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April 4, 2017

Mr. Steven B. Elmore
Director, Bureau of Drinking Water & Groundwater
Wisconsin Department of Natural Resources
101 S Webster Street
Madison, WI 53701-7921

Dear Mr. Elmore:

In response to your request dated December 8, 2015, I am forwarding interim drinking water health advisory levels (HALs) for 2-nitroaniline, 3- and 4-nitroaniline (combined), 2,4-diaminotoluene, and 2,6-diaminotoluene. These advisories were developed by our toxicology staff. The methods and studies used to derive them are explained in the attached background documents.

One of the compounds listed in your request, 5-methyl-2-nitroaniline (also known as 3-amino-4-nitrotoluene) was reviewed by DHS in 2011 in response a similar request from your agency. The present review did not identify any new information on this substance. As a result, our conclusion from 2011 remains: there is currently insufficient data to recommend a HAL for this substance. Three other methylnitroanilines were also listed in your recent request. Our review did not identify information on the potential health effects of these substances, and as such, there is currently insufficient data to recommend a HAL for these compounds.

Correspondence from September 2005 between the U.S. Army, the Wisconsin Department of Natural Resources, and our agency contained a chemical structure labeled "benzofuran," but the chemical is benzofurazan. Similarly, chemical structures labeled "3-methylbenzofuran" and "5-methylbenzofuran" are 4-methylbenzofurazan and 5-methylbenzofurazan, respectively. Regardless, our review did not identify any information on potential health effects and thus we are unable to recommend a HAL for these compounds also.

Compound	CAS-RN	Health Advisory Level
2-Nitroaniline	88-74-4	100 ug/L
3- and 4-Nitroaniline (combined)	99-09-2/100-01-6	2 ug/L
2,4-Diaminotoluene	95-80-7	0.01 ug/L
2,6-Diaminotoluene	823-40-5	300 ug/L
5-Methyl-2-nitroaniline	578-46-1	*Insufficient data to recommend a HAL*
3-Methyl-2-nitroaniline	601-87-6	*Insufficient data to recommend a HAL*
3-Methyl-4-nitroaniline	611-05-2	*Insufficient data to recommend a HAL*
3-Methyl-5-nitroaniline	618-61-1	*Insufficient data to recommend a HAL*
Benzofurazan*	273-09-6	*Insufficient data to recommend a HAL*
4-Methylbenzofurazan*	29091-40-5	*Insufficient data to recommend a HAL*
5-Methylbenzofurazan*	20304-86-3	*Insufficient data to recommend a HAL*

Please contact me if you have any questions regarding these recommendations.

Sincerely,

Jonathan G Meiman, MD
Chief Medical Officer
Bureau of Environmental and Occupational Health

Enclosures:

1. Interim drinking water health advisories for 2-, 3-, and 4-nitroaniline
2. Interim drinking water health advisories for 2,4-diaminotoluene
3. Interim drinking water health advisories for 2,6-diaminotoluene

cc: Chuck Warzecha, Deputy Administrator, Division of Public Health
Jeffrey Phillips, Director, Bureau of Environmental and Occupational Health
Bruce Rheineck, Chief, Groundwater Section, DNR Bureau of Drinking Water and Groundwater

Interim Drinking Water Health Advisory for 2-, 3-, and 4-Nitroaniline

Prepared by Roy Irving, PhD
Wisconsin Department of Health Services, April 4, 2017

Introduction:

2-nitroaniline (ONA), 3-nitroanlinine (MNA), and 4-nitroaniline (PNA) are structurally-similar isomers that are used as intermediates in the production of azo dyes. ONA is also used in the production of metal cutting fluids and anti-UV agents in plastics, while PNA is used to produce antioxidants and gasoline additives. Nitroanilines may also occur due to degradation of compounds, such as dinitrobenzene and dinitrotoluene.

Chemical Profile:

Chemical Name	2-Nitroaniline	3-Nitroaniline	4-Nitroaniline
CAS No.	88-74-4	99-09-2	100-01-6
Chemical Formula	$C_6H_6N_2O_2$	$C_6H_6N_2O_2$	$C_6H_6N_2O_2$
Molecular Weight	138.12	138.12	138.12
Density	1.442 g/cm^3	0.9011 at 25°C	1.4 g/cm^3
Solubility in Water	1170 mg/L at 20°C	1200 mg/L at 24°C	~600 mg/L at 25°C
Synonyms	o-Aminonitrobenzene, 1-amino-2-nitrobenzene, 2-aminonitrobenzene, o-nitroaniline, azoene fast orange GR base, azoene fast orange GR salt, azofix orange GR salt, azogene fast orange GR, azoic diazo component 6, 2-nitrobenzenamine, brentamine fast orange GR base, brentamine fast orange GR salt, CI azoic diazo component 6, CI 37025, Devol orange B, Devol orange salt B, Diazo fast orange GR, fast orange o base, fast orange base GR, fast orange base JR, fast orange GR base, fast orange GR salt, fast orange O salt, fast orange salt GR, fast orange salt JR, Hiltonil fast orange GR base, Hiltosal fast orange GR salt, Hindasol orange GR salt	Amarthol Fast Orange R Base, m-aminonitrobenzene, 1-amino-3-nitrobenzene, m-nitroaniline, azobase MNA, 3-nitrobenzenamine, CI azoic diazo component 7, CI 37030, Daito Orange Base R, Devol orange R, diazo fast orange R, fast orange M base, fast orange R base, Fast Orange MM base, Fast Orange R Salt, Hiltonil Fast Orange R Base, MNA, naphtoelan orange R base, nitranilin, m-nitraniline, m-nitroaminobenzene, m-nitrophenylamine, orange base IRGA I	1-amino-4-nitrobenzene, 4-nitraniline, 4-Nitrobenzenamine, azoamine red ZH, azofix red gg salt, Azoic diazo component 37, CI 37046, CI azoic diazo component 37, CI developer 17, Developer P, Devol Red GG, Diazo Fast Red GG, Fast Red 2G Base, Fast Red 2G Salt, Fast Red Base 2J, Fast Red Base GG, Fast Red GG Base, Fast Red GG Salt, Fast Red MP Base, Fast Red P Base, Fast Red P Salt, Fast Red Salt 2J, Fast Red Salt GG, Naphtoelan Red GG Base, NCI-C60786, Nitrazol CF Extra, Para-aminonitrobenzene, Para-nitroaniline, PNA, p-nitraniline, p-nitroaniline, p-Nitrophenylamine, Red 2G Base, Shinnippon fast red GG Base

Occurrence:

Estimated worldwide production of ONA is 20,000 to 25,000 tonnes per year (OECD/SIDS, 2001). MNA production is relatively low (no estimates of production were located). Between 1 and 10 million pounds of PNA is produced in the United States (DHHS, 2016).

ONA, MNA, and PNA are primarily used as intermediates in the synthesis of various dyes and pigments. In addition, PNA has been used in the production of antioxidants, gasoline gum inhibitors, pharmaceuticals, pesticides, and various dyes and pigments (e.g., Para-Red) (DHHS, 2016). Because they are solely used as intermediates in the production of other substances, exposure is expected to be low for the general population (OECD/SIDS, 1994). However, they could potentially be released into the environment through various waste streams (DHHS, 2016a, 2016b, 2016c).

In Wisconsin, ONA, MNA, and PNA have been detected in groundwater monitoring wells at the Badger Army Ammunition Plant and Fort McCoy U.S. Army installation (Correspondence from J Jonas to H Anderson, 2015). It has been hypothesized that detection of these compounds is due to dinitrotoluene (DNT) degradation at these sites.

Human Exposure Routes:

In occupational settings, the primary route of exposure to ONA, MNA, or PNA is expected to be dermal contact with the compound at workplaces where it is produced or used (DHHS, 2016a, 2016b, 2016c). In addition, exposure may occur through inhalation of ONA, MNA, or PNA dust. For PNA, the US Occupational Safety and Health Administration (OSHA) has set a permissible exposure limit of 6 mg/m^3 PNA (8 hour time weighted average), and the National Institute for Occupational Health and Safety set a recommended exposure limit of 3 mg/m^3 (10-hour time weighted average) (DHHS, 2016c). Both occupational guideline values were set to protect against risks from dermal exposure.

ONA, MNA, and PNA have been found in groundwater monitoring wells at sites where DNT contamination of soils and water has occurred. The primary route of human exposure to the NA isomers in groundwater is through ingestion of drinking water.

Acute Toxicity:

In rats exposed to ONA, LD_{50} values ranged from 1600 mg/kg body weight to 3560 mg/kg body weight (DHHS, 2016a). Studies in rabbits suggest that ONA is not irritating to the skin or eyes (DHHS, 2016a). ONA is not a sensitizing agent.

In one short-term study, groups of male and female SD rats were dosed with ONA (0, 1, 10, or 100 mg/kg/d) by gavage for 14 days (Komsta et al., 1989). Clinical signs, hematology, clinical chemistry, and histopathologic endpoints were evaluated. Methemoglobin (metHb) levels were not measured. No treatment-related effects were observed.

