction in the mean sensory of the sural nerve ($p=0.005$), as well as a non-significant reduction of sural amplitude was found in the previously exposed group to AA and NMA compared to the control group. VER latencies to the onset of the occipital potential were prolonged in both exposed groups compared to the control group ($p<0.05$). ERG 30 Hz flicker amplitude was reduced in the recently exposed group to AA and NMA compared to the referents ($p<0.05$). The results indicate slight subclinical, but persistent toxic effects in the sural nerve and the visual system in tunnel workers co-exposed to NMA and AA during grouting operations.	Goffeng (2008b)

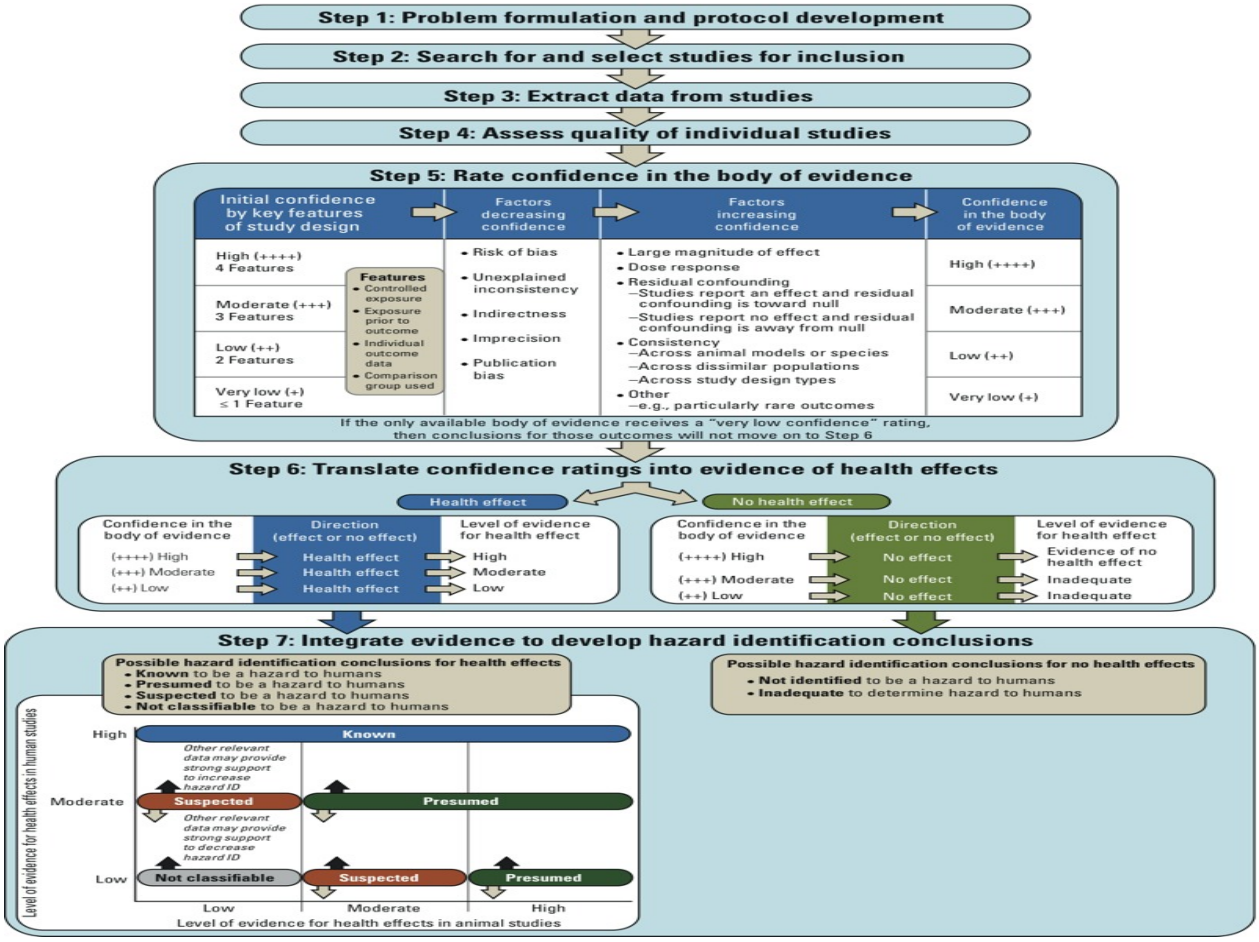
Detailed study summaries are available in Annex I of the CLH report.

