DISPOSITION OF PEER REVIEW COMMENTS FOR TOXICOLOGICAL PROFILE FOR N-NITROSODIMETHLYLAMINE (NDMA)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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Agency for Toxic Substances and Disease Registry

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Comments Provided by Peer Reviewer #1

General Comments

COMMENT 1: The draft profile is a well-written overview of the toxicological effects of NDMA. It follows the standard ATSDR approach in covering the various health- and exposure-related topics. At times it is quite brief but, in most cases, this is probably appropriate given the large number of studies and amount of information on this model mutagen and carcinogen. In spite of the large number of studies, there exist a considerable number of data gaps that make it difficult to confidently evaluate all important health endpoints and establish precise MRLs. Of particular note are the lack of high quality subchronic and chronic studies evaluating non-cancer effects as well as quality reproductive and developmental toxicity studies. This is a particular concern given the toxicity and widespread potential for exposure to NDMA. I recommend that the ATSDR submit this chemical to the National Toxicology Program for additional testing.

RESPONSE: *ATSDR will consider the Reviewer's suggestion to submit NDMA to the National Toxicology Program (NTP) for testing.*

ATSDR Charge Questions and Responses and Reviewer Comments

Chapter 1

QUESTION: Do you agree with those effects known to occur in humans as reported in the text? If not, please explain why and provide a copy of additional references you would cite and indicate where (in the text) these references should be included.

COMMENT 2: There currently are no effects occurring in humans included in Chapter 1. There are, however, multiple case reports of human poisoning by NDMA where symptoms including liver toxicity have been reported (e.g. Fussgaenger and Ditschuneit, 1980; Kimbrough, 1982). These are described to some degree later in the document. I would revise the text (beginning on line 367) to indicate these human studies, and briefly mention at least the liver effects that have been noted, as these are consistent with the effects that have been seen in test animals.

RESPONSE: *The text of Section 1.2 was revised to note the hepatic effects in human case reports.*

> Hepatic effects of NDMA have been observed in humans after acute poisoning incidents (Cooper and Kimbrough 1980; Freund 1937; Hamilton and Hardy 1974; Kimbrough 1982), and in at least one case, death was attributed to liver damage from NDMA exposure (Fussgaenger and Ditschuneit 1980; Pedal et al. 1982). The liver effects in animals exposed orally are well known.

QUESTION: Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

COMMENT 3: Yes. As indicated above, the liver has been seen to be the target organ in humans that have been poisoned by NDMA. In addition, there is evidence that the same mechanism of action occurs in both test animals and humans. As described in the Profile, NDMA is bioactivated, primarily by CYP2E1, to an alkylating agent which will bind to cellular nucleophiles including nucleophilic bases on DNA. The resulting DNA adducts are converted into mutations which eventually lead to cancer. Humans have this CYP enzyme and the same mechanisms should be operable in humans. Methylation of DNA has been seen in at least one human poisoned by NDMA (Herron and Shank, 1980), and liver

toxicity has been commonly seen in poisoned individuals (see discussion in text). NDMA has also been shown to induce mutations in metabolically active human lymphoblastoid cells (Dobo et al. 1997, 1998).

RESPONSE: *No response required.*

COMMENT 4: In this section, the Peto et al. studies have been described as inducing liver tumors in the rats. While true, this is somewhat misleading in that 4 different types of liver tumors were induced – tumors of the liver cells, mesenchyme (blood vessels), bile duct and Kupffer cells. These are generally considered different types of tumors and would not ordinarily be combined (McConnell et al. 1986). The induction of different tumor types should be explained in more detail in Chapter 2, but in this chapter, at a minimum, line 407 should be changed to indicate that NDMA induces several or multiple types of liver and lung tumors in rats and mice.

RESPONSE: *Section 1.2 was revised as suggested.*

> Oral exposure to NDMA induces several types of liver and lung tumors in rats and mice (Anderson 1988; Anderson et al. 1992a; Arai et al. 1979; Clapp and Toya 1970; Den Engelse et al. 1974; Ito et al. 1982; Keefer et al. 1973; Lijinsky and Kovatch 1989; Lijinsky and Reuber 1984; Magee and Barnes 1956; Peto et al. 1984, 1991a, 1991b; Takahashi et al. 2000; Takayama and Oota 1965; Terracini et al. 1966), and has also induced kidney tumors in these species (Lijinsky and Kovatch 1989; Takayama and Oota 1965; Terracini et al. 1966) and testicular tumors in rats (Terao et al. 1978).

QUESTION: Have exposure conditions been adequately described? If you disagree, please explain.

COMMENT 5: For the most part yes. However, I believe that additional information should be provided in this section (and more later in the document) about NDMA exposure through tobacco smoke. NDMA is present at much higher concentrations in tobacco smoke than in the tobacco itself (Tricker et al., 1991), and its concentration is on average 95 times higher in sidestream smoke as compared to mainstream smoke (IARC, 2004). This means that environmental tobacco smoke is a significant source of NDMA exposure which has the potential to affect non-smokers as well as smokers. Consistent with this, relatively high levels of NDMA have been found in environmental tobacco smoke-contaminated rooms (IARC, 2004).

RESPONSE: *The following text was added to Section 1.1.*

> NDMA is present at higher concentrations in tobacco smoke than in the tobacco products themselves (Tricker et al. 1991), and elevated NDMA concentrations in smoke-contaminated rooms suggests that exposure occurs in both smokers and nonsmokers (i.e., involuntary smoking) (IARC 2004). IARC (2004) reported that concentrations of NDMA in sidestream smoke were, on average, 95 times higher than in mainstream smoke.

Minimal Risk Levels (MRLs)

QUESTION: If no MRLs have been derived, do you agree that the data do not support such a derivation? Please explain.

COMMENT 6: While the studies are less-than ideal, I believe that it may be possible to derive an MRL for chronic exposure. For example, Table A-3 indicates that 0.022 mg/kg-day is a LOAEL based on decreased survival due to liver tumors. It indicates that a NOAEL was not determined but this is not

correct based on the doses and results in Table 2-2. Lower doses were administered to the male rats in the Peto et al study, and the highest of these, 0.011 mg/kg-day, would then be the NOAEL. This can also be confirmed by examining Table 7 in Peto et al. 1991 which indicates that no significant increase in tumors was seen at the 0.011 mg/kg-day dose and so there would also not be an increase in mortality due to liver tumors. The 0.011 mg/kg-day NOAEL could then be used to derive an MRL with an additional uncertainty factor being used, if desired, to account for the seriousness of the effect. While imprecise, this would allow the derivation of an MRL or a provisional MRL, which could help those making risk decisions. The key question is whether no MRL is better than an imprecise MRL. Ultimately, this may be a risk management decision.

RESPONSE: *ATSDR does not derive MRLs based on cancer endpoints or serious LOAELs (e.g., reduced survival). However, a new study identified by the Reviewer (Moniuszko-Jakoniuk et al. 1999) was used in conjunction with other data to derive a provisional acute-duration oral MRL.*

COMMENT 7: MRLs could also be derived from the LOAELs seen in acute and intermediate term studies by using an additional uncertainty factor to account for the use of a LOAEL rather than a NOAEL. An addition uncertainty factor could be used if desired, to account for the seriousness of the effect. This approach is not ideal but is done fairly commonly in assessing chemical risks.

RESPONSE: *ATSDR does not derive MRLs based on serious LOAELs.*

QUESTION: If MRLs have been derived, do you agree with the proposed MRL values? Explain. If you disagree, please specify the MRL value that you would propose.

COMMENT 8: MRLs were not derived.

RESPONSE: *No response needed.*

QUESTION: Do you agree/disagree with each component of the total uncertainty factor? Explain. If you disagree, please specify the uncertainty factor(s) that you propose.

COMMENT 9: MRLs were not derived.

RESPONSE: *No response needed.*

QUESTION: Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT 10: The induction of mutation is considered by many to represent a toxicological endpoint. This is pretty well accepted for germ cell mutations and is being increasingly recognized for somatic mutations. As a result, I would recommend the results of Butterworth et al. (1998) (and possibly others listed in Lambert et al. (2005)) be added to the appropriate MRL tables.

RESPONSE: *ATSDR develops MRLs based on noncancer endpoints; thus, mutations are not considered suitable endpoints for MRL derivation.*

COMMENT 11: The Roszczenko et al. (1996) study listed in Table A-2 has a very low LOAEL. I agree with the ATSDR that it should not be used to derive an intermediate duration MRL, because of the lack of histopathological data as well as from my perspective, a concern that the control levels were only measured at the beginning of treatment rather than at the end. In 1999, Roszczenko and colleagues also published another article entitled, "Influence of low concentrations of N- nitrosodimethylamine on the iron level and histopathological picture of rats liver, spleen and bone marrow" in Acta Poloniae Toxicologica (EID: 2-s2.0-0033366970). This may provide the necessary histopathology data and be help in establishing a MRL. I have requested a copy and will forward it when I receive it.

RESPONSE: *The Roszczenko paper cited by the Reviewer (Moniuszko-Jakoniuk et al. 1999) was retrieved along with a related paper by the same group of investigators (Roszczenko et al. 1996b). The three publications (Moniuszko-Jakoniuk et al. 1999; Roszczenko et al. 1996a, 1996b [all by the same group of investigators]), taken together, were used to derive a provisional acute-duration oral MRL for NDMA.*

Chapter 2. Health Effects

QUESTION: Do the health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature? If not, please suggest appropriate changes.

COMMENT 12: The Profile authors have done an impressive job summarizing the large amount of toxicological literature into easily interpretable Figures and Tables. I do believe that the conclusions in Chapter 2 reflect the findings in the published literature.

The Reviewer provided several recommended changes (see Comments 13-17).

RESPONSE: *No response needed.*

COMMENT 13: Lines 584-86. In addition to the two people who died, three other individuals were poisoned but survived. Two of the five poisoned were children I suggest that this be mentioned.

RESPONSE: *The text of Section 2.2 was revised as suggested.*

> Two of five people who consumed lemonade tainted with unknown quantities of NDMA (an adult male and a l-year-old boy) died within days, while the other three people (an adult female, adult male, and 2.5-year-old girl) survived (Cooper and Kimbrough 1980; Kimbrough 1982).

COMMENT 14: Table 2-2. NOAELs of 0.011 mg/kg-day should be added to entry 61 of the Table describing the rat studies by Peto et al.

RESPONSE: *ATSDR does not identify NOAELs for mortality or cancer.*

COMMENT 15: Section 2.7 beginning on line 7.42. I would indicate in this section that "slight to severe thrombocytopenia" was reported in the 5 individuals who were poisoned by NDMA (Kimbrough, 1980). It is mentioned below in the Hepatic section but it should also be mentioned in the Hematological section.

RESPONSE: *Section 2.7 was revised as suggested; the following sentence was added:*

In five individuals poisoned with unknown amounts of NDMA in lemonade, slight to severe thrombocytopenia was reported (Kimbrough 1982).

COMMENT 16: If the other Roszczenko article mentioned above is obtained and of adequate quality, it should be included in section 2.9.

RESPONSE: *The Roszczenko paper cited by the Reviewer (Moniuszko-Jakoniuk et al. 1999) was retrieved and added to Section 2.9 along with a related paper by the same group of investigators (Roszczenko et al. 1996b). Data from these studies were also added to Section 2.7 (Hematological) and Section 2.14 (Immunological).*

Section 2.7 (Hematological):
Administration of NDMA in drinking water to rats for 10 days resulted in dose-related increases in blood hemoglobin concentration at doses ≥0.0016 mg/kg/day, and a small but statistically significant increase in hematocrit at 0.0035 mg/kg/day (Roszczenko et al. 1996b); other hematology parameters were not measured. In a corollary study by the same group of investigators (Moniuszko-Jakoniuk et al. 1999), rats exposed via drinking water to 0.002 or 0.003 mg/kg/day for 10 days exhibited no changes in bone marrow histopathology.

When rats were exposed to 4 mg/kg/day NDMA by daily gavage for 15 days, significant decreases in platelet and reticulocyte counts were observed in conjunction with serious liver damage (Rothfuss et al. 2010). After 30 and 90 days of drinking water exposure to NDMA, rats showed increased blood hemoglobin concentrations at 0.0016 mg/kg/day (17–28%); however, hemoglobin concentration was significantly decreased (9%) after 30 days of exposure to 0.0035 mg/kg/day (Roszczenko et al. 1996b). Hematocrit was not affected at either dose in the 30-day experiment by Roszczenko et al. (1996b), and no other parameters were measured (a 90-day experiment at 0.0035 mg/kg/day was not conducted). Moniuszko-Jakoniuk et al. (1999) reported bone marrow histopathology changes in rats exposed to NDMA in drinking water for 30 or 90 days. After 30 days at 0.003 mg/kg/day and after 90 days at both 0.002 and 0.003 mg/kg/day, bone marrow changes included including focal necrosis of bone marrow, edema, degeneration, decrease in bone marrow megakaryocytes and migration to vascular sinus, and myelosclerosis (Moniuszko-Jakoniuk et al. 1999).

Section 2.9 (Hepatic):
A series of acute- and intermediate-duration studies in rats exposed to low concentrations of NDMA in drinking water was conducted by the same group of investigators (Moniuszko-Jakoniuk et al. 1999; Roszczenko et al. 1996a, 1996b). In these studies, groups of 7–8 male Wistar rats were exposed for 10, 30, or 90 days to concentrations of 10–50 μg/L (0.0007–0.0035 mg/kg/day). Each individual study evaluated limited endpoints, but taken together, the studies demonstrate liver effects at low doses after both acute and intermediate durations. After 10 days of exposure, doses of 0.0016–0.002 mg/kg/day resulted in effects on iron parameters (decreased total and latent iron binding capacity) and serum enzymes (≥2-fold increases in AST, ALT, ALP, and GGT) (Roszczenko et al. 1996a, 1996b), but no liver histopathology changes at doses up to 0.003 mg/kg/day (Moniuszko-Jakoniuk et al. 1999). After 30 days of exposure to ≥0.0016 mg/kg/day, similar perturbations of iron parameters were observed, and serum enzyme levels remained increased (Roszczenko et al. 1996a, 1996b). In addition, there was evidence for serious liver histopathology changes at 0.002 and 0.003 mg/kg/day, including degeneration, argyrophilic and collagenic fibers, and inflammatory infiltrations near portal biliary tract after 30 days (Moniuszko-Jakoniuk et al. 1999). The effects increased in severity to include steatosis and parenchymatosis after 90 days (Moniuszko-Jakoniuk et al. 1999).

Section 2.14 (Immunological):
Effects on splenic histology (megakaryocytes in red pulp and enhanced lymphatic "texture") were observed in rats exposed for 90 days to NDMA in drinking water at doses of 0.002 or 0.003 mg/kg/day; there were no changes after 30 days at either dose (Moniuszko-Jakoniuk et al. 1999).

COMMENT 17: Lines 863–864. This should be checked as I am not sure it is true. Peto and associates reported a linear relationship when they combined the four different types of liver tumors from both males and females with the results from both NDEA and NDMA. From my examination of the data for NDMA alone and when I examine the individual tumors separately for the male and the female rats, there seem to be doses where significant increases in effects are not seen (see Table 7 in Peto et al., 1991b).

RESPONSE: *The Reviewer is correct that there were doses (in the study by Peto et al. 1991b) at which no significantly increased incidence of liver tumors were seen. The text was revised to clarify the reason why neither a NOAEL nor a LOAEL for noncancer endpoints could be identified. ATSDR does not identify NOAELs or LOAELs for cancer endpoints.*

Significant dose-related trends were observed for several nonneoplastic or preneoplastic liver lesions, including hyperplastic nodules, cytomegaly, cysts, hepatocyte shrinkage (males only), and abnormality of glycogen-containing cells (females only). However, statistically significant increases in the incidence of these nonneoplastic changes (either individually or grouped) were seen only at doses ≥0.022 mg/kg/day. Because both increased liver tumor incidence and reduced survival due to tumors were observed at the same doses (≥0.022 mg/kg/day), neither a NOAEL nor a LOAEL for noncancer endpoints can be identified from these data.

QUESTION: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? Were the major study limitations sufficiently described in the text without going into lengthy discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT 18: I think the authors identified the relevant human studies and adequately described the studies, their results, and their limitations.

As indicated above, while not a designed human study, valuable information on NDMA toxicity can be obtained from the series of five individuals who were poisoned with NDMA as described by Kimbrough (1980). All five poisoned individuals showed "slight to severe thrombocytopenia, and changes in serum chemistry indicative of hepatotoxicity. This information should be added to the Hematological and Hepatic sections.

RESPONSE: *ATSDR assumes that the citation to which the Reviewer refers is Cooper and Kimbrough (1980) and/or Kimbrough (1982). Sections 2.7 (see Response to Comment 15) and 2.9 were revised as suggested. The text of Section 2.9 was revised as follows:*

Five members of a family who consumed unknown quantities of NDMA in lemonade became ill with nausea, vomiting, and serum chemistry changes associated with acute liver disease, as well as generalized bleeding and slight to severe thrombocytopenia (Cooper and Kimbrough 1980; Kimbrough 1982).

QUESTION: Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.

COMMENT 19: As indicated in the text, while a large number of studies were identified and evaluated, many of these were older and not performed using what would now be considered to be good laboratory procedures. Many did not test an adequate range of doses and as a result, no-observed-adverse effect levels could not be identified. This data set highlights the value of having results from studies that followed OECD or similar test guidelines when evaluating the hazard and dose responses of a chemical.

As indicated above, there is a study by Roszczenko and colleagues which evaluated the effects of low concentrations of NDMA in several tissues. If, when it is obtained, it is found to be of adequate quality, it should be added to the document.

RESPONSE: *The paper cited by the Reviewer (Moniuszko-Jakoniuk et al. 1999) was retrieved along with a related paper by the same group of investigators (Roszczenko et al. 1996b). These papers were added to the profile (Sections 2.7, 2.9, 2.14; see revised text in response to Comment 16).*

QUESTION: Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?

COMMENT 20: Yes, the most significant endpoint identified was liver cancer and increases in this cancer were seen in multiple species. The highest quality study appeared to be that of Peto et al. and it used rats, which appeared to be sensitive and give representative results.

RESPONSE: *No response needed.*

QUESTION: Has adequate attention been paid to dose-response relationships for both human and animal data? Please explain.

COMMENT 21: Yes. However, most of the human and animal studies provide only limited dose response information, particularly in the low dose region (below the LOAEL). As a result, no MRLs were identified. The Peto study is a good quality study which covered a wide range of doses and may allow a chronic MRL to be identified.