Little information is available regarding acute toxicity of MNA. In rats, oral LD_{50} values ranged from 535 mg/kg body weight to 900 mg/kg body weight (OECD/SIDS, 1994). In mice, two studies reported that the oral LD_{50} was approximately 310 mg/kg body weight (OECD/SIDS, 1994). MNA did not appear to be irritating to rabbit eyes when applied as a powder (DHHS, 2016b).

In rats, oral LD_{50} values for PNA range from 750 to 3249mg/kg body weight. In mice, the oral LD_{50} is 810 mg/kg body weight (DHHS, 2016c).

There were no studies found that investigated effects from acute oral exposure to ONA, MNA or PNA in humans. One case report describes an incident where workers were exposed through inhalation and dermal routes while sweeping up spilled PNA (Anderson, 1946). These workers became cyanotic and complained of headache, sleepiness, weakness and respiratory distress. Hematological endpoints were not assessed, but clinical signs improved after methylene blue treatment, which is used to treat methemoglobinemia. Aniline and a number of substituted anilines are associated with methemoglobinemia (NTP, 1993).

Chronic Toxicity:

ONA

No human or animal studies examining the effects of chronic oral exposure to ONA were identified.

MNA

No studies examining the effects of subchronic or chronic exposure to MNA in humans were identified. In addition, no studies that examined any subchronic or chronic exposure in animals were identified.

One short-term study on MNA was identified, an unpublished 28-day repeated dose study conducted by the Japanese Ministry of Health and Welfare (Onodera, ND). DHS was unable to obtain a copy of this report, so the following information was collected from the U.S. Environmental Protection Agency (EPA) Provisional Peer-Reviewed Toxicity Value (PPRTV) support document (EPA, 2009b) and OECD/SIDS summary (OECD/SIDS, 1994).

In this study, male and female rats were given MNA (0, 15, 50, or 170 mg/kg) by gavage for 28 days. An additional set of rats were given 0 or 170 mg/kg MNA by gavage for 28 days followed by 14-day recovery periods. Body weight was significantly lower in high-dose males compared to controls, and remained decreased at the end of the recovery period. Cyanosis was observed in male and female rats in the high-dose group.

At the end of the treatment period, metHb was increased in the female mid-dose group, and the high-dose groups for both sexes. Changes in other hematological endpoints were observed in treated animals. After the recovery period, most endpoints were similar to controls, although some remained increased in animals that received the highest doses of MNA.

Changes in clinical chemistry parameters (cholesterol, protein, albumin, BUN) were also noted and consistent with hemoconcentration.

Spleen and liver weight were increased in treated animals. Kidney, testes, and thyroid weight were also affected in some animals. Even after the recovery period, spleen, testes, and liver weights remained changed compared to controls. After the recovery period, epididymides weights were decreased in high-dose males and ovary weights were increased in high-dose females; these effects were not observed at the end of the treatment period.

Histopathological examination revealed hemosiderin deposition, extramedullary hematopoiesis, and congestion in spleens of treated animals. Erythroid hyperplasia of the bone marrow was observed in all groups. The severity of these effects in the spleen and bone marrow increased with dose. Other effects observed were hepatocyte swelling, hemosiderin deposition, and extramedullary hematopoieisis in the liver, renal lipofuscin was observed in the kidneys, and reduction of spermatogenesis. After the recovery period, all lesions were decreased in severity with the exception of hemosiderin. Based on the hematological effects and histopathological findings in the spleen and bone marrow, EPA identified a lowest observable adverse effect level (LOAEL) of 15 mg/kg-day (EPA, 2009b). Since this was the lowest dose of MNA tested, a no observable adverse effect level (NOAEL) was not determined.

PNA

There have been a number of animal studies investigating the chronic toxicity of 4-nitroaniline.

Nair et al. (1990) dosed groups of male and female Sprague-Dawley rats with 0, 0.25, 1.5, or 9 mg/kg PNA by daily gavage for 2 years. The high-dose treatment caused a slight increase (statistically significant positive trend) in mortality in males. In females, body weight in the high-dose group was increased compared to controls. In both sexes, increased metHb levels and decreased hemoglobin and red blood cell (RBC) counts were observed. Spleen and liver weight were increased in males. Accumulation of brown pigment (likely hemosiderin, an iron storage protein) was observed in the Kupffer cells of the liver and in the reticuloendothelial cells of the spleen. Hemosiderosis incidence in the liver was statistically significant in mid-dose males and both high-dose groups. Hemosiderosis incidence in the spleen was also statistically significant in mid- and high-dose males. There was a statistically significant, dose-related increase in severity of splenic hemosiderosis in females. Based on increased metHb in both sexes, and increased spleen weight and hemosiderosis in the liver and spleen of male rats, EPA identified a NOAEL of 0.25 mg/kg-day and a LOAEL of 1.5 mg/kg-day from this study (EPA, 2009c).

The National Toxicology Program (NTP) conducted a 2 year chronic toxicity and carcinogenicity study where male and female $B6C3F_1$ mice were dosed with 0, 3, 30, or 100 mg/kg-day of PNA by gavage for 5 days per week (NTP, 1993). The findings were generally consistent with those effects observed in rats.

Changes in hematological endpoints were observed, including increased metHb and sulfohemoglobin levels. In addition, liver and spleen weight were altered in some treated animals. Other observed effects were increased incidence of bone marrow hypercellularity,

hemosiderosis in the spleen and in the Kupffer cells of the liver, splenic congestion, and splenic extramedullary erythropoiesis. EPA identified a minimal LOAEL of 3 mg/kg-day (the lowest dose in this study) based on bone marrow hypercellularity in male mice and hemosiderosis of the spleen in female mice (EPA, 2009c).

Collectively, the results from these chronic toxicity studies suggest that PNA induces methemoglobinemia, which results in in hemolytic anemia and compensatory erythropoiesis. This mechanism of toxicity is supported by similar findings in a number of subchronic studies (Houser et al., 1983; Monsanto, 1981b; Nair et al., 1990; NTP, 1993), acute studies (Watanabe, 1979; SOCMA 1984) and *in vitro* studies (Watanabe 1979; French 1995).

Reproductive and Developmental Effects:

ONA

In a developmental study in rats, six mated female Charles River CD rats were given ONA (0, 50, 200, 400, 800, or 1200 mg/kg-d) by gavage from gestation day (GD) 6-15 (Monsanto Co., 1984). Mortality and clinical signs were evaluated daily and dams were sacrificed on GD 21 for necropsy and examination of uterine content. Hematological parameters were not analyzed in maternal blood and histopathological examination not performed.

Four out of six rats in the highest dose group died by GD11; necropsy revealed yellow coloration of the subcutaneous and abdominal fat was colored yellow indicating deposition of the test material. In the two highest dose groups, body weight gain and food consumption were reduced, and clinical signs were observed. Treatment had no effect on total implantations, litter size, fetal loss, or the incidence of external malformations. Mean fetal weights were reduced in the two high dose groups. Based on these results, US EPA identified a NOAEL of 400 mg/kg-day for both maternal and fetal effects (EPA, 2009a).

In a follow-up developmental study, groups of mated female rats were given ONA (0, 100, 300, or 600 mg/kg-d) by gavage on GD 6-15 (Monsanto Co., 1985). Mortality and clinical signs were evaluated daily and dams were sacrificed on GD 21 for gross pathologic examination; they were not examined for hematological and histopathological effects.

Maternal food consumption was reduced in all dosed groups, but maternal body weight was only reduced in the high dose group. No difference was observed between controls and treated groups for pregnancy rate, implantation rate, fetal resorptions, number of litters, fetal viability, mean litter weight, fetal sex distribution, and fetal body weight. One fetus in each of two litters in the high dose group had a heart abnormality (partial situs inversus), which the authors reported may have been treatment-related. EPA identified a NOAEL of 300 mg/kg-day based on critical effects of piloerection and pale or cold extremities in the dams and heart abnormalities in some fetuses (EPA, 2009a).

Two additional studies were identified that examined reproductive and developmental endpoints (Sisti, 2001a and 2001b). These studies are unpublished and DHS was not able to obtain a copy of the reports. As such, the following information was gathered from other ONA toxicity reviews by the Organization for Economic Cooperation and Development, OECD (OECD/SIDS, 2001) and the U.S. Environmental Protection Agency, EPA (EPA, 2009a).

In the developmental study (Sisti, 2001a), mated females were given ONA (0, 100, 200, or 400 mg/kg) by gavage on GD 0-19, and sacrificed on GD 20. No clinical signs were noted in

controls, and the only clinical signs noted in treated animals were matted fur and piloerection in the high-dose dams. Cyanosis, a potential indicator of methemoglobinemia, was not observed. Dose-dependent decreases (not statistically significant) in body weight were observed in the mid and high dose groups. Body weight gain was reduced in high-dose dams when compared to controls on GD 6 and GD20. There were no treatment-related differences observed in total implantations and resorptions, number of viable fetuses, sex ratios, fetal weight or fetal external malformations. From this study, EPA identified a maternal NOAEL and LOAEL of 200 and 400 mg/kg-day, respectively, based on clinical signs of toxicity in dams, and a NOAEL of 400 mg/kg-day for fetal toxicity.