Klimisch rating is not adapted for coting the quality of epidemiological studies. Therefore, assessment of the quality of the studies has been performed using OHAT (Office of Health Assessment and Translation (2015) in the framework of a practical exercice.

Weight of evidence assessment with OHAT approach

The OHAT approach for Systematic Review and Evidence Integration[2] provides an approach for assessing study quality or "risk of bias." The tool applies a parallel approach to the evaluation of risk of bias for human and experimental animal studies.

Figure 3 : Summary of OHAT approach (handbook, 2015)



The OHAT approach is divided into 7 different steps. Only the four first steps were followed here to cote the quality of the epidemiological studies in order to conclude on STOT RE classification for neurotoxicity. Indeed, going further in the next steps is limited as there are only three available epidemiological studies.

Steps 1, 2, 3 : are a review of the bibliography according to the PECO criteria (Population Exposure Comparator Outcome). Bibliographic research and criteria for inclusion of studies are according to 4 criteria:

- PECO:
  - P Population → professionals and general population
  - E Exposure → NMA exposure
  - C Comparator → exposed workers vs non exposed workers
  - O Outcome → neurological effects

[2] https://ntp.niehs.nih.gov/pubhealth/hat/noms/index-2.html

→ In total 3 studies of interest (Goffeng et al., 2008a & b; Kjuus et al., 2004) are retained and summarized in the table above.

Step 4 : consists of selecting studies reviewed in terms of risk of bias (RoB) by using a number of 11 questions which are selected depending on the type of study. The quality of the studies is assessed individually. The RoB questions are related to selection bias, confusion bias, performance bias, attrition / exclusion bias, detection bias, reporting and other bias. In this case the studies identified are cross-sectional studies and they are subject to 6 RoB questions. To each RoB question corresponds a response option described below (figure 4). Depending on the number of low or high bias risk responses the use of 3 key questions allow to gather studies in 3 groups: Tier 1 group (only « definitely » or « probably low » risk of bias), Tier 2 group (study does not meet criteria for « low or « high » risk of bias) and Tier 3 group (only « definitely high » or « probably high » risk of bias) group. Some studies which are Tier 3 group can be discussed to be excluded case by case.

	Tier 1		Tier 2	
Selection Bias	Goffeng et al., 2008a	Goffeng et al., 2008b		Kjuus et al., 2004
Did the study design or analysis account for important confounding and modifying variables ?	(++)	(++)		(+)
Can we be confident in the exposure characterization?	(+)	(+)		(-)
Can we be confident in the outcome assessment ?	(+)	(+)		(-)

Figure 4 : Grouping studies by Tier for NMA

Legend:
The response options for each RoB question are described in page 36 OHAT Handbook (January 9, 2015)

++	***Definitely Low** risk of bias*: There is direct evidence of low risk of bias practices (May include specific examples of relevant low risk of bias practices)
+	***Probably Low** risk of bias*: There is indirect evidence of low risk of bias practices OR it is deemed that deviations from low risk of bias practices for these criteria during the study would not appreciably bias results, including consideration of direction and magnitude of bias
– NR	***Probably High** risk of bias*: There is indirect evidence of high risk of bias practices OR there is insufficient information (e.g., not reported or "NR") provided about relevant risk of bias practices
––	***Definitely High** risk of bias*: There is direct evidence of high risk of bias practices (May include specific examples of relevant high risk of bias practices)

The questions are selected depending on the type of study (here: cross sectional studies); see table 5. OHAT Risk of Bias Tool. Source OHAT Handbook (January 9, 2015).

Conclusion of Weight of evidence using OHAT approach

In conclusion, based on the assessment of the quality of epidemiological studies using OHAT methodology, the observational studies in human are linked to definitively or probably low risk of bias (Goffeng, 2008a and b).

9.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

NMA was found to be neurotoxic from animal and human data.

In mice and rats, NMA induced peripheral neuropathy. The studies showed high mortality both in rat and mice at doses from 100 mg/kg bw/day.

Neurotoxicity occurred in rats exposed for 90 days at doses from 12.5 mg/kg bw/d, as shown by both neurobehavioural and morphological examinations (US NTP, 1989). No NOAEL could be derived from the rodent studies. Decreased forelimb and hind limb grip strength were observed from 25 mg/kg for female rats and from 12.5 mg/kg for male rats. A decreased startle response was seen for females at 25 mg/kg. Paralysis occurred at higher doses.

