Committee for Risk Assessment
RAC

Opinion on scientific evaluation of occupational exposure limits for
Nitrosamines

ECHA/RAC/OEL-O-0000007382-75-01/F

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OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON THE EVALUATION OF THE OCCUPATIONAL EXPOSURE LIMITS (OELs) FOR NITROSAMINES

In accordance with the Service Level Agreement[1] with the Directorate General for Employment, Social Affairs and Inclusion, the Committee for Risk Assessment (RAC) adopted by **consensus** on **30 November 2023** an opinion on the evaluation of the occupational exposure limits (OELs) for:

Chemical name(s):

- **N-Nitrosodiethylamine (diethylnitrosamine) (EC number 200-226-1; CAS RN 55-18-5)**
- **N-Nitrosodimethylamine (dimethylnitrosamine) (EC number 200-549-8; CAS RN 62-75-9)**
- **N-Nitroso di-n-propylamine (EC number 210-698-0; CAS RN 621-64-7)**
- **N-Nitrosodiethanoamine (2,2'-(Nitrosoimino)bisethanol) (EC number 214-237-4; CAS RN 1116-54-7)**

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Administrative information on the opinion

The Commission asked on 23 February 2022 the advice of RAC to assess the scientific relevance of occupational exposure limits for nitrosamines, in support of the preparation of proposals for amendment of Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens mutagens or reprotoxic substances at work (CMRD), and in line with the 2017 Commission Communication '*Safer and Healthier Work for All' - Modernisation of the EU Occupational Safety and Health Legislation and Policy*[2].

ECHA has prepared a scientific report concerning occupational limit values for nitrosamines at the workplace. This scientific report was made available[3] on **18 April 2023** and interested parties were invited to submit comments by **16 June 2023.**

RAC developed its opinion on the basis of the scientific report submitted by ECHA. During the preparation of the opinion, the scientific report was further developed as an Annex to ensure alignment with the opinion.

[1] Service level agreement with the Directorate General for Employment, Social Affairs and Inclusion (DG EMPL - Ares (2022)711149)
[2] http://ec.europa.eu/social/main.jsp?langId=en&catId=148&newsId=2709&furtherNews=yes
[3] https://echa.europa.eu/oels-pc-on-oel-recommendation

Summary of the RAC Opinion after the assessment of the scientific relevance of OELs for nitrosamines

The main conclusions of the draft opinion of RAC on the assessment of the scientific relevance of Occupational Exposure Limits (OELs) for nitrosamines are provided below; this is the outcome of the RAC evaluation to derive limit values for the inhalation route and the evaluation for dermal exposure and a skin notation.

Derived Limit Values[4]

OEL as 8-hour TWA:	None proposed
STEL:	None proposed
BLV:	None proposed
BGV:	None proposed

Notations

Notations:	Skin

Cancer exposure-risk relationship

NDMA Air concentration		NDELA* Air concentration		Excess life-time cancer risk (cases per 100 000 exposed)
mg/m³	ppm	mg/m³	ppm	
7×10^{-7}	2×10^{-7}	8×10^{-6}	1×10^{-6}	4
7×10^{-6}	2×10^{-6}	8×10^{-5}	1×10^{-5}	40
7×10^{-5}	2×10^{-5}	8×10^{-4}	1×10^{-4}	400
7×10^{-4}	2×10^{-4}	8×10^{-3}	1×10^{-3}	4000

* Excess life-time cancer risk for NDELA, which is based on oral data, is corrected by a factor of 100, to adjust for the indicated higher potency of NDMA following inhalation compared to oral exposure.

RAC notes that, in the future, "*the European Commission and its relevant stakeholders will aim to set limit values for non-threshold substances between the predetermined "upper risk level" and the "lower risk level""*. The (ACSH, 2022) opinion agreed that the upper risk level is 4:1 000 (corresponding to 4 predicted cancer cases in 1 000 employees) and the lower risk level is 4:100 000, assuming exposure over 8 hours per day, 5 days a week over a 40-year working life period.

[4] The naming conventions of limit values and notations used here follow the 'Methodology for the Derivation of Occupational Exposure Limits' (SCOEL 2013; version 7) and the Joint ECHA/RAC – SCOEL Task Force report (2017b). https://echa.europa.eu/documents/10162/13579/jtf_opinion_task_2_en.pdf/db8a9a3a-4aa7-601b-bb53-81a5eef93145

RAC OPINION

Background

This draft opinion concerns **nitrosamines**, with specific focus on:

- N-Nitrosodiethylamine (diethylnitrosamine, **NDEA**) (EC number 200-226-1; CAS RN 55-18-5)
- N-Nitrosodimethylamine (dimethylnitrosamine, **NDMA**) (EC number 200-549-8; CAS RN 62-75-9)
- N-Nitroso di-n-propylamine (**NDPA**, EC number 210-698-0; CAS RN 621-64-7)
- N-Nitrosodiethanoamine (**NDELA**, 2,2'-(Nitrosoimino)bisethanol) (EC number 214-237-4; CAS RN 1116-54-7)
- N-Nitrosomorpholine (**NMor**, EC number 627-564-6; CAS RN 59-89-2)

Originally, the evaluation of NMor was not requested by the European Commission. However, as an outcome of the open consultation, NMor was identified as an additional relevant agent, because it was detected in various occupational settings (especially in the rubber industry) and sufficient data exist to perform an exposure-health risk relationship analysis.

The relevance of other nitrosamine compounds for occupational exposure and cancer risk has also been considered. See further section 1 of Annex 1.

This evaluation takes previous reviews into account, in particular, from:

- AGS, 2015
- DECOS, 1999
- EFSA, 2022
- EMA, 2020
- ATSDR, 2023 on NDMA

In addition, a recent publication by Blum et al. (2023) on the use of the benchmark-dose (BMD) approach to derive occupational exposure limits (OELs) for N-nitrosamines has been considered.

<u>Harmonised Classification (Regulation EC No 1272/2008)</u>

- NDMA, NDPA and NDELA have a harmonised classification in the EU as Carc. Cat. 1B.
- NDEA and NMor do not have a harmonized classification. However, NDEA has been notified by REACH registrants as Carc. Cat. 1A/1B, and NMor as Carc. Cat. 2 and Muta. Cat. 2.

Key conclusions of the evaluation

- Nitrosamines are chemicals containing a nitroso group attached to an amine. The number of known nitrosamines is about 300 and they are present in e.g. tobacco smoke, processed food, as drug contaminants, and can form endogenously in the gastrointestinal tract.
- The nitrosamines under focus are no loger used in industry[5] but can be formed as by-products by the reaction of nitrosamine precursors (generally secondary amines) with nitrosating agents such as nitrogen oxides.
- Rubber industry involving vulcanisation is a typical industry field with exposure to nitrosamines. Vulcanisation accelerators are the main source of nitrosamines in this industry field.
- Nitrosamines are typically present as mixtures of different nitrosamines compounds at workplaces (see Table 16 and Table 17 in Annex 1). In the rubber industry NDMA, NDEA and NMor are the most commonly detected nitrosamines compounds. NDELA has been measured only in a specific sector (metal processing MWF - as per Table 16 and Table 17; section 5.3.2 and section 5.5 of Annex 1).
- Nitrosamines are metabolised in the body to diazonium ions, which are alkylating agents resulting in the formation of DNA adducts and direct DNA damage.
- Although some reports suggest a threshold for the genotoxicity of alkylating agents at low exposure levels based on DNA repair capacity, RAC considers that the available data is too limited to define thresholds for nitrosamines. Therefore, a default linear approach is proposed for the derivation of OELs for nitrosamines.
- The carcinogenicity of nitrosamines has been unequivocally confirmed in animal studies, with several organs and tissues being affected (primarily the liver, and the gastrointestinal and respiratory tracts). Studies in human population support a carcinogenic effect of nitrosamines. However, they do not provide a robust database to derive an exposure risk relationship (ERR) for any specific nitrosamine.

Five cancer exposure-risk relationships were estimated for four nitrosamines as presented in Table 1.

Table 1. Excess life-time cancer exposure-risk relationships for NDMA, NDEA, NMor and NDELA.

Excess life-time cancer risk (Cases per 100 000 exposed)	**Air concentration** (mg/m^3)				
	NDMA (inhalation study)	**NDMA** (oral study)	**NDEA** (oral study)	**NMor** (oral study)	**NDELA**** (oral study)
	BMDL as PoD*	BMDL as PoD	BMDL as PoD	T25 as PoD	BMDL as PoD
4	7×10^{-7}	8×10^{-5}	2.8×10^{-5}	1.6×10^{-5}	8×10^{-6}
40	7×10^{-6}	8×10^{-4}	2.8×10^{-4}	1.6×10^{-4}	8×10^{-5}
400	7×10^{-5}	8×10^{-3}	2.8×10^{-3}	1.6×10^{-3}	8×10^{-4}
4000	7×10^{-4}	8×10^{-2}	2.8×10^{-2}	1.6×10^{-2}	8×10^{-3}
Key study:	Klein et al. (1991)	Peto et al. (1991)	Peto et al. (1991)	Lijinsky et al. (1988)	Lijinsky and Kovatch (1985)

[5] NDPA is part of the request from the Commission, although it does not occur in occupational setting.

* PoD: point of departure (for the calculations)
** Excess life-time cancer risk for NDELA, which is based on oral data, is corrected by a factor of 100, to adjust for the indicated higher potency of NDMA following inhalation compared to oral exposure.

Note: For NDPA, data were too limited to allow the estimation of carcinogenic dose-response relationship.