Sisti also conducted a reproductive toxicity study (Sisti, 2001b) where ONA (0, 50, 150, or 400 mg/kg) was given by gavage to male and female Sprague Dawley rats 4 weeks prior to mating through postpartum day 4 (for female rats), or through gestation of females (for male rats). The total duration of exposure was 9 weeks. This study apparently was conducted using standardized testing guidelines from OECD (OECD Testing No. 422, OECD, 1996).

Matted fur was observed in high-dose rats. Cyanosis was not observed. Body weight was significantly reduced in mid- and high-dose adults at several times throughout the treatment period. A significant increase in pup mortality was observed in litters from high-dose dams that had reduced body weight gain. Litter size and litter weight were significantly reduced on postpartum day 4 in the high-dose group compared to controls. From this study, EPA identified a NOAEL of 150 mg/kg-day and a LOAEL of 450 mg/kg-day for maternal toxicity (effects on body weight gain and clinical signs). A reproductive NOAEL of 150 mg/kg-day and a LOAEL of 450 mg/kg-day were also identified based on increased pup mortality and reduced litter weight (EPA, 2009a).

MNA

One reproductive and developmental toxicity study on MNA was identified, an unpublished 28 day repeated dose study conducted by the Japanese Ministry of Health and Welfare (Mizutani, ND). DHS was unable to obtain a copy of this report, so the following information was collected from the EPA PPRTV support document (EPA, 2009b) and OECD/SIDS summary (OECD/SIDS, 1994).

In this short-term reproductive/developmental toxicity study, groups of rats were given MNA (0, 5, 15, or 50 mg/kg) by gavage from 14 days before mating through a 14-day mating period and a 14 day post-mating period (for males) or through day 4 of lactation (females). Mortality and clinical signs were monitored daily and mating performance, fertility, and reproductive parameters were evaluated. After treatment, complete gross pathological and histopathological examinations were conducted on the ovaries, testes, and epididymides. Hematology, clinical chemistry, and organ weights were not evaluated. Pups were evaluated for body weight (at birth and postnatal day 4), pup survival, and external, internal and skeletal malformations.

During delivery, one female in the high-dose group died, but no treatment-related clinical signs of toxicity were noted before death. One female in the mid-dose group and two in the high-dose group had difficult labors and lost their litters. The authors noted that in the high-dose group, one female and one male had pale extremities "late" in the dosing period. There were consistent decreases in body weight of adult females and males, but this was not statistically significant. Mating performance and fertility were unaffected by treatment. Live birth index was decreased

in mid- and high-dose groups, although this may be related to litter loss described above. All other reproductive parameters were comparable to controls.

Pathological examination found effects in the liver and spleen of adult males and in the spleen of adult females. Hepatomegaly was observed in some high-dose males. Enlarged and/or dark colored spleen was observed in some animals in the mid-dose group (3 males, 1 female) and high-dose group (all males, 8 females). No treatment-related histopathological changes were observed in ovaries, testes or epididymides of parental animals.

Based on pathological findings in the spleens of male rats and potential reproductive toxicity in female rats, EPA identified a NOAEL of 5 mg/kg-day and a LOAEL of 15 mg/kg-day for the parental generation in this study (EPA, 2009a). EPA advised caution in interpreting the parental NOAEL since hematology was not evaluated in this study and another 28-day repeat dose study (Onodera, ND – details described above) was not able to identify a NOAEL for hematological effects (EPA, 2009b). From this study, EPA also identified a NOAEL of 50 mg/kg-day for fetal effects.

PNA

PNA was tested for reproductive toxicity in rats (Nair et al., 1990) through a 2-generation reproductive toxicity study. Groups of Sprague Dawley rats were given doses of PNA (0, 0.25, 1.5, or 9 mg/kg-day) by daily gavage for 14 weeks (F_0 generation) or 18 weeks (F_1 generation) before mating and during mating, gestation, and lactation. The only effect observed was a statistically significant decrease in pregnancy rate in the F_0 generation, but this effect was not seen in the F_1 generation. Because this effect was not observed in the F_1 generation, the authors concluded that this was not a treatment-related effect.

In a developmental study in rabbits, mated does were given PNA (0, 15, 75, or 125 mg/kg) by gavage on gestational days 7-19 (Monsanto Co., 1982). Increases in mortality and incidence of body weight loss were observed in the high-dose group. No treatment-related effects were observed in fetuses. From this study, EPA identified a NOAEL of 75 mg/kg-day and a LOAEL of 125 mg/kg-day for maternal toxicity, based on mortality and weight loss. The NOAEL for developmental effects was 125 mg/kg-day, the highest dose tested.

In another developmental study, groups of Sprague-Dawley rats were given PNA (0, 25, 85, or 250 mg/kg) by gavage on gestational days 6-19 (Monsanto Co., 1980). Maternal weight gain was reduced in the high dose group compared to controls. Yellow staining of the fur, increased incidence of gross discoloration and surface irregularities in the spleen, and increased spleen weight were observed in dams in the mid- and high-dose groups. The percentage of resorptions was increased and the percentage of live fetuses was decreased in the high-dose group. A dose-related decrease in fetal weight was observed and was statistically significant in the mid- and high-dose groups. The incidence of fetal malformations was increased in the high-dose group. External malformations were mainly of the tail and digits; internal malformations observed were in the kidneys and skeletal system.

From this study, EPA identified a NOAEL of 25 mg/kg-day and a LOAEL of 85 mg/kg-day for both maternal (altered spleen weight and appearance of the spleen) and fetal toxicity (decreased fetal weight) (EPA, 2009c).

Carcinogenicity:

No carcinogenicity studies on ONA or MNA were located. Neither the EPA nor the International Agency for Research on Cancer (IARC) have classified PNA with respect to carcinogenicity.

For PNA, NTP evaluated carcinogenicity endpoints as part of a 2-year study in mice (NTP, 1993). In male mice, significant positive trends were reported for incidence of hemangiosarcomas in the liver and vascular neoplasms (hemangiomas and hemangiosarcomas combined) at all sites. Although tumor incidence was not greater than concurrent controls by pair-wise comparisons, incidence was statistically increased compared to historical controls. These effects were not observed in females. Although incidence of hemangioma or hemangiosarcoma (combined) at all sites was elevated in female mice, the increases were not significantly different from concurrent or historical controls. Based on this information, NTP concluded that there was no evidence of carcinogenic activity in female mice, and equivocal evidence for carcinogenic activity in male mice.

Nair et al. (1990) investigated carcinogenic activity in a 2-year rat study. Although several types of spontaneously occurring neoplasms were observed, tumor incidence was similar between control and treated rats, and no dose-response relationship in tumor incidence was observed. The authors concluded that there was no evidence of treatment-related carcinogenicity in rats given PNA orally at the dose levels in this study.

Mutagenicity/Genotoxicity:

S. typhimurium reverse mutation assays have been used to investigate the potential mutagenicity of ONA, MNA, and PNA (Assmann et al., 1997; Chiu et al., 1978; Chung et al., 1996; Corbett et al., 1985; Dellarco and Prival, 1989; Garner and Nutman, 1977; Haworth et al., 1983; Kawai et al. 1987; Le et al., 1985; Pai et al., 1985; Shahin, 1985; Shimizu and Yano, 1986; Thompson et al., 1983). PNA has had mutagenic activity in these assays, and the results suggest that metabolic activation is required. Similarly, MNA was mutagenic in these assays when metabolic activation was present, but results were inconsistent when metabolic activation was absent. In assays with ONA, results were inconsistent across studies when metabolic activation was present and ONA did not induce mutations when metabolic activation was absent. ONA and MNA did not induce mutations in a reverse mutation assay using *E. coli* and metabolic activation (Thompson et al., 1983). MNA was weakly mutagenic in the Kada *Bacillus subtilis* rec assay without metabolic activation present (Shimizu and Yano, 1986).

There are a few studies that have examined genotoxicity of these compounds in mammalian cells. Of the three isomers, PNA genotoxicity has been the most extensively investigated.

PNA induced mutations in L5178Y mouse lymphoma cells without metabolic activation and sister chromatid exchanges in Chinese hamster ovary (CHO) cells with metabolic activation (NTP, 1993). Two studies also reported induction of sister chromatid exchanges by PNA in the absence of metabolic activation (Galloway et al., 1987; Chung et al., 1996). Chromosomal aberrations were observed in human lymphocytes treated with PNA *in vitro* (Huang et al., 1996) and in CHO cells with metabolic activation (NTP, 1993). PNA covalent binding to RNA was observed in cultured human granulocytes activated to undergo a respiratory burst (Corbett and Corbett, 1988). PNA was not mutagenic in the CHO forward gene mutation assay (Monsanto Co., 1984) or the *D. melanogaster* sex-linked recessive lethal mutation assay (Zimmering et al., 1989). A mouse micronucleus assay did not provide evidence of clastogenicity (Monsanto Co.,

1989). In addition, two metabolites of PNA, 1,4-benzenediamine and 2-amino-5-nitrophenol have been shown to be mutagenic *in vitro*, although data is limited on their potential mutagenicity *in vivo* (NTP, 1993).