Neurotoxicity occurred in mice exposed for 90 days at doses from 25 mg/kg/d. Decreased forelimb grip strength in male and female mice were observed at dose from 25 mg/kg bw/d. No lesions were apparent in the brainstem, spina cord, or peripheral nerves. However, dose-related decreases in forelimb grip strength were seen in male and female mice at week 6 and 13, and decreases in hindlimb grip strength were noted at week 13 at doses from 25 mg/kg bw/d. An exaggerated startle response was seen for female mice given 100 mg/kg bw/d. A reduction in rotarod performance was seen at week 6 for male and female mice receiving 100 mg/kg/d and for male mice receiving 25 mg/kg bw/d performance at 13 week was significantly reduced for mice receiving 100 mg/kg bw/d compared with that for vehicle controls. Motor activity was not affected in animals given NMA. These findings in mice are consistent with the development of peripheral neuropathy observed at lower doses in rats.

Therefore, peripheral nerve system is the specific target organ organ after a repeated exposure to NMA in rodents. No histopathological examination was performed in the studies in rats and mice.

Overall, neurological effects were reported in both rat and mice. This results confirm the results obtained by QSAR analysis using DEREK modelisation concluding that neurotoxicity of NMA in mammal is plausible.

PNS symptoms, generally mild and in almost all cases reversible, were reported in some human cases in a study considered of low quality (probably low to probably high risk of bias) according to OHAT criteria (Kjuus 2002, 2004). Some demyelinating and axonal changes in peripheral nerves of tunnel workers linked to co-exposure to NMA and AA during grouting operations were reported. These changes are considered as slight subclinical, but persistent toxic effects based on the results of examination of neurotoxicity effects in the sural nerve and the visual system (Goffeng, 2008a, 2008b). They are reported in studies with definitively to probably low risk of bias by OHAT methodology. These effects observed in human cases are consistent to those observed in animal studies with NMA.

The link between exposure (co-exposure to NMA and AA) and PNS symptoms has been investigated using hemoglobin (Hb) adducts of AA (AA Val) as a biomarker of internal dose

(Hagmar, 2001; Kjuus *et al.*, 2002, 2004). Strong dose-response associations between AA Val and PNS symptoms have been found. NMA forms the same hemoglobin adducts as AA, but at equivalent exposure, the concentration of adducts formed by NMA is 3 times less than the concentration of adducts formed by AA. These results confirm the link between NMA and neurological symptoms but with a lower expected potency compared to AA.

Human data issued from studies with definitively to probably low risk of bias (2 studies; OHAT approach) in combination with reliable animal data (1 study in rats and mice; klimisch score) with NMA support the evidence of a neurotoxic potential of NMA.

9.12.2 Comparison with the CLP criteria

Table 19: Results of toxicity studies relevant for STOT RE in comparison to the CLP criteria

Conclusion	**CLP criteria**
<u>Non animal data</u> DEREK modelisation concludes that neurotoxicity of NMA in mammal is plausible. <u>Animal data</u> The target organ of NMA is the peripheral nerve system (repeat exposure) from appropriate 90-day oral studies in rats and mice. Decreased forelimb and hind limb grip strength were observed from 25 mg/kg for female rats and from 12.5 mg/kg for male rats. A decreased startle response was seen for females at 25 mg/kg. Paralysis occurred at higher doses but no histopathology examination was performed for neurotoxicity. A LOAEL of 12.5 mg/kg bw/d was identified in rat (male/female) based on the decreased forelimb and hind limb grip strength in male rats. No NOAEL can be derived since neurotoxicity was observed at all doses tested. Similar neurobavioural effects without specific lesions were reported in a 90-day study in mice from 25 mg/kg bw/day. In mice, the NOAEL for neurotoxicity was 12.5 mg/kg bw/d. <u>Human data</u> Neurotoxicity was reported in two human observational studies definitively to probably low risk of bias. The observed neurological effects are judged consistent with the description of significant effects reported in the guidance on the application of the CLP criteria (version 4.1 – June 2015); annex 1	STOT RE Category 1 (H372): Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Guidance value to assist on category 1 classification based on 90-day oral studies in rats: c ≤ 10 mg/kg bw/day

