Committee for Risk Assessment

RAC

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

Adopted

8 June 2018

8 June 2018

CLH-O-0000001412-86-211/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: N-(hydroxymethyl)acrylamide; methylolacrylamide; [NMA]

EC Number: 213-103-2

CAS Number: 924-42-5

The proposal was submitted by France and received by RAC on 13 April 2017.

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

France has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at *http://echa.europa.eu/harmonised-classification-and-labelling-consultation/* on 20 June 2017. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by 4 August 2017.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: Stine Husa

Co-Rapporteur, appointed by RAC: Christine Bjørge

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on 8 June 2018 by consensus.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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 current Annex VI entry										
Dossier submitters proposal	TBD	N-(hydroxymethyl)acrylamide (NMA)	213-103-2	924-42-5	Muta. 1B Carc. 1B STOT RE 1	H340 H350 H372 (peripheral nervous system)	GHS08 Dgr	H340 H350 H372 (peripheral nervous system)			
RAC opinion	TBD	N-(hydroxymethyl)acrylamide; methylolacrylamide; [NMA]	213-103-2	924-42-5	Muta. 1B Carc. 1B STOT RE 1	H340 H350 H372 (peripheral nervous system)	GHS08 Dgr	H340 H350 H372 (peripheral nervous system)			
Resulting Annex VI entry if agreed by COM	TBD	N-(hydroxymethyl)acrylamide; methylolacrylamide; [NMA]	213-103-2	924-42-5	Muta. 1B Carc. 1B STOT RE 1	H340 H350 H372 (peripheral nervous system)	GHS08 Dgr	H340 H350 H372 (peripheral nervous system)			

GROUNDS FOR ADOPTION OF THE OPINION

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 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 examined.

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)

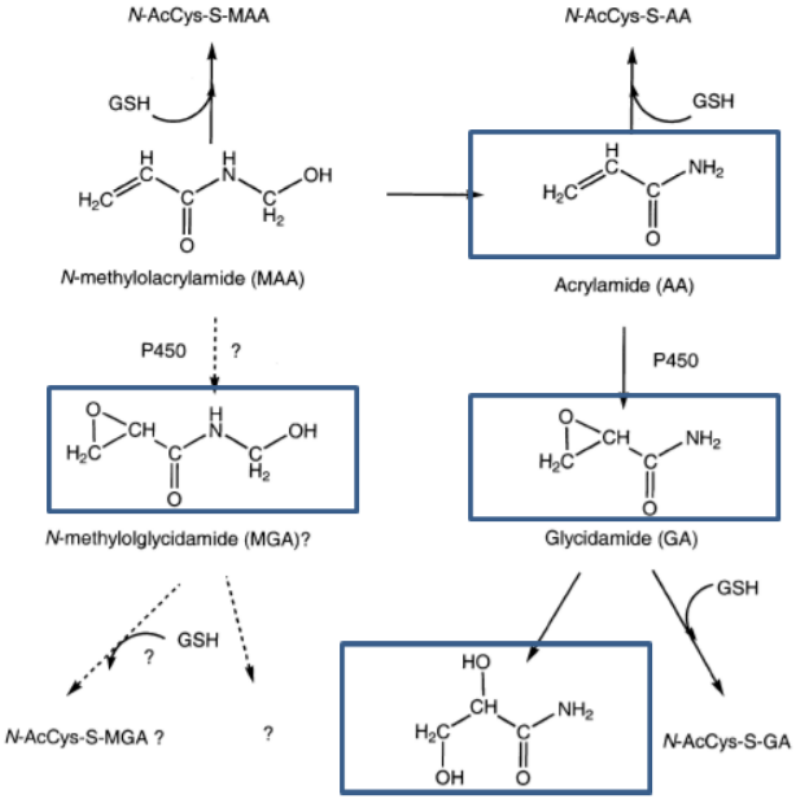
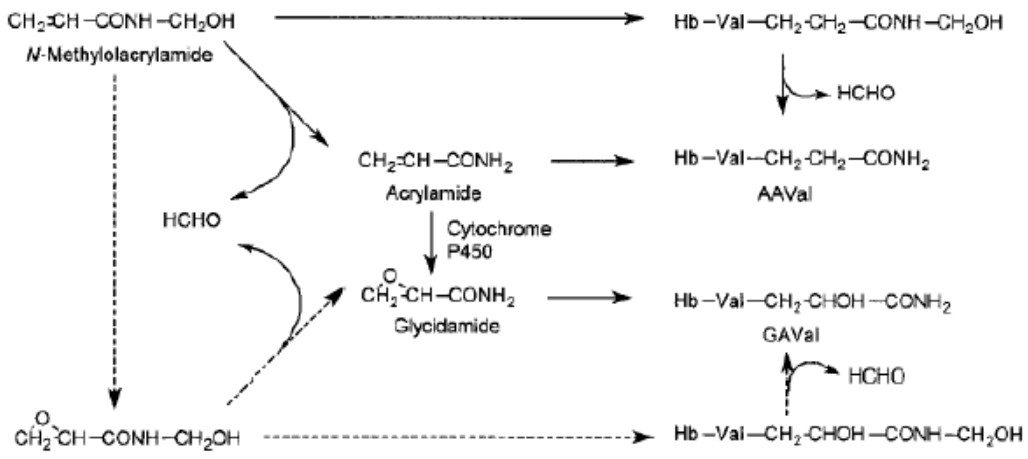


Figure. Possible routes of reaction of NMA to produce AA Val and GA Val (From Fennel et al., 2003)



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.