ONA did not induce unscheduled DNA synthesis in primary cultured hepatocytes (Yoshimi et al., 1988; Thompson et al., 1983). ONA induced a small increase in bone marrow micronuclei in male, but not female, mice (Blakey et al., 1994).

In a study in cultured rat hepatocytes, MNA did not induce unscheduled DNA synthesis in cultured rat hepatocytes (Thompson et al., 1983). MNA induction of chromosomal aberrations in Chinese hamster CHL cells *in vitro* was observed in the absence and presence of metabolic activation (OECD/SIDS, 1994). A single oral dose of 300 mg/kg MNA resulted in positive results in an *in vivo* micronucleus test in mice, but inhibition of bone marrow cell proliferation was not observed (OECD/SIDS, 1994). These effects were not seen at lower doses.

Collectively, these studies suggest that PNA has mutagenic and clastogenic properties. Although the data are more limited for MNA, the available results suggest that the compound may also have mutagenic potential. In its review of these studies, EPA concluded that ONA does not appear to be genotoxic; however, EPA acknowledged that the data are limited (EPA, 2009a).

Interactive Effects:

A primary adverse effect associated with aniline and other substituted aniline compounds is methemoglobinemia (NTP, 1993). Methemoglobinemia has been associated with ONA, MNA, and PNA, but the relative potency of each of the nitroaniline isomers to induce methemoglobinemia is unclear because the limited data available has shown inconsistent results, possibly due to species/strain differences.

In vitro studies have shown that ONA, MNA and PNA induce metHb formation when incubated with hemoglobin (Watanabe, 1976) or freshly drawn sheep erythrocytes (French et al., 1995).

In an *in vitro* study with freshly drawn sheep erythrocytes, MNA and PNA increased metHb formation to a similar extent. When an NADP-based bioactivation system was present, both MNA and PNA generally induced metHb formation to a greater extent, but PNA had about twice the activity of MNA (French et al., 1995).

In vivo, a single dose (150 mg/kg or 600 mg/kg, by gavage) of MNA or PNA, but not ONA, increased metHb levels in Sprague Dawley rats 6 hours after dosing (SOCMA, 1984). In a second study, when male Wistar rats were given nitroaniline isomers (100 uM) by injection; metHb levels 5 hours after injection were increased compared to controls for all 3 isomers (Watanabe, 1976). When MNA and PNA were given to rats at 50% of the LD_{50}, PNA was more potent than MNA in inducing methemoglobinemia and sulfhemoglobinemia (Vasilenko et al. 1974).

Repeat oral dosing studies (described above) demonstrate the ability of PNA to increase metHb levels; however, the information is much more limited for MNA. The available studies on PNA and MNA do not use the same animal models nor do they involve the same treatment duration, which means that direct comparisons of subchronic and chronic toxicity are not possible. There are no data from repeat oral dosing studies on ONA potential for methemoglobinemia induction. However, dermal and inhalation studies in animals have reported increased metHb levels or cyanosis due to ONA treatment (EPA, 2009a).

Even though the available data do not allow for direct comparisons of the isomers' ability to induce methemoglobinemia, there is sufficient evidence to suggest that all three isomers can cause metHb formation. No studies have examined the combined effects of exposure to multiple nitroaniline isomers; however, based on similar ability to induce methemoglobinemia, the effects from exposure to multiple nitroanilines, as well as other metHb inducers (e.g., other aniline isomers, nitrates) are likely to be additive.

In addition, individuals with pre-existing conditions like anemia, hematopoietic disorders, or low levels of metHb reductase may be more susceptible to the effects of ONA, MNA, and PNA (EPA, 2009b, 2009c).

Environmental Fate:

Through their use in the production of various substances, ONA, MNA, PNA may be released to the environment through various waste streams. (DHHS, 2016a, 2016b, 2016c).

Atmospheric

Based on its vapor pressure, ONA and MNA are expected to exist as a vapor in the ambient atmosphere. PNA is expected to exist in both vapor and particulate phases in the ambient atmosphere. ONA, MNA, and PNA in the air will react with photochemically-produced hydroxyl radicals in the air, resulting in degradation. Because ONA, MNA, and PNA can absorb sunlight at certain wavelengths, they may degrade through direct photolysis by sunlight as well. Particulate-phase PNA can be removed from the air by wet or dry deposition (DHHS, 2016a, 2016b, 2016c).

Aquatic

ONA, MNA, and PNA are soluble in water, and based on their soil organic carbon/water partition coefficient (K_{oc}) values, it is unlikely that they will adsorb to suspended soils and sediments. The nitroaniline isomers are not expected to readily volatilize from water. Aromatic amines are susceptible to degradation through reactions with photochemically-produced radicals. Half-lives for aniline are approximately 19-30 hours of sunlight depending on the type of oxidant. The nitro moiety in ONA, MNA, and PNA may cause these reactions to proceed somewhat slower. Bioconcentration of the three isomers in aquatic organisms is expected to be low (DHHS, 2016a, 2016b, 2016c).

Terrestrial

Based on their K_{oc} values, ONA, MNA, and PNA may have high mobility in soil. At the same time, the amine moiety in the nitroaniline isomers can bind strongly to humus or organic matter in the soil, suggesting that mobility may vary among soil types. ONA, MNA, and PNA are not expected to readily volatilize from soil. Studies suggest that the nitroaniline isomers are not readily biodegraded (DHHS, 2016a, 2016b, 2016c).

Analytical Laboratory Methods (from DHHS, 2016a, 2016b, 2016c)

Method: EPA-EAD 1625
Procedure: Gas chromatography/mass spectrometry
Matrix: Water
Detection Limit: Not provided for ONA, MNA, or PNA

Method: EPA-OSW 8901

Procedure: Gas chromatography with either electron capture detection or nitrogen-phosphorus detection
Matrix: Water, soil, and waste matrices
Detection Limit: Not provided for ONA, MNA, or PNA

Method: EPA-OSW 8270D
Procedure: Gas chromatography /mass spectrometry with ion trap detector
Matrix: Solid waste matrices, soils and groundwater
Detection Limit: 50 ug/L (ONA and MNA), 20 ug/L (PNA)

Method: DOE OM100R
Procedure: Gas chromatography/mass spectrometry with ion trap detector
Matrix: Solid waste matrices, soils, and groundwater
Detection Limit: 200 ug/L (ONA), 71 ug/L (MNA), 54 ug/L (PNA)

Method: EPA 8131
Procedure: Gas chromatography
Matrix: Aqueous matrices
Detection Limit: 1.0 ug/L (ONA), 3.3 ug/L (MNA), 11.0 ug/L (PNA)

Method: EPA 8410
Procedure: Gas chromatography/fourier transform infrared
Matrix: Wastewater, soils and sediment, and solid wastes
Detection Limit: 20 ug/L (ONA, MNA, and PNA)

Regulatory Summary:

ONA	
MCL	None
MCLG	None
LOAEL	450 mg/kg-d (EPA, 2009a; Sisti, 2001a,b)
NOAEL	150 mg/kg-d (EPA, 2009a; Sisti, 2001a,b)
Screening chronic reference dose (RfD)	0.015 mg/kg-d or 1×10^{-2} mg/kg-d (EPA, 2009a)
MNA	
MCL	None
MCLG	None
LOAEL	15 mg/kg-day (EPA, 2009b; Onodera, ND)
NOAEL	N/A[1]
Screening subchronic RfD	0.001 mg/kg-day (EPA, 2009b)

[1] The reproductive/developmental study identified a NOAEL of 5 mg/kg-day. However, EPA advised caution about interpreting this because hematological, clinical chemistry, and histopathological endpoints were not evaluated (EPA, 2009b).

PNA

MCL	None
MCLG	None
LOAEL	1.5 mg/kg-day (EPA, 2009c; Nair et al., 1990)
NOAEL	0.25 mg/kg-day (EPA, 2009c; Nair et al., 1990)
$BMDL_{1SD}$	0.37 mg/kg-d (EPA, 2009c based on data from Nair et al., 1990)
Chronic provisional RfD	0.004 mg/kg-day (EPA, 2009c)
Provisional oral slope factor	0.02 per mg/kg-day (EPA, 2009c)

Recommendations and Conclusions

Recommended Drinking Water Health Advisory Level for 2-Nitroaniline (ONA)

The EPA screening chronic reference dose (RfD) for ONA (0.01 mg/kg-day) was used as an Acceptable Daily Intake value to derive a recommended drinking water health advisory level as follows:

$$\text{Health Advisory Level (ug/L)} = \frac{\text{Acceptable Daily Intake x Body Weight (kg) x 100 \%}}{\text{Water Intake (L/d)}}$$

where:

Body weight = 10 kg
Water Intake = 1 L/d

$$\frac{\text{0.01 mg/kg-d x 10 kg x 100 \%}}{\text{1 L/d}} = \text{0.1 mg/L or 100 ug/L}$$

Therefore, DHS recommends 100 ug/L as a drinking water health advisory level for ONA.