(3.9.2.7.3) *"significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g., sight, hearing and sense of smell)."* In conclusion, neurological effects occurred in animal at a dose of 12.5 mg/kg bw/day in male rats which is slightly higher than 10 mg/kg bw/day (threshold for STOT RE 1 classification). However, doses lower than 10 mg/kg bw/day are not tested in the 90-day study in rats. Similar effects were reported in mice at higher doses. Human data confirm the neurological effects of NMA reported in experimental studies. In addition, the known neurotoxic properties of AA, a structural analoguous of NMA, support the evidence of a neurotoxic potential of NMA. In this context, the weight of evidence integrating both animal and human data with NMA and the analogy with AA allows to conclude that **NMA is hazardous to humans regarding neurotoxicity and that there are sufficient evidence to propose a Category 1 for NMA.**	
The effects are reported in human cases and animal studies. The effects are observed at doses tested which are slighty above the limit of 10 mg/kg bw/d according to CLP criteria. However, the weight of evidence integrating both human and animal data and neurotoxicity potential known for acrylamide allow to conclude that there are sufficient evidence to propose a Category 1 for NMA.	Category 2 (H373): Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. In exceptional cases human evidence can also be used to place a substance in Category 2. Guidance values to assist on category 2 classification based on 90-day oral studies in rats: $10 < c \leq 100$ mg/gk bw/day

9.12.3 Conclusion on classification and labelling for STOT RE

Neurotoxic effects were observed in rats after NMA exposure at all tested doses starting from 12.5 mg/kg bw/day. Even if this is slightly higher than the threshold for STOT RE 1 classification, this category is judged adequate for NMA considering the weight of evidence integrating both human and animal data.

It can be discussed if these results are due to NMA itself or due to AA as an impurity or a metabolite since AA is already classified as STOT RE 1. Indeed, there are some existing NMA composition which contains AA as an impurity at levels higher than the generic concentration limit for mixture classification (0.1%). In this context, purity of NMA tested in studies reported for

STOT RE classification has been checked but no adequate information can be found on the level of AA in the batch tested. Therefore, the influence of AA on the results of the available studies performed with NMA is uncertain. In addition, there were some investigations on the biotransformation of NMA to AA. At this time, although some data suggest that NMA could be metabolized into AA, there is no clear evidence of this transformation. Anyway, when using DEREK modelisation, it has been found that NMA can have intrinsic neurotoxic properties. In conclusion, it has been considered that the neurotoxic effects are related to an intrinsic property of NMA or of its metabolites and thus NMA is proposed to be classified STOT RE 1.

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

For the evaluation of STOT RE, the DS summarised the available 16-d and 90-d studies conducted on both rats and mice (Burcher, 1990a,b). In addition, one 90-d study (rats) of lower quality was included. As regards human data, the DS summarised four studies of occupationally exposed workers.

Experimental animal data

In a 16-d oral gavage study (non-guideline) with Fisher 344 rats, ataxia was reported after 7 days in the 100 mg/kg bw/day dose group (Burcher, 1990a). At 200 mg/kg bw/day, 3/5 males and 2/5 females died. Clinical signs observed in this dose group were ataxia, muscle tremors and hyper-irritability. In the highest dose group (400 mg/kg bw/day), all rats died within 4 days. In the same study, B6C3F1 mice were also exposed to NMA by oral gavage. Ataxia was seen in the 200 mg/kg bw/day dose group and in the 400 mg/kg bw/days dose group all male mice and 4/5 female mice died on the 2nd day of treatment.

In a 90-d gavage study (non-guideline) with Fischer 344 rats, neurotoxicity occurred at doses ≥ 12.5 mg/kg bw/day (Burcher, 1990b). This was observed as decreased forelimb and hindlimb grip strength from 12.5 mg/kg bw/day for male rats and from 25 mg/kg bw/day for female rats. At doses ≥ 50 mg/kg bw/day, hindlimb ataxia progressing to paralysis was observed in males and females. Further, at doses ≥ 100 mg/kg bw/day all animals died, and in the 200 mg/kg bw/day dose group, deaths occurred before the 6th study week. In the same study, B6C3F1 mice were also exposed to NMA by gavage. Peripheral neuropathy, observed as decreased forelimb grip strength in both males and females, occurred from 25 mg/kg bw/day, however no histopathological lesions were seen in the brainstem, spinal cord or peripheral nerves. All mice in the 200 mg/kg bw/day dose group died before the end of the study. The DS concluded that the findings both for rats and mice are consistent with development of peripheral neuropathy, occurring at lower doses in rats than in mice.