RESPONSE: *The effects identified at the lowest doses in the study by Peto et al. (1991a, 1991b) were cancer and reduced survival due to cancers. ATSDR does not derive MRLs based on cancer endpoints or serious LOAELs (e.g., reduced survival). ATSDR has derived a provisional acute-duration oral MRL based on new studies identified by the Reviewer.*

QUESTION: Are you aware of any studies that are not included in the profile that may be important in evaluating the toxicity of the substance? Please provide a copy of each study and indicate where in the text each study should be included.

COMMENT 22: In a 15-day study, Rothfuss et al. (2010) measured, hematological effects, clinical chemistry and liver pathology as well as the genotoxicity endpoints (Comet and blood MN) in male rats administered NDMA orally. This study should be added to the relevant sections.

RESPONSE: *The 15-day study by Rothfuss et al. (2010) was added to the oral LSE table, Sections 2.3 (Body Weight), 2.7 (Hematological), 2.9 (Hepatic), and Table 2-5 (Genotoxicity In Vivo).*

> *Section 2.3:*
> Decreased body weight gain was reported in male rats after 15 days of exposure to NDMA at 2 mg/kg/day, and at 4 mg/kg/day, absolute body weight was also decreased (magnitude of change was not reported); severe liver effects accompanied the body weight changes (Rothfuss et al. 2010).
>
> *Section 2.7:*
> When rats were exposed to 4 mg/kg/day NDMA by daily gavage for 15 days, significant decreases in platelet and reticulocyte counts were observed in conjunction with serious liver damage (Rothfuss et al. 2010).
>
> *Section 2.9:*
> In other intermediate-duration studies with rats exposed to higher doses, characteristic hepatic effects (as described above) were produced by treatment with NDMA in the diet at doses of ≥2 mg/kg/day for 15 days (Rothfuss et al. 2010), 7.2 mg/kg/day for 4–12 weeks (Khanna and Puri 1966), 10 mg/kg/day for 62–95 days (Barnes and Magee 1954), and 3.9 mg/kg/day for 40 weeks (Magee and Barnes 1956). No liver histopathology changes were observed at 0.5 mg/kg/day for 15 days (Rothfuss et al. 2010).

COMMENT 23: Judging from the summary in WHO (2008), there are a large number of genotoxicity studies describing the induction of chromosome aberrations and micronuclei by NDMA in various tissues including some that arose in the offspring of Syrian hamsters administered NDMA during pregnancy (Inui et al., 1979). These or at least representative examples of key genotoxicity endpoints (chromosome aberrations, micronuclei, mutation) should be included in Table 2-5 and included in the text (see next paragraph and response to #10 below).

RESPONSE: *Table 2-5 includes a large number of studies of chromosome aberrations and micronuclei in tissues of animals administered NDMA, including the cited study (Inui et al. 1979) and many of the studies cited by the World Health Organization (WHO 2008). The following studies cited by WHO (2008) were added to Table 2-5: Barbin et al. 1983; Braithwaite and Ashby 1988; Brambilla et al. 1987; Mirsalis and Butterworth 1980; Mirsalis et al. 1989; Pool et al. 1990; Webster et al. 1996; and Wild 1978. The text of Section 2.19 was revised to include discussion of the key genotoxicity endpoints, as shown in Response to Comment 24.*

COMMENT 24: There are also a large number of studies in which NDMA has been tested by oral gavage or in the diet for the induction of mutations in transgenic mice. Most of these do not appear in the document and should be included in the in vivo Genotoxicity section (see summary compilation on pages 137-139 in Lambert et al. 2005.) Similarly, Delker et al. (2008) is another more recent study which should be added. These mutational endpoints are among the most important and relevant in genotoxicity testing and the text needs to be expanded to reflect this. As indicated above, mutagenic effects themselves can be considered to be an adverse effect and one of these studies (e.g. Butterworth et al. 1998) could potentially be used to derive a MRL.

RESPONSE: *ATSDR develops MRLs based on noncancer endpoints; thus, mutations are not considered suitable endpoints for MRL derivation. Studies of NDMA-induced mutations in transgenic mice cited in Lambert et al. (2005) were added to Table 2-5, as was the study by Delker et al. (2008). The text of Section 2.19 was expanded to include discussion of the transgenic rodent studies and other key genotoxicity endpoints:*

In vitro assays have demonstrated increased mutation frequencies in bacteria, yeast, and mammalian cell systems incubated with NDMA with metabolic activation (see Table 2-4). Increases in the frequency of chromosomal aberrations have been observed in several rat cell types, Chinese hamster lung, ovary, and fibroblast cells, and in human fibroblast cells. As with the mutation assays, the positive results were seen in the presence of exogenous metabolic activation or in metabolically competent cell systems. *In vitro* tests for micronuclei have shown mixed results; increases in micronuclei were observed in human lymphoblastoid cells (Crofton-Sleigh et al. 1993) and in human hepatoma (HepG2) cells (Valentin-Severin et al. 2003) tested without metabolic activation, and in Chinese hamster lung cells tested with activation (Matsushima et al. 1999). Assays with other human cell types and with rat and mouse cells yielded negative results (see Table 2-4). In a large number of other in vitro tests, NDMA was shown to induce sister chromatid exchanges and DNA damage, repair synthesis, or unscheduled synthesis (see Table 2-4).

NDMA has been tested extensively for mutagenicity in transgenic rodent models including the Big Blue® and Big Blue® cII rat and Big Blue®, Big Blue® cII, and Muta™ mouse (reviewed by Lambert et al. 2005; see also Table 2-5). In these studies, NDMA was administered orally (diet or gavage) or via i.p. injection for one or more days at doses between 1.8 and 54 mg/kg/day. Tissues, including liver, lung, kidney, bone marrow, spleen, bladder, and forestomach were sampled for mutations from 1 to 183 days after exposure. In these experiments, NDMA has consistently yielded increased mutations in the liver regardless of species, exposure route, duration, sampling time, and transgene (*lacI, cII, lacZ*). In mice, increased mutation frequencies were also observed in the lung and kidney (reviewed by Lambert et al. 2005).

Studies that have examined the spectrum of mutations induced by NDMA have shown that the most common mutations in the Muta™ (*lacZ*) and Big Blue® (*lacI*) mouse are GC→AT transitions, primarily at non-CpG sites (Delker et al. 2008; reviewed by Lambert et al. 2005). GC→AT transitions can be produced if O6-methylguanine adducts are not repaired, and this particular type of mutation is associated with an increased risk of cancer. Other mutations shown in these analyses included A:T→T:A transversions as well as single and multiple base pair deletions and frameshift mutations (Delker et al. 2008; reviewed by Lambert et al. 2005).

There is some evidence that younger animals may be more susceptible to NDMA mutagenicity. In one study, NDMA administration increased the mutation frequency in the livers of Big Blue (lacI) mice when administered as five daily doses of 2 mg/kg/day beginning at 3 weeks of age, but not when administered under the same conditions beginning at 6 weeks of age (reviewed by Lambert et al. 2005). The authors suggested that the difference in response could stem from age-related differences in metabolic activation, DNA adduct removal rates, or rates of mutation fixation. Delker et al. (2008) treated this same strain with three daily doses of 7 mg/kg/day beginning at 12 weeks of age and observed a significant increase in mutation frequency in the liver.

Along with the results in transgenic rodents, other *in vivo* studies have provided additional evidence for the genotoxicity of NDMA. As shown in Table 2-5, exposure to NDMA has resulted in mutations in rat kidney, mouse intestine and lymphocytes, *Drosophila melanogaster*,

and fish liver; chromosomal aberrations or aneuploidy in rat liver, hamster fibroblasts, and *Drosophila*; and micronuclei in several species and tissues. In addition, NDMA has induced DNA methylation and adducts, DNA damage, and unscheduled DNA synthesis, especially in the liver, in a number of species (see Table 2-5).

Taken together, the *in vitro* and *in vivo* genotoxicity data demonstrate unequivocally that one or more metabolites of NDMA is genotoxic to the liver in a wide range of species. In accordance with this finding, the liver is the primary target of NDMA carcinogenesis, suggesting that genotoxicity plays a role in the mechanism by which NDMA induces cancer.

COMMENT 25: Unscheduled DNA synthesis is a specific type of assay and the studies measuring this endpoint should probably be labeled as such, rather than simply DNA synthesis/repair as shown in Table 2-5.

RESPONSE: *The entries in Tables 2-4 and 2-5 were re-categorized as unscheduled DNA synthesis, replicative DNA synthesis, DNA repair synthesis, or inhibition of DNA synthesis.*

COMMENT 26: As described by Stott and Watanabe (1980), NDMA did not bind to the sperm heads of male mice following in vivo administration by iv, ip or oral gavage, which was consistent with its lack of activity in the rodent dominant lethal assay. According to the authors, "The relatively low rate of testicular mixed-function oxidase activity required for DMN alkylations activation and the relatively rapid spontaneous decomposition of DMN metabolites may account for the low level of "activated" DMN in the vicinity of the sperm heads and thus of binding."

RESPONSE: *The information in the comment was added to Section 2.19 (Genotoxicity):*

NDMA has not shown genotoxic activity in germ cells *in vivo* or *in vitro*. No increase in unscheduled DNA synthesis was seen in spermatocytes of rats exposed to NDMA by inhalation (Doolittle et al. 1984). In addition, NDMA was negative for dominant lethal mutations in ICR/Ha Swiss mice exposed by i.p. injection (Epstein et al. 1972). In CF-1 mice exposed by i.p., intravenous (i.v.), or oral administration, ^{14}C-NDMA did not alkylate sperm heads at doses from 4 to 14 mg/kg (Stott and Watanabe 1980). These study authors suggested that the lack of binding might stem from relatively low levels of the active NDMA metabolites in the testes resulting from low enzyme activity and short half-life of the metabolites.

COMMENT 27: DNA adducts were identified an individual who was poisoned with NDMA. This should be mentioned in Section 2.19 and line 1336 modified.

RESPONSE: *Section 2.19 was revised as suggested:*

Methylated DNA adducts (7-methylguanine and O6-methylguanine)were detected in the liver of a 23-year-old man who died from a suspected NDMA poisoning (Herron and Shank 1980). No other studies of genotoxicity in humans exposed to NDMA were located.

COMMENT 28: For the 1st entry in Table 2-4, I believe the entry in the "Without" column should be "NT or –". If the current entry is correct, it would be surprising, given NDMA's requirement for bioactivation to exert its toxic effects.

RESPONSE: *The result under the "Without" metabolic activation column for the first entry in Table 2-4 (gene mutation in Salmonella typhimurium) was corrected to "NT or –."*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the substance isomers? Please provide a copy if this is a new reference.

COMMENT 29: See above responses.

RESPONSE: *See responses to Comments 22–24.*

QUESTION: Were all appropriate NOAELs and/or LOAELs identified for each study (both in the text and the Levels of Significant Exposure (LSE) tables and figures)? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

COMMENT 30: Based on the studies that I reviewed, appropriate NOAELs and LOAELs were identified, with the exception of the Peto et al. studies and the other studies identified above. I believe that it should be possible to identify a NOAEL in the Peto et al. studies and use it to derive a chronic MRL.

RESPONSE: *The effects identified at the lowest doses in the study by Peto et al. (1991a, 1991b) were cancer and reduced survival due to cancers. ATSDR does not derive MRLs based on cancer endpoints or serious LOAELs (e.g., reduced survival).*

QUESTION: Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables? If not, please explain why and suggest appropriate changes.

COMMENT 31: The categorizations seem appropriate to me.

RESPONSE: *No response needed.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain. If citing a new reference, please provide a copy and indicate where (in the text) it should be included.

COMMENT 32: I believe that, where known, the most important mechanisms have been identified. However, the discussion of mutagenicity and genotoxicity in Section 2.19 seems wholly inadequate to me, especially as mutation almost certainly plays an important role in the carcinogenicity of NDMA and may be considered a relevant effect in its own right. I believe that more discussion is warranted, even if it is simply a few paragraphs. There is also a need to provide some interpretation or "weigh" the genotoxicity results, not simply list them in a table where less important endpoints (sister chromatid exchanges, DNA damage) are given the same weight as more important ones (in vivo mutation, chromosome aberrations and micronuclei in mammalian models). See Eastmond (2016) for recommendations on weighing the various endpoints.

RESPONSE: *The text of Section 2.19 has been expanded to discuss the most important genotoxicity endpoints, as show in Response to Comment 24.*

QUESTION: Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.

COMMENT 33: The conclusions seemed appropriate to me.

RESPONSE: *No response needed.*

Chapter 3. Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions

QUESTION: Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.

COMMENT 34: The discussion seems adequate to me.

The Reviewer provided a few minor suggestions (see Comments 35-41).

RESPONSE: *See responses to suggestions below.*

COMMENT 35: Line 1454. The Vss for the human (~926 mL/kg) should be expressed in ml/kg units so that it can be compared with those in Table 3-2.

RESPONSE: *The Vss in units of mL/kg was added to Section 3.1.2 as suggested.*

Gombar et al. (1990) used these data to derive an allometric equation for body weight scaling of V_{ss}, and estimated a NDMA V_{ss} of 64,800 mL (~926 mL/kg) for a 70-kg human.

COMMENT 36: Line 1484. Add ", a competitive inhibitor of CYP2E1," following ethanol.

RESPONSE: *The text of Section 3.1.2 was revised as suggested.*

Coadministration of ethanol, a competitive inhibitor of CYP2E1, increased the concentrations of NDMA in blood and all tissues; in the group receiving 50 ppm NDMA with ethanol for 4 weeks, concentrations were 218, 64, 444, 182, and 72 ppb in blood, kidney, lung, brain, and liver, respectively.

COMMENT 37: Line 1490. The information showing elevated levels in various tissues seems inconsistent with information on the previous page that NDMA doesn't accumulate in the sampled tissues (e.g. line 1457, 1465 and 1480. When these types of inconsistencies are seen, it would be helpful if an explanation for differences were provided.

RESPONSE: *The sentence indicating elevated tissue concentrations pertains to levels of radioactivity, while the statements pertaining to lack of accumulation are referring to unchanged NDMA. The text of Section 3.1.2 was revised for clarity as follows:*

Daugherty and Clapp (1976) reported that 15 minutes after oral administration of ^{14}C-NDMA to mice, the relative amounts of radioactivity in the homogenates of heart, forestomach, esophagus, liver, and lung were 1, 2, 3, 10, and 70, respectively. The differences in tissue levels reported in this study are likely due to the study authors' measurement of radioactivity (including

metabolites); studies that measured unchanged NDMA (e.g., Anderson et al. 1986) showed little variation in tissue concentrations.

COMMENT 38: Line 1528. I believe that Magee and Hultin (1962) which is the source of the information in George (2019) should be added as a reference.

RESPONSE: *The citation in Section 3.1.3 was corrected.*

The methyldiazonium ion is an alkylating agent that methylates macromolecules including nucleic acids and proteins (Magee and Hultin 1962).

COMMENT 39: Figure 3-1. The "n" is missing on alpha-hydroxylation.

RESPONSE: *The spelling error in Figure 3-1 was corrected.*

COMMENT 40: Line 1645. The half-life for biliary excretion listed is 0.014 hrs. This is not credible as it is equivalent to less than one minute (0.84 min). I checked the Alaneme and Maduagwu (2004) article and confirmed that 0.014 hr. is listed in the table. However, that value also seems to be inconsistent with the data for the diets shown in Figure in their article. Unfortunately, the line for the normal diet is missing from their figure.

RESPONSE: *The reported half-life of 0.014 hours was deleted from Section 3.1.4 due to the apparent error in the primary report. There was not enough information in the publication to yield an estimate of the half-life because the line for normal diet is missing from Figure 2 of the paper.*

QUESTION: Have all available pharmacokinetic/pharmacodynamic models and supporting data been presented? If not, please explain.

COMMENT 41: PBPK/PBPD models were not found in the literature and therefore not presented in the Profile. I am not aware of any other relevant models.

RESPONSE: *No response needed.*

QUESTION: Is there adequate discussion of the differences in toxicokinetics between humans and animals? Is there adequate discussion of the relevance of animal toxicokinetic information for humans?

COMMENT 42: Yes. There is a useful paragraph discussing animal to human extrapolation.

RESPONSE: *No response needed.*

Children and Other Populations that are Unusually Susceptible

QUESTION: Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be? Please provide any relevant references.

COMMENT 43: Epstein et al. (1972) reported that i.p. injection of NDMA did not induce dominant lethal mutations in mice. This was noted as being in contrast with other alkylating agents that did induce dominant lethal mutations. While this suggests that NDMA may not induce mutations in male germ cells, the results should be interpreted with caution as the study design and analyses were unusual. However, consistent with this report, Stott and Watanabe (1980) reported that NDMA did not bind to sperm heads of male mice following in vivo administration by iv, ip or oral gavage. The authors commented that the relatively low rate of CYP activity in the testes which is required for NDMA bioactivation and alkylation, combined with the relatively rapid spontaneous decomposition of NDMA metabolites, may have resulted in a low level of bioactivated NDMA in the vicinity of the sperm heads, and, as a result, binding was not detected.

In contrast, Anderson et al. (1989) reported that administration of NDMA by ip injection could result in transplacental carcinogenesis in mice. The article also summarizes previous studies that had investigated NDMA metabolism in utero and other studies potentially related to transplacental carcinogenesis by NDMA. The authors may want to include some of this information in the Profile, following line 1711.

A later report by Anderson et al. (2000) provided additional detail on the potential risks of carcinogenesis initiated during the perinatal period. They noted that susceptibility to perinatal carcinogenesis is influenced by the ability of the developing child or organism to "activate chemically stable carcinogens to reactive DNA-damaging intermediates. This process is most often dependent on cytochrome P450s, which in rodents make their appearance during the last several days of gestation. In mice, metabolism of NDMA increased steadily in fetal livers from GD 16, where it was 3% of adult levels, through day 19 (13%), postnatal day 1 (25%) and day 4 (50%), reaching adult levels by day 7". The authors further indicated that in human embryos, NDMA demethylase activity was detectable by 5-6 weeks after conception, and increased 3-fold by 11-12 weeks, indicating that similar to mice, the embryos are capable of bioactivating NDMA in utero.

Consistent with the above indication of transplacental effects, Inui et al. (1979) reported that the oral administration of NDMA to pregnant Syrian hamsters resulted in chromosome aberrations, mutation and morphological transformation in the developing embryos.