- The most conservative and reliable estimate of cancer exposure-risk relationship was derived from an inhalation study with NDMA in rats (Klein et al., 1991). Estimated cancer exposure-risk relationships based on oral exposure studies were rather similar for NDMA, NDEA and NMor. Only NDELA is of lower potency, as described further in the Opinion.
- In addition to carcinogenicity, nitrosamines can cause non-cancer liver effects. One study suggested effects on iron-binding capacity at low exposure levels. Based on RAC evaluation, these effects are unlikely at 8h TWA levels of 0.08 µg/m^3 (8 x 10^{-5} mg/m^3) (see later in this Opinion). This value should be considered when setting the binding limit value for nitrosamines to ensure that also the risk of non-cancer effects is minimised.
- Also reproductive effects have been suggested but the data is limited.
- Considering that the potency differences between the main types of nitrosamines present in rubber industry (i.e. NDMA, NDEA and NMor) are not pronounced, RAC recommends to (i) set the same limit value on the basis of the inhalation study dose-response of NDMA (since this is the most conservative PoD derived for NDMA, RAC considers that this PoD is conservative enough to compensate for the uncertainty regarding differences in potency of NDEA, NDMA, and NMor), and (ii) to apply it for the combined exposure to nitrosamines (sum of several nitrosamines measured using the method by DFG (2022)).
- For NDELA, detected typically in metal processing industry, a separate dose-response has been derived, since the available data indicate its lower carcinogenic potency compared to NDMA/NDEA/NMor. RAC recommends to (i) set a specific limit value based on the dose-response derived for NDELA and (ii) to apply it when NDELA is detected in the workplace air.
- Also, for NDELA, RAC proposes to apply a factor of 100 to the cancer exposure-risk relationship based on oral data for NDELA, to adjust for the indicated higher potency of NDMA following inhalation compared to oral exposure.
- There are only few biomonitoring studies on the exposure of general population and workers to nitrosamines. Based on the available data it is not possible to set BGV or BLV for nitrosamines.
- There is evidence suggesting the ability of nitrosamines to pass through the skin. Therefore, a ‘skin’ notation is recommended.

Carcinogenicity and mode of action

Carcinogenicity (see section 7.7 of Annex 1 for full discussion)

- **Human data**. IARC concluded that NDEA and NDMA are probably carcinogenic to humans (Group 2A, based on inadequate evidence in humans and sufficient evidence in animal studies), and that NDPA, NDELA, and NMor are possibly carcinogenic to humans (Group 2B, based on inadequate evidence in humans and sufficient evidence in animal studies).
  Epidemiological data on inhalation exposure in workers, dietary intake, and consumption of drugs contaminated with nitrosamines, indicate that exposure to nitrosamines both in occupational settings and in general population may be associated with a cancer risk of various types:
  - In **workers**, these included oesophageal, rectal, skin, pancreatic, lung, bladder, liver, stomach, salivary gland, laryngeal, brain, and prostate cancers, as well as leukaemia, multiple myeloma, and non-Hodgkin lymphoma. Occupational exposures mainly involved NDELA, NDMA, NMor, or total nitrosamines.
  - In general population, **dietary exposure** to NDMA, NDEA, NPyr, NPip, or NMAMBA, was associated with increased risk of carcinoma at different sites, including oral cavity, oesophagus, stomach, colorectum, pancreas, brain, lung, and liver.
  - Exposure to nitrosamines as **drugs' contaminants** (primarily to NDMA) was linked to an increased risk of bladder, oesophagus, stomach, liver, pancreas, and colorectal cancer. However, negative data for the association with this type of exposure were also reported.

  The available epidemiological studies have significant limitations, primarily related to a deficient exposure assessment to nitrosamines and/or a lack of adjustment for major confounding factors, such as smoking or co-exposure to other carcinogens, and they are therefore not robust enough to allow a dose-response relationship estimation.

  For example, in studies of workers exposed to nitrosamines in metalworking fluids (MWF), the exposure information was on MWF as such, while nitrosamines' content in an MWF was not measured and adjustment for co-exposure to other toxic substances (such as steel, iron, aluminium, sulphur, biocide, asbestos, or solvents) was not possible.
  In rubber industry workers, the exposure to a specific nitrosamine was not specified, or the exposure assessment was based on the total nitrosamines (see Table 46 in Annex 1). Also, in some studies it was noted that the workers were co-exposed to other toxic substances, such as pesticides.
  Regarding dietary exposure in the general population, EFSA (2023) pointed out that in all of these studies, selection bias, information bias, and confounding were present. Nitrosamines intake was estimated from data obtained from food frequency and food history questionnaires, which are imperfect measures of exposure and thus misclassification of exposure is likely to occur. EFSA also noted that due to concomitant exposure to nitrosamines from other sources (e.g. smoking, occupation) and/or other unmeasured factors (e.g. Helicobacter for gastric cancer, fruits and vegetables intake, chemicals contained in meat other than nitrosamines), these studies cannot be used for the risk assessment.
  Overall, RAC conclude that epidemiological studies support an association between nitrosamines and cancer, but they do not provide a robust database for quantitative derivation of an exposure-risk relationship. Other national and EU bodies (AGS, DECOS, EFSA, EMA) have come to a similar conclusion.

- **Animal data**. There is a significant body of unequivocal data from animal studies showing that nitrosamines are potent carcinogens following oral, inhalation, dermal, or parenteral exposure, in different rodent and non-rodent species, including primates, both following acute and chronic exposure. The most prevalent tumour types were

hepatic, gastrointestinal, and respiratory (also following non-inhalation exposure), and increased incidence of neoplastic effects in other organs (such as kidneys, brain, testes, urinary tract, mammary glands, or haematopoietic system) was also occasionally observed.

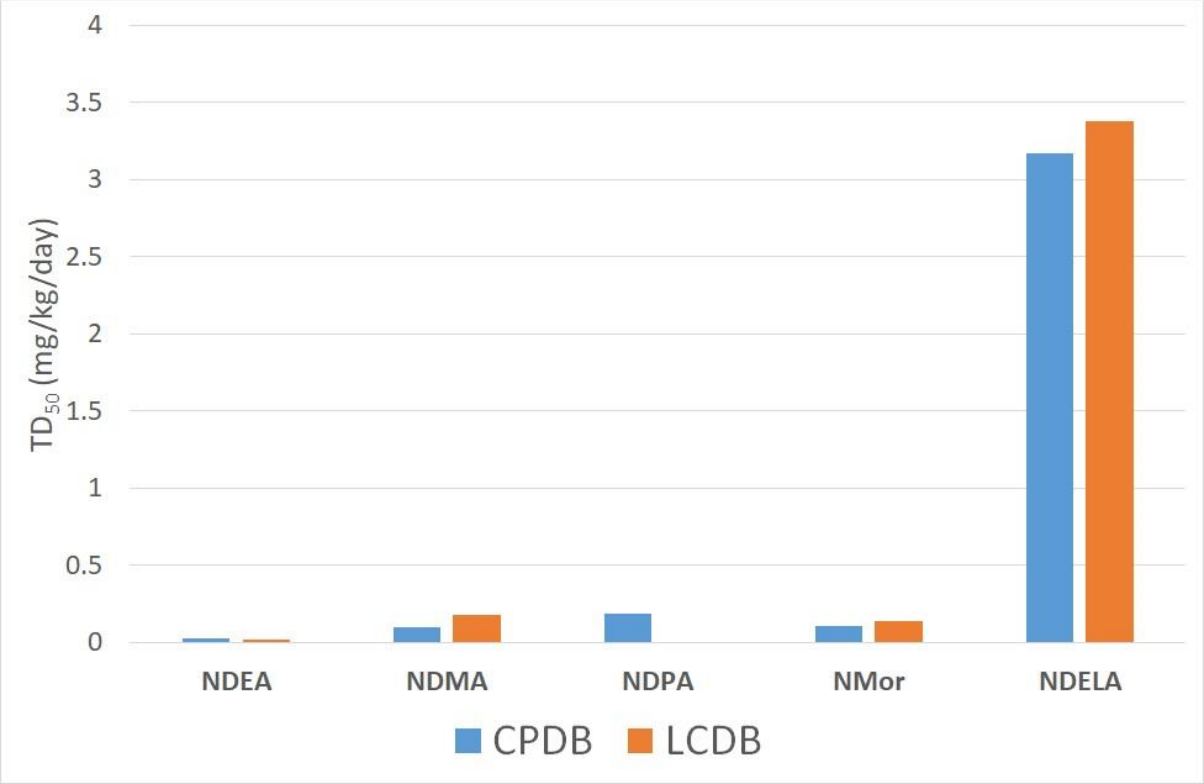
- **Carcinogenic potency**. Different sources of information (Carcinogenic Potency Database, CPDB[6]; key/relevant studies in the rat; Lhasa-generated TD_{50} values) have compared the carcinogenic potencies of nitrosamines. Overall, they indicate similar potency (within one order of magnitude) for NDEA, NDMA, NDPA, and NMor, with somewhat higher potency of NDEA compared to NDMA, NDPA, and NMor. On the other hand, NDELA showed one to two orders of magnitude lower potency (i.e., higher TD_{50} values) compared to the other four evaluated nitrosamines (see Table 31 in Annex 1).
  The way of derivation of TD_{50} values[7] from CPDB and Lhasa-generated TD_{50} values is described in section 7.7.2.1 in Annex 1.

The carcinogenic potency ranking based on CPDB (as presented in Figure 1 below) was also used in the EMA (2020) report (on nitrosamine impurities in human medicinal products). The EMA report points out that while the accuracy of the TD_{50} strongly depends on the study quality and size, most of the studies reported for nitrosamines have only one or two dose groups and low number of animals per group (i.e., less than 50).
Nonetheless they are still included in a harmonic mean TD_{50} in CPDB reports if other conditions are met. Comparable studies in the same rat strain with the same number of dose groups are available only for NDMA and NDEA.

Another limitation of the CPDB is that it is not an exhaustive database. For example, inhalation studies for NDMA are not included in the database. The Norwegian Institute for Air Research (Harju et al. 2011) also warned about using TD_{50} approach for quantitative cancer risk assessment. It seems that the linear extrapolation of TD_{50} could markedly underestimate or overestimate the true risk.[8]

Other ranking approaches are also available (see section 7.7.2.1 of Annex 1), but overall, they do not differ markedly, in terms of orders of magnitude, from the ranking presented above. EFSA (2022) concluded that "NDEA, NMEA, NDMA and possibly NMor are in the group of highest concern" regarding their carcinogenic potency. RAC agreed with this conclusion.

[6] https://www.nlm.nih.gov/databases/download/cpdb.html
[7] The reference value is the TD_{50}, which is the daily dose which will induce tumours in half of the animals that would have remained tumour-free if not exposed (i.e., 'zero' dose).
[8] When starting with a dose causing 50% effect, the uncertainties derived when extrapolating to the lower doses are expected to be much larger than when starting with a dose causing 25% effect. Therefore, it is better to start with lower dose descriptors, such as T25, BMD10, or BMD05.



CPDB: Carcinogenic Potency Database; LCDB: Lhasa Carcinogenicity Potency Database

Figure 1. CPDB and LCDB TD_{50} values for carcinogenicity in rats for evaluated nitrosamines, were taken from the LCDB Summary reports.