Recommended Drinking Water Health Advisory Level for 3-Nitroaniline (MNA) and 4-Nitroaniline (PNA)

As mentioned above, no studies were located that examined the toxic effects of MNA over a subchronic or chronic exposure duration. A single short-term study (28 days) was conducted by the Japanese Ministry of Health; the results suggest that, similar to PNA, MNA can increase metHb levels, cause subsequent anemia, and trigger compensatory hematopoieisis. These findings are supported by the results of several *in vitro* and acute *in vivo* studies that demonstrate the ability of MNA to induce methemoglobinemia.

In its review of the toxicity information on MNA, EPA found that the limitations with the study and the available toxicity database on MNA were significant enough to preclude derivation of a provisional peer-reviewed toxicity value (EPA, 2009). However, EPA developed a screening subchronic RfD based on a LOAEL from the short-term study and using an uncertainty factor of 10,000, which is composed of a 10-fold factor for interspecies differences, a 10-fold factor for intraspecies variability, a 3-fold factor for the use of a study with a less-than-subchronic exposure duration, a 10-fold factor for the use of a LOAEL to calculate a RfD, and a 3-fold factor for database insufficiencies. To use the EPA screening subchronic RfD to derive a recommended drinking water health advisory, which is intended to protect against chronic exposure, an additional uncertainty factor would need to be incorporated. The use of the resulting

large uncertainty factor would be unprecedented in the development of a drinking water health advisory level.

As such, the similarities and differences between MNA and the other isomers were considered to explore the possibility of recommending a drinking water health advisory level that applies to a group of the isomers. In this assessment, DHS considered the following:

- As possible breakdown products of various dinitrotoluene isomers, it is plausible that MNA may occur in the environment together with ONA and PNA.
- Evidence suggests that MNA may cause similar non-cancer effects (i.e., methemoglobinemia induction) as ONA and PNA.
- EPA developed a provisional oral slope factor for PNA based on a study demonstrating equivocal evidence of carcinogenicity in male mice. This was supported by a battery of *in vitro* assays that suggest that PNA has mutagenic and clastogenic properties.
- No studies were located that examined potential carcinogenicity of ONA or MNA.
- MNA has been assessed with a smaller variety of i*n vitro* genotoxicity tests than PNA, but where similar tests have been performed, the results are generally similar, suggesting that MNA may also have mutagenic and clastogenic properties. *In vitro* genotoxicity testing of ONA does not provide evidence that the compound is genotoxic.

Were DHS to recommend a drinking water health advisory that applies to PNA, the EPA provisional oral slope factor for PNA (0.02 per mg/kg-day) would be used. Based on the similarities between MNA and PNA described above, it is appropriate to develop a group drinking water health advisory level that is based on the provisional oral slope factor for PNA and applies to both MNA and PNA. This is calculated as follows:

$$\text{Health Advisory Level (ug/L)} = \frac{\text{Target Cancer Risk x Body Weight (kg)}}{\text{Oral Slope Factor (per mg/kg/d) x Water Intake (L/d)}}$$

where:

Target Cancer Risk = 10^{-6} (1 in 1 million cancer risk)
Body weight = 70 kg
Water Intake = 2 L/d

$$\frac{10^{-6} \text{ x } 70 \text{ kg}}{0.02 \text{ per mg/kg-day x } 2 \text{ L/d}} = 0.002 \text{ mg/L or } 2 \text{ ug/L}$$

For the reasons described above, **DHS recommends a drinking water health advisory level of 2 ug/L that applies to the summed concentration of PNA and MNA.**

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Interim Drinking Water Health Advisory for 2,4-Diaminotoluene

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Introduction

The primary use of 2,4-diaminotoluene (2,4-DAT) has been as an intermediate in the production of 2,4-toluene diisocyanate, which in turn is used to produce polyurethane. 2,4-DAT has also been used in the production of dyes to color hair, silk, wool, cotton, paper, varnishes, wood stains, furs, and leather (NTP 2013). Other applications include the preparation of impact resins, polyamides with superior wire-coating properties, antioxidants, hydraulic fluids, urethane foams, and fungicide stabilizers, and as a photographic developer (US DHHS 2016).

Chemical Profile

Chemical Name	2,4-Diaminotoluene
CAS No.	95-80-7
Chemical Formula	$C_7H_{10}N_2$
Molecular Weight	122.167
Density	1.521 at 25°C
Solubility in Water	3.18×10^4 mg/L at 25°C; very soluble in water, ethanol, ethyl ether; soluble in acetone, carbon disulfide
Synonyms	4-Methylbenzene-1,3-diamine; 4-Methyl-1,3-benzenediamine; 1,3-Diamino-4-methylbenzene; 2,4-Diamino-1-methylbenzene; 5-Amino-o-toluidine; 3-Amino-p-toluidine; 2,4-Diaminotoluen; Diaminotoluene; 2,4-Diamino-1-toluene; 2,4-Diaminotoluol; 4-Methyl-m-phenylenediamine; 2,4-Tolamine; M-Toluenediamine; 2,4-Toluenediamine; 2,4-Toluylenediamine; Tolylene-2,4-diamine; M-Toluylendiamin; M-Toluylenediamine; M-Tolyenediamine; M-Tolylenediamine; 4-M-Tolylenediamine; CI Oxidation Base; CI Oxidation Base 20; CI Oxidation Base 35; CI Oxidation Base 200; CI 76035; Nako TMT; NCI-CO23O2; Pelagol J; Pelagol grey J; Tertral G; Pontamine developer TN; Renal MD; MTD; TCA; TDA; Zoba GKE; Zogen developer H; Azogen developer H; Benzofur MT

Occurrence

According to the Environmental Protection Agency's (EPA's) Toxics Release Inventory, 2,4-DAT is primarily released to air, with total releases prior to 2003 ranging from 500 to 4,000 pounds. However, there have been some large releases to soil and groundwater. Over 6,000 pounds were released to an off-site nonhazardous-waste landfill in 1991 and 54,000 pounds to an off-site underground injection well in 1998. Since 2003, most 2,4-DAT waste has been sent to off-site hazardous and nonhazardous waste landfills. In 2007, releases totaled 18,220 pounds, of which 17,000 lb was released to an off-site hazardous-waste landfill and nearly all of the rest to air (NTP 2014).

In Wisconsin, 2,4-DAT has been detected in groundwater monitoring wells at the Badger Army Ammunition Plant at levels as high as 380 ppb, but has not been detected at the U.S. Army installation at Fort McCoy or the Barksdale Dupont site. The presence of 2,4-DAT at this site likely resulted from the microbial degradation of residual 2,4-dinitrotoluene in soil and groundwater (Bradley et al., 1997; Cheng et al., 1996; Freedman et al., 1996; Liu et al., 1984; Noguera and Freedman, 1996, 1997).

Human Exposure Routes

Occupational exposure to 2,4-DAT can occur through inhalation of air in polyurethane manufacturing plants (IARC 1978). For those living near waste sites or military bases with 2,4-DAT-contaminated soil and/or groundwater, the primary route of exposure would be through ingestion of drinking water from contaminated wells. Dermal or inhalation exposures from contaminated groundwater or soil are potential secondary routes of exposure, but would be unlikely to result in acute effects at the concentrations previously detected.

Acute Toxicity

In humans, acute exposures to 2,4-DAT can cause severe skin, eye and respiratory irritation (Von Oettingen 1941; WHO 1987). Inhalation of 2,4-DAT fumes can trigger asthma and cause coughing, labored breathing, and respiratory distress (WHO 1987). Exposure to high levels can cause dizziness, convulsions, fainting and death (US EPA 2000). No statistically significant dose-dependent contact hypersensitivity response to 2,4-DAT was demonstrated in mice when the site of sensitization was prepared using shaving and dermabrasion with or without adjuvant (NTP 1990).

2,4-DAT is also an ocular and dermal irritant in animals. In rabbits, instillation of 100 µg 2,4-DAT in the eye and dermal application of 500 mg 2,4-DAT caused severe irritation and blisters after 24 hours (WHO 1987). The acute toxic effects of 2,4-DAT following ingestion are marked by central nervous system depression, severe jaundice and methemoglobinemia, 6 to 8 hours after exposure (Selye 1973; Waring and Pheasant 1976).

The acute oral toxicity (LD_{50}) values for 2,4-DAT in rodents following single-dose intraperitoneal injection ranged from 80 to 480 mg/kg (Weisburger et al. 1978; Grantham et al.

1980). Industry studies have found that the LD_{50} values for 2,4-DAT are 179 to 212 mg/kg for male rats, 380 mg/kg for male mice and approximately 500 mg/kg for rabbits (US EPA 1986).