There is almost no discussion about the potential for increased susceptibility of children to NDMA. This may be because of the limited amount of direct evidence. Two of the five cases reported by Kimbrough (1980) as having been poisoned by NDMA were children and one of them died. However, not enough information is known about the doses to determine whether they were unusually susceptible. Given the relatively high breathing rates and drinking water consumption rates of children, one would expect them to be at a somewhat increased risk of exposure. In contrast, one would expect that infants and young children would be at decreased risk given their reduced levels of CYP2E1 as compared with adults (Alcorn and McNamara, 2003). However, repair of O6-methylguanine was reported to be lower in newborn mice than adults given the same ip injection of NDMA (Anderson et al., 2000). In addition, cell proliferation is likely to be higher in developing children. These would suggest an increased risk for NDMA mutagenesis. Interestingly, while young animals exhibited increased proliferation in their livers, similar approx. 2-fold increases in mutant frequency in the liver were seen in young and old transgenic mice orally administered NDMA (Tinwell et al., 1994).

RESPONSE: *The following sections of the text were revised to incorporate the information provided in the comment. Where possible, primary sources of data were used in lieu of reviews (i.e., Alcorn and McNamara 2003):*

Section 2.18 (Cancer):

O6-methylguanine adducts were detected in fetal tissues of patas monkeys exposed to NDMA during pregnancy (Chhabra et al. 1995). In addition, Anderson et al. (1989) reported significantly increased incidences of hepatocellular carcinomas in offspring of C3H/HeNCr MTV- mice given 7.4 mg/kg NDMA by i.p. injection on GD 16 or 19. These studies provide support for the findings of Aleksandrov (1974), who reported tumors (sites unspecified) in the offspring of rats exposed orally to NDMA on GD 21.

Section 2.19 (Genotoxicity):

NDMA has not shown genotoxic activity in germ cells *in vivo* or *in vitro*. No increase in unscheduled DNA synthesis was seen in spermatocytes of rats exposed to NDMA by inhalation (Doolittle et al. 1984). In addition, NDMA was negative for dominant lethal mutations in ICR/Ha Swiss mice exposed by i.p. injection (Epstein et al 1972). In CF-1 mice exposed by i.p., (intravenous) i.v., or oral administration, ^{14}C NDMA did not alkylate sperm heads at doses from 4 to 14 mg/kg (Stott and Watanabe). These study authors suggested that the lack of binding might stem from relatively low levels of the active NDMA metabolites in the testes resulting from low enzyme activity and short half-life of the metabolites. Despite the lack of germ cell genotoxicity in these studies, NDMA did induce O6-methylguanine adducts in patas monkey fetuses (Chhabra et al. 1995), chromosomal aberrations and micronuclei in the embryos of treated pregnant hamsters (Inui et al. 1979), and transplacental carcinogenesis in mice exposed by i.p. injection (Anderson et al. 1989).

Section 3.2 (Children and Other Populations that are Unusually Susceptible):

Data on NDMA levels measured in human infant blood or tissues have not been reported. Infants may be exposed to NDMA in infant formula, drinking water, food, and air (particularly in indoor environments with ambient tobacco smoke). Infants may also be exposed to very low levels of leaching from rubber baby bottle nipples or pacifiers; Sections 5.5 and 5.6 provide further information on these potential exposures. Two older studies (Lakritz and Pensabene 1984; Uibu et al. 1996) reported detections of NDMA in human breast milk, but more recent data are not available. Studies of animals exposed during pregnancy demonstrate that NDMA crosses the placenta (Althoff et al. 1977; Chhabra et al. 1995) and can be excreted in breast milk (Chhabra et al. 2000; Diaz Gomez et al. 1986).

The susceptibility of infants and children to NDMA toxicity is complex, with some factors suggesting decreased susceptibility (e.g., reduce metabolic activation) and others suggesting increased susceptibility (e.g., reduced ability to repair DNA adducts).

Age-Related Pharmacokinetic Differences. Bioactivation of NDMA results from its oxidative metabolism, primarily via CYP2E1. The expression and activity of CYP2E1 varies by age, with lowest levels seen in infants. Vieira et al. (1996) evaluated CYP2E1 protein and ribonucleic acid (RNA) levels in hepatic microsomes from humans of various ages. The study authors observed no detectable CYP2E1 protein, and very little messenger RNA (mRNA), in hepatic microsomes from human fetuses. Within the first 24 hours after birth, CYP2E1 levels reached approximately 20% of adult activity; levels increased steadily over the first year of life, reaching about 80% of adult levels by 1 year of age (Vieira et al. 1996). Few differences in CYP2E1 activity are seen among children and adults. In a study of older children and adults, Blanco et al. (2000) observed no significant difference in CYP2E1 activity toward ethoxycoumarin in livers from humans <10, 10–60, or >60 years old.

Age-related differences in NDMA metabolic capacity have been seen in animals. No CYP2E1 protein was detected in livers from rat fetuses obtained at GD 10 or 20, but CYP2E1 was detectable in neonatal (4-day-old) rat liver (Borlakoglu et al. 1993). CYP2E1 mRNA levels did

not differ with age. NDMA-demethylase activity was not detectable in fetal rat liver microsomes but increased more than 3-fold between PND 4 and 60 (Borlakoglu et al. 1993). In mice, hepatic NDMA-demethylase activity was present as early as GD 16 (3% of adult levels) and increased steadily after birth, reaching adult levels by PND 7 (Anderson et al. 2000; Jannetti and Anderson 1981).). Yoo et al. (1987) observed increased NDMA-demethylase activity (and mutagenicity) in liver microsomes from weanling rats compared with adult rats; no age differences were seen in hamsters.

Consumption of alcohol during pregnancy may increase the bioactivation of NDMA in infants. When pregnant rats were exposed to ethanol, hepatic CYP2E1 content was significantly increased in both maternal and fetal liver; the increase in the fetal liver was more than 2-fold compared with fetuses of rats that did not receive ethanol (Carpenter et al. 1997). Fetal liver microsomes from dams exposed to ethanol also showed increased N-nitrosodimethylamine demethylase activity (1.5-fold higher compared with controls) (Carpenter et al. 1997).

Age-Related Differences in Susceptibility. Factors that may increase the susceptibility of infants and children (relative to adults) to the toxic effects of NDMA include increased cell proliferation associated with growth and lower capacity to repair DNA adducts, both of which may lead to greater mutation frequency in developing organisms. Coccia et al. (1988) observed markedly higher (>4-fold) levels of O6-methylguanine adducts in newborn mice compared with adult mice after i.p. administration of the same dose of NDMA. These authors also measured the activity of O6-methylguanine DNA methyltransferase (an enzyme that repairs DNA adducts induced by alkylating agents) and reported levels almost 4 times higher in adult mice compared with newborn mice, consistent with the differences in adduct levels (Coccia et al. 1988).

There is some evidence that younger animals may be more susceptible to NDMA mutagenicity. In one study, NDMA administration increased the mutation frequency in the livers of Big Blue (lacI) mice when administered as five daily doses of 2 mg/kg/day beginning at 3 weeks of age, but not when administered under the same conditions beginning at 6 weeks of age (reviewed by Lambert et al. 2005). The authors suggested that the difference in response could stem from age-related differences in metabolic activation, DNA adduct removal rates, or rates of mutation fixation. No difference in the fold-change in mutation frequency was observed in lac I transgenic mice exposed to a single oral dose of 10 mg/kg NDMA at 8–12 weeks of age or 72 weeks of age (Tinwell et al. 1994a).

Transgenerational Effects. Available studies have not shown evidence for NDMA-induced germ cell mutagenicity or dominant lethal mutations (Doolittle et al. 1984; Epstein et al. 1972; Stott and Watanabe 1980); however, two studies suggested that NDMA may induce transplacental carcinogenesis after oral administration in rats (Aleksandrov 1974) or i.p. administration in mice (Anderson et al. 1989). Aleksandrov (1974) did not report data in control animals or specific tumor types, limiting the utility of this study. When pregnant C3H/HeNCr MTV- mice were treated by i.p. administration on GD16 or 19, NDMA induced significant increases in hepatocellular carcinomas in male and female offspring and a significant increase in sarcomas in male offspring (Anderson et al. 1989). In contrast, Beebe et al. (1993) did not observe increases in lung or liver tumors in offspring of pregnant Swiss mice exposed by the same route at a higher dose on GD19. Beebe et al. (1993) sacrificed the offspring at 1 year of age, while Anderson et al. (1989) did not sacrifice animals until they were moribund (average age 17–21 months), which may explain the disparate findings.

QUESTION: Is there a discussion of populations at higher risk of susceptibility? Do you agree with the choice of populations? Please explain and provide any additional relevant references.

COMMENT 44: There is a discussion of populations with higher susceptibility, and I agree with the choice of populations presented. There is evidence that obese individuals have higher CYP2E1 activity (Emery et al. 2003) and therefore may be at higher risk upon exposure to NDMA. I would recommend that this potentially susceptible population be mentioned. Similarly, moderate to heavy consumers of alcohol exhibit elevated levels of CYP2E1 (Liangpunsakul et al., 2005) and would therefore be expected to be at higher risk of NDMA-related toxicities. I would also suggest that this group of drinkers be mentioned, as well as the likely complexity of the interactions.

The relationship between alcohol consumption and NDMA risk is likely to be complicated as co-exposure to the two agents would, as described in the Profile, likely result in reduced NDMA bioactivation in the liver, leading to a potential for more extra-hepatic bioactivation. However, alcohol consumption in days to weeks prior to NDMA exposure could result in enzyme induction, leading to increased levels of CYP2E1 and more overall NDMA bioactivation in the consuming individuals. The enhancement of liver effects in animals is described later in the Interactions section but its potential effect in humans should also be mentioned.

RESPONSE: *The following text was added to Section 3.2:*

> Increased CYP2E1 activity has been demonstrated in obese individuals (Emery et al. 2003) and moderate to heavy consumers of alcohol (Liangpunsakul et al. 2005), suggesting a potential for greater bioactivation of NDMA in these individuals.

Biomarkers of Exposure and Effect

QUESTION: Are the biomarkers of exposure specific for the substance? Please explain.

COMMENT 45: Yes, urinary NDMA is a specific biomarker of exposure. Although rapidly metabolized, NDMA has been shown to increase substantially in individuals taking ranitidine, a widely used pharmaceutical, which was postulated to be converted within the body to NDMA (Zeng and Mitch, 2016). Urinary NDMA has also been seen within patients with schistosomiasis and those with bladder cancer believed to be caused by schistosomiasis.

RESPONSE: *The text of Section 3.3.1 was revised to include discussion of urinary NDMA as a specific biomarker of exposure.*

> Biomarkers of internal exposure to NDMA include urinary NDMA, urinary methylmercapturic acid, and methylated DNA adducts. It should be noted that none of these biomarkers distinguishes between exogenous and endogenously-formed NDMA, and only urinary concentrations of NDMA itself are specific to NDMA (other methylating agents will yield methylmercapturic acid and methylated DNA adducts).
>
> As discussed in Section 3.1, NDMA is primarily cleared from the blood via metabolism, and little is excreted unchanged. However, urinary excretion of NDMA is known to increase with exposure. Zeng and Mitch (2016) showed that, after intake of 150 mg ranitidine (a pharmaceutical product from which NDMA may form in the gut), the 24-hour rate of urinary excretion of NDMA (measured by gas chromatography-ion trap-mass spectrometry) in volunteers increased by about 400-fold compared with samples collected before the ranitidine exposure. The authors noted that the magnitude of urinary NDMA is not indicative of the systemic exposure

because the majority of NDMA is metabolized in the body with only a small fraction excreted unchanged in urine.

QUESTION: Are the biomarkers of effect specific for the substance? Please explain.

COMMENT 46: I am not aware of biomarkers of effect that are uniquely specific for NDMA. Some biomarkers such as N7-methylguanine and O6-methyl guanine are specific for methylating agents but not NDMA per se. As indicated above and in the Profile, these adducts were measured by Herron and Shank (1980) in a male who had been poisoned by NDMA. A number of the common genetic toxicity endpoints (chromosome aberrations, micronuclei, HPRT mutations) can also be biomarkers of effect but are also not specific for NDMA.

RESPONSE: *No response needed.*

Interactions with Other Chemicals

QUESTION: Is there adequate discussion of the interactive effects with other substances? Does the discussion concentrate on those effects that might occur at hazardous waste sites? Please explain and provide any additional references.

COMMENT 47: Yes, in general, the potential effects of ethanol to competitively inhibit NDMA metabolism in the liver and enhance bioactivation in extra-hepatic tissues are adequately described. However, chronic ethanol consumption is known to induce CYP2E1 in humans which would be expected to enhance NDMA bioactivation. This potential interaction has been described in the text for animals (line 1854) but its potential impact on humans who chronically consume alcohol as a potentially sensitive group has not.

RESPONSE: *See Response to Comment 44. In addition, the following text was added to Section 3.4:*
In humans, moderate to heavy consumption of alcohol increases hepatic CYP2E1 activity (Liangpunsakul et al. 2005), which may increase the bioactivation of NDMA and its toxic effects in these individuals.

QUESTION: If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? Please explain and provide any additional references.

COMMENT 48: Interaction with alcohol is known and one mechanism involved in the interaction is described. As indicated above, I also recommend including enzyme induction by alcohol as another mechanism of interaction.

RESPONSE: *See response to Comment 47.*

Chapter 4. Chemical and Physical Information

QUESTION: Are any of the values or information provided in the chemical and physical properties tables wrong or missing? Please explain and provide any additional references.

COMMENT 49: In searching various sources, I was able to find the same values for most of the properties. However, I should mention that I found discrepant values for a number of them and am not sure which ones are the correct ones. The melting point was listed as -50C in WHO, (2008). The Henry's law constant was a particular problem as most sources had different values. Some of this could be normal variability in the estimates or differences in units, but I recommend that the values be checked. Haruta et al. (2011) had a measured value (dimensionless Henry's law constant (KH') of 1.0 × 10-4) which, I would recommend including, if it checks out, since it is an experimental value rather than a calculated one.

RESPONSE: *ATSDR has added this value to Chapter 4.*

QUESTION: Is information provided on the various forms of the substance? Please explain.

COMMENT 50: I am not aware of any other forms of NDMA.

RESPONSE: *No response needed.*

Chapter 5. Potential for Human Exposure

QUESTION: Is the information on production, import/export, use, and disposal of the substance complete? Please explain and provide any additional relevant references.

COMMENT 51: The information seems adequate. I recommend the following change.

Line 2015. Replace "any" with "significant". NDMA is used in research labs so small amounts are either manufactured in the U.S. or imported.

RESPONSE: *ATSDR has re-written the sentence in Section 5.2.2 as follows:*
It is unlikely that there are significant quantities of NDMA directly imported or exported to or from the United States.

QUESTION: Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and technically sound information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.

COMMENT 52: The information provided seems sufficient to me. Recent evidence indicates that NDMA can be formed during the grilling of meats (Kim et al. 2019). I suggest that this source be added to the end of Line 2075.

RESPONSE: *The text of Section 5.3 was revised to add the information suggested by the Reviewer.*
NDMA may be released to the air during the grilling of meats such as beef, pork, and duck (Kim et al. 2019).

QUESTION: Does the text cover pertinent information relative to transport, partitioning, transformation, and degradation of the substance in all media? Do you know of other relevant information? Please provide references for added information.

COMMENT 53: The information provided seems adequate to me and I do not know of other relevant information.

RESPONSE: *No response needed.*

QUESTION: Does the text provide information on levels monitored or estimated in the environment, including background levels? Are proper units used for each medium? Does the information include the form of the substance measured? Is there an adequate discussion of the quality of the information? Do you know of other relevant information? Please provide references for added information.

COMMENT 54: In general, the description on the levels in the environment seem adequate. I would include information on the levels of NDMA in environmental tobacco smoke-contaminated indoor air (reported to be between 0.01 and 0.1 $\mu g/m^3$; Environment Canada, 1999) and those found in various work-rooms, conference rooms, restaurants and bars where people smoked which ranged from less than 10 ng/m^3 to 240 ng/m^3 (Jenkins et al., 2000 as cited in IARC, 2004).

RESPONSE: *ATSDR could not locate the Environment Canada (1999) reference cited by the Reviewer to verify the information in the comment. The text of Section 5.5.1 was revised to include the additional information from IARC (2004) on indoor air concentrations in smoky rooms, as follows:*

> In their review of the chemical composition of tobacco smoke, IARC (2004) noted that NDMA concentrations in indoor spaces where people were smoking (restaurants, bars, conference rooms) ranged between <0.01 and 0.24 $\mu g/m^3$.

QUESTION: Does the text describe sources and pathways of exposure for the general population and occupations involved in the handling of the substance, as well as populations with potentially high exposures? Do you agree with the selection of these populations? If not, why? Which additional populations should be included in this section?

COMMENT 55: Yes, sources and pathways of exposure for the general population are described. Fristachi and Rice (2007), Chowdury (2014) and Park et al. (2015) contain additional information on the concentration of NDMA in foods and food types. I agree with the selection of populations with elevated exposures. In Section 5.7 I would also include individuals who, for prolonged periods of time, have taken NDMA-contaminated pharmaceuticals or pharmaceuticals such as ranitidine which can be converted into NDMA in the body.

RESPONSE: *Data from Park et al. (2015) were not included because the food samples were collected in Korea and were not relevant to U.S. exposures. The papers by Fristachi and Rice (2007) and Chowdury (2014) were not cited because they used earlier data on food concentrations; the profile cites a more recent and comprehensive review of published food concentration data by Lee (2019). Section 5.7 was revised to add individuals taking NDMA-contaminated medications, as follows:*

> It appears that those segments of the general population with potentially high exposure to NDMA from exogenous sources would include tobacco smokers and nonsmokers who come in contact with tobacco smoke for extended periods of time (reviewed by Smith et al. 2000), people who consume large quantities of foods or beverages containing NDMA or its precursors (e.g., nitrites) (Baxter et al. 2007; Fan and Lin 2018; Lee 2019; Stuff et al. 2009), and individuals who have taken medications containing NDMA or its precursors (FDA 2019a, 2019b, 2020a) for prolonged periods of time.

Chapter 6. Adequacy of the Database

QUESTION: Do you know of other studies that may fill a data gap? Please provide any relevant references.

COMMENT 56: Please see the responses above describing studies which will fill data gaps. With regards to the Exposures in Children, there are two additional references of NDMA in infant formula found in Fristachi and Rice (2007).