RAC also recognises that for the members of a "high-concern" nitrosamines group (NDEA, NMEA, NDMA, NDPA, and NMor), the available data are not sufficiently robust to allow for more precise ranking that could be potentially used to adjust separate limit values for specific nitrosamines in this group.

Mode of Action (see section 8.1 of Annex 1 for full discussion)

When entered into the body, nitrosamines like NDMA and NDEA undergo α-hydroxylation by CYP450 monooxygenases to form dealkylated primary nitrosamines and further to diazonium ions and aldehydes. These diazonium ions are alkylating agents able to bind to DNA and form DNA adducts. Especially O-alkylations, like O^6-alkylguanine are generally considered highly mutagenic lesions. Primary DNA adducts typically formed by NDMA, NDEA, NDPA and NDELA are listed in Table 39 of Annex 1.

For of NDMA, methylguanines, N7-Me-Gua and O^6-Me-Gua are the major adducts produced whereas NDEA produces ethyl adducts N7-Et-Gua, O^6-Et-Gua, O^2-Et-Thy, and N3-Et-Ade. NDMA was shown to be excreted in urine mainly as metabolites.

NDPA is metabolized via α-, β-, and γ-hydroxylation of the propyl group with α-hydroxylation being the most important metabolic route resulting in the production of various propyl/hydroxypropyl and butyl/ hydroxybutyl DNA adducts.

NDELA is metabolized both via α-, and β-hydroxylation. β-hydroxylation producing N-nitroso-(2-hydroxyethyl)glycine (which has been detected in the urine of rats treated with NDELA with 6% share of the total excretion), N-Nitroso-2-hydroxymorpholine (NHMor) and glyoxal. According to Li and Hecht (2022), β-hydroxylation may not play a significant role in the carcinogenicity of NDELA. α-hydroxylation, on the other hand, results in the formation of 2-hydroxyethyldiazonium ion, which may result in the formation of genotoxic hydroxyethylguanine adducts. In contrast to NDMA, the majority of NDELA is excreted unchanged to the urine. These metabolic differences can be hypothesised to explain (at

least partly) the quantitative differences in the carcinogenic potency between NDMA/NDEA and NDELA.

Also adducts formed by common cyclic nitrosamines, including NMor have been rather well characterized (Li and Hecht, 2022), and NMor has been shown to form various 2-ethoxyacetaldehyde purine adducts, like N7-(2-oxoethoxyethyl)guanine and O^6-(2-oxoethoxyethyl)guanine.

Repair of these DNA lesions depends on type of adduct formed. For example, O^6-alkylguanine and O^4-alkyl-thymine adducts are mainly repaired via dealkylation by DNA alkyl transferase (methyl-guanine-methyltransferase, MGMT). MGMT activity shows wide inter and intraspecies variability and variability between different tissues. Other repair mechanisms involved in the repair of nitrosamine caused DNA damages include base excision repair, direct damage reversal by ALKBH demethylase (similar direct reversal mechanism as MGMT), and nucleotide excision repair.

As can be expected based on their alkylating properties, various nitrosamines have shown genotoxic effects in various bacterial and mammalian cell systems as well as *in vivo*. The available data on the *in vitro* and *in vivo* mutagenicity and genotoxicity of NDEA, NDMA, NDPA and NDELA are described in section 7.6 of Annex 1 and show overall positive responses.

(Jenkins et al., 2015, Johnson et al., 2021) have suggested that there might be a threshold for the genotoxicity of alkylating agents at low exposure levels based on the repair capacity of the relevant DNA adducts. Based on this, Johnson et al (2021) conducted BMD modelling for NDMA and NDEA using *in vivo* mutagenicity data (increased *lacI* or *gpt* mutations in rat liver), to derive mutant frequency dose-responses, and calculate permitted daily exposure limits for the two substances. A 50% increase in mutations was used as a critical effect size. BMD modelling was also performed on carcinogenicity data from the Peto et al, 1991 studies. Several uncertainty/modifying factors were applied on the mutagenicity BMD values-based PDE which was compared to carcinogenicity-based PDEs.

Blum et al. (2023) used data on the liver adducts formation to support the setting of health-based OEL for NMDA, although the PoD finally used for their derivation of an OEL was based on tumour data from Peto et al 1991: the key data on adduct formation referred to was the study by Souliotis et al. (2002), investigating DNA adduct formation in female Wistar Furth/NCr rats after exposure to NDMA for up to 180 days (doses: 0.2, 0.3, 0.4, 0.52, 1.06, 1.58, 2.11, and 2.64 ppm corresponding to 28, 42, 56, 73, 148, 221, 295, and 372 µg/kg). The authors reported rapid, dose-dependent accumulation of N7- and O^6-methylguanine in liver and white blood cells DNA, together with an increase in DNA replication, and subsequently a NOEL for induction of DNA replication at 28 µg/kg (0.2 ppm).

However, RAC notes that it is not possible to identify the NOEL for adduct induction since the lowest dose of 28 µg/kg (0.2 ppm) already caused a rapid increase in O^6-methylguanine adducts. During the exposure period, O^6-methylguanine adduct levels decreased to zero in the five lowest exposure groups, which was hypothesised to be due to a reduction in water intake. In the drinking water study by Peto et al 1991, a decrease in water intake up to 70% of the level of controls was also seen. The doses in Souliotis et al. (2002) are similar to those used in Peto et al. (1991) in which an increase in tumour induction was seen starting from 22 µg/kg in female rats (leading to a $BMDL_{10}$ of 42 µg/kg).

Overall, RAC concludes that nitrosamines are alkylating agents resulting in cancer mediated by genotoxic MoA. RAC acknowledges the possibility for a threshold for the genotoxicity of nitrosamines at low levels. However, RAC considers that the available data do not allow the identification of such threshold. Even in the study by Souliotis et al (2002), using several different dose levels, a rapid increase in adducts was already seen at the lowest dose level, which corresponds to the level in which increase in tumour induction was seen in the study by Peto et al., 1991. Therefore, RAC considers that it is not possible to use a MoA-based threshold-approach for the derivation of OELs for nitrosamines.

Cancer Risk Assessment

RAC considers that, as already mentioned in the previous section (Carcinogenicity and mode of action), human data do not provide a robust database for quantitative derivation of an exposure risk relationship due to significant limitations, especially regarding quantification of exposure and lack of adjustment for major confounding factors.

Cancer risk assessment based on animal chronic studies is, therefore, proposed (see section 9.1 of Annex 1 for full discussion).

AGS (Ausschuss für Gefahrstoffe, 2015) and DECOS (Health Council of the Netherlands, 1999) also based their assessment for nitrosamine-related occupational cancer risk on animal data, namely on the Klein et al. (1991) inhalation study in rats:

- AGS derived workplace air concentrations corresponding to tolerable (4:1 000) and acceptable (4:10 000 or 4:100 000) cancer excess risks for NDMA, using as starting point a BMD_{10} value of 18.66 µg/m^3 (0.019 mg/m^3) for human equivalent exposure at the workplace, calculated from the nasal tumour incidences observed in the Klein et al. (1991) study. Lifetime excess risk of 4 per 100 000 was estimated at exposure to 7.5 x 10^{-6} mg/m^3, assuming a linear dose-response relationship without a threshold value.
- DECOS estimated a so-called health-based calculated occupational cancer risk for NDMA of 4:100 000 for 40 years of occupational exposure to be 2 x 10^{-6} mg/m^3. They relied on the lowest concentration (120 µg/m^3, i.e. 0.12 mg/m^3) resulting in tumorigenesis in Klein et al. (1991) study, assuming a linear dose-response relationship.

RAC was able to estimate a carcinogenic dose-response relationship for four out of five evaluated substances: NDMA, NDEA, NDELA, and NMor. For NDPA, data were too limited to allow the estimation.

Cancer risk assessment for NDMA

A number of oral (via drinking water, diet, oral gavage) and parenteral (intraperitoneal, subcutaneous) studies are available for NDMA, but only three inhalation studies were identified: Klein et al. (1989, 1991), Moiseev and Benemansky (1975), and Druckrey et al. (1967).

The key inhalation study is considered to be by Klein et al. (1989, 1991), and the key oral study by Peto et al. (1991).

1) Inhalation study with NDMA

The key study chosen for the exposure-risk relationship for NDMA following inhalation exposure is **Klein et al. study (1989, 1991)**.

This is a non-guideline study in which female Sprague-Dawley rats (Hanover, FRG), aged eight weeks at the beginning of the experiment, were exposed to NDMA (four or five times per week, 4 or 4-5 h/day; please see the explanation on uncertainties below) for 207 days at 0, 0.04 ppm (0.12 mg/m^3), 0.2 ppm (0.6 mg/m^3), and 1 ppm (3 mg/m^3), in stainless-steel inhalation boxes and cages as a whole-body exposure, without food, water or bedding material. The 4-h exposure was followed by 2 h of enhanced air flow to diminish NDMA contamination of the boxes, cages and animals' fur. The concentrations of NDMA and exhaled CO_2 were determined continuously for each box. The absorbed daily dose and the total dose of NDMA in mg/kg body weight were calculated by using a mean breathing volume of 6 L for a rat (based on CO_2 measurements) and NDMA concentration measurements.

The results are reported in two separate publications: Klein et al. (1989), which is an interim report (up to 772nd day of experiment, out of which the exposure lasted for

207 days in the period from day 140 to 660 of experiment), and Klein et al. (1991), which is a final report (until all animals in the experiment died).

Klein et al. (1991) study is considered by RAC as the most sensitive available animal study on nitrosamines, despite the limitations which are discussed below. Since this is an inhalation study, several advantages are acknowledged: the inhalation exposure route is the most relevant in occupational settings; the first-pass effect is avoided; the tumours were observed in nasal tissues, i.e., tissues of first contact. The PoD in this study is one order of magnitude lower than the PoD derived from an oral study with NDMA.

As mentioned above, AGS and DECOS also based their assessment of NDMA on Klein et al. (1991) study. AGS considered that first-pass effect with oral exposure could introduce a significant uncertainty in the risk assessment based on oral studies, and DECOS, after reviewing the available oral, inhalation and parenteral studies, considered Klein et al. study as the most sensitive and most reliable study for estimation of the potential risk of cancer at the workplace.