In a supplementary 90-d neurotoxicity study of lower quality with male Wistar rats (non-guideline) exposed via drinking water, a NOAEL of 11 mg/kg bw/day was set based on depression of the [^{3}H]colchicine-binding to neurotubulin of sciatic nerves and in the spinal cord of both the cervical and lumbar regions, but not in the brain or in the cerebellum (Tanii and Hashimoto, 1983).

Human data

The DS included summaries of four studies presenting human data from workers exposed to NMA.

In a supportive study by Hagmar *et al*. (2001), health effects following occupational exposure to AA and NMA were assessed using Hb adducts as a biomarker for the internal dose. 213 workers were included in the study population. Many workers developed health effects (alterations of the peripheral nervous system).

In a study by Kjuus *et al*. (2002, 2004), slight effects on the peripheral nervous system were shown for 24 tunnel workers exposed to AA and NMA during grouting. Apart from a possible delayed axonal effect on sensory fibres in the sural nerve, the effects largely seemed to be reversible within 16 months post exposure.

In a study by Goffeng *et al*. (2008a), possible persisting visual system effects in tunnel workers exposed to NMA and AA were examined. Slightly reduced light sensitivity and reduced colour discrimination among exposed workers compared to controls were observed.

In another study by Goffeng *et al*. (2008b), nerve conduction, visual evoked response (VER) and electroretinography (ERG) were evaluated in tunnel workers exposed to NMA and AA. The study indicated slight sub-clinical but persistent toxic effects in the sural nerve and the visual system in exposed tunnel workers.

The DS concluded that NMA can be considered to be neurotoxic in both human and animal studies and that classification as STOT RE 1 for effects on the peripheral nervous system is justified.

Comments received during public consultation

Comments were received from two MSCAs. One MSCA supported the proposed classification for STOT RE based on the animal data, however they had some doubts regarding if category 1 or 2 would be the most appropriate. Another MSCA was of the opinion that information e.g. regarding the reduction in grip strength observed in the animal studies were too limited to come to a conclusion. Both MSCAs considered the value of the human data difficult to assess due to the co-exposure to acrylamide.

Assessment and comparison with the classification criteria

Experimental animal data

Two oral gavage studies with rats and mice were included by the DS (table below).

Table. Summary of repeated dose toxicity studies in rats and mice.

Study	Strain, duration of treatment, route	Dose level	NOAEL/LOAEL	Effects	STOT RE guidance values
Bucher *et al*., (1990a), NTP (1989), No guideline,	Rats (Fisher 344), 16-d study, oral	0, 25, 50, 100, 200, 400 mg/kg	NOAEL; Male: 50 mg/kg bw/day Female: 100	<u>100 mg/kg bw/day:</u> 10% reduced bw in males Ataxia after 7 days in males <u>200 mg/kg bw/day:</u>	Category 1: ≤ ~50 mg/kg bw/day

GLP NMA, purity > 98%	gavage, 5 males/5 females per dose	bw/day	mg/kg bw/day	3/5 males and 2/5 females died Ataxia, muscle tremors, hyperirritability 27% (males)/20% (females) reduced bw <u>400 mg/kg bw/day:</u> All died within 4 days	Category 2: ≤ ~500 mg/kg bw/day
Bucher *et al*. (1990a), NTP (1989), No guideline, GLP, NMA, purity > 98%	Mice (B6C3F1), 16-day study, oral gavage, 5 males/5 females per dose	0, 25, 50, 100, 200, 400 mg/kg bw/day	NOAEL: Male/female: 100 mg/kg bw/day	<u>200 mg/kg bw/day:</u> Ataxia <u>400 mg/kg bw/day:</u> All males and 4/5 females died on 2nd day Ataxia in surviving female	Category 1: ≤ ~50 mg/kg bw/day Category 2: ≤ ~500 mg/kg bw/day
Bucher *et al*. (1990b), NTP (1989), No guideline, GLP, NMA, purity > 98%	Rats (Fisher 344), 90-day study, oral gavage, 10 males/10 females per dose	12.5, 25, 50, 100, 200 mg/kg bw/day	NOAEL: not derived LOAEL: 12.5 mg/kg bw/day	LOAEL based on decreased forelimb/hindlimb grip strength in males. <u>Mortality:</u> All rats ≥ 100 mg/kg bw/day died. 200 mg/kg bw/day; all died before the 6th week of the study <u>Clinical signs:</u> From 12.5 mg/kg bw/day; decreased forelimb and hindlimb grip strength From 50 mg/kg bw/day; hind limb ataxia (from 8th week) progressing to paralysis (11th week). From 100 mg/kg bw/day: hindlimb ataxia (3rd week) progressing to hindlimb paralysis (6th or 7th week). Motor activity was not significantly different at any dose group.	Category 1: ≤ 10 mg/kg bw/day Category 2: ≤ 100 mg/kg bw/day
Bucher *et al*. (1990b), NTP (1989), No guideline, GLP, NMA, purity > 98%	Mice (B6C3F1), 90-day study, oral gavage, 10 males/10 females per dose	12.5, 25, 50, 100, 200 mg/kg bw/day	NOAEL: not derived LOAEL: 12.5 mg/kg bw/day	LOAEL based on the decreased relative testis weight in male mice and decreased forelimb grip strength in female mice <u>Mortality</u>: All mice in the highest dose group died before the end of the study <u>Clinical signs:</u> 12.5 mg/kg bw/day: decreased relative testis weight in male mice and decreased forelimb grip strength in female mice 25 mg/kg bw/day: Decreased forelimb grip strength in male/female. Reduction in rotarod performance in males. 100 mg/kg bw/day: exaggerated startle response in females. Reduction in rotarod performance in females. Motor activity was not	Category 1: ≤10 mg/kg bw/day Category 2: ≤100 mg/kg bw/day