RESPONSE: *The references cited by Fristachi and Rice (2007) for estimates of concentrations in infant formula are dated from 1980 to 1981 (in contrast, the data cited by Hrudey et al. 2013 are from a 2011 publication) and are not likely to represent current conditions; thus, these references were not added to the profile.*

QUESTION: Do you agree with the identified data needs? Please explain.

COMMENT 57: Yes. There is a need for high quality subchronic, chronic, reproductive and developmental toxicity studies, particularly ones that span a range of doses that exhibit no effects to clear effects. As indicated above, I believe that ATSDR should recommend NDMA to the NTP for testing.

RESPONSE: *ATSDR will consider the Reviewer's suggestion to recommend NDMA to the NTP for testing.*

COMMENT 58: Line 2653. Revise to …suggesting that, in humans and other large animals, organs and tissues…

RESPONSE: *The text of Section 6.2 was revised as suggested.*

> Toxicokinetic studies have shown that greater amounts of unchanged NDMA escapes first-pass metabolism and reaches systemic circulation in larger species such as dogs, pigs, and monkeys than in rats and mice (Gombar et al. 1987, 1988, 1990; Hino et al. 2000; Mico et al. 1985; Streeter et al. 1990a, 1990b), suggesting that in humans and other large animals, organs and tissues other than the liver may receive larger doses and/or exhibit significant toxicity.

COMMENT 59: Line 2658. Revise to …..acute poisoning and recovery, or subsequent death.

RESPONSE: *The text of Section 6.2 was revised as suggested.*

> The only information available concerning effects of NDMA in humans exposed for acute durations comes from cases of acute poisoning and recovery or subsequent death.

COMMENT 60: Line 2662-64. Revise to "Studies of hepatic and other non-hepatic effects in …..inform dose response assessment and identify additional target organs in humans."

RESPONSE: *The text of Section 6.2 was revised as suggested.*

Studies of hepatic and other non-hepatic effects in occupationally-exposed humans for whom reliable exposure estimates are available could inform dose-response assessment and identify additional target organs in humans.

COMMENT 61: Line 2695. As indicated above, a measured value for the Henry's law constant is now available (Haruta et al., 2011).

RESPONSE: *The text of Section 6.2 was revised to omit the data need for a measured Henry's law constant.*

Many physical and chemical properties are available for NDMA; however, a measured value for K_{oc} constant at ambient temperature is not available.

QUESTION: Are the data needs presented in a neutral, non-judgmental fashion? Please note any bias in the text.

COMMENT 62: Yes.

RESPONSE: *No response needed.*

Chapter 7. Regulations and Guidelines

QUESTION: Are you aware of any additional regulations or guidelines that should be included? Please provide citations.

COMMENT 63: No

RESPONSE: *No response needed.*

QUESTION: Are there any that should be removed? Please explain.

COMMENT 64: No.

RESPONSE: *No response needed.*

COMMENT 65: I recommend that the units of mg/kg-day be used rather than mg/kg/day. I believe that it is more accurate and facilitates calculations.

RESPONSE: *The units of mg/kg/day are consistent with ATSDR guidance for toxicological profile development. ATSDR will consider the Reviewer's suggestion for future revisions of the guidance document.*

Comments provided by Peer Reviewer #2

General Comments

COMMENT 1: My expertise lies in NDMA formation, occurrence and removal from water, and my comments are aligned mostly within this area. Comments are provided for each chapter. Overall, I found the document not very comprehensive in the literature that was cited. Most of the literature was > 10 years old despite hundreds of papers on NDMA in drinking water, wastewater, groundwater, river water and associated human exposures.

RESPONSE: *See responses to specific comments below.*

Chapter 1

COMMENT 2: The opening sentences state "For most people, the largest source of exposure to NDMA is through endogenous production (within the body) from precursors that occur naturally in the body or in the diet. " Key here is the required presence of nitrite in the diet too to drive the NDMA formation reactions (e.g., DOI: 10.1289/ehp.106-1533225, DOI, https://doi.org/10.1186/s12940-019-0525-z, DOI: 10.1097/EDE.0000000000001112, DOI: 10.3390/ijerph15071557), where sources of nitrite include nitrate in drinking water. Clearly stating the precursors are important.

RESPONSE: *The sentence in Section 1.1 was changed to:*

> For most people, the largest source of exposure to NDMA is through endogenous production (within the body) from precursors (presence of nitrite in foods including drinking water) that occur naturally in the body or in the diet.

COMMENT 3: NDMA is also present in ground-level fogs (e.g., DOI: 10.1021/es101698q) and could be inhaled. Including specific references would be helpful.

RESPONSE: *The text in Section 1.1 was changed to read:*

> NDMA has been found in ground-level fogs (Hutchings et al. 2010) and could be inhaled.

COMMENT 4: Line 333 is vague: "at low levels in a large number", as "low" is not a meaningful term. Replace "low" with "parts per trillion".

RESPONSE: *ATSDR has changed the text in Section 1.1 as suggested by the Reviewer.*

> An extensive survey in the United States (EPA 2016) showed NDMA detection at parts per trillion levels in a large number of public water systems (PWSs).

COMMENT 5: Line 334. It should be stated that NDMA is present in drinking and wastewaters primarily due to reactions of disinfectants (chloramines, ozone) with amine-based organic molecules. (DOI: 10.1016/j.watres.2013.04.050, https://doi.org/10.5942/jawwa.2015.107.0013, https://doi.org/10.1016/j.chemosphere.2015.10.023, https://doi.org/10.1021/acs.est.6b00602).

RESPONSE: *ATSDR has added the following sentence to Section 1.1*

> It occurs primarily due to reactions of disinfectants such as chloramines and ozone with amine-based organic molecules in the water.

COMMENT 6: Line 336 should include "polymers used in water/wastewater treatment" and this general reference: https://doi.org/10.1021/acs.est.5b04254, DOI: 10.1039/D0EW00392A.

RESPONSE: *ATSDR has amended the text in Section 1.1 to read:*

> NDMA has been detected in a variety of other media including foods and beverages, pharmaceutical products, toiletries and cosmetics, tobacco products, rubber products, polymers used in water/wastewater treatment, pesticides, and sewage sludge.

COMMENT 7: Line 337 "were not located. " here are some references: DOI: 10.5487/TR.2015.31.3.279, but many reports post 1990 exist.

RESPONSE: *ATSDR removed the following sentence from Section 1.1: "For most of these media, including foods, the vast majority of published NDMA levels were from samples collected before 1990, and more recent data were not located." The reference cited by the Reviewer (Park et al. 2015) was not added to the profile because the food samples were collected in Korea and do not represent U.S. exposure levels.*

COMMENT 8: Line 342 would benefit from these references: doi: 10.1634/theoncologist.2020-0142, https://doi.org/10.1021/es500997e.

RESPONSE: *No change was made. Chapter 1 represents a generic overview. Specific references are included in later chapters.*

COMMENT 9: Section 1.3. Several US states (CA, MA) and Canada have health advisory levels for NDMA in drinking water. These are not official MRLs, but somewhere in the introduction would seem appropriate to note. Eg.:
https://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/NDMA.html

https://www.canada.ca/en/health-canada/services/publications/healthy-living/guidelines-canadian-drinking-water-quality-guideline-technical-document-n-nitrosodimethylamine-ndma.html

NDMA is a drinking water DBP with a carcinogenic risk level of 10-6 at lifetime exposure levels of 0.7 ng/L (USEPA, 2002). The Environmental Protection Agency (EPA) is currently considering regulating NDMA in the United States as indicated by its inclusion in Unregulated Contaminant Monitoring Rule 2 (UCMR2). Canada and two U.S. states (CA, MA) have already begun regulating NDMA in the form of notification or guidance at levels of 10-40 ng/L (California Department of Public Health, 2013; Health Canada, 2011; Massachusetts Department of Energy and Environmental Affairs, 2004).

RESPONSE: *The focus of Chapter 7 is U.S. federal regulations and guidelines, with the exception of the International Agency for Research on Cancer (IARC) cancer classifications and WHO air and water guidelines; state and international regulations and guidelines (other than IARC and WHO) are not included in Chapter 7 or Table 7-1. No change was made.*

Chapter 2. Health Effects

COMMENT 10: My expertise is in exposures through drinking water, and it is just noteworthy that the animal studies were performed at very high NDMA concentrations. Additionally, it is unclear which studies, if any, measured or adjusted for nitrate/nitrite in the diet and the potential impacts on endogenous production of NDMA. Considering the protein type of intake and nitrite/nitrate presence may be helpful in reducing the uncertainty of these hazard assessments.

RESPONSE: *ATSDR agrees that this information would reduce uncertainty in the hazard assessments; however, these data were not reported in the animal studies reviewed. No change was made.*

Chapter 3. Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions

COMMENT 11: No specific comments

RESPONSE: *No response needed.*

Chapter 4. Chemical and Physical Information

COMMENT 12: No specific comments

RESPONSE: *No response needed.*

Chapter 5. Potential for Human Exposure

COMMENT 13: Section 5.1 has odd and inconsistent formatting

RESPONSE: *Section 5.1 is formatted according to ATSDR style guidance. No changes were made.*

COMMENT 14: Section 5.1 & 5.2 have unusual references and would benefit from citations provided with comments above for Chapter 1.

RESPONSE: *Consistent with ATSDR guidance, Section 5.1 is a bulleted overview and does not include citations. As appropriate, citations provided by the Reviewer in comments on Chapter 1 were added to the profile in appropriate sections of Chapter 5. Vermeer et al. (1998) and van Breda et al. (2019) were added to Section 5.6. Krasner et al. (2013) and Sgroi et al. (2016) were added to Section 5.2.1. Hutchings et al. (2010) was added to Section 5.5.1. Atkinson et al. (2020); Hanigan et al. (2015); Zeng et al. (2016); and Zeng and Mitch (2015) were added to Section 5.5.2.*

The paper by Barry et al. (2020) was an epidemiology study of nitrite/nitrate intake and bladder cancer; the study did not include any estimates of NDMA formation or intake, so it was not added to the profile. Similarly, Ward et al. (2018) was a review of epidemiology studies of nitrite/nitrate intake and cancer and was not added. The Park et al. (2015) study was not included because it reported NDMA in food samples collected in Korea; these data are not relevant to U.S. exposures. Liu et al. (2014) was not added to the profile because these authors conducted computational experiments to inform the mechanism by which NDMA is formed during chloramination; no relevant data were reported. The

review by Adamson and Chabner (2020) was not added to the profile as it did not report relevant information that was not already in the profile.

COMMENT 15: Sections 5.1 and 5.2 should include something acknowledging this: NDMA in drinking water has become more prevalent because potable water utilities switched from predominantly free chlorine to chloramines in the early 2000s in response to USEPA Maximum contaminant levels (MCLs) for regulated disinfection by-products (THM, HAA). Consequently, NDMA is observed as a DBP in chloraminated drinking water systems more so than free chlorine based systems; exceptions exist for utilities with ammonia present in water. (e.g., https://doi.org/10.1039/C8EW00442K, DOI: 10.1016/j.watres.2013.04.050, DOI: 10.1016/j.chemosphere.2017.10.089).

RESPONSE: *ATSDR has added the following text to Section 5.2.1:*

> NDMA in drinking water has become more prevalent because potable water utilities switched from predominantly free chlorine to chloramines for disinfection purposes in the early 2000s in response to EPA Maximum Contaminant Levels (MCLs) for regulated disinfection byproducts. The goal was to reduce the level of four halomethanes (chloroform, bromodichloromethane, dibromochloromethane, and bromoform) as well as five haloacetic acids (mono-, di-, and trichloroacetic acid, bromoacetic acid, and dibromoacetic acid), which can form by reaction of chlorine or bromine with natural organic matter. Consequently, NDMA is observed as a disinfection byproduct in chloraminated drinking water systems more than free chlorine-based systems. The formation of NDMA from precursors and strategies for its removal are discussed in multiple reviews (Krasner et al. 2013, 2018; Sgroi et al. 2018).

COMMENT 16: Overall – this paper would help the chapter: Krasner, S. W., Mitch, W. A., McCurry, D. L., Hanigan, D., Westerhoff, P. Formation, Precursors, Control, and Occurrence of Nitrosamines in Drinking Water: A Review. Water Research, 2013.

RESPONSE: *ATSDR has added data from this study to Section 5.5.2, as follows:*

> A review by Krasner et al. (2013) discusses the formation of NDMA and other nitrosamines from various precursors in water treatment facilities and the different methods to limit the formation of NDMA, such as physical removal of precursors by biologically activated carbon and granular activated carbon or the degradation of such compounds by ozonation or increased pre-chlorination time. The most important precursors are amine-containing coagulation polymers and effluent-impacted source waters (Krasner et al. 2013).

COMMENT 17: NDMA is also included in lists of drinking water treatment chemicals by NSF International (NSF/ANSI 61).

RESPONSE: *No change was made to the profile based upon this comment as ATSDR has drinking water and beverage guidelines for NDMA based upon data from the EPA, WHO, and the Food and Drug Administration (FDA), which are included in Chapter 7 of the profile.*

COMMENT 18: Line 2129 is missing a lot of more recent literature (e.g., https://doi.org/10.1021/es070818l, DOI: 10.1016/j.chemosphere.2012.06.025, https://doi.org/10.1016/j.chemosphere.2016.05.090, https://doi.org/10.1016/j.ijggc.2014.11.004, https://doi.org/10.1016/j.watres.2010.03.014, DOI: 10.1021/es070818l).

RESPONSE: *ATSDR has amended Section 5.4.1 to include data from the references the Reviewer has cited. Additional text now reads:*

> Because NDMA has strong absorbance at approximately 227 and 254 nm wavelengths and a large quantum yield at these absorption frequencies photolysis by ultraviolet (UV) irradiation at water reuse and drinking water facilities, is a treatment technique to reduce NDMA levels (Sczcuka et al. 2020; Sharpless and Linden 2003). UV-based advanced oxidation processes utilize irradiation of aqueous solution in conjunction with hydrogen peroxide or photocatalysts such as titanium dioxide, which produce powerful oxidizing agents (hydroxyl radicals), to assist in the degradation of NDMA at water treatment facilities (Fujioka et al. 2017; Sczcuka et al. 2020). Sakai et al. (2012) studied the effects of UV wavelength on the degradation kinetics of NDMA in water. Three different light sources were studied: a 222-nm Kr Cl excimer UV lamp, a 254-nm mercury UV lamp, and a 230–270-nm filtered medium pressure (FMP) mercury UV lamp. It was concluded that a higher degradation efficiency of irradiated NDMA solutions was observed using the 222-nm lamp and FMP lamp as opposed to the 254-nm lamp but water quality parameters such as the amount of naturally occurring organic matter could affect the degradation efficiency. Nitrosamines such as NDMA have been shown to undergo direct photolysis under environmental conditions with the half-life on the order of several minutes (Sorensen et al. 2015). Direct photolysis of NDMA under simulated environmental conditions (wavelengths >290 nm) was investigated by Plumlee and Reinhard (2007). Using a light source that simulated Southern California midsummer, midday sun (intensity 765 W/m^2), the direct photolysis half-life of NDMA was determined to be 16 minutes; however, increasing amounts of dissolved organic matter decreased the degradation rate of NDMA since these substances also absorb photons in the environmental UV spectrum. The direct photolysis half-life of NDMA in infiltration basins (advanced purified, recycled water) at initial levels up to 9.0 ng/L prior to sunrise declined to below the detection limit (<1.5 ng/L) by 10:00 A.M. due to natural photolysis, and the half-life ranged from 33 to 86 minutes depending upon the intensity of solar irradiation (Reny et al. 2021). Chen et al. (2010) used experimental photolysis data to derive a quantitative structure-activity relationship (QSAR) for the rate of photolysis of NDMA and several other disinfection byproducts produced in water treatment facilities.

COMMENT 19: Nitrosamines are emitted from amine-based CO2 capture systems (e.g., https://doi.org/10.1080/09593330.2018.1536172, https://www.sepa.org.uk/media/155585/review-of-amine-emissions-from-carbon-capture-systems.pdf.

RESPONSE: *ATSDR has added data from this reference to Section 5.3.1, as follows:*

> The use of amine-containing solvents in post-combustion CO_2 capture (PCCC) plants to reduce greenhouse emissions from anthropogenic point sources such as fossil fuel fired power plants can result in atmospheric emissions of NDMA and other nitrosamines (SEPA 2015; Sorensen et al 2015).

The first citation (Aqeel and Lim 2018) was unable to be retrieved at this time so was not cited

COMMENT 20: line 2138 should include more recent references on NDMA fate in soils: https://doi.org/10.1016/j.scitotenv.2020.144287, DOI: 10.1016/j.watres.2011.03.053.

RESPONSE: *The first citation (Reny et al. 2021) pertains to transformation and biodegradation and was therefore added to Section 5.4.2, as follows:*

> The direct photolysis half-life of NDMA in infiltration basins (advanced purified, recycled water) at initial levels up to 9.0 ng/L prior to sunrise declined to below the detection limit (<1.5 ng/L) by 10:00 A.M. due to natural photolysis, and the half-life ranged from 33 to 86 minutes depending upon the intensity of solar irradiation (Reny et al. 2021).

The second citation (Van Huy et al. 2011) pertains to occurrence in water and was added to Section 5.5.2, as follows:

> NDMA was monitored for, and detected in, both groundwater and river water in Tokyo, Japan (Van Huy et al. 2011). Levels were <0.5–5.2 ng/L (median: 0.9 ng/L) in groundwater and <0.5–3.4 ng/L (2.2 ng/L) in river water.

COMMENT 21: The USEPA 2nd Unregulated contaminant monitoring rule included NDMA among 6 nitrosamines in drinking water. This data seems absent from Section 5.5 (e.g., DOI: 10.5942/jawwa.2015.107.0009).

RESPONSE: *No change required; data from the second Unregulated Contaminant Monitoring Rule (UCMR) (cited as EPA 2016) were already included in the first two paragraphs of Section 5.5.2.*

COMMENT 22: Line 2252 should include more recent reviews: https://doi.org/10.1039/C8EW00442K, DOI: 10.1016/j.watres.2013.04.050, DOI: 10.1016/j.chemosphere.2017.10.089).