There are several uncertainties stemming from the study reports, as well as limitations related to the study's methodology:

- While the exposure conditions are described in detail, duration of exposure is not clearly described in the reports. In Klein et al. (1989), NDMA exposure was reported as 4 h per day, 5 days per week, while in Klein et al. (1991), the exposure was reported as 4-5 h per day, 4 times per week. Therefore, AGS (2015) directly contacted the authors of the study to clarify that the animals were exposed 207 times for 4-5 h/d within approximately 530 days. Nevertheless, it is still not clear in which way the 207 exposure-days were distributed over approximately a 530-day period. There is an uncertainty to which extent dosing-free intervals could have influenced tumour development (e.g., due to not saturated metabolic capacity due to exposure-free days).
- Since it seems that whole-body, and not nose-only exposure method was applied, some extent of oral exposure cannot be ruled out.
- It is not clear how many animals were histopathologically examined. Namely, Klein et al. (1991) stated that *"tumour-bearing animals were sacrificed by ether inhalation, and their organs were excised and fixed in formalin"*. It is not stated whether the animals which died of natural death during the experiment were histopathologically examined. Also, it is not described how often and in which way alive animals were examined for the presence of tumour(s). This could lead to an underestimation of the true incidence of tumours, both in exposed and in control animals.
- Although in the interim report, one case of nasal tumour (squamous cell carcinoma) was reported in the control group, zero incidence of nasal tumours was stated in the final report.
- The earliest days of nasal tumours' manifestation do not match between the two reports, although these days are within the time-period covered by both reports (up to 772 study days).
- Only female sex was used, with only 36 animals per group (while, for example, 50 animals per sex/group is recommended in the OECD Test Guideline). Additionally, in the highest-dose group, survival started to rapidly decline after approximately the 440th day of the study. It is questionable, therefore, whether the number of animals is sufficient in terms of biological and statistical evaluation.

These uncertainties are expected to underestimate, rather than overestimate the risk.

Table 2. Nasal tumours findings in Klein et al. (1989, 1991)

		NDMA		
	Control	**0.04 ppm** (0.12 mg/m^3)	**0.2 ppm** (0.6 mg/m^3)	**1 ppm** (3 mg/m^3)
N of rats	36	36	36	36
INTERIM REPORT (772nd day of the study; i.e. ~2 years)				
N of dead rats (%)	25 (69)	23 (64)	29 (81)	36 (100)
Calculated NDMA *daily* uptake (mg/kg bw/day)	0	0.01	0.04	0.18
Calculated NDMA *total* uptake (mg/kg bw)	0	1.3-2	3-8	13-37
N of rats with nasal tumours / N histopathologically examined? [a]	1/14[a]	3/9[a]	12/16[a]	12/26[a]
Type of nasal tumour:				
aesthesioneuroblastoma		1	2	7
mucoepidermoidal carcinoma		2	10	5
squamous cell carcinoma	1		2	1
Nasal tumour manifestation days	489	470-638	488-662	189-579
FINAL REPORT (1200th day of the study; i.e. ~3 years)				
Median age at death (days)	795	860	772	524
N of rats with nasal tumours	0[b]	13	31	19
aesthesioneuroblastoma		2	2	9
mucoepidermoidal tumours [c]		11	30	7
mucoepidermoidal carcinoma		2	8	3
squamous cell carcinoma			2	1
neurogenic sarcoma				1
osteogenic sarcoma				2
Nasal tumour manifestation days	-	568-897	356-972	198-579

[a] The publication does not state what the second number (after slash) represents.
[b] However, in the interim report, one control rat had diagnosed squamous cell carcinoma.
[c] Including mucoepidermoidal carcinoma.

In several rats, more than one nasal tumour type was reported in the same animal. Therefore, the total number of rats with nasal tumours is less than the sum of specific tumours listed in the table above. However, an occurrence of multiple types of nasal tumours in the same animal was reported only for some cases, so the possibility of mistakes in the reporting cannot be excluded.

Survival was not markedly decreased after NDMA exposure at 0.04 ppm (0.12 mg/m^3) and 0.2 ppm (0.6 mg/m^3) but was significantly lower at 1 ppm (3 mg/m^3). The median survival time of animals given 1 ppm (3 mg/m^3) NDMA was nine months less than that of the control group, due to NDMA toxicity (as discussed by the study authors). On the other hand, the median survival of animals given 0.04 ppm (0.12 mg/m^3) was two months longer than that of controls.

The mean body weight in rats exposed to 0.04 ppm (0.12 mg/m^3) and 0.2 ppm (0.6 mg/m^3) did not differ significantly from the control. However, the rats treated with 1 ppm (3 mg/m^3) had significantly lower body weight gain (Figure 2 in Klein et al. (1991) publication). E.g., at the end of exposure period, rats at 1 ppm of NDMA weighed 270 g compared to 350 g in the control group.

Increased incidence of nasal tumours, i.e., aesthesioneuroblastoma (olfactory neuroblastoma), and mucoepidermoidal tumours including carcinoma, was observed already at the lowest dose, i.e. 0.04 ppm (0.12 mg/m^3). Except for aesthesioneuroblastoma, a clear dose-response was not observed due to lower incidences at the top dose (1 ppm, i.e. 3 mg/m^3), but this could be explained by marked mortality in this dose group, which started to be pronounced at the beginning of the second year of the study.

The incidence of tumours in other organs did not appear to be related to treatment, although there was one case of hepatocellular carcinoma each in the low-dose and medium-dose groups, two cases of liver adenoma at the low-dose, and one case at the medium-dose, while these tumours were not reported in the controls.

Table 3. Non-nasal tumours in Klein et al. study (1991)

Finding	**Control**	**NDMA dose**		
		0.04 ppm (0.12 mg/m^3)	**0.2 ppm** (0.6 mg/m^3)	**1 ppm** (3 mg/m^3)
Respiratory tract				
Adenocystic lung carcinoma				1
Tracheal adenoma			1	
Digestive tract				
Hepatocellular carcinoma		1	1	
Hepatic adenoma		2	1	
Cholangiocarcinoma				1
Cholangioma	12	11	7	8
Pancreatic carcinoma	2			
Pancreatic insuloma	2	1		
Intestinal tumour	1 (adenocarc.)	1 (myoma)		
Endocrine glands				
No. of tumour-bearing animals	30	30	28	12
Pituitary adenoma	19	20	19	
Suprarenal gland cortical adenoma	18	19	14	10
Suprarenal gland pheochromocytoma	9	11	7	
Thyroid adenoma	12	15	12	3
Mammary gland				
No. of tumour-bearing animals	24	22	18	6
Adenoma, fibroma, fibroadenoma	26	28	15	3
Adenocarcinoma	14	9	8	
Fibrosarcoma		2		
Squamous-cell carcinoma			1	
Other tumours	Neurogenic sarcoma in the abdominal cavity; Skin (2 squamous-cell carcinomas, 1 haemangioma, 1 sebaceous adenoma); Rhabdomyoma; Squamous-cell carcinoma of the oral mucosa	Neurogenic sarcoma in the abdominal cavity (2); Leukaemia; Lymphoma; Uterine myoma; Squamous-cell carcinoma of the oral mucosa (2)	Histiocytic sarcoma of the abdominal cavity	Neurogenic sarcoma in the abdominal cavity; Ependymoma of the cerebrum; Astrocytoma of the cerebrum; Theca-cell tumour of the ovary; Adenocarcinoma of the oral mucosa

In several rats, more than one tumour type was reported in the same animal.

The dose-response correlations on nasal tumour incidences reported by Klein et al. (1991) were not suitable as such for benchmark dose modelling (BMD) due to non-linear dose-response when all dose levels were included (EFSA BMD tool showed that the AIC of the best model (minimum AIC) was more than two units larger than that of the full model, i.e., it was almost 10 units larger than that of the full model)[9].

Therefore, other options to estimate additional lifetime cancer risk are presented:

a) T25 approach,

b) BMD approach with the top dose omitted or

c) BMD approach with the top dose included with an assumption that the high dose results in a 100% incidence, i.e., 36/36 animals with a tumour.

Both BMD approaches were modelled by RIVM (2014). The authors of the RIVM report assumed that the early deaths in the high dose group resulted in an unrealistically low tumour incidence, since the animals died before developing a tumour.

a) T25 approach

T25 was used as the PoD, using the LOAEC of 0.12 mg/m^3 related to the nasal cavity tumours. Additional lifetime cancer risks were calculated according to the ECHA Guidance on information requirements and chemical safety assessment, Chapter R.8.

As explained in Annex 1 of the opinion, T25 was calculated as:

T25 = LOAEC * [reference incidence/(incidence at LOAEC – control incidence)] * (1-control incidence) / 1

With the LOAEC = 0.12 mg/m^3 for nasal cavity tumours and the reference incidence = 0.25

T25 = 0.12 mg/m^3 * [0.25 / (13/36 – 0/36)] * (1 – 0/36)/1 = **0.08 mg/m^3 NDMA**

The T25 value was adjusted to correspond to worker exposure conditions (40 years, 48 weeks/year, 8 h/day, and correction for the inhalation volume for workers at light physical activity). No allometric scaling is needed for inhalation exposure.

$T25_{(worker)}$ = 0.08 mg/m^3 * (75 years/40 years) * (52 weeks/48 weeks) * (4 days/5 days) * (4.5h/8 h) * (6.7 m^3/10 m^3) = **0.049 mg/m^3**

Additional lifetime cancer risks were calculated according to a linearised approach (high to low dose extrapolation):

Exposure concentration representing **1*10^{-5} risk**: 0.049 mg/m^3 / 25000 = **2*10^{-6} mg/m^3** (corresponding to 0.0000006 ppm)

i.e., Exposure concentration representing **4*10^{-5} risk**: **8*10^{-6} mg/m^3**

Assuming linearity, excess life-time cancer risks were calculated and are presented in Table 40 of Annex 1.

b) BMD approach – top-dose omitted

It was noted that the nasal tumour incidence at the top dose was lower than at the mid-dose, which influences the dose-response curve (nasal tumour incidences were 0/36, 13/36, 31/36, and 19/36 at 0, 0.12, 0.6, and 3 mg/m^3 dose levels, respectively). Therefore, BMD modelling was applied only on the incidences observed at low and mid-dose (0.04 and 0.2 ppm NDMA, corresponding to 0.12 and 0.6 mg/m^3) data, omitting the top-dose (1 ppm, corresponding to 3 mg/m^3) incidence, using EFSA Open Analytics software for BMD analysis, which uses the R-package PROAST, version 70.0, for the

[9] EFSA (2017) https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2017.4658

underlying calculations (quantal response, extra-risk: BMD10%, 90% confidence interval) on the total nasal tumour incidences. This yielded 7/7 accepted models, with the lowest BMDL of approximately 0.003 mg/m^3 (from the gamma model) assuming the benchmark response (BMR) of 10%.
A comparable outcome was earlier reported by RIVM (2014) for the Klein et al. (1991) data.