HUMAN HEALTH HAZARD EVALUATION

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, GLP NMA, purity > 98%	Rats (Fisher 344), 16-d study, oral gavage, 5 males/5 females per dose	0, 25, 50, 100, 200, 400 mg/kg bw/day	NOAEL; Male: 50 mg/kg bw/day Female: 100 mg/kg bw/day	<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> 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 1: ≤ ~50 mg/kg bw/day 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

Study	Strain, duration of treatment, route	Dose level	NOAEL / LOAEL	Effects	STOT RE guidance values
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 significantly different at any dose group.	Category 1: ≤10 mg/kg bw/day Category 2: ≤100 mg/kg bw/day
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.

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.

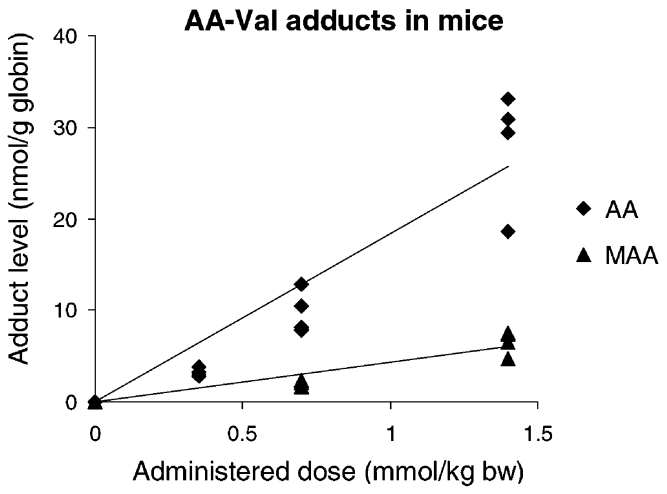
Table: Number of dead implants for three different mating intervals (Witt et al., 2003)

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)

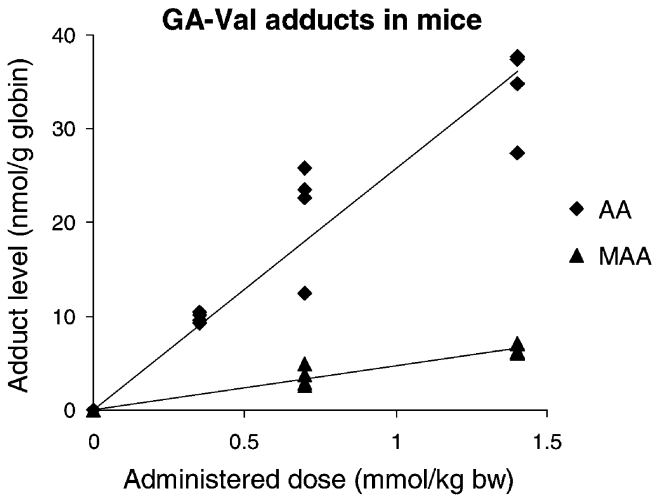
In the first micronucleus assay (Paulsson *et al*., 2002, similar to OECD TG) NMA and AA was given to male CBA mice and male Sprague-Dawley rats via i.p. for 13 weeks at doses of 0.35, 0.7 and 1.4 mmol/kg bw/day corresponding to 0, 35, 71 and 142 mg/kg bw/day NMA and 25, 50 and 100 mg/kg bw/day AA. The 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):

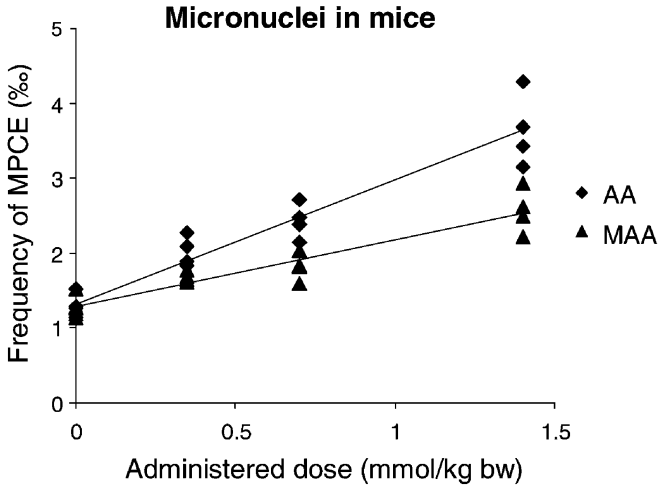


GA: Glycidamide, AA: Acrylamide, MAA the same as NMA

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

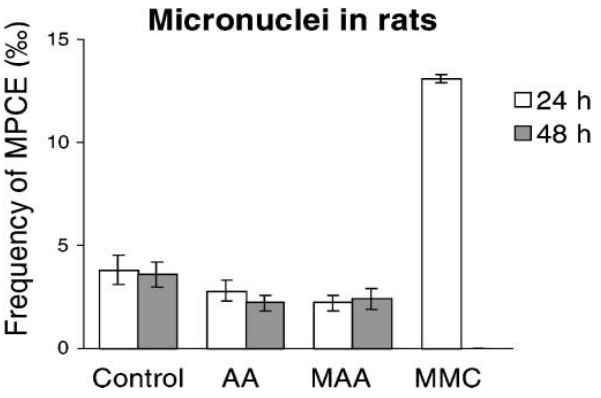
An increase in MN frequency in peripheral blood erythrocytes was also reported in male mice (see figure below).

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; H340 is justified.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

NMA was tested in two carcinogenicity studies by gavage, one in rats and one in mice. Both 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. 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]

Males	Control	25 mg/kg bw/day	50 mg/kg bw/day	HCD
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]

Males	Control	25 mg/kg bw/day	50 mg/kg bw/day	HCD
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.

Additional references

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Törnqvist M, Mowrer J, Jensen S, Ehrenberg L (1986). Monitoring of environmental cancer initiators through haemoglobin adducts by a modified Edman degradation method. Anal. Biochem. 154, 255–266.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).