RESPONSE: *Data from all three citations (Krasner et al. 2013, 2018; Sgroi et al. 2018) were added to the profile and the following text was added to Section 5.5.2:*

> A review by Krasner et al. (2013) discusses the formation of NDMA and other nitrosamines from various precursors in water treatment facilities and the different methods to limit the formation of NDMA, such as physical removal of precursors by biologically activated carbon and granular activated carbon or the degradation of such compounds by ozonation or increased pre-chlorination time. The most important precursors are amine-containing coagulation polymers and effluent-impacted source waters (Krasner et al. 2013). In a separate study of 21 full-scale drinking water plants, ozonation of raw or settled water was shown to be an effective method of degrading NDMA precursors and increasing the free pre-chlorination time from <3 minutes of treatment to over 1 hour potential from 21 to 90% (Krasner et al. 2018). Hanigan et al. (2012) studied the ability of activated carbon to adsorb precursors and reduce the NDMA formation potential from river water and effluent from a wastewater treatment plant and found that the NDMA formation potential was in the range of 37–91%, depending upon the concentration of the activated charcoal used. While ozonation can facilitate the degradation of NDMA-forming precursors, it may also result in the formation of NDMA in the treatment of wastewater or highly contaminated surface water (Sgroi et al. 2014, 2016, 2018; Vaidya et al. 2021).

COMMENT 23: Line 2252 should acknowledge that a small portion of polymers added during drinking water treatment are responsible for NDMA formation (e.g., https://pubs.rsc.org/en/content/articlehtml/2020/ew/d0ew00392a).

RESPONSE: *This information has been noted in several sections of the profile. The reference (Atkinson et al. 2020) was added to the end of the following sentence in Section 5.5.2*

> The formation of NDMA in potable water supplies has been attributed to precursors contained in natural organic matter, tertiary and quaternary amines, anion exchange resins and cationic coagulant polymers (such as polydiallyldimethylammonium chloride), and/or in source waters impacted by wastewater contamination (which may include tertiary amine-based drugs, cosmetics, or toiletries) (Atkinson et al. 2020; Dai and Mitch 2013; EPA 2016; Zeng and Mitch 2015; Zeng et al. 2016).

COMMENT 24: ozone can also be responsible for NDMA formation (https://doi.org/10.1016/j.chemosphere.2020.128333, https://doi.org/10.1021/es5011658

RESPONSE: *ATSDR has added the following text to Section 5.5.2*

> While ozonation can facilitate the degradation of NDMA-forming precursors, it may also result in the formation of NDMA in the treatment of wastewater or highly contaminated surface water (Sgroi et al. 2014, 2016, 2018; Vaidya et al. 2021).

COMMENT 25: Line 2027 should include: DOI: 10.1016/j.watres.2012.09.034

RESPONSE: *ATSDR believes that the Reviewer intended to suggest the addition of this reference (Soltermann et al. 2013) to the water monitoring section regarding NDMA formation in pools. The text of Section 5.5.2 was amended to read:*

> UV treatment is often used as a disinfection technique in large pool maintenance. However, Soltermann et al. (2013) reported that UV treatment (at wavelengths of 254 nm) of swimming pool water containing chlorinated dimethylamine and monochloramine resulted in slightly increased NDMA formation instead of the expected decreases.

COMMENT 26: In the drinking water section note that controlling the "removal of NDMA formation organic precursors" has been shown to reduce NDMA formation in treated drinking water, and use of granular or powder activated carbon is among the more effective methods to remove known and unknown organic precursors (e.g., https://doi.org/10.1021/acs.estlett.5b00096, DOI: 10.1021/es302922w)

RESPONSE: *ATSDR has added these references (Hanigan et al. 2015 and 2012) to Section 5.5.2, as follows:*

> Hanigan et al. (2015) found that the pharmaceutical agent, methadone, which is often used to treat heroin withdrawal symptoms, has a high potential to form NDMA in water treatment facilities.
>
> *and*
>
> Hanigan et al. (2012) studied the ability of activated carbon to adsorb precursors and reduce the NDMA formation potential from river water and effluent from a wastewater treatment plant and found that the NDMA formation potential was in the range of 37–91%, depending upon the concentration of the activated charcoal used.

COMMENT 27: NDMA is readily, and commonly, photolyzed by UV light at water reuse and drinking water facilities, and is a treatment technique to reduce NDMA levels in water (e.g., DOI: 10.1016/j.chemosphere.2012.06.025, https://doi.org/10.1021/es0488941, DOI: 10.1002/1522-2675(200205)85:5<1416::AID-HLCA1416>3.0.CO;2-I, DOI: 10.1021/es025814p, DOI: 10.1021/acs.est.0c05704, https://doi.org/10.1007/s40726-017-0052-x)

RESPONSE: *ATSDR has added the suggested citations and revised the text of Section 5.4.2 to add the following:*

> Because NDMA has strong absorbance at approximately 227 and 254 nm wavelengths and a large quantum yield at these absorption frequencies, photolysis by ultraviolet (UV) irradiation at water reuse and drinking water facilities, is a treatment technique to reduce NDMA levels

(Sczcuka et al. 2020; Sharpless and Linden 2003). UV-based advanced oxidation processes utilize irradiation of aqueous solution in conjunction with hydrogen peroxide or photocatalysts such as titanium dioxide, which produce powerful oxidizing agents (hydroxyl radicals), to assist in the degradation of NDMA at water treatment facilities (Fujioka et al. 2017; Sczcuka et al. 2020). Sakai et al. (2012) studied the effects of UV wavelength on the degradation kinetics of NDMA in water. Three different light sources were studied: a 222-nm Kr Cl excimer UV lamp, a 254-nm mercury UV lamp, and a 230–270-nm filtered medium pressure (FMP) mercury UV lamp. It was concluded that a higher degradation efficiency of irradiated NDMA solutions was observed using the 222-nm lamp and FMP lamp as opposed to the 254-nm lamp but water quality parameters such as the amount of naturally occurring organic matter could affect the degradation efficiency. Nitrosamines such as NDMA have been shown to undergo direct photolysis under environmental conditions with the half-life on the order of several minutes (Sorensen et al. 2015). Direct photolysis of NDMA under simulated environmental conditions (wavelengths >290 nm) was investigated by Plumlee and Reinhard (2007). Using a light source that simulated Southern California midsummer, midday sun (intensity 765 W/m2), the direct photolysis half-life of NDMA was determined to be 16 minutes; however, increasing amounts of dissolved organic matter decreased the degradation rate of NDMA since these substances also absorb photons in the environmental UV spectrum. The direct photolysis half-life of NDMA in infiltration basins (advanced purified, recycled water) at initial levels up to 9.0 ng/L prior to sunrise declined to below the detection limit (<1.5 ng/L) by 10:00 A.M. due to natural photolysis, and the half-life ranged from 33 to 86 minutes depending upon the intensity of solar irradiation (Reny et al. 2021).

COMMENT 28: Line 2522 could include: Key here is the required presence of nitrite in the diet too to drive the NDMA formation reactions (e.g., DOI: 10.1289/ehp.106-1533225, DOI, https://doi.org/10.1186/s12940-019-0525-z, DOI: 10.1097/EDE.0000000000001112, DOI: 10.3390/ijerph15071557), where sources of nitrite include nitrate in drinking water. Clearly stating the precursors are important.

RESPONSE: *ATSDR has revised the text of Section 5.6 as follows:*

Mean urinary NDMA levels in the control week were 287 ng per 24-hour period, but increased to 871 ng per 24-hour period in the second week when the subjects were consuming a diet high in nitrate concentration (Vermeer et al. 1998). Subjects consuming either a diet of processed red meat or unprocessed white meat (3.75 g/kg body weight) for 2 weeks showed significantly greater urinary excretion of apparent total N-nitroso compounds in the second week when they used drinking water high in nitrate levels as opposed to the first week when nitrate levels in drinking water were kept low (van Breda et al. 2019).

In addition, ATSDR reviewed the text of Section 5.7 as follows:

It appears that those segments of the general population with potentially high exposure to NDMA from exogenous sources would include tobacco smokers and nonsmokers who come in contact with tobacco smoke for extended periods of time (reviewed by Smith et al. 2000), people who consume large quantities of foods or beverages containing NDMA or its precursors (e.g., nitrites; Baxter et al. 2007; Fan and Lin 2018; Lee 2019; Stuff et al. 2009), and individuals who have taken medications containing NDMA or its precursors (FDA 2019a, 2019b, 2020a) for prolonged periods of time.

Chapter 6. Adequacy of the Database

COMMENT 29: line 2712 – change "are lacking" to "are limited". Line 2712-2717 are already done and available in above references

RESPONSE: *ATSDR has changed the wording in Section 6.2 to "are limited" as suggested.*
Kinetic data regarding photolysis in water and on soil surfaces, biodegradation in water under aerobic and anaerobic conditions, and biodegradation in soil under anaerobic conditions are limited.

COMMENT 30: line 2747 – more work is needed to calculate the contribution of drinking water presence of NDMA to human exposure, relative to other sources.

RESPONSE: *ATSDR added the following sentence to Section 6.2 under Exposure Levels in Humans:*
More work is needed to improve estimates of the contribution of NDMA in drinking water to human exposure, relative to other sources, and the contribution of dermal exposure in swimming pools or bathing activities.

COMMENT 31: line 2747 - Additional information related to nitrate in drinking water on endogenous NDMA formation is needed (e.g., https://pubmed.ncbi.nlm.nih.gov/30822653/). This is true for children too (e.g., line 2754)

RESPONSE: *ATSDR added the following sentence to Section 6.2 under Exposure Levels in Humans*:
Additional information related to the impact of nitrate in drinking water on endogenous NDMA formation in humans (including children) is needed.

COMMENT 32: Dermal exposure data in pool water, bathing seems lacking; and detection of NDMA in blood.

RESPONSE: *ATSDR has included dermal exposure in swimming pools and bathing as a data need. There seems sufficient data on NDMA blood levels, so this was not included as a data need.*

Comments provided by Peer Reviewer #3:

General Comments

COMMENT 1: In performing this review I was obliged to assume that the draft authors have accurately represented and interpreted cited references unless I had occasion to have reviewed the cited references myself.

RESPONSE: *No response needed.*

ATSDR Charge Questions and Responses and Reviewer Comments

Chapter 1

QUESTION: Do you agree with those effects known to occur in humans as reported in the text? If not, please explain why and provide a copy of additional references you would cite and indicate where (in the text) these references should be included.

COMMENT 2: Yes, but the draft report's coverage of occupational epidemiology is inadequate – details of deficiencies are provided in comments on Chapter 2.

RESPONSE: *See responses to Comments 11 and 20.*

QUESTION: Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

COMMENT 3: Yes. The mechanisms reported in animal studies are relevant to humans.

RESPONSE: *No response needed.*

QUESTION: Have exposure conditions been adequately described?

COMMENT 4: Yes, some minor comments provided in the annotated text of the report.

RESPONSE: *See responses to annotated comments below.*

COMMENT 5: The overview provided by Chapter 1 needs to explain that NDMA is only one of a large number of volatile N-nitrosamines, many of which exhibit similar toxicological mechanisms. It is true that NDMA is the most commonly found volatile N-nitrosamine in media such as drinking water and it has likely been the subject of the largest number of toxicological studies, but readers should not be encouraged to believe that the non-detection of NDMA necessarily means the absence of any volatile N-nitrosamines in any sample. Understanding would also be improved by explaining that there are a group of clearly toxic volatile N-nitrosamines commonly found in tobacco smoke, generally referred to as tobacco specific nitrosamines. NDMA is not considered to be a tobacco specific nitrosamine.

RESPONSE: *The following statement was added to Section 1.1:*

NDMA is the most well-studied of several volatile N-nitrosamines that exhibit similar toxic properties (including several others that are found in tobacco smoke).

Minimal Risk Levels (MRLs)

QUESTION: If no MRLs have been derived, do you agree that the data do not support such a derivation? Please explain.

COMMENT 6: Yes. The ATSDR definition of MRL appears to be such that the evidence base for NDMA is not adequate to derive MRLs, even though the mechanisms of toxicity for NDMA are very well understood.

RESPONSE: *No response needed.*

QUESTION: If MRLs have been derived, do you agree with the proposed MRL values? Explain. If you disagree, please specify the MRL value that you would propose.

COMMENT 7: Not applicable.

RESPONSE: *No response needed.*

QUESTION: Do you agree/disagree with each component of the total uncertainty factor? Explain. If you disagree, please specify the uncertainty factor(s) that you propose.

COMMENT 8: Not applicable.

RESPONSE: *No response needed.*

QUESTION: Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT 9: Not applicable.

RESPONSE: *No response needed.*

Chapter 2. Health Effects

QUESTION: Do the health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature? If not, please suggest appropriate changes.

COMMENT 10: Yes, with the qualifiers noted below.

RESPONSE: *See responses to remaining comments.*

QUESTION: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)?

COMMENT 11: The only occupational epidemiology study cited in the draft was Hidajat et al. (2019). This study is likely the best available study for addressing the inevitable validity challenges (inadequate power, inadequate specificity, confounding, etc.) specificity that are faced by occupational epidemiology. There were numerous occupational epidemiology studies that were not cited or discussed. These are listed below. That list of occupational epidemiology studies includes 12 on cancer, 1 on respiratory illness, and 1 on fatal, non-alcohol-related chronic liver disease. Many of these studies address NDMA as only one of a number of occupational contaminant exposures.

The Reviewer provided the following List of Occupational Epidemiology Studies including NDMA as an exposure that were not cited nor apparently even reviewed for citation:

All these references, except for Kersemaekers et al. (1995), Straif et al. (1999) and Xu et al. (2018) were about occupational cancers. Kersemaekers et al. (1995) is about reproductive disorders, Straif et al. (1999) is about death from non-alcohol chronic liver disease and Xu et al. (2018) is about respiratory illness.

Andreotti, G; Silverman, DT. 2012. Occupational risk factors and pancreatic cancer: A review of recent findings. Molecular Carcinogenesis. 51(1, S1): 98-101. DOI: 10.1002/mc.20779.

Betenia, N; Costello, S; Eisen, EA. 2012. Risk of cervical cancer among female autoworkers exposed to metalworking fluids. Scandinavian Journal Work, Environment & Health. 38(1): 78-83. DOI: 10.5271/sjweh.3193.

Calvert, GM; Ward, E; Schnorr, TM; Fine, LJ. 1998. Cancer risks among workers exposed to metalworking fluids: A systematic review. American Journal Industrial Medicine. 33(3): 282-292. DOI: 10.1002/(SICI)1097-0274(199803)33:3<282::AID-AJIM10>3.0.CO;2-W.

Cocco, P; Ward, MH; Dosemeci, M. 1999. Risk of stomach cancer associated with 12 workplace hazards: analysis of death certificates from 24 states of the United States with the aid of job exposure matrices. Occupational Environmental Medicine. 56 (11): 781-787. DOI: 10.1136/oem.56.11.781.

Cocco, P; Ward, MH; Dosemeci, M. 1998. Occupational risk factors for cancer of the gastric cardia - Analysis of death certificates from 24 US states. Journal Occupational Environmental Medicine. 40(10): 855-861. DOI: 10.1097/00043764-199810000-00004.

Friesen, MC (Friesen, Melissa C.); Costello, S (Costello, Sadie); Eisen, EA. 2009. Quantitative Exposure to Metalworking Fluids and Bladder Cancer Incidence in a Cohort of Autoworkers. American Journal Epidemiology. 169(12): 1471-1478. DOI: 10.1093/aje/kwp073.

Kersemaekers, WM; Roelveld, N; Zielhuis, GA. 1995. Reproductive disorders due to chemical-exposure among hairdressers. Scandinavian Journal Work, Environment & Health. 21(5): 325-334. DOI: 10.5271/sjweh.46.

Krstev, S; Dosemeci, M; Lissowska, J; Chow, WH; Zatonski, W; Ward, MH. 2005. Occupation and risk of stomach cancer in Poland. Occupational Environmental Medicine. 62(5): 318-324. DOI: 10.1136/oem.2004.015883.

Richardson, DB; Terschuren, C; Hoffmann, W. 2008. Occupational risk factors for non-Hodgkin's lymphoma: A population-based case-control study in Northern Germany. American Journal Industrial Medicine. 51 (4): 258-268. DOI: 10.1002/ajim.20552.

Straif, K; Weiland, SK; Bungers, M; Holthenrich, D; Taeger, D; Yi, S; Keil, U. 2000. Exposure to high concentrations of nitrosamines and cancer mortality among a cohort of rubber workers. Occupational Environmental Medicine. 57 (3): 180-187. DOI: 10.1136/oem.57.3.180.

Straif, K; Weiland, S.; Werner, B.; Wienke, A.; Keil, U. 1999. Elevated mortality from nonalcohol-related chronic liver disease among female rubber workers: Is it associated with exposure to nitrosamines? American Journal Industrial Medicine. 35:264–271.

Sullivan, PA; Eisen, EA; Woskie, SR; Kriebel, D; Wegman, DH; Hallock, MF; Hammond, SK; Tolbert, PE; Smith, TJ; Monson, RR. 1998. Mortality studies of metalworking fluid exposure in the automobile industry: VI. A case-control study of esophageal cancer. American Journal Industrial Medicine. 34(1): 36-48. DOI: 10.1002/(SICI)1097-0274(199807)34:1<36::AID-AJIM6>3.0.CO;2-O.

Xu, YY; Karedal, M; Nielsen, J; Adlercreutz, M; Bergendorf, U; Strandberg, B; Antonsson, AB; Tinnerberg, H; Albin, M. 2018. Exposure, respiratory symptoms, lung function and inflammation response of road-paving asphalt workers. Occupational Environmental Medicine. 75 (7): 494-500. DOI: 10.1136/oemed-2017-104983.

RESPONSE: *As shown above, the Reviewer identified 13 publications as occupational epidemiology studies for NDMA. None of the studies were identified in literature searches performed for the profile as they were not indexed to NDMA, but rather to nitrosamines. All of the publications were reviewed for relevance to the NDMA profile.*

Cancer: *Ten studies pertained to cancer endpoints. Calvert et al. (1998) was a systematic review of the epidemiology of cancer among workers exposed to metalworking fluids. Three other publications identified by the Reviewer were primary studies of cancer associations with exposure to nitrosamines in metalworking fluids (Betenia et al. 2012; Friesen et al. 2009; Sullivan et al. 1998). Available information suggests that the primary nitrosamine in metalworking and cutting fluids is N-nitrosodiethanolamine (NDELA) (Ducos and Gaudin 2003; Hopf et al. 2019; Tricker et al. 1989). ATSDR was not able to locate any information suggesting the existence of NDMA in metalworking fluids or vapors/aerosols from these materials. Searches of PubMed using terms for "NDMA" and "metalworking" or "cutting fluids" revealed no results. Since there is no evidence of exposure to NDMA in workers using metalworking fluids, the review and three primary studies of this population (Calvert et al. 1998), Betenia et al. 2012; Friesen et al. 2009; Sullivan et al. 1998) were not added to the profile.*

Andreotti and Silverman (2012) published a review of occupational exposures associated with pancreatic cancer. These authors cited three studies of rubber and tire industry workers that reported positive associations with pancreatic cancer (Delzell and Monson 1985; Li and Yu 2002; Solenova 1992). The Reviewer recommended the consideration of one other study of cancer in rubber industry workers (Straif et al. 2000).