Chronic Toxicity

No information is available regarding the long-term effects of 2,4-DAT in humans. Chronic animal studies conducted by the National Cancer Institute (NCI) in rats and mice have reported some non-cancer toxicities (NCI 1979). In the NCI studies, groups of 50 male and 50 female F344 rats were exposed via diet to a time-weighted average dose of 79 ppm (low dose; both sexes) or 171 ppm (high dose; females) and 179 ppm (high dose; males) for up to two years, depending upon survival. Dietary exposures were converted to 3.95 mg/kg/day for the low-dose rats, 8.55 mg/kg/day for the high-dose female rats, and 8.80 mg/kg/day for the high-dose male rats by multiplying the dose in parts per million by the EPA-derived food factor of 0.05, which is the fraction of a species body weight consumed per day as food (US EPA 1980).

In rats, there was a significant dose-related decrease in body weight gain and survival rate, necessitating termination of the initial high-dose groups after 79 weeks for the males and after 84 weeks for the females (NCI 1979). Although chronic renal disease is usually present in aging F344 rats, renal disease in the rats exposed to dietary 2,4-DAT for 79 to 103 weeks was appreciably more severe and occurred at an earlier age than in the control rats (NCI 1979). In the same rats, histopathological lesions in the liver of both sexes and dose groups were observed at increased incidences and ranged from focal fatty changes to severe diffuse degeneration (NCI 1979).

In the same study, 50 male and 50 female B6C3F1 mice were exposed to dietary levels of 100 or 200 ppm of 2,4-DAT for 101 weeks (NCI 1979). Dietary exposures were converted to 13 and 26 mg/kg/day after multiplying by the food factor of 0.13 for mice (US EPA 1980). Mean body weights were decreased compared with controls, except in the low-dose male group (NCI 1979). The survival rate of the mice was unaffected. The incidence of liver hyperplasia was significantly elevated above controls in both male and female mice at both dose levels (NCI 1979). Despite the identification of some non-cancer toxicities in rats and mice treated with 2,4-DAT, EPA has not evaluated the toxicological literature to determine whether there is adequate data to derive a reference dose (RfD) for 2,4-DAT.

Carcinogenicity

US EPA:	Group B2, probable human carcinogen
IARC:	Group 2B, possibly carcinogenic to humans
NTP:	Reasonably anticipated to be a human carcinogen

No studies were located that evaluated the relationship in humans between cancer and exposure specifically to 2,4-DAT. In rats and mice, dietary administration of 2,4-DAT produced a significant increase in the incidence of many tumor types, including liver, mammary gland, subcutaneous fibromas, and lymphomas (US EPA 1986).

One study treated 25 male Charles River/CD rats with 2,4-DAT at 300 and 625 mg/kg, along with 25 male and female CD-1 mice at 500 and 1000 mg/kg for 18 months (Weisburger et al. 1978). The rats and mice used in this study had a high incidence of spontaneous tumors. However, there was a statistically significant increase in subcutaneous fibromas in male rats and hepatocellular carcinomas and vascular tumors in male and female mice compared with controls (Weisburger et al. 1978).

Studies by the NCI on the carcinogenicity of 2,4-DAT in rats and mice (NCI 1979) confirmed the report by Weisburger et al. The NCI study administered 2,4-DAT at time-weighted average doses of 3.95 and 8.8 mg/kg/day to 50 male Fisher 344 rats, and doses of 3.95 and 8.55 mg/kg/day (transformed from ppm) to 50 female Fisher 344 rats for 103 weeks (NCI 1979). The researchers observed dose-related development of hepatocellular carcinomas or neoplastic nodules in treated rats of both sexes. In addition, NCI reported that carcinomas and adenomas of the mammary gland occurred in female rats at incidences that were dose related and significantly greater than those in the controls in both the high- and low-dose groups.

Groups of 50 male and 50 female B6C3F1 mice were similarly administered 2,4-DAT at 13 or 26 mg/kg/day (transformed from ppm) for 101 weeks (NCI 1979). In male mice, tumor incidence was not significantly increased compared with that in the control animals. However, the incidence of hepatocellular carcinomas in treated female mice was dose-related and significantly higher than that in the controls. Numbers of lymphomas were also higher in low-dose female mice.

On the basis of these results, EPA concluded that 2,4-DAT was carcinogenic for Fisher 344 rats of both sexes and for female B6C3F1 mice (NCI 1979). EPA has also concluded that there was sufficient evidence of carcinogenicity in experimental animals, but insufficient evidence in humans, deeming 2,4-DAT a "probable human carcinogen". EPA has calculated a provisional oral cancer slope factor of 3.2 $(mg/kg/d)^{-1}$ based on the increased incidence of mammary tumors in female rats (US EPA 1986).

Mutagenicity/Genotoxicity

Studies in *Salmonella typhimurium* strains TA1538 and TA98 have found 2,4-DAT to be a potent mutagen after metabolic activation, but only a weakly mutagen in bacteria without activation (WHO 1987). 2,4-DAT was found to be a weak mutagen in *Drosophila melanogaster* and nonmutagenic in *Neurospora crassa*, but these results may have been due to the lack of a mammalian metabolic activation system in these studies (US EPA 1986).

In mammalian cells, 2,4-DAT was mutagenic at the thymidine kinase locus in mouse lymphoma L5178Y cells without metabolic activation and mutagenic to Chinese hamster ovary AT3-2 cells both with and without metabolic activation; however, negative results were observed at the *hgprt* locus in both cell types (Coppinger et al. 1984). In the presence of 2,4-DAT, hamster embryo cells were morphologically transformed *in vitro* without exogenous metabolic activation (Pienta and Kawalek 1981). Injection of male mice with 2,4-DAT at 40 mg/kg did not induce obvious chromosomal strand breaks in isolated bone marrow cells 30 and 48 hours post-treatment (Soares and Lock 1980).

Reproductive and Teratogenic Effects

In humans occupationally exposed to 2,4-DAT, several studies did not report any statistically significant reproductive or developmental effects (US EPA 1986). Developmental and reproductive effects were observed in animals, including a significant decrease in the number of births and increases in maternal deaths, stillbirths, and resorptions (US EPA 1986).

In DBA/2J mice, administration of 2,4-DAT orally or via intraperitoneal injection at 40 mg/kg body weight for 48 hours, followed by eight week mating trials did not reveal any treatment-related effects on sperm morphology or fertility, as measured by the dominant lethal assay (Soares and Lock 1980). However, in male Sprague Dawley rats, long-term exposure to 2,4-DAT in the feed impaired reproductive performance and capacity (Thysen et al. 1985a; Thysen et al. 1985b). Exposure to dietary levels of approximately 15 mg/kg of 2,4-DAT for 10 weeks decreased fertility and exerted an inhibitory effect on sperm production in male rats. Eleven weeks after treatment, the sperm count remained significantly depressed, suggesting irreversible damage to the germinal components in the testes. After 10 weeks of treatment and 11 weeks after treatment was stopped, data from hormone analyses showed significant decreases in serum-testosterone and an elevation of serum-luteinizing hormone concentrations, which were associated with a reduction in seminal vesicle weight. Histological changes found in the reproductive organs from treated males were correlated with these physiological changes. At approximately 5 mg/kg body weight, 2,4-DAT did not cause any of these toxic responses.

Repeated skin application of 2,4-DAT at 2 mL/kg body weight throughout gestation induced a low incidence of skeletal changes in rats (Burnett et al., 1976). Another study found a statistically significant increase in the number of resorptions, maternal deaths and stillbirths in CD-1 mice treated treated by gavage with 150 mg/kg/day of 2,4-DAT on days 7 through 14 of pregnancy, compared to controls (Smith 1983).

Interactive Effects

No data on interactive effects of 2,4-DAT with other chemicals could be located.

Environmental Fate

Atmospheric

When 2,4-DAT is released to air, it may photolyze and react with photochemically generated hydroxyl radicals, with an estimated half-life of 8 hours (NTP 2014).

Terrestrial and Aquatic

Because 2,4-DAT is soluble in water and has a low soil sorption partition coefficient, it will most likely leach into the subsurface when released to soil. However, it is not likely to volatilize from

either water or soil. When 2,4-DAT is released to water, it is most likely to remain in solution, where it is subject to biodegradation and photooxidation (US DHHS 2016).

Analytical Laboratory Methods

Method: EPA-EAD 1625
Procedure: gas chromatography/mass spectrometry
Analyte: 2,4-diaminotoluene
Matrix: water
Detection Limit: not provided (US DHHS 2016).

Method: EPA-RCA 8270D
Procedure: gas chromatography/mass spectrometry
Analyte: 2,4-diaminotoluene
Matrix: solid waste matrices, soils, air sampling media and water
Detection Limit: 10 μg/L (US DHHS 2016).