Table 4. Results of BMD modelling for Klein et al. (1991) data with omitted top-dose

Model	Log likelihood	AIC*	accepted	BMDL	BMDU	BMD	convergence
null	-73	148		NA	NA	NA	NA
full	-38.05	82.1		NA	NA	NA	NA
two.stage	-38.12	82.2	yes	23.3	40.8	30.6	yes
log.logist	-38.05	82.1	yes	12.9	70.2	40.2	yes
Weibull	-38.05	82.1	yes	5.21	56.1	24.9	yes
log.prob	-38.05	82.1	yes	14.2	73.2	42.7	yes
gamma	-38.05	82.1	yes	**2.57**	59.3	23.8	yes
LVM: Expon. m3-	-38.05	82.1	yes	5.16	64.7	30	yes
LVM: Hill m5-	-38.05	84.1	yes	5.12	107	35.8	yes

BMDL, BMDU, and BMD are expressed in µg/m^3. *Akaike information criterion

All models had log likelihood very similar to the full model, and similar AIC values.

Although RAC recognises the large (10-fold) difference between the lowest BMDL and respective BMD, due to the limitations of Klein et al. (1991) study described above, RAC proposes using $BMDL_{10}$, instead of BMD, as a PoD.

According to the BMD guidance[10], the lowest BMDL value of all accepted models is normally used as the Reference Point (RP), in the absence of models' averaging.

The **lowest $BMDL_{10}$ of 0.003 mg/m^3** (3 µg/m^3) was, therefore, used as a PoD for the cancer ERR calculations.

Adjustment to correspond to worker exposure conditions (40 years, 48 weeks/year, 8 h/day, and correction for the inhalation volume for workers at light physical activity) (no allometric scaling is needed for inhalation exposure) gave:

$BMDL_{(worker)}$= 0.003 mg/m^3 * (75 years/40 years) * (52 weeks/48 weeks) * (4 days/5 days) * (4.5 h/8 h) * (6.7 m^3/10 m^3) = 0.0018 mg/m^3

Additional lifetime cancer risks were calculated as follows according to a linearised approach (high to low dose extrapolation):

Exposure concentration representing **1*10^{-5} risk**: 0.0018 mg/m^3 / 10000 = **1.8*10^{-7} mg/m^3** (corresponding to 0.00000006 ppm)

i.e., Exposure concentration representing **4*10^{-5} risk**: **7*10^{-7} mg/m^3**

Assuming linearity, excess life-time cancer risks were calculated and are presented in Table 5 below, and in Table 40 in Annex 1**Error! Reference source not found.**.

Table 5: Cancer exposure-risk relationship (nasal cavity tumours) after working life exposure to a given 8-hour air concentration of NDMA for five working days a week over a 40-year working life period.

NDMA concentration in air (mg/m^3)	NDMA in air (ppm)	Excess life-time cancer risk (Cases per 100 000 exposed)
0.0000007	0.0000002	4

[10] EFSA (2017) https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2017.4658

NDMA concentration in air (mg/m³)	NDMA in air (ppm)	Excess life-time cancer risk (Cases per 100 000 exposed)
0.000007	0.000002	40
0.00007	0.00002	400
0.0007	0.0002	4000

c) BMD approach – top-dose group incidence set at 100%

This approach was also presented in the RIVM report (2014). This scenario seemed like a reasonable worst case since already 31 out of 36 animals developed tumours at a five times lower dose (in the mid dose group). Using the same software as stated above, 6/7 fitted models were accepted (Table 6).

As in the approach b) (top-dose omitted), the lowest $BMDL_{10}$ of approximately 0.003 mg/m³ (3 µg/m³; from the gamma model) was obtained – similar outcome as in the RIVM report (2014).

Table 6. Results of BMD modelling for Klein et al. (1991) data with top-dose incidence set at 100%.

Model	Log likelihood	AIC*	accepted	BMDL	BMDU	BMD	convergence
null	-98.92	199.84		NA	NA	NA	NA
full	-38.05	84.1		NA	NA	NA	NA
two.stage	-38.12	82.24	yes	23.3	40.7	30.6	yes
log.logist	-38.48	82.96	yes	20.4	73.2	45.2	yes
Weibull	-38.06	82.12	yes	6.69	56.2	25.3	yes
log.prob	-38.22	82.44	yes	20.5	74.7	45.9	yes
gamma	-38.06	82.12	yes	**3.11**	59.6	23.9	yes
LVM: Expon. M3-	-38.08	82.16	yes	6.44	65.2	29	yes
LVM: Hill m5-	-38.09	84.18	no	NA	NA	29.2	no

BMDL, BMDU, and BMD are expressed in µg/m³. *Akaike information criterion

Since both BMD approaches gave similar estimates, which were more conservative than the T25 approach, **RAC agreed to use the "BMD approach – top-dose omitted" for cancer risk assessment of NDMA following inhalation exposure**, as the simpler one of the two BMD approaches.

RAC also reviewed two earlier inhalation studies:

First study **Moiseev and Benemansky (1975)** exposed male and female Balb/C mice (30 – 68 per group/sex) and Wistar rats (36 – 51 per group/sex) to 0.005 mg/m³ or 0.2 mg/m³, continuously for 17 months and 25 months respectively. Every 3 months, 4-6 animals per group were sacrificed. All animals which died or were sacrificed were histopathologically examined[11]. Increased incidence (statistically significant in most cases)

[11] Methodology section of Moiseev and Benemansky (1975) study (translated from Russian): "*Balb/C mice and Wistar rats were used in the experiment. Exposure was carried out in 200 L inhalation chambers, at NDMA concentration of 0.005 mg/m3 and 0.2 mg/m3. Analytical control was carried out for the first six months daily, and later - 2-3 times a week from each cell. The exposure was carried out continuously 24h per day for 17 months in mice and 25 months for rats, which accounts for more than 2/3 of the average life span for respective species. The control groups of animals were kept under normal conditions. With the aim of detecting the neoplasms, every 3 months 4-6 animals*

in liver, lung, and kidney tumours in both species and sexes was observed at the higher dose only, i.e. at 0.2 mg/m^3 (Table 7).

RAC notes the study limitations in methodology and reporting: only two doses were tested; the methodology is very briefly described (e.g. a method for measuring the concentration of NDMA in an inhalation chamber is not stated); animal survival and general toxicity of NDMA in exposed groups are not reported; interim sacrifice every 3 months was markedly reducing the number of animals per group during the study. Therefore, RAC considers that these limitations render this study inadequate for cancer risk assessment. However, the study results also identify the carcinogenic potential of NDMA at a similar dose level as observed in Klein et al. (1991). Although the pattern of tumour types (adenoma, adenocarcinoma, and sarcoma in kidneys and lungs; hepatic adenomas, haemangiomas, and sarcomas) is different than the one observed in Klein et al., both are in line with other studies with nitrosamines, following either oral or parenteral exposure. It should be noted that it is unclear whether nasal tissues were histopathologically examined in Moiseev and Benemansky (1975) study.

Table 7. Tumours findings in Moiseev and Benemansky (1975) study

NDMA dose (mg/m^3)	Mice Rats	Sex	Number of animals		**Tumour incidence**					Sum of all tumours
			Total	With tumour	Lung	Liver	Kidney	Mammary gland	Other type	
0	Mice	M	36	3	1	0	0	2	0	3
	Mice	F	45	9	2	0	0	7	0	9
	Rats	M	40	9	3	2	2	1	1	9
	Rats	F	37	13	2	1	0	7	3	13
0.005	Mice	M	47	2	0	0	1	0	1	2
	Mice	F	30	7	2	2	1	1	1	7
	Rats	M	36	9	2	0	1	1	5	9
	Rats	F	51	14	3	1	1	8	1	14
0.2	Mice	M	33	9	5*	0	3*	0	1	9
	Mice	F	68	22	14*	6*	1	2*	0	23
	Rats	M	31	25	6	10*	28*	1	2	47
	Rats	F	30	17	6*	2	4*	1	0	13

* Statistically different from control (Chi square test), $P < 0.05$; Sex: M – males, F – females.

Although not specifically stated by the study authors, it can be assumed that multiple tumour types were occasionally observed in the same animal (e.g., in the kidneys, the following tumour types were observed: adenomas, haemangiomas, adenosarcomas, adenocarcinomas, spindle cell sarcomas), since the number of animals with tumour(s) was lower than the sum of all tumours observed in a corresponding study group of rats and mice. It is not clear, however, why the sum of all observed tumours is lower than the number of animals with tumours in female rats dosed at 0.2 mg/m^3.

RAC notes that the tumour types in the two studies differ (incidence of kidney and liver tumours (Moiseev and Benemansky) versus nasal tumours (Klein et al)) and their onset may also differ. Nasal tumours may be considered more sensitive, although it is unknown

were sacrificed. The organs of animals which died or were sacrificed were fixed in a 10% solution and embedded in paraffin. Sections were stained with hematoxylin-eosin and picrofuchsin solution according to van Gieson. When determining the tumours, the relevant guidelines were used. Data on the incidence of tumours were statistically analysed by chi-square test and by the percentage comparison method."

whether nasal tract was investigated histopathologically in the Moiseev and Benemansky study.

Second study **Druckrey et al. (1967)** lifetime inhalation study in BD rats reported increased incidences of nasal tumours (esthesioneuroblastoma, squamous cell carcinoma) at 150 mg/m^3 (in 8 out of 12 animals) and 300 mg/m^3 (in 4 out of 6 animals) (as reported in ATSDR, 2019). Due to the high doses applied, a low number of animals per group, only two exposure levels, and no incidences for the control group, this study is not considered adequate for risk assessment.

2) Oral study with NDMA

The oral study by Peto et al. (1991) is the most comprehensive, well described, chronic, oral study, performed with inbred Colworth rats (n=60/sex/dose; control group n=240/sex). The animals were exposed to NDMA or NDEA in drinking water, at increasing concentrations ranging from 0.033-16.896 ppm (estimated at 0.001–0.697 mg/kg bw/day in males and 0.002-1.224 mg/kg bw/day in females), with a total of 15 dose groups (Peto et al., 1991a, 1991b).