NDMA exposure in rubber industry workers has been documented, and NDMA is one of two primary nitrosamine exposures in the industry; the other is N-nitrosomorpholine (Hidajat et al. 2019b; Tricker et al. 1989). Therefore, studies of rubber industry workers may inform the hazard identification for NDMA. In addition to the studies identified by the Reviewer and by Andreotti and Silverman (2012), there is a large database of cancer epidemiology studies of workers in the rubber industry. In order to provide a

more complete evaluation of the data on cancers in rubber industry workers, a meta-analysis on this specific topic (Boniol et al. 2017) was added to the profile in lieu of individual epidemiological studies. The meta-analysis featured a comprehensive list of cancer sites and a thorough evaluation of the literature (all of the human studies [published before 2017] identified in a targeted PubMed search for the terms "nitrosamine," "rubber," and "cancer" were considered by Boniol et al. (2017). Boniol et al. (2017) reviewed 234 publications, including the studies recommended by the Reviewer (Straif et al. 2000 and the three primary studies identified by Andreotti and Silverman 2012), and selected 46 cohort and 59 case-control studies for inclusion. Importantly, Boniol et al. (2017) excluded case-control studies of nitrosamine exposure that were not specific to the rubber industry; this ensured that nitrosamine exposures in other industries, which may not include exposure to NDMA, were excluded. In addition, Boniol et al. (2017) cross-referenced the studies reporting results for the same cohort, ensuring that the studies of the same cohort were not included multiple times in the analysis. For example, Boniol et al. (2017) reported that Straif et al. (2000) is a duplicate of Weiland et al. (1996), which was updated by Vlaanderen et al.(2013; included in meta-analysis).

Noncancer: *Three studies identified by the Reviewer pertained to noncancer endpoints (Kersemaekers et al. 1995; Straif et al. 1999; Xu et al. 2017). Kersemaekers et al. (1995) conducted a literature review of reproductive outcomes among women working as hairdressers. The authors noted briefly that nitrosamines, including NDMA, had been found in hair salon products in the 1980s. However, the authors noted that there were few data on nitrosamine exposures in this setting, and that the available data showed the nitrosamine concentrations to be low relative to other chemicals to which hairdressers were exposed (solvents, glycol ethers, formaldehyde, phthalates). Kersemaekers et al. (1995) did not describe or cite any studies assessing the relationships between reproductive outcomes and exposure to NDMA or nitrosamines among hairdressers. Given that the paper is a review rather than a primary source, it did not identify relevant primary human data, and is more than 25 years old, the Kersemaekers et al. (1995) review was not added to the NDMA profile.*

Xu et al. (2017) evaluated lung function and respiratory symptoms in pavers using crumb rubber modified asphalt and conventional asphalt. These authors measured and reported personal exposure sampling results for the sum of nine nitrosamines (including NDMA). The expectation was that higher levels of nitrosamine exposure would be observed in samples from workers using crumb rubber modified asphalt, but the results showed no difference in total nitrosamine concentration from conventional and crumb rubber asphalt paving. The authors noted that N-nitrosopiperidine was detected in all samples and that N-nitrososphenylamine was detected in some samples; they did not report whether NDMA was detected or not. The authors suggested that there was another source of nitrosamines in the asphalt besides crumb rubber, stating "Since N-nitroso-dimethylamine and N-nitroso-morpholine are the most common detected nitrosamines in the rubber industry, including production of tyres, [the] two detected nitrosamines in our study may suggest that the source was not only reused rubber tyres added in the asphalt mixture, but other additives."(p 499). Based on this information, it appears that NDMA was not among the primary nitrosamines to which the pavers were exposed (and it is uncertain whether NDMA was present at all). A PubMed search for NDMA and the terms "asphalt" or "paving" identified no studies, providing additional support for the finding that NDMA is not a key exposure for pavers. Based on this information, Xu et al. (2017) was not included in the NDMA profile.

Straif et al. (1999) investigated the association between liver cirrhosis and work in rubber production and was added to the Section 2.9 (Hepatic) of the profile. To ensure adequate coverage of noncancer effects in this population, a PubMed search for the terms "nitrosamine," "disease," and "rubber" was conducted; this search identified two additional studies that were also added to the profile (Hidajat et al. 2020; Jonsson et al. 2009). Hidajat et al. (2020) reported noncancer disease mortality associations with estimated NDMA exposures among U.K. rubber factory workers; this study was added to Sections 2.4 (Respiratory) and 2.6 (Gastrointestinal). Jonsson et al. (2009) reported NDMA (and other nitrosamine)

exposure levels, respiratory and ocular symptoms, and immunologic markers among Swedish rubber industry workers; results of this study were added to Sections 2.4 (Respiratory), 2.12 (Ocular), and 2.14 (Immunological).

Section 2.4 (Respiratory):
An occupational epidemiology study of reported increased odds of self-reported respiratory symptoms, including nose bleeds, burning or dry throat, hoarseness, and severe dry cough among 172 Swedish rubber production workers, when compared with 118 unexposed subjects (Jonsson et al. 2009). Median breathing zone NDMA concentrations in the workplaces ranged between 0.24 and 8.2 μg/m^3 (Jonsson et al. 2009). Hidajat et al. (2019a) reported significantly increased subdistribution hazard ratios (SHRs) (based on competing risk survival analysis) for mortality from respiratory diseases with increasing cumulative NDMA exposure in a cohort of 36,442 U.K. rubber workers followed for 49 years. The SHR for the highest quartile of cumulative NDMA exposure was 1.41 (95% confidence interval [CI] 1.30, 1.53). Results were not adjusted for smoking status. The authors noted that confounding by unmeasured smoking status was unlikely (based on sensitivity analyses) but could not be ruled out entirely.

Section 2.5 (Cardiovascular):
Higher risks of mortality from circulatory and cerebrovascular diseases and ischemic heart disease (SHRs up to 1.48) were reported in a large cohort of 36,441 male U.K. rubber factory workers followed for 49 years (Hidajat et al. 2020).

Section 2.6 (Gastrointestinal):
Increased risks of digestive diseases with increasing NDMA exposure were reported among U.K. rubber industry workers in a 49-year follow-up study (Hidajat et al. 2019a). In this study, SHRs across quartiles of cumulative NDMA exposure ranged up to 1.60 (95% CI 1.31, 1.95).

Section 2.9 (Hepatic):
A large cohort mortality study of 36,144 male U.K. rubber factory workers reported an increased risk of mortality from liver disease for workers in the third quartile of cumulative NDMA exposure (SHR of 2.22; 95% CI 1.24, 3.99) (Hidajat et al. 2020). In the highest quartile, the SHR was elevated (1.35) but the CI included 1.0. The results were not adjusted for alcohol intake.

In a cohort of 2,875 German female rubber workers with occupational exposure to nitrosamines, the rate of mortality from non-alcoholic cirrhosis of the liver was elevated compared with the rate in the general population of German women (Straif et al. 1999). All 10 of the cases of non-alcoholic cirrhosis occurred among women employed in production of technical rubber goods, and the risk of death from this cause increased with earlier year of hire and longer duration of employment in rubber good production (Straif et al. 1999). Straif et al. (1999) reported that the highest documented nitrosamine concentration in the facilities included in their study was NDMA at 170 μg/m^3. The study authors did not report concentrations of other nitrosamines in the women's workplaces; however, the other primary nitrosamine measured in rubber production facilities is N-nitrosomorpholine, which often occurs at exposure levels similar to NDMA (de Vocht et al. 2007; Hidajat et al. 2019b; Jonsson et al. 2009; Straif et al. 2000; Tricker et al. 1989).

Section 2.12 (Ocular):
In a group of 172 Swedish rubber industry workers exposed to NDMA concentrations up to 8.4 μg/m^3, odds of self-reported itching, runny, or burning eyes were increased when compared with 118 unexposed subject (Jonsson et al. 2009). No other studies reporting ocular effects in humans exposed to NDMA were identified in the literature searches.

Section 2.14 (Immunological):
One study of immune system markers in humans exposed to NDMA during work in rubber production (Jonsson et al. 2009) was located; no other immunological studies in humans were identified. In a group of 172 Swedish rubber industry workers exposed to NDMA and several other nitrosamines, blood levels of eosinophils and immunoglobulin G (IgG) were significantly increased (14 and 11%, respectively) when compared with 118 unexposed subjects. There were no significant differences in leukocyte or neutrophil counts or in levels of α1-antitrypsin, C-reactive protein, or IgA, IgM, or IgE. Across the eight facilities where the exposed workers were employed, median detected breathing zone concentrations of NDMA ranged between 0.24 and 8.2 $\mu g/m^3$ (Jonsson et al. 2009). Other nitrosamines, including N-nitrosomorpholine, N-nitrosodiethylamine, N-nitrosodi-n-butylamine, N-nitrosopiperidine, and N-nitrosopyrrolidine, were detected less frequently and at lower concentrations.

Section 2.18 (Cancer):
There is a substantial number of studies of cancer in workers in the rubber industry, and NDMA is known to be one of two primary nitrosamine exposures in this industry, the other being N-nitrosomorpholine (de Vocht et al. 2007; Hidajat et al. 2019b; Jonsson et al. 2009; Straif et al. 2000; Tricker et al. 1989). The large database of cancer studies in rubber industry workers was evaluated in a meta-analysis by Boniol et al. (2017). With the more recent study published by Hidajat et al. (2019a), the meta-analysis provides a synopsis of the relevant data from epidemiology of cancer in rubber industry employees.

Boniol et al. (2017) conducted a comprehensive meta-analysis of cancer associations with employment in rubber manufacturing, using the IARC definition for exposure to rubber manufacturing. These authors reviewed 234 publications and selected 46 cohort and 59 case-control studies for inclusion. Boniol et al. (2017) excluded case-control studies of nitrosamine exposure that were not specific to the rubber industry; thus, nitrosamine exposures in other industries, which may not include exposure to NDMA, were excluded. In addition, Boniol et al. (2017) cross-referenced studies that reported results for the same cohort, ensuring that the studies of a given cancer in the same cohort were not included multiple times in the analysis. Summary relative risk estimates were estimated using a random effects model.

Boniol et al. (2017) evaluated studies of 32 individual cancer sites. Their analysis showed increased summary relative risks for bladder cancer (1.36, 95% CI 1.18, 1.57), leukemia (1.29, 95% CI 1.11, 1.52), cancers of the lymphatic and hematopoietic systems not otherwise specified (1.16, 95% CI 1.02, 1.31), and cancer of the larynx (1.46, 95% CI 1.10, 1.94). A borderline increased summary relative risk was calculated for lung cancer (1.08, 95% CI 0.99, 1.17); risks for other cancer sites were not increased. Significant heterogeneity between studies was observed for all of the above cancer sites except for cancers of unspecified cancers of the lymphatic and hematopoietic systems. The increased risks for bladder cancer, lung cancer, and leukemia were not changed when the trim and fill method to correct potential publication bias was applied to the data. Stratification of studies by participants' date of first employment showed that there were no increases in risks for bladder cancer, lung cancer, or leukemia among workers who began work after 1960, although the numbers of studies of recent employment were small.

QUESTION: Were the major study limitations sufficiently described in the text without going into lengthy discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT 12: The study limitations discussed did not address all the substantial limitations raised by Hidajat et al. (2019). The report draft states: "Although individual smoking histories were not available for the participants, the authors reported that smoking was unlikely to be an important confounder based on an analysis using smoking prevalence among a cohort of more recent rubber industry workers." Hidajat et al. (2019) included lack of smoking history among 7 cited study limitations (vs. 2 "main" limitations cited in the draft report plus the missing smoking history). Hidajat et al. (2019) stated about the smoking history limitation: "Fourth, information on important confounders such as smoking and other lifestyle factors were unavailable, although additional simulations indicated smoking was unlikely to be a significant confounding factor." Given the importance of smoking behaviour as a confounder to any cancer epidemiological study, readers should not be left with the impression that an opinion by the study authors means that they have eliminated confounding from smoking in their cancer findings.

RESPONSE: *The text of Section 2.18 was revised to remove the statement suggesting that confounding from smoking had been eliminated, and to add the remaining limitations cited by the study authors.*

> This study (Hidajat et al. 2019a) had a number of strengths, including large cohort size with lengthy (49-year) follow-up and quantitative cumulative exposure estimates based on historic exposure measurements. The limitations noted by the study authors were: (1) the subject's individual employment histories prior to 1967 and during follow-up were not available (suggesting the possibility of exposure misclassification); (2) the 15-year lag time assumed in the analysis may not be suitable for blood cancers with shorter lag times; (3) some cancers may have been undercounted due to the use of underlying cause of death on death certificates; (4) information on confounders such as smoking history was not available for the subjects; (5) there was potential for selection bias because only workers who lived to 35 years of age were eligible for inclusion; (6) measurement error in individual exposure assessment was possible due to the use of a job-exposure matrix; and (7) there were correlations between NDMA and other exposures in the industry (other nitrosamines, nitrosomorpholine, rubber dust and fumes), as well as the possibility of cross-contamination across departments. These limitations make it difficult to establish clear associations between NDMA exposure and mortality from specific cancers.

QUESTION: Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?

COMMENT 14: Yes, the species used cover the range from rodents to primates.

RESPONSE: *No response needed.*

QUESTION: Has adequate attention been paid to dose-response relationships for both human and animal data? Please explain.

COMMENT 15: Yes, but adequate human quantitative evidence of dose-response is not available, nor is it likely attainable.

RESPONSE: *No response needed.*

QUESTION: Are you aware of any studies that are not included in the profile that may be important in evaluating the toxicity of the substance? Please provide a copy of each study and indicate where in the text each study should be included.

COMMENT 16: No.

RESPONSE: *No response needed.*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the substance isomers? Please provide a copy if this is a new reference.

COMMENT 17: No.

RESPONSE: *No response needed.*

QUESTION: Were all appropriate NOAELs and/or LOAELs identified for each study (both in the text and the Levels of Significant Exposure (LSE) tables and figures)? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

COMMENT 18: N/A.

RESPONSE: *No response needed.*

QUESTION: Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables? If not, please explain why and suggest appropriate changes.

COMMENT 19: N/A.

RESPONSE: *No response needed.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain. If citing a new reference, please provide a copy and indicate where (in the text) it should be included.

COMMENT 20: The failure to cite Straif et al. (1999) albeit with its small sample size, lack of discussion of the human evidence for non-alcohol fatal chronic liver disease is a deficiency. The same with Xu et al. (2018) for respiratory outcomes and Kersemaekers et al. (1995) for reproductive disorders. The full citation for Kersemaekers et al. (1995), Straif et al. (1999) and Xu et a. (2018) are provided in the list of citations at the end of this document and copies have been provided with the submission of this review.

RESPONSE: *The studies cited by the Reviewer do not contain mechanistic information. As noted in response to Comment 10 above, Kersemaekers et al. (1995) was a review and was not added to the profile. Xu et al. (2017) assessed lung function in asphalt workers with measured exposures to nitrosamines but no evidence of exposure to NDMA. Straif et al. (1999) assessed liver disease in a cohort of rubber industry workers with occupational exposure to nitrosamines; this study was added to Section 2.9 because NDMA is known to have been a primary nitrosamine exposure in this industry (Tricker et al. 1989).*

Section 2.9 was revised to add a description of the study by Straif et al. (1999):

> In a cohort of 2,875 German female rubber workers with occupational exposure to nitrosamines, the rate of mortality from non-alcoholic cirrhosis of the liver was elevated compared with the rate in the general population of German women (Straif et al. 1999). All 10 of the cases of non-alcoholic cirrhosis occurred among women employed in production of technical rubber goods, and the risk of death from this cause increased with earlier year of hire and longer duration of employment in rubber good production (Straif et al. 1999). Straif et al. (1999) reported that the highest documented nitrosamine concentration in the facilities included in their study was NDMA at 170 μg/m^3; the study authors did not report concentrations of other nitrosamines in the women's workplaces; however, the other primary nitrosamine measured in rubber production facilities is N-nitrosomorpholine, which often occurs at exposure levels similar to NDMA (de Vocht et al. 2007; Hidajat et al. 2019b; Jonsson et al. 2009; Straif et al. 2000; Tricker et al. 1989).

QUESTION: Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.

COMMENT 21: Yes.

RESPONSE: *No response needed.*

Chapter 3. Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions

QUESTION: Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.

COMMENT 22: No. The largest issue for consideration of NDMA as an exogenous contaminant with potential human health impacts is the premise that NDMA (and other volatile N-nitrosamines) are endogenously produced because, if that production dominates relevant human exposure, then control of external exposure to NDMA may have limited ability to reduce human health effects. The draft introduces this important topic in the opening of Section 3.1 including the following paragraph: "As discussed further in Section 5.6, NDMA is produced endogenously through both acid-catalyzed nitrosation of amine precursors (primarily in the stomach) and through biologically-catalyzed nitrosation in other tissues including the oral cavity, intestine, liver, blood, and bladder (Hrudey et al. 2013). Estimates of the amount of NDMA produced endogenously vary widely. Using three different methods and available literature on measured human NDMA blood levels, O6-methylguanine DNA adducts, and urinary excretion levels, Hrudey et al. (2013) estimated the rate of endogenous production to be approximately 1 mg/day (equivalent to 0.014 mg/kg/day for a 70-kg adult)."This text in the draft overlooks that Fristachi & Rice (2007), as EPA staffers, first raised the issue that endogenous production of NDMA may greatly exceed any exogenous exposure with the resulting impact this understanding places on development of environmental regulations. The evidence that endogenous generation of NDMA could be important was not discovered by either Hrudey et al. (2013) or Fristachi & Rice (2007), but both papers fully cite the original research upon which they relied upon for estimating the relative quantitative importance endogenous formation of NDMA. The last sentence of the quote from the draft above does not convey an accurate sense of the uncertainty of the estimate for endogenous production of NDMA that Hrudey et al. (2013) conveyed by their statement: "Despite the multiple possible sources of error in the estimates of endogenous formation of NDMA we have derived, it seems clear that formation rates approaching 1 mg/day are usual and in some individuals such formation can be significantly greater. Notably, Tannenbaum(121) estimated that up to 670 μg/day of NDMA was formed endogenously using similar methodology."