				significantly different at any dose group.	
Tanii and Hashimoto, 1983 No guideline, no GLP	Male Wistar rats Exposure: 90 days via drinking water. 4 males/dose group	0, 33.9, 54.6, 87.4, 139.4 mg/mL	NOAEL: 11 mg/kg bw/day	NOAEL based on depression of the [^{3}H]colchicine-binding to neurotubulin of sciatic nerves and in the spinal cord of both the cervical and lumbar regions, but not in the brain or in the cerebellum	Category 1: ≤10 mg/kg bw/day Category 2: ≤100 mg/kg bw/day

A 16-d oral gavage study in rats and mice (no guideline, GLP), used doses of 0, 25, 50, 100, 200 and 400 mg/kg bw/day and 5 males/5 females per dose group. Rats developed ataxia at 100 mg/kg bw/day, and males showed a 10% reduction in body weight at this dose. At 200 mg/kg bw/day, 3/5 males and 2/5 females died. Clinical signs seen were ataxia, muscle tremors and hyperirritability. Final body weights were 27%/20% lower than controls for males/females. Histopathological observations included hyperplasia of the bronchiolar and tracheal epithelium, dysplasia of the nasal and tracheal epithelium, centrilobular hepatocellular necrosis, lymphoid depletion of the spleen and myelin degeneration of the lumbar ventral spinal nerve. At the highest dose of 400 mg/kg bw/day, all rats died within 4 days. Mice showed ataxia from 200 mg/kg bw/day and at 400 mg/kg bw/day all males and 4/5 females died on the 2nd day. The surviving female showed ataxia. Further, at this dose the incidence of bronchial epithelial hyperplasia (mild) appeared to be dose related in males and females. Sinusoidal congestion of the liver and vacuolar degeneration of myocardial fibres were seen in males and females.

A 90-d oral gavage study in rats and mice (non-guideline, GLP), used doses of 0, 12.5, 25, 50, 100 and 200 mg/kg bw/day and 10 males/10 females per dose group. Rats developed ataxia (hindlimb) during the 8th week of treatment, progressing to hindlimb paresis during the 11th week at 50 mg/kg bw/day. At 100 mg/kg bw/day, hindlimb ataxia started in the 3rd week of dosing, progressing to paralysis in the 6th-7th week. Males exhibited burrowing behaviour after gavage beginning on the 4th week. All rats at this dose level died. At 200 mg/kg bw/day the rats showed generalised irritability during 1st week. All rats died before the 6th week. Only animals surviving to the 3rd week showed hindlimb ataxia progressing to hindlimb paralysis. A summary of forelimb and hindlimb grip strength presented as percent of control, is shown in the table below. A statistically significantly decreased hindlimb grip strength was seen from 12.5 mg/kg bw/day in male rats at 13 weeks. Motor activity was not significantly different in any dose group. At 50 mg/kg bw/day, the female rats showed significantly lower startle response scores compared to controls. Further, at this dose females showed increased landing foot spread at the 6th week (not measured on the 13th week, no other group showed this effect). Histopathological lesions in the brain were only seen at the highest dose in rats, including focal or multifocal necrosis of small neurons in the granular cell layer of the cerebellum.