Mortality increased in a dose-related manner, both in NDMA- and NDEA-exposed animals of both sexes. In the higher-dosed groups the increase was primarily due to death from nitrosamine-induced tumours of the oesophagus or liver (including a few tumours of the Kupffer cells), to the sacrifice of animals that were severely ill from these diseases, or to the sacrifice of animals in which liver abnormalities were thought to have been palpated. Still, survival was rather satisfactory (average survival times in the lower 7 dose groups (0.033 – 1.584 ppm, i.e. 0.001 – 0.048 mg/kg bw/day) and the control group was 33 months in males and 30 months in females, and at dose levels up to 2.64 ppm (i.e. 0.08 mg/kg bw/day) more than 75% of males were alive after 2 years of the study, and more than 75% of females after 1.5 years of the study).

Target organ for NDMA tumorigenesis was liver, due to intrahepatic activation of nitrosamines to unstable intermediates which produce promutagenic DNA adducts. The incidences of any liver tumour (summed across cell type and fatal/incidental) were statistically significantly increased at doses ≥0.022 mg/kg/day (0.528 ppm) (as reported in ATSDR, 2019). At low dose ranges (<1 ppm, i.e. 0.03 mg/kg/day), an approximately linear relationship between dose and liver neoplasms was observed (Figure 2, as an illustrative example). Details of the study are described in Annex 1 (see section 7.7.2.2.2 and Table 35).

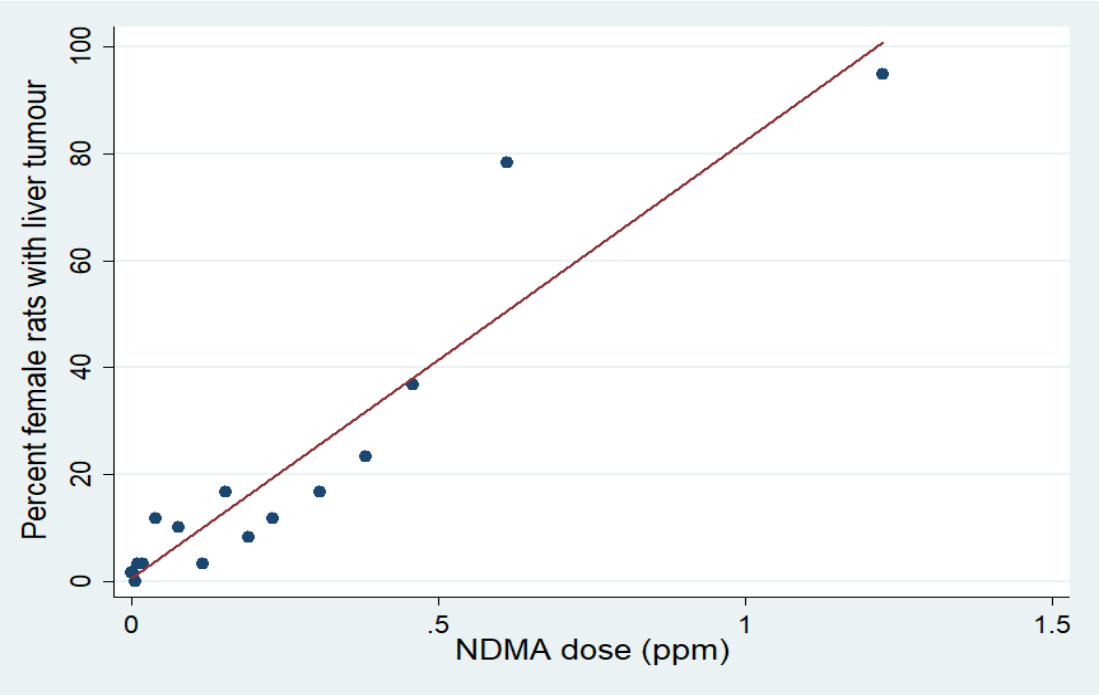


Figure 2. Incidence of all malignant liver cancers (fatal+incidental; bile duct/Kupffer cell/liver cell/mesenchyme/other) in female rats in Peto et al. (1991)

Peto et al. (1991) noted a limitation in the early study conduct, namely, the rats were palpated weekly and sacrificed if palpable liver lesions were present, in order to monitor the onset of malignancies at a stage well before they were likely to cause death. However, 28 animals (of 4080) developed palpable cysts or nodules that led to their premature sacrifice before any malignant disease was macroscopically evident, and 27 animals were sacrificed in error, with livers that appeared normal at autopsy. These errors occurred mostly during the early months of the study (the palpation criteria for sacrifice became stricter later in the study) and they were more common in the high- than in the low-dosed groups, presumably because the staff responsible for palpation were not "blind" to treatment. Nevertheless, this error is expected to have little or no effect on the dose-response relationship in the animals exposed to lower doses.

For the cancer risk assessment, the BMD approach was applied, using EFSA Open Analytics software (quantal response, with model averaging, sex as a covariate, extra-risk: $BMD_{10\%}$, 95%CI) on the total malignant liver cancers in male rats in the above-described study. A BMDL of 0.0421 mg/kg bw/day was derived, assuming the benchmark response (BMR) of 10%.

The calculations were performed according to the ECHA Guidance on information requirements and chemical safety assessment, Chapter R.8 (as explained in Annex 1 of the opinion):

Conversion of the oral rat dose to the corresponding air concentration using the standard breathing volume for the rat (0.38 m^3/kg bw for 8 h exposure of workers):

BMDL(inhalation) = (0.0421 mg/kg bw/d) / (0.38 m^3/kg bw/d) = **0.11 mg/m^3** (8 h)

Correction for exposure duration (considering 40 years of work, 5 days/week), and inhalation volume (rats in rest vs worker light activity) using default values:

BMDL(worker) = 0.11 mg/m^3 ∗ (75 years/40 years) * (52 weeks/48 weeks) * (7 days/5 days) * (6.7 m^3/10 m^3) = **0.21 mg/m^3**

Since a high degree of absorption is assumed for oral exposure (see Annex 1, section 7.1.2), no correction for bioavailability is needed.

Additional lifetime cancer risks were calculated according to a linearised approach (high to low dose extrapolation):

Exposure concentration representing **$1*10^{-5}$ risk**: 0.21 mg/m^3 / 10 000 = **$2*10^{-5}$ mg/m^3** (corresponding to 0.000006 ppm)

i.e., Exposure concentration representing **$4*10^{-5}$ risk**: **$8*10^{-5}$ mg/m^3**

As this concentration is higher than the one derived from the inhalation study, no further ERR derivation is presented.

Cancer risk assessment for NDEA

Since no long-term inhalation study was available for NDEA, the oral study by Peto et al (1991a,b), described above, is used for the cancer risk assessment for NDEA. Significant increases in several tumour types were observed (primarily liver and oesophagus), with the total malignant liver tumours identified as the most sensitive ones.

For the cancer risk assessment, the BMD approach was applied, using EFSA Open Analytics software (quantal response, with model averaging, sex as a covariate, extra-risk: BMD10%, 95%CI) on the total malignant liver cancers in female rats exposed to NDEA (Peto et al. 1991a,b). A BMDL of 0.0146 mg/kg bw/d was calculated (assuming the BMR of 10%) and used for the ERR calculations:

Conversion of the oral rat dose to the corresponding air concentration using the standard breathing volume for the rat (0.38 m^3/kg bw for 8 h exposure):

BMDL(inhalation) = (0.0146 mg/kg bw/d) / (0.38 m^3/kg bw/d) = **0.038 mg/m^3** (8 h)

Correction for exposure duration (considering 40 years of work, 5 days/week), and inhalation volume (rats in rest vs worker light activity) using default values:

BMDL(worker) = 0.038 mg/m^3 ∗ (75 years/40 years) * (52 weeks/48 weeks) * (7 days / 5 days) * (6.7 m^3/10 m^3) = **0.073 mg/m^3**

Since a high degree of absorption is assumed for oral exposure (see Annex 1, section 7.1.2), no correction for bioavailability is needed.

Additional lifetime cancer risks were calculated according to a linearised approach (high to low dose extrapolation):

Exposure concentration representing **$1*10^{-5}$ risk**: 0.073 mg/m^3 / 10 000 ≈ **$7*10^{-6}$ mg/m^3** (corresponding to 0.000002 ppm)

i.e., Exposure concentration representing **$4*10^{-5}$ risk ≈ $3*10^{-5}$ mg/m^3**

Assuming linearity, excess life-time cancer risks were calculated and are presented in Annex 1 (Table 41).

Cancer risk assessment for NDELA

Since no inhalation experiments are available, the oral study by Lijinsky and Kovatch (1985) was identified as the key study, with total liver tumours in female rats (LOAEL 0.879 mg/kg/day) as the most sensitive endpoint.

This is a well reported study in which F344 rats of both sexes (the Frederick Cancer Research Facility) received NDELA in drinking water at dose levels of 28, 64, and 160 mg/L. The 160 mg/L solution was given to 27 male and 27 female rats for 50 weeks; the 64 mg/L solution was given to 20 male and 20 female rats for 50 weeks, and to 20 male and 20 female rats for 100 weeks; and the 28 mg/L solution was given to 39 male and 39 female rats for 100 weeks. In the control group, there were 20 animals per sex. After treatment, the animals were allowed to die or killed when moribund, necropsied and organs histopathologically examined.

NDELA treatment did not affect the survival rate in females, but a lower survival rate in males in all exposed groups compared to controls was observed at week 100. At week 50,

the survival rate ranged from 95-100% in all groups, and at 100 weeks, the survival rate ranged from 55-64% in exposed males (80% in control males), and from 75-81% in exposed females (only 55% in control females).

Although many of the treated animals had malignant liver tumours, they did not apparently cause rapid death of the animals.

For the cancer risk assessment, the BMD approach was applied, using EFSA Open Analytics software (quantal response, with model averaging, sex as a covariate, extra-risk: BMD10%, 90%CI) on the total malignant liver cancers in female rats exposed to NDELA (Lijinsky and Kovatch, 1985). A BMDL of 0.45 mg/kg bw/day was calculated (assuming the BMR of 10%) and used for the ERR calculations:

Conversion of the oral rat dose to the corresponding air concentration using the standard breathing volume for the rat (0.38 m^3/kg bw for 8 h exposure of workers):

BMDL(inhalation) = (0.45 mg/kg bw/d) / (0.38 m^3/kg bw/d) = **1.2 mg/m^3** (8 h)

Correction for exposure duration (considering 40 years of work, 5 days/week), and inhalation volume (rats in rest vs worker light activity) using default values:

BMDL(worker) = 1.2 mg/m^3 * (75 years/40 years) * (52 weeks/48 weeks) * (7 days/5 days) * (6.7 m^3/10 m^3) = **2.3 mg/m^3**

Since a high degree of absorption is assumed for oral exposure (see Annex 1, section 7.1.2), no correction for bioavailability is needed.