Regulatory Summary

MCL	None
MCLG	None
LOAEL	3.95 mg/kg/day
NOAEL	None
Uncertainty factor	3000
RfD	1.3×10^{-3} mg/kg/day
Cancer classification	EPA Group B2, probable human carcinogen; IARC Group 2B, possibly carcinogenic to humans; NTP, reasonably anticipated to be a human carcinogen
Provisional cancer slope factor	$3.2\ (mg/kg/day)^{-1}$ for oral exposure (US EPA 1986)

Recommendations and Conclusions

Non-cancer

In the two-year NCI bioassay, significant and dose-related decreases in body weight gain and survival in rats were the most sensitive non-cancer toxic endpoints observed (NCI 1979). The decreases in body weight gain and survival in rats occurred at all doses, including the lowest dose of 2,4-DAT administered (3.95 mg/kg/day). Although EPA has not evaluated the toxicological literature to determine whether there is adequate data to derive an RfD for 2,4-DAT, the Wisconsin Department of Health Services (DHS) has calculated an RfD of 1.3×10^{-3} mg/kg/day, based on a lowest-observed-adverse-effect level (LOAEL) of 3.95 mg/kg/day and application of a cumulative uncertainty factor of 3000 (10 for extrapolation from rats to humans,

10 for protection of sensitive individuals, 10 for use of a LOAEL instead of a NOAEL and 3 for deficiencies in the database).

Consistent with the methods outlined in Chapter 160 of the Wisconsin State Statutes, the following Health Advisory Level was calculated:

$$\text{Health Advisory Level } (\mu g/L) = \frac{\text{Acceptable Daily Intake x Body Weight (kg) x 100\%}}{\text{Water Intake (L/d)}}$$

where:
Body weight = 10 kg
Water Intake = 1 L/d

$$\frac{0.0013 \text{ mg/kg/d x 10 kg x 100 \%}}{1 \text{ L/d}} = 0.013 \text{ mg/L or } 13 \ \mu g/L$$

Cancer

The U.S. EPA derived a provisional oral cancer slope factor based on the increased incidence of mammary tumors in female rats seen in the two-year NCI bioassay (US EPA 1986). Consistent with the methods outlined in Chapter 160 of the Wisconsin State Statutes, the following Health Advisory Level was calculated:

$$\text{Health Advisory Level } (\mu g/L) = \frac{\text{Target Cancer Risk x Body Weight (kg)}}{\text{Oral Slope Factor (per mg/kg/d) x Water Intake (L/d)}}$$

where:
Target Cancer Risk = 10^{-6} (one-in-a-million cancer risk)
Body Weight = 70 kg
Water Intake = 2 L/d

$$\frac{10^{-6} \text{ x 70 kg}}{3.2 \text{ per mg/kg/d x 2 L/d}} = 0.011 \ \mu g/L$$

Conclusion

DHS recommends the use of 0.011 µg/L as the Health Advisory Level for 2,4-DAT, as it is lower and more conservative than that calculated for non-cancer effects.

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Interim Drinking Water Health Advisory for 2,6-Diaminotoluene

Prepared by Ryan Wozniak
Wisconsin Department of Health Services, April 4, 2017

Introduction

Most 2,6-diaminotoluene (2,6-DAT) is used as part of mixture of diaminotoluenes known as Meta-DAT, composed of either an 80:20 or 65:35 mixture of 2,4- and 2,6-DAT, respectively (WHO 1987). Meta-DAT is used primarily in the synthesis of toluene diisocyanates, which in turn are used to produce polyurethane. 2,6-DAT is also used as an intermediate in the synthesis of several dyes used for textiles and furs. In 2002, the reported production plus imports of 2,6-DAT in the US was between 10,000 to 500,000 lbs (US EPA 2002).

Chemical Profile

Chemical Name	2,6-Diaminotoluene
CAS No.	823-40-5
Chemical Formula	$C_7H_{10}N_2$
Molecular Weight	122.167
Density	1.0913 at 25°C
Solubility in Water	3.17×10^4 m/L at 25°C; very soluble in water, ethanol, benzene
Synonyms	2,6-Toluenediamine; Toluene-2,6-diamine; 2,6-Toluylenediamine; 2,6-Tolylenediamine; 2-Methyl-1,3-benzenediamine; 2,6-Diamino-1-methylbenzene; 2-Methyl-m-phenylenediamine; 2-Methyl-1,3-phenylenediamine; NCI-C50317

Occurrence

An estimated 6000 metric tons of diaminotoluenes (DATs) are disposed into authorized landfills, and roughly 1400 metric tons of DATs are released to the environment during the production of the chemicals (WHO, 1987). More recent data from the US EPA Toxics Release Inventory database showed that 3967 pounds of DATs were released into the environment in 2013, with the vast majority being released into the air (US DHHS 2016). Additionally, total off-site waste transfer amounted to 8,168,463 pounds in the same year (US DHHS 2016).

2,6-DAT has not been detected in the groundwater in Wisconsin. However, it has been hypothesized that 2,6-DAT could be produced from the microbial degradation of 2,6-dinitrotoluene (2,6-DNT) at sites with residual 2,6-DNT contamination in soil and groundwater (Yang et al. 2008). Due to the high water solubility of 2,6-DAT, leakage of 2,6-DAT from landfills or storage sites and accidental spillage during shipping and handling may also lead to surface and groundwater contamination (WHO 1987).

Human Exposure Routes

In occupational settings, the primary exposure routes are expected to be dermal contact or inhalation of 2,6-DAT at polyurethane foam processing plants. Air samples collected near the demolding step of the polyurethane foam manufacturing process detected 2,6-DAT at concentrations ranging from 60 to 110 ppm (Lewandowski 2005). For those living near waste sites or military bases with 2,6-DAT-contaminated soil and/or groundwater, the primary route of exposure would be through ingestion of drinking water from contaminated wells. Dermal or inhalation exposures from contaminated groundwater or soil are potential secondary routes of exposure.

Acute Toxicity

In humans, acute exposures to 2,6-DAT can cause severe skin, eye and respiratory irritation (WHO 1987). In animals, most of the available data on the acute and sub-chronic toxicity of 2,6-DAT comes from a National Cancer Instute (NCI) study published in 1980 (NCI 1980). For rats, the single oral dose that caused lethality was 3000 mg/kg for males and 1000 mg/kg for females (NCI 1980). Death occurred at all doses in mice, with half the mice (one out of two) dying at the lowest dose of 100 mg/kg (NCI 1980). Male and female rats exposed to 2,6-DAT in their diet for 14 days had reductions in their respective body weights of 10 and 52 percent at concentrations of 1000 ppm, and 47 and 88 percent at concentrations of 3000 ppm, compared to controls (NCI 1980). There was elevated mortality among mice given a diet containing 2,6-DAT at 3000 ppm for 14 days. No effect was seen on body weights of either rats or mice after 14 days on a diet containing 300 ppm 2,6-DAT (NCI 1980).

In a 13-week sub-chronic study in rats and mice given 2,6-DAT in their diet, lower weight gain was noted in male rats at 100 ppm (the lowest dose) and in female rats at 1000 ppm (NCI 1980). This effect was also noted in mice, at 300 ppm for males and at 1000 ppm for females, with no effect on weight gain at 100 ppm (NCI 1980). The rats developed thyroid hyperplasias at exposures of 3000 ppm or higher. Renal hyperpigmentation and a papilloma in the forestomach were observed in the mice at 1000 ppm (NCI 1980).

Chronic Toxicity and Carcinogenicity

No studies of the chronic toxicity or carcinogenicity of 2,6-DAT in humans were located in the available literature. In animals, a 2-year bioassay conducted by the NCI using F344 rats and B6C3F1 mice is the only long-term study that has investigated the chronic toxicity and carcinogenicity of 2,6-DAT (NCI 1980). In the NCI study, rats (n=50/sex) and mice (n=50/sex) were exposed to 2,6-DAT dihydrochloride in their diet for 103 weeks. Rats were exposed to 0, 250 or 500 ppm of 2,6-DAT in their diet, while mice were exposed to 0, 50 or 100 ppm. At sacrifice or upon death of the animal, gross examination was performed on all major tissues, and 26 organs and tissues were collected for histologic examination. Using body weight data graphically supplied in the study report, allometric equations for food consumption, and adjusting for molecular weight of the dihydrochloride salt, doses of 2,6-DAT were estimated as

0, 12 or 25 mg/kg/day in male rats; 0, 15 or 30 mg/kg/day in female rats; and 0, 5 or 10 mg/kg/day in male and female mice (US EPA 1988).

Chronic Toxicity

Chronic treatment with 2,6-DAT at the doses listed above did not affect survival of rats, which was adequate for assessment of late-developing tumors (NCI 1980). In comparison to controls, body weight was reduced through much of the study in high-dose males (7 percent decrease in time-weighted average body weights), and low and high-dose females (9 and 11 percent, respectively) (NCI 1980). No treatment-related clinical abnormalities were reported at any dose level in male or female rats and the incidence of nonneoplastic lesions in treated rats did not differ significantly from controls (NCI 1980). The small decreases in body weight in treated rats are not considered an adverse effect, making the high dose of 500 ppm (25 mg/kg/day in males and 30 mg/kg/day in females) a no-observed-adverse-effect level (NOAEL) in this study.

In mice, treatment with 2,6-DAT did not result in any effects on survival, clinical signs of toxicity or body weight gain (NCI 1980). No nonneoplastic changes, either at the gross or microscopic level, could be attributed to treatment with 2,6-DAT (NCI 1980). The high dose of 100 ppm (10 mg/kg/day in both males and females) is a NOAEL in this study.