However, from 25 mg/kg bw/day, the incidence of axon filament and myelin sheath degeneration of the brain stem, spinal cord and/or peripheral nerves were increased. At this dose also effects on the urinary bladder were seen (haemorrhage and oedema), however it is unclear if this was a direct effect of the test substance or whether it was secondary to peripheral nerve injury.

Table. Forelimb and hindlimb grip strength in rats.

Dose (mg/kg bw/day	Forelimb/hindlimb grip strength (% of control) Week 6		Forelimb/hindlimb grip strength (% of control) Week 13	
	Males	Females	Males	Females
12.5	100/96	92/88	97/82*	90/94
25	104/91	96/88	92/64*	86*/85
50	94/85**	86**/65*	67*/35*	57*/24*
100	42*/26*	19*/19*	28/13***	-/-

*p < 0.01, **p < 0.05

*** only one animal examined, no statistical analysis performed

In the same 90-d oral gavage study in rats and mice (non-guideline, GLP), using doses of 0, 12.5, 25, 50, 100 and 200 mg/kg bw/day and 10 male/10 female mice per dose group, all mice in the top dose died before the end of the study. There were no marked changes in body weight in the dosed animals compared to controls, however from 12.5 mg/kg bw/day decreased relative testis weights were seen. Decreased forelimb and hindlimb grip strength was observed starting from 12.5 mg/kg bw/day in female mice (see the table below). At 100 mg/kg bw/day, female mice showed an exaggerated startle response.

Table. Forelimb and hindlimb grip strength, mice.

Dose (mg/kg bw/day	Forelimb/hindlimb grip strength (% of control) Week 6		Forelimb/hindlimb grip strength (% of control) Week 13	
	Males	Females	Males	Females
12.5	89/89	91/91	90/97	88*/86
25	82*/82	83*/85	84*/70*	87*/82**
50	74*/79	77*/83	79*/66*	78*/73*
100	83*/54*	81*/46*	92/47*	86*/59*

*p < 0.01, **p < 0.05

In a study of lower quality (non-guideline, no GLP), Wistar rats (4 males/dose) were exposed to NMA (0, 33.9, 54.6, 87.4 and 139.4 mg/mL) in drinking water for 60-90 days (Tanii and Hashimoto, 1983). A NOAEL of 11 mg/kg bw/day was set based on depression of the [^{3}H]colchicine-binding to neurotubulin in the sciatic nerves and in the spinal cord of both the cervical and lumbar regions, but not in the brain or in the cerebellum.

Human data

In a study by Hagmar *et al*. (2001), the health effects of occupational exposure of tunnel construction workers to NMA and AA was assessed using Hb-adducts of AA as a biomarker for the internal dose. The level of Hb-adducts is considered to give a valid estimate of the average exposure to AA during preceding months covering 120 days (corresponding to the lifespan of red blood cells) (Törnquist *et al*., 1986, Bergmark, 1997). 210 workers were exposed dermally and by inhalation to Rhoca Gil® containing NMA (37%), AA (1.5%) and 0.9% formaldehyde for approximately 2 months. Air quality measurements reported close levels of AA and NMA of 0.27 mg/m^3 and 0.34 mg/m^3 respectively. However, it was anticipated that during grouting activities the main route of exposure was through dermal absorption. These workers underwent a health examination starting with a self-administered questionnaire on medical history, smoking habits etc. Increased levels of Hb-adducts were seen in 163 workers up to a maximum of 17.7 nmol/g globin (normal background range 0.02-0.07 nmol/g). A dose response-relationship was found between Hb-adduct levels and PNS symptoms, and 39% of the workers with Hb-adduct levels exceeding 1 nmol/g globin experienced tingling or numbness in the hands or feet. A NOAEL of 0.51 nmol/g globin was established. The PNS symptoms were in general mild and reversible during a 18-month follow up after exposure. It is uncertain if the PNS symptoms observed were related to NMA or AA exposure or both.