The following full citations were provided by the Reviewer.

Fristachi A; Rice G. 2007. Estimation of the total daily oral intake of NDMA attributable to drinking water. Journal Water Health. 5(3): 341-355.

Tannenbaum, S.R. 1980. A model for estimation of human exposure to endogenous N-nitrosodimethylamine. Oncology 37: 232-235. DOI: 10.1159/000225442.

RESPONSE: *Fristachi and Rice (2007) cited an estimate of endogenous production reported by Vermeer et al. (1998). This study was added to Section 3.1, along with another estimate of endogenous exposure (Krul et al. 2004), to better show the uncertainty in reported estimates.*

> In a study of volunteers in which urinary NDMA was measured before and after consuming fish meals rich in amines along with the acceptable daily intake of nitrate, Vermeer et al. (1998) estimated endogenous production of NDMA to be 174 µg/day (about 0.0029 mg/kg/day). Krul et al. (2004) employed an in vitro model of the human gastrointestinal tract to estimate NDMA formation occurring with gradual intake of nitrate at a range of doses from 0.1 to 10 times the acceptable daily intake. The study authors estimated cumulative NDMA amounts of 1.3–422 µg when a rapid decrease in gastric pH was simulated and 1.8–42.7 µg when gastric pH was modeled at slow decrease.

QUESTION: Have all available pharmacokinetic/pharmacodynamic models and supporting data been presented? If not, please explain.

COMMENT 23: Yes, except as discussed in Comment 24 below.

RESPONSE: *See Response to Comment 24.*

QUESTION: Is there adequate discussion of the differences in toxicokinetics between humans and animals? Is there adequate discussion of the relevance of animal toxicokinetic information for humans?

COMMENT 24: The draft found no toxicokinetic studies on humans thereby making discussion of differences between humans and animals unnecessary. Given the limited evidence available, the draft should have considered Krul et al. 2004, Levallois et al. 2000, and Veid et al. 2011, even though these are limited in their application.

The following full citations were provided by the Reviewer.

Krul, CAM; MJ Zeilmaker; RC Schothorst; R Havenaar. 2004. Intragastric formation and modulation of N-nitrosodimethylamine in a dynamic in vitro gastrointestinal model under human physiological conditions. Food Chemical Toxicology 42: 51-63. DOI: 10.1016/j.fct.2003.08.005.

Levallois P; Ayotte P; van Maanen JM; DeRosiers T; Gingras S; Dallinga JW; Vermeer IT; Zee J; Poirier G. 2000. Excretion of volatile nitrosamines in a rural population in relation to food and drinking water consumption. Food Chemical Toxicology. 38(11):1013-1019.

Veid, J; Karttunen, V; Myohanen, K; Myllynen, P; Auriola, S; Halonen, T; Vahakangas, K. 2011. Acute effects of ethanol on the transfer of nicotine and two dietary carcinogens in human placental perfusion. Toxicology Letters. 205(3): 257-264. DOI: 10.1016/j.toxlet.2011.06.014.

RESPONSE: *Krul et al (2004) was added to Section 3.1; Veid et al. (2011) was added to Section 3.1.2; and Levallois et al. 2000 was added to Section 3.1.4. No change to Section 3.1.6 was needed.*

Krul et al. (2004) employed an *in vitro* model of the human gastrointestinal tract to estimate NDMA formation occurring with gradual intake of nitrate at a range of doses from 0.1 to 10 times the acceptable daily intake. The study authors estimated cumulative NDMA amounts of 1.3–422 µg when a rapid decrease in gastric pH was simulated and 1.8–42.7 µg when gastric pH was modeled at slow decrease.

Co-treatment of perfused human placentas with ethanol and NDMA did not alter the placental transfer of NDMA (Veid et al. 2011).

NDMA was not detected (detection limit of 10 ng/L) in the urine of 59 nonsmokers who consumed drinking water containing 2 mg nitrate/L (geometric mean) (Levallois et al. 2000). Only one of the eight nitrosamines analyzed in the urine samples was detected: N-nitrosopiperidine (Levallois et al. 2000).

Children and Other Populations that are Unusually Susceptible

QUESTION: Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be? Please provide any relevant references.

COMMENT 25: Yes. Given that metabolism of NDMA plays a role in health outcomes, it is relevant that animal evidence shows that the expression of cytochrome P450s and related enzymes that are involved in the metabolism of NDMA, e.g., many P450s, including CYP2E1, are not measureable in the fetal liver (Borlakoglu et al., 1993, Carpenter et al., 1997). Weanling rats and hamsters have higher NDMA demethylase activity that mature rats (Yoo et al. 1987). In humans, the activity of NDMA demethylase reaches adult levels above the age of 10 (Blanco et al. 2000).

The following full citations were provided by the Reviewer.

Blanco, JG; PL Harrison; WE Evans; MV Relling. 2000. Human cytochrome P450 maxiumal activities in pediatric versus adult liver. Drug Metabolism & Disposition. 28(4): 379-382.

Borlakoglu, JT; A Scott; CJ Henderson and CR Wolf. 1993. Expression of P450 isoenzymes during rat liver organogenesis. International Journal Biochemistry 25(11): 1659-1668.

Carpenter, SP; DD Savage; ED Schultz; JL Raucy. 1997. Ethanol-mediated transplacental induction of CYP2E1 in fetal rat liver. Journal Pharmacology Experimental Therapeutics. 282: 1028-1036.

Yoo, J-SH; SM Ning; CJ Patten; CS Yang. 1987. Metabolism and activation of N-nitrosodimethylamine by hamster and rat microsomes: Comparative study with weanling and adult animals. Cancer Research. 47: 992-998.

RESPONSE: *The text of Section 3.2 has been expanded to address age-related variations in NDMA bioactivation as follows:*

> ***Age-Related Pharmacokinetic Differences.*** Bioactivation of NDMA results from its oxidative metabolism, primarily via CYP2E1. The expression and activity of CYP2E1 varies by age, with lowest levels seen in infants. Vieira et al. (1996) evaluated CYP2E1 protein and ribonucleic acid

(RNA) levels in hepatic microsomes from humans of various ages. The study authors observed no detectable CYP2E1 protein, and very little messenger RNA (mRNA), in hepatic microsomes from human fetuses. Within the first 24 hours after birth, CYP2E1 levels reached approximately 20% of adult activity; levels increased steadily over the first year of life, reaching about 80% of adult levels by 1 year of age (Vieira et al. 1996). Few differences in CYP2E1 activity are seen among children and adults. In a study of older children and adults, Blanco et al. (2000) observed no significant difference in CYP2E1 activity toward ethoxycoumarin in livers from humans <10, 10–60, or >60 years old.

Age-related differences in NDMA metabolic capacity have been seen in animals. No CYP2E1 protein was detected in livers from rat fetuses obtained at GD 10 or 20, but CYP2E1 was detectable in neonatal (4-day-old) rat liver (Borlakoglu et al. 1993). CYP2E1 mRNA levels did not differ with age. NDMA-demethylase activity was not detectable in fetal rat liver microsomes but increased more than 3-fold between PND 4 and 60 (Borlakoglu et al. 1993). In mice, hepatic NDMA-demethylase activity was present as early as GD 16 (3% of adult levels) and increased steadily after birth, reaching adult levels by PND 7 (Anderson et al. 2000; Jannetti and Anderson 1981). Yoo et al. (1987) observed increased NDMA-demethylase activity (and mutagenicity) in liver microsomes from weanling rats compared with adult rats; no age differences were seen in hamster liver microsomes.

Consumption of alcohol during pregnancy may increase the bioactivation of NDMA in infants. When pregnant rats were exposed to ethanol, hepatic CYP2E1 content was significantly increased in both maternal and fetal liver; the increase in the fetal liver was more than 2-fold compared with fetuses of rats that did not receive ethanol (Carpenter et al. 1997). Fetal liver microsomes from dams exposed to ethanol also showed increased N-nitrosodimethylamine demethylase activity (1.5-fold higher compared with controls) (Carpenter et al. 1997)

QUESTION: Is there a discussion of populations at higher risk of susceptibility? Do you agree with the choice of populations? Please explain and provide any additional relevant references.

COMMENT 26: Yes, co-consumption of alcohol.

RESPONSE: *The following sentence was added to Section 3.2:*

Increased CYP2E1 activity has been demonstrated in obese individuals (Emery et al. 2003) and moderate to heavy consumers of alcohol (Liangpunsakul et al., 2005) suggesting a potential for greater bioactivation of NDMA in these individuals.

Biomarkers of Exposure and Effect

QUESTION: Are the biomarkers of exposure specific for the substance? Please explain.

COMMENT 27: The discussion of Biomarkers of Exposure in Section 3.3.1 is odd. From lines 1787 to 1801 the draft report discusses methylated DNA adducts with the citation of only 2 references (Gallo et al. 2008 & Hrudey et al. 2013). This is odd given the discussion in Chapter 2 of Mechanisms from lines 1279 to 1332 that cites 14 references, including Souliotis et al. (1995, 2002) and Chhabra et al. (1995) that discuss the formation of O6-methylguanine by exposure to NDMA. O6-methylguanine, even with its declared limitations of specificity to NDMA discussed by several papers, IS a biomarker of exposure to NDMA. The relevant papers to be discussed about using O6-methylguanine as a

biomarker of exposure to NDMA, in addition to Souliotis et al. (1995, 2002) and Chhabra et al. (1995), should include Georgiadis et al. (2000, 2010) and Kyrtopoulis (1998).

The following full citations were provided by the Reviewer.

Kyrtopoulos, S.A. 1998. DNA adducts in humans after exposure to methylating agents. Mutation Research. 405:135-143.

Georgiadis, P., E. Samoli, S. Kaila, K. Katsouyanni, and S. A. Krytopoulos. 2000. Ubiquitous presence of O6-methylguanine in human peripheral blood and cord blood DNA. Cancer Epidemiology Biomarkers Prevention. 9: 299-305.

Georgiadis, P., S. Kaila, P. Makedonopoulou, E. Fthenou, L. Chatzi, V. Pietsa, and S.A. Kyrtopoulos. 2011. Development and validation of a new, sensitive immunochemical assay for O6-methylguanine in DNA and its application in a population study. Cancer Epidemiology Biomarkers Prevention. 20(1): 82-90. DOI: 10.1158/1055-9965.EPI-10-0788.

RESPONSE: *The discussion of Biomarkers of Exposure (Section 3.3.1) was revised to incorporate the suggested citations and to point the reader to the additional information in Section 2.18 (Cancer Mechanisms):*

> The O^6-methylguanine adduct is postulated to derive primarily from endogenous production of NDMA, and measurements in humans have been used as one method to estimate endogenous production (Georgiadis et al. 2000; Hrudey et al. 2013). In a review examining the use of these adducts as biomarkers of nitrosamine exposure, Gallo et al. (2008) concluded that measurement of N^7- and O^6-methylguanine adducts in lymphocytes could be used as biomarkers for exogenous and endogenous nitrosamine exposure for the purpose of epidemiology studies. Immunoassay methods are recommended due to increased sensitivity and high throughput potential (Gallo et al. 2008; Georgiadis et al. 2010). It was noted, however, that these adducts are short-lived and may not represent long-term exposure (Gallo et al. 2008). Animal studies have demonstrated the presence of O^6-methylguanine adducts in liver (Souliotis et al. 1995, 2002), blood leukocytes (Kyrtopoulos 1998; Souliotis et al. 1995, 2002) and fetal tissues following oral exposure to NDMA (Chhabra et al. 1995). A discussion of the relevance of these DNA adducts to carcinogenesis is provided above in *Mechanisms* under Section 2.18 (Cancer).

QUESTION: Are the biomarkers of effect specific for the substance? Please explain.

COMMENT 28: Methylated DNA adducts discussed above as biomarkers of exposure are mutations that persist and can cause mutations leading to adverse health effects so they might also be reasonably interpreted as biomarkers of effect.

RESPONSE: *The text of Section 3.3.2 was revised to include the following paragraph:*

> Methylated DNA adducts (described further as biomarkers of exposure in Section 3.3.1) may be considered biomarkers of preneoplastic changes induced by NDMA or other methylating agents. In particular, the O6-methylguanine adduct induced by NDMA exposure is persistent and is known to induce mutations leading to tumors. Mutations (consisting of G:C to A:T transitions) derived from these adducts have been detected in lung tumors of mice exposed to NDMA and in transgenic mice exposed to NDMA (reviewed by WHO 2008).

Interactions with Other Chemicals

QUESTION: Is there adequate discussion of the interactive effects with other substances? Please explain and provide any additional references.

COMMENT 29: Overall, yes. The statement: "NDMA can be formed endogenously via acid-catalyzed nitrosation of amine precursors in the gastrointestinal tract, especially the stomach (Hrudey et al. 2013)" cites an indirect reference, not the authentic source for this statement: Mirvish (1975).

The following full citation about endogenous formation of NDMA was provided by the Reviewer.

Mirvish SS. 1975. Formation of N-nitroso compounds: Chemistry, kinetics, and in vivo occurrence. Toxicology Applied Pharmacology. 31(3): 325-351.

RESPONSE: *The citation for the statement in Section 3.4 was revised to Mirvish (1975).*
NDMA can be formed endogenously via acid-catalyzed nitrosation of amine precursors in the gastrointestinal tract, especially the stomach (Mirvish 1975).

QUESTION: Does the discussion concentrate on those effects that might occur at hazardous waste sites? Please explain and provide any additional references.

COMMENT 30: Although this is not a particularly important issue for NDMA, there is an adequate discussion of interactions with "heavy metals".

RESPONSE: *No response needed.*

QUESTION: If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? Please explain and provide any additional references.

COMMENT 31: Yes.

RESPONSE: *No response needed.*

Chapter 4. Chemical and Physical Information

QUESTION: Are any of the values or information provided in the chemical and physical properties tables wrong or missing? Please explain and provide any additional references.

COMMENT 32: Not that I am aware of.

RESPONSE: *No response needed.*

QUESTION: Is information provided on the various forms of the substance? Please explain.

COMMENT 33: N/A.

RESPONSE: *No response needed.*

Chapter 5. Potential for Human Exposure

QUESTION: Is the information on production, import/export, use, and disposal of the substance complete? Please explain and provide any additional relevant references.

COMMENT 34: Yes, as far as I am aware.

RESPONSE: *No response needed.*

QUESTION: Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and technically sound information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.

COMMENT 35: Yes, as far as I am aware.

RESPONSE: *No response needed.*

QUESTION: Does the text cover pertinent information relative to transport, partitioning, transformation, and degradation of the substance in all media? Please provide references for added information.

COMMENT 36: Yes

RESPONSE: *No response needed.*

QUESTION: Do you know of other relevant information? Please provide references for added information.

COMMENT 37: No.

RESPONSE: *No response needed.*

QUESTION: Does the text provide information on levels monitored or estimated in the environment, including background levels?

COMMENT 38: Yes.

RESPONSE: *No response needed.*

QUESTION: Are proper units used for each medium?

COMMENT 39: Yes.

RESPONSE: *No response needed.*

QUESTION: Does the information include the form of the substance measured?

COMMENT 40: N/A.

RESPONSE: *No response needed.*

QUESTION: Is there an adequate discussion of the quality of the information?

COMMENT 41: Adequate.

RESPONSE: *No response needed.*

QUESTION: Do you know of other relevant information? Please provide references for added information.

COMMENT 42: Not that I am aware of.

RESPONSE: *No response needed.*

QUESTION: Does the text describe sources and pathways of exposure for the general population and occupations involved in the handling of the substance, as well as populations with potentially high exposures? Do you agree with the selection of these populations? If not, why? Which additional populations should be included in this section?

COMMENT 43: The description for populations with potentially high exposures includes only mention of Spiegelhalder et al, 1984 for an inadequate mention of occupational exposures in the draft report. References about occupational exposure to volatile nitrosamines that should have been discussed include: deVocht et al. (2007), Ducos et al. (1988), Oury et al. (1997). Reh & Fajen (1996), Rounbehler et al. (1979), Spiegelhalder & Preussmann (1983).

The following citations regarding occupations with exposures to nitrosamines were provided by the Reviewer.

de Vocht, F.; Burstyn, I; Straif, K; Vermeulen, R; Jakobsson, K; Nichols, L; Peplonska, B; Taeger, D; Kromhout, H. 2007. Occupational exposure to NDMA and NMor in the European rubber industry. Journal Environmental Monitoring. 9: 253–259. DOI: 10.1039/b615472g.

Ducos, P; Gaudin, R; Maire, C; Mavelle, T; Bouchiki, B; Debry, G. 1988. Occupational exposure to volatile nitrosamines in foundries using the "Ashland" core-making process. Environmental Research. 47: 72-78.

Oury, B.; Limasset, JC; Protois, JC. 1997. Assessment of exposure to carcinogenic N-nitrosamines in the rubber industry. International Archives Occupational Environmental Health. 70: 261-271.

Reh, BM; Fajen, JM. 1996. Worker exposures to nitrosamines in a rubber vehicle sealing plant. American Industrial Hygiene Association Journal. 57: 918-923.

Roundbehler, DP; Krull, IS; Goff, EU; Mills, KM; Morrison, J; Edwards, GS; Fine, DH; Fajen, JM; Carson, GA; Rheinhold, V. 1979. Exposure to N-nitrosodimethylamine in a leather tannery. Food Cosmetics Toxicology. 17: 487-491.

Spiegelhalder, B; Preussmann, R. 1983. Occupational nitrosamine exposure. 1. Rubber and tyre industry. *Carcinogenesis*. 4(9): 1147-1152.