Additional lifetime cancer risks were calculated according to a linearised approach (high to low dose extrapolation):

Exposure concentration representing **1*10^{-5} risk**: 2.3 mg/m^3 / 10 000 = **2*10^{-4} mg/m^3**

i.e., Exposure concentration representing **4*10^{-5} risk**: **8*10^{-4} mg/m^3**

Assuming linearity, excess life-time cancer risks were calculated and are presented (corrected by a factor of 100) in Table 42, Annex 1.

Cancer risk assessment for NMor

Since no inhalation experiments are available, the oral study by Lijinsky et al. (1988) was identified as the key study, with total liver tumours in female rats (LOAEL: 0.0035 mg/kg/day) as the most sensitive endpoint.

This is a well reported study in which female F344 rats (the Frederick Cancer Research Facility) received NMor in drinking water at dose levels of 0.07 mg/L or 0.18 mg/L for 100 weeks (100 rats/group); 0.45, 1.1, 2.6, or 6.4 mg/L for 50 or 100 weeks (96 rats/group, half of which were treated for 50 weeks and half for 100 weeks); 16 mg/L for 50 weeks (24 rats); 40 mg/L for 40 weeks (24 rats); or 100 mg/L for 25 weeks (24 rats). The animals were exposed 5 days per week. Control animals (N=80) received deionised water. At the end of the treatment, the animals were allowed to die or were killed when moribund. Each animal was dissected, and all lesions and major tissues and organs were histopathologically examined.

At 90 weeks, the survival rate was >80% at dose levels below 16 mg/L. Most of the rats in the study died with tumours. In controls and in the lower dose groups, tumours common for female F344 rats predominated (mononuclear cell leukaemia, pituitary neoplasms, neoplasms of the adrenal and mammary glands). The groups given the higher doses of NMor had increased incidence of liver, oesophagus, and oral cavity tumours. These tumours were rare in control group.

BMD modelling of the dose-response relationship reported by Lijinsky et al. (1988) was attempted but T25 resulted in a slightly lower estimate compared to BMDL (0.018 vs. 0.019 mg/kg bw/d). T25 was used as a PoD based on a LOAEL of 0.0035 mg/kg/day related to total liver tumours in female rats:

T25 = LOAEC * [reference incidence/(incidence at LOAEC – control incidence)] * (1-control incidence) / 1

LOAEC = 0.0035 mg/kg/day for liver tumours in female rats; reference incidence = 0.25

T25 = 0.0035 mg/kg bw/d x [0.25 / (6/100 – 1/80)] * [(1 – 1/80)/1] = **0.018 mg/kg bw/d NMor**

This dose of 0.018 mg/kg bw/d was used for the cancer risk calculations:

Conversion of the oral rat dose to the corresponding air concentration using the standard breathing volume for the rat (0.38 m^3/kg bw for 8 h exposure of workers):

$T25_{(inhalation)}$ = (0.018 mg/kg bw/d) / (0.38 m^3/kg bw/d) = **0.047 mg/m^3** (8 h)

Correction for exposure duration (considering 40 years of work, 5 days/week), and inhalation volume (rats in rest vs worker light activity) using default values:

$T25_{(worker)}$ = 0.047 mg/m^3 * (75 years/40 years) * (52 weeks/48 weeks) * (7 days/5 days) * (6.7 m^3/10 m^3) = **0.09 mg/m^3**

Since a high degree of absorption is assumed for oral exposure (see Annex 1, section 7.1.2), no correction for bioavailability is needed.

Additional lifetime cancer risks were calculated according to a linearised approach (high to low dose extrapolation):

Exposure concentration representing **1*10^{-5} risk**: 0.09 mg/m^3 / 25 000 = **4*10^{-6} mg/m^3** (corresponding to 0.0000008 ppm)

i.e., Exposure concentration representing **4*10^{-5} risk**: **1.6*10^{-5} mg/m^3**

Assuming linearity, excess life-time cancer risks were calculated and are presented in Annex 1, Table 43.

Conclusion of the cancer risk assessment

As presented in Table 8 below, five cancer exposure-risk relationships were estimated for four nitrosamines.

Table 8. Summary of excess life-time cancer risk

Excess life-time cancer risk (Cases per 100 000 exposed)	**Air concentration** (mg/m^3)				
	NDMA (inhalation study)	**NDMA** (oral study)	**NDEA** (oral study)	**NMor** (oral study)	**NDELA*** (oral study)
	BMDL as PoD	BMDL as PoD	BMDL as PoD	T25 as PoD	BMDL as PoD
4	7 x 10^{-7}	8 x 10^{-5}	2.8 x 10^{-5}	1.6 x 10^{-5}	8 x 10^{-6}
40	7 x 10^{-6}	8 x 10^{-4}	2.8 x 10^{-4}	1.6 x 10^{-4}	8 x 10^{-5}
400	7 x 10^{-5}	8 x 10^{-3}	2.8 x 10^{-3}	1.6 x 10^{-3}	8 x 10^{-4}
4000	7 x 10^{-4}	8 x 10^{-2}	2.8 x 10^{-2}	1.6 x 10^{-2}	8 x 10^{-3}
Key study:	Klein et al. (1991)	Peto et al. (1991)	Peto et al. (1991)	Lijinsky et al. (1988)	Lijinsky and Kovatch (1985)

* Excess life-time cancer risk for NDELA, which is based on oral data, is corrected by a factor of 100, to adjust for the indicated higher potency of NDMA following inhalation compared to oral exposure.

For NDPA, data were too limited to allow for dose-response relationship modelling, e.g. strain and/or sex of animals were not reported, exposure period was not specified or was rather short, number of dose levels was very low (only one or two), tumour incidence data were not available, or the route of exposure for subcutaneous or intraperitoneal.

The estimates based on oral exposure are rather similar between NDMA, NDEA and NMor. Similar estimates for NDEA and NMor, despite the higher potency of NDEA (see section Carcinogenicity and mode of action above), could be explained by these values being derived from different experiments and in different rat strains.

The highest estimate was obtained for NDELA, which is in line with its lower potency ranking described earlier in the Opinion. Although it can be hypothesized that differences in metabolism and adduct formation may explain the lower potency of NDELA, our knowledge of its metabolism is rather limited.

There is a question whether the higher carcinogenic potency of NDEA compared to other nitrosamines should be taken into account. Namely, if the nasal cavity cancer risk (by inhalation exposure in Klein et al. 1991) was two orders of magnitude higher for NDMA compared to liver cancer risk in an oral study by Peto et al. (1991), the same could be expected for NDEA, NMor and other nitrosamines, for which only oral carcinogenicity data are available.
Therefore, the nasal cavity cancer risk of NDEA could be expected to be higher than that of NDMA, due to a higher potency of NDEA.

A possible approach could be to apply a factor of 100 to the cancer exposure-risk relationships for NDEA and NMor, which are based on oral data, to adjust for the indicated higher potency of nitrosamines following inhalation compared to oral exposure.

However, RAC points out that nitrosamines' potency ranking is linked to high uncertainty, as described in previous sections. Comparable studies in the same rat strain with the same number of dose groups are available only for NDMA and NDEA, while the accuracy of the TD_{50}-based potency ranking strongly depends on the study quality and size (the limitations of this approach are already noted in the opinion). As already mentioned above, additional lifetime cancer risk estimates for NDMA and NDEA based on the same oral study (Peto et al., 1991) are of the same order of magnitude ($8*10^{-5}$ mg/m^3 and $2.8*10^{-5}$ mg/m^3, respectively, at $4*10^{-5}$ risk level).

Therefore, **for NDMA, NDEA, and NMor**, RAC agreed to use the **cancer exposure-risk relationship derived for NDMA based on inhalation data with BMDL as PoD**. This is the most conservative PoD derived for NDMA, since it is based on an inhalation (rather than oral) exposure route, and it is the lowest value obtained for NDMA by different modelling approaches for cancer exposure-risk relationship estimation. RAC considers that this PoD is conservative enough to compensate for the uncertainty regarding differences in potency of NDEA, NDMA, and NMor.

For **NDELA**, which showed up to three orders of magnitude lower potency compared to NDEA, NDMA, and NMor, RAC agreed to **apply a factor of 100 to the cancer exposure-risk relationship** based on oral data for NDELA, to adjust for the indicated higher potency of nitrosamines following inhalation compared to oral exposure. RAC, nevertheless, points out that in the absence of experimental inhalation data for NDELA, the application of this adjustment factor introduces some degree of uncertainty as it remains unknown whether the difference between oral and inhalation toxicity observed for NDMA applies equally to the other nitrosamines.

Derived Limit Values

8h-TWA - Non-cancer liver effects (see section 9.2.2 of Annex 1 for full discussion)

There are limited data available on the non-cancer effects of nitrosamines in humans. Some reports from rubber industry suggest associations between nitrosamine exposure and respiratory effects or mortality due to circulatory, respiratory, and digestive diseases, but the effect of other confounding exposures can not to be excluded.

One study suggested elevated mortality from nonalcohol-related chronic liver disease among female rubber workers (Straif et al., 2019). There are also case reports of liver diseases caused by acute or repeated exposure to high levels of NMDA (ATSDR, 2023). This is in line with animal data since liver effects have been consistently observed also in animal studies with NDMA, NDEA, NDPA. The lowest LOAEL for these effects is 0.002 mg/kg/day for NDMA observed in the studies by Moniuszko-Jakoniuk et al. (1999) and Roszczenko et al. (1996). The effects observed included increases in liver enzyme (AST, ALT, ALP and GGT) levels, increased incidence of degeneration, argyrophilic and collagenic fibres and increased inflammatory infiltrations near portal biliary tract, steatosis and parenchymatosis in the liver. In addition, a decrease of the latent iron-binding capacity was observed in these studies at exposure levels ≥0.0016 mg/kg/day. This was identified as the most sensitive endpoint observed in these studies. These effects observed already after two weeks of exposure were recently used by ATSDR (2023) to derive minimal risk level (MRL) for short-term exposure of NDMA.