In a study by Kjuus *et al*. (2004), 24 tunnel workers co-exposed to NMA (26-29%) and AA (up to 1.5%) from grouting were examined. These 24 were selected from a group of a total of 73 workers due to the highest exposure to acrylamide containing grout. The reference group consisted of tunnel workers not exposed to NMA; 8 for Hb-adduct measurements and 50 for neurophysiological measurements. Exposure was assessed based on a qualitative information, as there were no measurements of NMA and AA in the working environment. Hb-adducts of AA were measured. The mean levels in exposed non-smokers were 0.082 nmol/g Hb and in exposed smokers 0.225 nmol/g Hb. Unexposed non-smokers/smokers in comparison had 0.033/0.154 nmol/g Hb. The blood samples were collected 60-143 (mean 84) days after cessation of exposure. Symptoms and nerve conduction properties were examined 4 and 16 months after cessation of exposure. Slight PNS effects which largely seemed to be reversible within 16 months were observed. Three workers showed possible delayed axonal effects on sensory fibres in the sural nerve. In addition, some effects on photoreceptors in the central part of the retina were observed. It is uncertain if the effects observed were related to NMA or AA exposure or both, because it is not possible to distinguish between AA and NMA induced Hb-adducts of AA when humans are exposed simultaneously to both substances.

Goffeng *et al*. (2008a) investigated possible persisting visual system effects in 44 tunnel workers previously exposed to NMA (26-29%) and AA (up to 1.5%) from grouting. The previous studies by Kjuus *et al*. (2004) and Hagmar *et al*. (2001) had looked at neurotoxicity effects following exposure to AA and NMA, and found that they indicated an effect on the PNS. Goffeng *et al*. (2008a) looked at a possible effect on the visual system as a part of the CNS investigations. The exposures were both dermally and by inhalation, however there were no measurements of NMA/AA in the working environment. The study also included a control group consisting of 44 workers not previously exposed to NMA containing grouts, but with experience working in tunnels. Exposures were assessed

based on information from questionnaires. This study indicated a slightly reduced light sensitivity and a reduction in colour discrimination in exposed workers compared to unexposed workers 2-10 years after exposure. However, the study has to be treated with caution since no exposure response relationship was observed and there was a potential heterogenicity between the control and exposed groups. It is uncertain if the effects observed were related to NMA or AA exposure or both.

In another study by Goffeng *et al*. (2008b), nerve conduction, visual evoked response (VER) and electroretinography (ERG) were evaluated in 44 tunnel workers previously exposed to NMA (26-29%) and AA (up to 1.5%) from grouting (exposure more than 2 years prior to examination). In addition, 24 more recently exposed tunnel workers (16 months post exposure) were included in the study. 49 tunnel workers not previously exposed to NMA were included in the control group. Exposure was assessed by questionnaires. The results indicated a slight sub-clinical but persistent effect in the sural nerve and the visual system of the exposed tunnel workers. It is uncertain if the effects observed were related to NMA or AA exposure or both.

In summary, the animal studies showed decreased forelimb and hindlimb grip strength at the lowest tested dose of 12.5 mg/kg bw/day in a 90-days rat and mice studies. Strictly when evaluating the animal data according the guidance values (Category 1: ≤ 10 mg/kg bw/day and Category 2: ≤ 100 mg/kg bw/day), this could be considered to fall within a classification for STOT RE 2 with the peripheral nervous system as the target organ. However, since no NOAEL value was derived from the studies, possible effects below 10 mg/kg bw/day cannot be excluded.

In addition to the animal studies, there are two human epidemiological studies showing effects on the peripheral nervous system of tunnel workers co-exposed to NMA and AA, observed as tingling or numbness in the hands or feet and indications of demyelinisation and axonal changes in peripheral nerves. The symptoms were reported to be mainly reversible within 16-18 months after end of exposure. Further, two other studies reported signs of persisting subclinical effects on the sural nerve and the visual system.

Based on the epidemiological studies it is difficult to distinguish between effects resulting from exposure to AA (which has an existing classification in Annex VI to the CLP Regulation as STOT RE 1) and NMA, since the grouting agent which the tunnel workers were exposed to contained approximately 1.5-5% AA and 26-31% NMA. However, studies have shown that there is a correlation between Hb-adducts formed after exposure to AA or NMA and PNS symptoms.

According to the CLP regulation, a classification as STOT RE 1 can be based on reliable and good quality evidence from human cases or epidemiological studies or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations.

Taking the human epidemiological studies showing effects on the nervous system, persisting up to 16-18 months after cessation of exposure, into account and supported by the neurological findings in the animal studies, RAC concludes that classification of NMA as STOT RE 1; H372 (peripheral nervous system) is justified.

9.13 Aspiration hazard

Not evaluated.

10 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated.

11 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated.

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13 ANNEXES

See separated annex I file for detailed study summaries.