RESPONSE: *The recommended citations were added to Section 5.7, along with information from two more recent studies of occupational exposure in the rubber industry (Hidajat et al. 2019a; Jonsson et al. 2009) with measured concentrations in air.*

> Occupational settings in which there is potential for exposure to NDMA include, but are not limited to, leather tanneries, rubber and tire industries, rocket fuel industries, dye manufacturers, soap, detergent and surfactant industries, foundries (core-making), fish-processing industries (fish-meal production), pesticide manufacturers, and warehouse and sale rooms (especially for rubber products) (Ducos et al. 1988; de Vocht et al. 2007; Oury et al. 1997; Reh and Fajen 1996; Roundbehler et al. 1979; Spiegelhalder and Preussman 1983; Tricker et al. 1989).
> Nitrosamines such as NDMA may form in the air of occupational settings when nitrogen oxides, which are ubiquitous in air react with amines and moisture. Exposure may result from inhalation or dermal contact. N-nitrosamines including NDMA were monitored for in the breathing zone of 96 workers employed at eight different companies in the rubber industry in Sweden (Jonsson et al. 2009). Total nitrosamine levels ranged from below the detection limits to 36 $\mu g/m^3$. For NDMA, the median levels ranged from below the detection limit of 0.19 $\mu g/m^3$ (3-hour sampling time) to 8.2 $\mu g/m^3$. A comprehensive study which examined levels of nitrosamines in air samples in the British rubber industry using the EU-EXASRUB database over the period of 1977–2002 reported that the arithmetic mean of measured NDMA levels over all job descriptions was 0.32 $\mu g/m^3$ (N=2,023), while the reported geometric mean was 0.16 $\mu g/m^3$; 88.7% of the samples were below the detection limits (Hidajat et al. 2019a).

QUESTION: Do you agree with the selection of these populations? If not, why? Which additional populations should be included in this section?

COMMENT 44: No. The foregoing occupational exposure assessments (see Comment No. 43) define occupations with measured exposures to nitrosamines.

RESPONSE: *See Response to Comment 43.*

Chapter 6. Adequacy of the Database

QUESTION: Do you know of other studies that may fill a data gap? Please provide any relevant references.

COMMENT 45: Yes.

The Reviewer also provided additional full citations for Occupational Epidemiology Studies (see Comment 11); Section 3.1 TOXICOKINETICS (see Comments 22 and 24); Section 3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE (see Comment 25);

Section 3.3.1 Biomarkers of Exposure (see Comment 27); Section 3.4 INTERACTIONS WITH OTHER CHEMICALS (see Comment 29); and Section 5.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES (see Comment 43).

RESPONSE: *See Responses to Comments numbered above.*

QUESTION: Do you agree with the identified data needs? Please explain.

COMMENT 46: No. The conclusion that: "No biomarkers of exposure specific to NDMA have been identified" is not entirely accurate. This statement is presumably justified on the basis that the DNA adduct O6-methylguanine is not limited to NDMA, but biomarkers are usually not perfectly specific. Even with its limitations, O6-methylguanine should be mentioned with those limitations fully explained.

RESPONSE: *The text of Section 6.2 was revised as follows:*

> O6-methylguanine DNA adducts have been used as a biomarker of exposure to NDMA, although exposures to other compounds can also produce these adducts.

QUESTION: Are the data needs presented in a neutral, non-judgmental fashion? Please note any bias in the text.

COMMENT 47: No. The bias is towards asking for additional data even when the impact of obtaining additional data is limited to negligible. The best example is the statement in the section Food Chain Bioaccumulation: "Based on this limited amount of information, it is speculated that human exposure to NDMA through diet is not the result of food chain bioaccumulation. Monitoring for the accumulation of NDMA in organisms from several trophic levels could be used to support this conclusion." NDMA lacks any physical / chemical / stability properties that would support any expectation of bioaccumulation through food chains.

RESPONSE: *The sentence recommending collection of data on food chain bioaccumulation was deleted from Section 6.2, and the preceding sentence was revised as follows:*

> Based on this information and the physical-chemical properties of NDMA, it is expected that human exposure to NDMA through diet is not the result of food chain bioaccumulation.

Chapter 7. Regulations and Guidelines

QUESTION: Are you aware of any additional regulations or guidelines that should be included? Please provide citations.

COMMENT 48: Yes. See below, both citations are the result of detailed scientific reviews of NDMA.

Guidelines for Canadian Drinking Water Quality (2011): Guideline Technical Document N-Nitrosodimethylamine (NDMA) is available on Internet at the following address: www.healthcanada.gc.ca.

Australian Drinking Water Guidelines 6. 2011 Version 3.5 Updated August 2018. N-Nitrosodimethylamine (NDMA), p.800. https://www.nhmrc.gov.au/about-us/publications/australian-drinking-water-guidelines.

RESPONSE: *The focus of Chapter 7 is U.S. federal regulations and guidelines, with the exception of the IARC cancer classifications and WHO air and water guidelines; international regulations and guidelines (other than IARC and WHO) are not included in Chapter 7 or Table 7-1.*

QUESTION: Are there any that should be removed? Please explain.

COMMENT 49: No.

RESPONSE: *No response needed.*

Additional Comments

COMMENT 50: p.39, line 857 – NDEA is not included in APPENDIX F ACRONYMS, ABBREVIATIONS AND SYMBOLS.

RESPONSE: *ATSDR does not require that all acronyms used in the profile be listed in Appendix F. The NDEA acronym was removed from the text in Section 2.9 and replaced with the full word.*

> Histopathology examinations were performed on grossly-observed lesions; apart from these, only the liver and esophagus (target for N-nitrosodiethylamine, which was also tested) were routinely examined microscopically.

COMMENT 51: p.40, lines 898-904 – multiple acronyms and terms were not included in Appendix F.

RESPONSE: *The acronyms (TGF, CTGF, FGF, NF) were removed from the text of Section 2.9 and replaced with full words.*

> Generation of reactive oxygen species results, exacerbating the injury to hepatocytes and leading to lymphocyte release of proinflammatory cytokines (e.g., transforming growth factor β1 and nuclear factor-κB) and activation of Kupffer cells. Oxidative stress and lipid peroxidation deplete hepatic antioxidants and antioxidant enzymes including catalase and glutathione peroxidase.
>
> Injury to endothelial cells results in the release of fibrogenic mediators including fibroblast growth factor 1 and connective tissue growth factor as well as induction of hedgehog signaling (promotes hepatic regeneration). In addition, release of Factor VIII (a blood-clotting protein also known as anti-hemophilic factor) from injured endothelial cells may result in aggregation of platelets, which triggers further production of inflammatory (transforming growth factor β1) and fibrogenic (platelet-derived growth factor) mediators.

COMMENT 52: p.50, Table 2-3 notes below table: HPFS, NHS, not included in Appendix F.

RESPONSE: *The acronyms are defined in the notes below Table 2-3 as that is the only place in the profile where the acronyms appear. No change was made.*

COMMENT 53: p.57, Table 2-4 - multiple acronyms and terms were not included in Appendix F.

RESPONSE: *The only acronyms in Table 2-4 were defined in the notes below the table (DNA and NT). Other elements of the table that may resemble acronyms are cell line identifiers (e.g., MCL-5, HepG2, H4IIEC3). No change was made.*

COMMENT 54: p.69, Figure 3-1: "alpha-hydroxylation" is mis-spelled as "alpha-hydroxylatio" in the Figure itself.

RESPONSE: *The spelling of hydroxylation was corrected in Figure 3-1.*

COMMENT 55: p.70, multiple acronyms and terms used on this page were not included in Appendix F.

RESPONSE: *All acronyms included on the relevant page of Section 3.1.3 were defined at first use in the text, consistent with ATSDR guidance. No change was made.*

COMMENT 56: p.78, line 1843: "psuedolobule" is presumably intended to be "pseudolobule."

RESPONSE: *The spelling of pseudolobule was corrected in Section 3.4.*

COMMENT 57: p.80, multiple acronyms and terms used on this page were not included in Appendix F.

RESPONSE: *All acronyms included on the relevant page of Section 3.4 were defined at first use in the text, consistent with ATSDR guidance. No change was made.*

COMMENT 58: NDBA, NDPhA, NPYR are not included in APPENDIX F ACRONYMS, ABBREVIATIONS AND SYMBOLS.

RESPONSE: *These acronyms were deleted from Section 5.5.3 and replaced with the full chemical names:*

> Three nitrosamines (N-nitrosodibutylamine, N-nitrosodiphenylamine, and N-nitrosopyrrolidine) were detected in some of the sediment cores; however, NDMA was not detected (10.2 ng/g detection limit) in any of the sediments tested.

COMMENT 59: p.C9, Figure B-1: There is NO mention of "Epidemiology studies", presumably these were intended to be included under "Toxicology studies" because a small number of epidemiology studies have been cited in the draft, but this representation is NOT consistent with prevailing terminology with respect to epidemiology vs. toxicology, these scientific lines of inquiry are NOT the same and one is NOT a subset of the other.

RESPONSE: *The box in Figure B-1 labelled "Toxicology Studies" was re-labelled "Health Effect Studies" to reflect toxicology, epidemiology, and case report studies.*

Annotated Comments on the Toxicological Profile

Responses to Reviewer comments that were not considered editorial or stylistic are presented below.

COMMENT 60: Referring to the overview statement in Section 1.1—The document needs to explain that NDMA is one of only dozens of volatile N-nitrosamines which exhibit similar toxic properties while noting that NDMA is most widely studied and occurs most commonly.

RESPONSE: *The following statement was added to Section 1.1:*

> NDMA is the most well-studied of several volatile N-nitrosamines that exhibit similar toxic properties (including several others that are found in tobacco smoke).

COMMENT 61: Referring to the occurrence of NDMA as a byproduct of disinfection in water treatment plants during the chlorination or chloramination of drinking water and wastewater in Section 1.1—The extensive evidence on this topic shows that this mechanism of NDMA formation is valid, but these studies also show, along with the massive EPA UCMR2 database, that levels of NDMA are very low relative to other exposure routes. The point that NDMA exposure levels via disinfected drinking water are low.

RESPONSE: *The sentence in Section 1.1 was changed to read:*

> NDMA commonly occurs at low levels as a byproduct of disinfection in water treatment plants during the chlorination or chloramination of drinking water and wastewater.

COMMENT 62: Referring to the statement in the summary of health effects in Section 1.2 that only one study of human exposure in an occupational setting was located—This comment jumped off the page as an evidently extraordinary claim as stated. A quick Google Scholar search of "occupational NDMA exposure" yielded 1830 results. While recognizing that only a small number of these hits will be worth citing and discussing, the claim that there is only one study of human exposure in an occupational setting is clearly wrong. The review discusses this matter in more detail and a list of studies that should have been reviewed is provided.

RESPONSE: *The sentence in Section 1.2 was revised for clarity. Additional studies recommended by the Reviewer were incorporated into the profile, as discussed in response to Comment 11.*

> A few studies of human noncancer effects associated with occupational exposure to NDMA were located; most of the epidemiological studies examined associations between estimated dietary intake of NDMA and cancers.

COMMENT 63: Referring to the statement of NDMA as a very potent carcinogen in the Cancer subsection of Section 1.2—The term "potent" is commonly used in describing carcinogens, but it is rarely defined when it is used. The most defensible usage for this term is to refer to the cancer slope factor for carcinogens that have such quantitative parameters about how little of any given carcinogen is capable of causing cancer. Using this definition, there is a range of at least 100,000,000 in the magnitude of known cancer slope factors. With such an enormous range, what does and adjective modifier of "very potent" mean? (Flamm W.G., Lake L.R., Lorentzen R.J., Rulis A.M., Schwartz P.S., Troxell T.C. (1987) Carcinogenic Potencies and Establishment of a Threshold of Regulation for Food Contact Substances. In: Whipple C. (eds) De Minimis Risk. Contemporary Issues in Risk Analysis, vol 2. Springer, Boston, MA. https://doi.org/10.1007/978-1-4684-5293-8_8).

RESPONSE: *The text of Section 1.2 was revised to omit the term "potent," as follows:*

In animals exposed orally, NDMA has induced increased incidences of lung tumors in mice after a single 5 mg/kg dose (Anderson et al. 1992a).

COMMENT 64: Referring to bold text in Section 2.1 and elsewhere in the profile—A various places throughout the document, text is presented in bold. The purpose / meaning of this presentation is not clear.

RESPONSE: *The bold text is templated, recurring text used generally in all toxicological profiles (per ATSDR guideline template); the bolding is removed before the profile is finalized. No change was made to the document.*

COMMENT 65: Referring to discussion of Factor VIII in the Mechanisms of Hepatotoxicity subsection of Section 2.9—This term is provided without definition. Presumably, this refers to: Factor VIII (FVIII) is an essential blood-clotting protein, also known as anti-hemophilic factor (AHF).

RESPONSE: *A definition of Factor VIII was added to Section 2.9.*

In addition, release of Factor VIII (a blood-clotting protein also known as anti-hemophilic factor) from injured endothelial cells may result in aggregation of platelets, which triggers further production of inflammatory (transforming growth factor β1) and fibrogenic (platelet-derived growth factor) mediators.

COMMENT 66: Referring to the statement in Section 2.18that the cancer database for NDMA includes only one occupational cohort study— See comment 62.

RESPONSE: *The text of Section 2.18 was revised to clarify the available cancer epidemiology data for NDMA.*

Available epidemiological data pertaining to cancers associated with NDMA include occupational studies, studies of drugs contaminated with NDMA, and studies of dietary exposure to NDMA. Only one occupational epidemiology study (Hidajat et al. 2019a) identified in the literature reported associations between cancer and exposure to NDMA itself.

COMMENT 67: Referring to Table 2-3 in Section 2.18—There is no explanation for the criteria used to adopt the descriptors used in this table, i.e. "No association" vs. "Positive association" if this is based on a p value on an OR, it should be stated. Likewise there is a huge range in the meaning of "positive association". This is normally dealt with by quoting what is being used as the criterion so that the reader can apply her/his own judgement as to meaning.

RESPONSE: *In Table 2-3, the words "no association" and "positive association" were replaced with visual depictions (↑ for significant association and ↔ for no association) and a footnote was added to explain their meaning (there were no studies reporting significant negative associations).*

[a]↑: significant association (confidence limits for the effect estimate do not include 1.0); ↔: no significant association (confidence limits for the effect estimate include 1.0).

COMMENT 68: Referring to the statement "NDMA is also a potent carcinogen in mice" in Section 2.18—See Comment 63. The term "potent" is commonly used in describing carcinogens, but it is rarely defined when it is used.

RESPONSE: *The term "potent" was deleted from the following sentence in Section 2.18.*

NDMA is also a carcinogen in mice.

COMMENT 69: Referring to Figure 3-1—alpha-hydoxylation [top left] is mis-spelled.

RESPONSE: *The spelling error in Figure 3-1 was corrected.*

COMMENT 70: Referring to the statement of ingestion of NDMA in Section 5.1—Inconsistent terminology used. Nitrosamine levels in malt beverages are described as "low levels" when the values shown in Table 5-3, p.96 are much higher than those reported for drinking water

RESPONSE: *The text of Section 5.1 was revised to remove the reference to "low levels."*

The general population may also be exposed to trace amounts of NDMA through ingesting foods containing nitrosamines such as cured or smoked meats and fish; ingesting foods containing alkylamines, which can form NDMA in the stomach; ingesting drinking water or malt beverages containing NDMA; and inhalation of tobacco smoke.

COMMENT 71: Referring to the statement of formation of NDMA as an unintentional byproduct of the chlorination of wastewater and drinking water in Section 5.1—This statement uses better terminology than the note above where drinking water and malt beverages are mentioned in the same sentence. That said, the relevance of NDMA formed in wastewater is far less for human exposure than in drinking water, but that comparative ranking is not evident in this statement.

RESPONSE: *The text of this bullet in Section 5.1 was revised to clarify the relative exposure to wastewater relative to drinking water, as follows:*

Very low levels of NDMA may form as an unintentional byproduct of the chlorination of drinking water at treatment plants that use chloramines and chlorine for disinfection. NDMA may also be formed in wastewater, but human exposure to wastewater is expected to be very limited.

COMMENT 72: Referring to the disposal discussion in Section 5.2—This is odd terminology. I could not check the reference by accessing Castegnaro et al. 1982 remotely without buying it from IARC. Physical access to my Faculty library for this IARC publication is not viable right now.

RESPONSE: *The text of Section 5.2 was revised to use more common terminology.*

Contaminated solid materials should be enclosed in sealed plastic bags that are labeled to indicate the presence of a carcinogen, with the name and amount of carcinogen.

COMMENT 73: Referring to the inclusion of fish processing facilities in Section 5.3—I am not seeing this occupational exposure for nitrosamines in references that I have been able to access. EPA (2014) refers to the 1989 ATSDR Tox Profile for NDMA as its source for this list of industrial sources. A

review of that document does not reveal a traceable original source for this claim that fish processing facilities are an industrial source for NDMA.

RESPONSE: *The paper that identifies fish processing facilities as a source of occupational NDMA exposure is Tricker et al. (1989), a review that cites the National Institute for Occupational Safety and Health (NIOSH) (Rounbehler and Fajen 1982) for the data on fish processing facilities. The Tricker et al. (1989) citation was added to the text of Sections 1.1, 5.3, and 5.7 to clarify the original source of the information.*

COMMENT 74: Referring to the discussion of Baxter et al (2007) in Section 5.4.4—This statement stood out, leading to a review of Baxter et al. (2007). It did devote a substantial discussion to the possibility that NDMA could originate in contaminated source waters. However, Baxter et al. (2007) did not have the benefit of the extensive study of NDMA as a drinking water contaminant which has been published since 2007, particularly the massive EPA UCMR2 database (1198 water systems with over 18,000 analyses performed) to understand what levels of NDMA could be expected in disinfected drinking water. These data, along with at least 8 other published surveys show that NDMA levels are rarely above a few ng/L (i.e., 0.00x µg/L) showing that drinking water contributions to NDMA levels in beer are most likely to be negligible. The statement in this highlighted sentence is not informative and is misleading based on current knowledge.

RESPONSE: *The statement referred to in the comment ("The authors also suggested that NDMA could originate in contaminated source waters used to produce the beverages [Baxter et al. 2007]") was deleted from Section 5.4.4.*

COMMENT 75: Referring to the data needs for chronic MRL derivation in Section 6.2—The reality is that the "many" studies referred to all have limited power, limited specificity and considerable confounding.

RESPONSE: *The data needs text for chronic MRL derivation in Section 6.2 was revised to omit the reference to "many" cancer epidemiology studies, as it is not pertinent to chronic MRL derivation.*

> Reliable epidemiological studies examining associations between oral intake of NDMA and noncancer endpoints were not located.

COMMENT 76: Referring to the data needs for food chain bioaccumulation in Section 6.2—This suggestion would be a poor use of research resources given the well-documented knowledge of the physical/chemical/stability properties of NDMA.

RESPONSE: *The sentence recommending collection of data on food chain bioaccumulation was deleted from Section 6.2, and the preceding sentence was revised as follows:*

> Based on this information and the physical-chemical properties of NDMA, it is expected that human exposure to NDMA through diet is not the result of food chain bioaccumulation.