The starting point for the MRL derivation was $BMDL_{1SD}$[12] 0.0014 mg/kg/d, calculated on the basis of this data. For the purpose of this assessment (8h TWA), ECHA used the same data (Roszczenko et al., 1996) to calculate the BMDL for the 18% BMR (which is the level showing statistical difference). BMDLs of 0.0006-0.0009 mg/kg/d were derived using EFSA Open Analytics software and PROAST[13]. If the lowest BMDL of 0.0006 mg/kg/d is taken as a starting point, it can be calculated to correspond to an occupational exposure air level of:

BMDL = 0.001 mg/m^3 (0.0006 mg/kg/d / 0.38 m^3/kg bw x 6.7 m^3/ 10 m^3)
(ECHA guidance R8)

To calculate a level causing no risk for these effects, an assessment factor (AF) of 2.5 is used for remaining interspecies differences and an AF of 5 for intraspecies differences, resulting in an 8h TWA of 0.00008 mg/m^3 (0.08 µg/m^3).

Since the adversity of these effects (decreased latent iron-binding capacity) is unsure and the effects were not more severe after longer exposure duration, no assessment factor to account for study duration was considered necessary.

Liver effects (histopathological findings), observed by Moniuszko-Jakoniuk et al (1999) at the dose-levels of ≥0.002 mg/kg/day, were not suitable for benchmark dose modelling. However, if a LOAEL of 0.002 mg/kg/day is taken as a starting point for the calculation of threshold level for non-cancer effects of NMDA, this would correspond to a worker inhalation exposure to the air levels of 0.0035 mg/m^3 (= 0.002 mg/kg bw/d / 0.38 m^3/kg bw) *x* (6.7 mg/m^3 / 10 mg/m^3).

If standard assessment factors of 2.5 for interspecies differences, and a factor of 5 for worker intraspecies differences, as well as a factor of 2 to cover for sub-chronic to chronic extrapolation, this will result in 0.00014 mg/m^3 (0.14 µg/m^3).

No further assessment factors are considered necessary since the use of Moniuszko-Jakoniuk et al (1999) and Roszczenko et al. (1996) data as a starting point is already considered a sufficiently conservative approach.

In other studies, non-cancer liver effects have been seen only at 10-times higher exposure levels. These studies include the chronic carcinogenicity study by Peto et al. (1991a,b), in which dose-dependent increases in the incidence of non-neoplastic and pre-neoplastic lesions in the liver (including hyperplastic nodules, cytomegaly, cysts, hepatocyte shrinkage and abnormality of glycogen-containing cells) were seen with both NDMA and NDEA.

[12] (95% lower confidence limit on the BMD associated with 1 SD change from control mean)
[13] PROAST https://www.rivm.nl/en/proast (copyright RIVM National Institute for Public Health and the Environment).

A clear increase in these effects were seen at the dose of 0.044 mg/kg/day for both substances and 0.022 mg/kg/day can be considered as a NOAEL. The recent evaluation by ATSDR (2023) had, however, interpreted 0.022 mg/kg/day as a LOAEL and the lower dose (0.01 mg/kg/day) as NOAEL. At the dose of ≥0.022 mg/kg/day, an increased liver tumour incidence and a reduced survival due to tumours were observed. In a most recent study by Souliotis et al. (2002, see above) no hepatocellular alterations or necrosis of the liver were seen at the doses up to 0.372 mg/kg/bw.

RAC considers that at the air levels below 0.00008 mg/m^3 (0.08 µg/m^3), which is the level derived based on iron binding capacity, the risk of non-cancer target organ effects is negligible. This level corresponds to an excess cancer risk of about 5:1000 NDMA exposed workers (see section 9.1.2 of Annex 1, and Table 8 above).
As a consequence, **a BOEL based on cancer risk will also protect from non-cancer effects, provided that the value will not exceed 0.00008 mg/m³.** This value should be considered when setting the binding limit value for nitrosamines to ensure that also the risk of non-cancer effects is minimised.

Although NDEA has been a up to 2-3 times more potent hepatocarcinogen than NDMA in the study by Peto et al. 1991, non-cancer hepatotoxic effects were seen at the same dose as for NDMA. The limit value derived for non-cancer effects based on the studies by Moniuszko-Jakoniuk et al. (1999) and Roszczenko et al. (1996) is conservative enough to reach a conclusion and therefore protective also from the hepatotoxicity of NDEA.

8h-TWA - Reproductive effects (see section 9.2.2 of Annex 1 for full discussion)

None of the nitrosamines included in this assessment are classified for reproductive effects. However, there are some limited data on the reproductive toxicity of NDMA. One study is available on NDEA. One study with one dose (0.5 mg/kg bw/d) of NDMA or NDEA suggests effects on testosterone levels and testicular histopathology in rabbits. In the study by Anderson et al. (1978) increased incidences of foetal/perinatal deaths were seen in mice treated with 0.026 mg/kg bw/day of NMDA 75 days prior to mating, during pregnancy and lactation. In the exposed group, higher number of male pups when compared to female pups were observed.

The dose tested was the lowest dose tested in the available reproductive toxicity tests corresponding to inhalation exposure of workers to air levels of 0.026 mg/m^3, which is 10-times higher than the PoD derived for hepatotoxic effects above. However, there are some unclarities/deficiencies in the study. For example, there was no difference in the total number of living pups between controls and exposed groups, and the majority of the foetal deaths were due to one litter, in which all pups died. It should be also noted that at these dose levels, liver effects have been already suggested in other studies. The study is therefore not considered appropriate for limit value setting.

Monitoring methods and recommendations

No health-based limit value can be set for the carcinogenic effects of nitrosamines, and therefore RAC has only derived dose-response relationships for the key nitrosamine compounds typically measured at workplaces.

The rubber industry is a typical industry field in which exposure to nitrosamines may occur. The exposure to nitrosamines in rubber industry is always mixed exposure to several nitrosamine compounds. Based on the existing measurement data, the proportion of individual nitrosamine compounds may vary even within this industry. NDMA and NMor are nitrosamines most commonly measured and detected in rubber industry but also NDEA and other nitrosamines have been detected.

Since the toxicological effects of nitrosamines are the same, key effects being direct DNA damage, and cancer and non-cancer liver effects, the effects of nitrosamines can be considered additive. Therefore, in the risk assessment the combined exposure to several nitrosamines needs to be taken into account.

Considering that the potency differences among the main types of nitrosamines, i.e. NDMA, NDEA and NMor are relatively small, the same limit value derived on the basis of the dose-response of NDEA or NMor (see above section - Cancer Risk Assessment) are recommended to be used for the combined exposure to nitrosamines (sum of nine nitrosamines, see below). This is in line with the recent recommendation by EFSA (2023) which concludes that the available data is too limited for the setting of potency factors and therefore assumes equal potency for all dietary relevant nitrosamines (NDMA, NMEA, NDEA, NDPA, NDBA, NMA, NSAR, NMor, NPip and NPyr).

The current measurement methods for the measurement of nitrosamines in air allow the measurement of 7-9 different nitrosamines in the same analysis. The GC-TEA based method described by DFG (2022) has a LOQ of 0.01 µg/m^3 (1 x 10^{-5} mg/m^3) for 400 L sample over 3-4 hours and has been validated for NDMA, NMEA, NDEA, NDiPA, NDPA, NDBA, NPip, NPyr and NMor. In rubber industry, where several of these nitrosamines may be present in air, it is recommended to use this approach.

In the metal industry where metal working fluids are used, NDELA has been the main nitrosamine observed although the presence of other nitrosamines cannot be excluded. NDELA is clearly less potent than the other nitrosamines mentioned above, and this is reflected in the separate dose-response derived (see above section - Cancer Risk Assessment). There is also a separate GC-TEA based method for the measurement of NDELA in air (DGUV, 1992) with a LOQ of:

0.035 µg/m^3 (3.5 x 10^{-5} mg/m^3) or 0.17 µg/m^3 (1.7 x 10-^{5} mg/m^3) NDELA.

Accordingly, RAC considers that a separate OEL for NDELA is justified.

In case both NDELA and other nitrosamines are detected, both OELs should be considered and complied with. Since NDELA shares the same mode of action (although being less potent) as other nitrosamines, possible combined effects (and subsequent risk) should be considered.

Short term limit value (STEL)

Based on the available data no STEL is considered necessary for nitrosamines.

Biological guidance and limit values

No biological limit or guidance value can be proposed for nitrosamines.

Biological Monitoring (see section 6.2 of of Annex 1 for full discussion)

Although it is possible to measure several nitrosamines in urine, there are limited data available on the background nitrosamine levels in urine and correlations between air levels and urinary nitrosamine levels. RAC notes that only NDELA exposure has been measured in occupational settings, namely in activities involving exposure to metalworking fluids. However, these studies date back to time before year 2000.

Similarly, the available data on background nitrosamine levels in general population is limited. The scientific publications summarised in Annex 1 are rather old and it is unclear how well they represent the exposure of present-day European population. In US, NHANES survey from 2013-2014 suggests that the levels of nitrosamines in the general population are very low[14]. As part of this large biomonitoring survey, urinary levels of NDEA, NMor and NMEA were bio-monitored from more than 2000 individuals representing general population. The levels remained mostly below the limits of detection.

[14] https://www.cdc.gov/exposurereport/index.html

However, it is noted that there is a large variability in the results between the studies from different regions: when in NHANES cohort P95 level of NDEA in general population was <LOQ of 5 ng/l, in Taiwanese cohort levels of 0.12 ± 0.06 ng/mL (=120± 60 ng/l) were reported in non-smoking adults. Considering that there are no recent European data, no BGV can be proposed.

Notations

NDMA and NDELA have been tested for skin penetration *in vitro*, showing an average 48h absorption of 23.6% and 2.6% for NDELA and NDMA. Lower absorption of NDMA was most probably attributable to its high volatility. NDMA has induced lung adenomas in mice after dermal exposure. Also, the diffusion of NDPA through rat skin *in vitro* has been demonstrated. Based on this evidence a 'skin' notation is proposed.

ATTACHMENTS

Annex 1 - The ECHA scientific report gives the detailed scientific grounds for the opinion.

Annex 2 - The RCOM reflects the comments received on the ECHA scientific report, and the responses provided by ECHA and RAC (excluding confidential information).