Carcinogenicity

In rats, there was a marginally significant dose-related trend for increased incidence of hepatic neoplastic nodules or hepatocellular carcinomas in males (0/50, 2/50, 4/50 in the control, low- and high-dose groups, respectively); however, the researchers did not consider this to be a treatment-related effect, as none of the treatment groups were significantly different from controls in pairwise comparisons (NCI 1980). Similarly, a dose-related trend was observed for the incidence of animals with islet-cell adenomas of the pancreas in male rats (0/45, 1/46, 4/45 in the control, low- and high-dose groups, respectively), but was not considered treatment-related by the researchers because pairwise comparisons did not attain statistical significance. Other neoplasms occurred with similar incidence in treated and control rats. The researchers concluded that 2,6-DAT was not carcinogenic to rats in this bioassay, although it is not clear that the maximum tolerated dose (MTD) was achieved.

In mice, a slight increase in the incidence of vascular neoplasms of the spleen and liver was seen in male mice (1/50, 5/50, 3/50 in the control, low- and high-dose groups, respectively), but no dose-related trend was seen, and the differences were not statistically significant (NCI 1980). Also in male mice, there was a significant trend for increased lymphomas relative to controls (2/50, 8/50, 2/50 in the control, low- and high-dose groups, respectively); however, the only apparent change was in the low-dose group, and the increase in this group was not statistically significant after adjustment for multiple comparisons. Female mice showed a significant trend for hepatocellular carcinoma (0/50, 0/49, 3/49 in the control, low- and high-dose groups, respectively), but the researchers did not consider this change to be treatment related because pairwise comparisons were not statistically significant. This, the researchers concluded that 2,6-DAT was not carcinogenic to mice in this bioassay, but acknowledged that the MTD was not achieved.

Mutagenicity/Genotoxicity

2,6-DAT is a potent mutagen in *Salmonella typhimurium* when tested with metabolic activation, but it is not mutagenic without metabolic activation (Ashby and Tennant 1988; Cheung et al. 1996; Cunningham et al. 1989; Dybing and Thorgeirsson 1977; Florin et al. 1980; George and Westmoreland 1991; Sayama et al. 1989). In mammalian cells treated *in vitro*, 2,6-DAT was negative in assays for unscheduled DNA synthesis (UDS) in primary cultured rat hepatocytes treated (Butterworth et al. 1989; Selden et al. 1994), but was positive for UDS in primary cultured human hepatocytes (Butterworth et al. 1989). Assays for induction of micronuclei in Chinese hamster ovary cells with or without S9 (Miller et al. 1995) and cell transformation in primary hamster embryo cells (Greene and Friedman 1980) were also positive. An assay for DNA fragmentation in cultured rat hepatocytes was negative (Allavena et al. 1992), but low levels of covalent binding to DNA were reported in another study in cultured rat hepatocytes (Furlong et al. 1987).

In vivo, 2,6-DAT did not induce mutations in *lac*I transgenic male mice (Hayward et al. 1995). Assays for UDS in hepatocytes isolated from male rats treated with 150 mg/kg of 2,6-DAT by gavage in corn oil (Mirsalis et al. 1982) or 300 mg/kg by gavage in water (George and Westmoreland 1991) were negative, but positive results were obtained in hepatocytes from males rats treated with 2000 mg/kg by gavage in aqueous carboxymethylcellulose suspension (Allavena et al. 1992). A bone marrow micronucleus assay in rats was negative at oral doses of 1000-2000 mg/kg in aqueous carboxymethylcellulose suspension (Allavena et al. 1992), but weak positive results were found in rats treated with 300 to 600 mg/kg by gavage in water in another study (George and Westmoreland 1991), and intraperitoneal (i.p.) doses as low as 31 mg/kg produced significant increases in bone marrow micronuclei in mice (Shelby et al. 1993). Assays for detection of DNA fragmentation (alkaline elution/ electrophoresis) showed no evidence of DNA damage in the liver, kidney, bladder, colon, stomach, lung, brain or bone marrow of mice treated by oral gavage with 60 mg/kg (Sasaki et al. 1999) or in the liver of rats treated with 1000 mg/kg orally (Allavena et al. 1992), but did find significant increases in DNA fragments in rat liver after oral dosing with 2000 mg/kg (Allavena et al. 1992). Assays for formation of DNA adducts in liver were negative in rats treated with up to 500 mg/kg i.p. (La and Froines 1993; Taningher et al. 1995). 2,6-DAT (50 mg/kg i.p. 5 days/week for 6 weeks) did not promote preneoplastic liver foci in partially hepatectomized male rats initiated with diethylnitrosamine (Taningher et al. 1995).

Reproductive and Teratogenic Effects

No studies investigating the reproductive effects of 2,6-DAT in humans or animals could be located. One study was located that investigated the teratogenic effects of 2,6-DAT in rats and rabbits (Knickerbocker et al. 1980). Rats were administered oral doses of 10, 30, 100, and 300 mg/kg, while rabbits received oral doses of 3, 10, 30, and 100 mg/kg on gestational days 6 through 15 (Knickerbocker et al. 1980). In rats, the three highest doses of 2,6-DAT administered (30, 100, and 300 mg/kg) produced an increase in fetuses with hemorrhagic abdomens, the two highest doses (100 and 300 mg/kg) resulted in an increase in the occurrence of incomplete vertebrae, and the highest dose (300 mg/kg) resulted in missing sternebrae and incomplete closure of the skull in fetuses (Knickerbocker et al. 1980). Thus, the no-observed-adverse effect

level (NOAEL) for teratogenic effects in rats is 10 mg/kg. In rabbits treated with 2,6-DAT during gestation, no skeletal or soft-tissue abnormalities were observed in their offspring, but increased resorptions, along with decreased fetal body weights and survival rates were reported at a dose of 100 mg/kg, resulting in a NOAEL of 30 mg/kg (Knickerbocker et al. 1980).

Interactive Effects

No data on interactive effects of 2,6-DAT with other chemicals could be located.

Environmental Fate

Atmospheric

If released to air, a vapor pressure of 2.46×10^{-3} mm Hg at 25°C indicates 2,6-DAT will exist solely as a vapor in the atmosphere. Vapor-phase 2,6-DAT will be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 0.6 hours (US DHHS 2016).

Terrestrial and Aquatic

If released to soil, 2,6-DAT is expected to have low mobility and to adsorb more strongly to soils containing organic carbon and clay than their neutral counterparts. 2,6-DAT will not volatilize from dry soil surfaces based upon its vapor pressure. If released into water, 2,6-DAT is expected to adsorb to suspended solids and sediment. Volatilization from water surfaces is not expected to be an important fate process. The potential for bioconcentration of 2,6-DAT in aquatic organisms is low. Hydrolysis is not expected to be an important environmental fate process since this compound lacks functional groups that hydrolyze under environmental conditions (pH 5 to 9) (US DHHS 2016).

Analytical Laboratory Methods

High-performance liquid chromatography with ultraviolet and electrochemical detection can be used to measure 2,6-DAT in water samples (Riggin and Howard 1983).

Regulatory Summary

MCL	None
MCLG	None
LOAEL	None
NOAEL	25 mg/kg/day
Uncertainty factor	1000
Provisional chronic RfD	0.03 mg/kg/day
Cancer classification	None
Cancer slope factor	None

Recommendations and Conclusions

Non-cancer

A provisional chronic reference dose (RfD) of 0.03 mg/kg/day for 2,6-DAT was derived by applying an uncertainty factor of 1000 (10 for extrapolation from rats to humans, 10 for protection of sensitive individuals, and 10 for deficiencies in the database, including lack of reproductive and developmental toxicity studies) to the oral chronic NOAEL of 25 mg/kg/day in rats from the NCI study.

Confidence in the principal study is low. The study included exposure throughout the two-year study period and adequate numbers of male and female rats and mice in each dose group, but only two treated groups. The study was performed as a cancer bioassay, and included only limited evaluation of non-cancer endpoints. Statistical analysis of findings was performed only for cancer-related endpoints. The study did not identify a target organ effect or LOAEL in either species, indicating that doses tested were too low. Confidence in the database is low, as the only supporting study is the subchronic range-finding study described in the same report. Overall confidence in the provisional chronic RfD is low.

Consistent with the methods outlined in Chapter 160 of the Wisconsin State Statutes, the following Health Advisory Level was calculated:

$$\text{Health Advisory Level } (\mu g/L) = \frac{\text{Acceptable Daily Intake x Body Weight (kg) x 100\%}}{\text{Water Intake (L/d)}}$$

where:
Body Weight = 10 kg
Water Intake = 1 L/d

$$\frac{0.03 \text{ mg/kg/d x } 10 \text{ kg x } 100\%}{1 \text{ L/d}} = 0.30 \text{ mg/L or } 300 \ \mu g/L$$

Cancer

NCI evaluated the carcinogenic effects of 2,6-DAT in rats and mice in two-year feeding studies (NCI 1980). No treatment-related neoplasms were seen in males or females of either species. However, it is not clear that doses used in these studies were appropriate. In rats, the only effects were small changes in body weight in both dose groups that were not clearly adverse or related to treatment. In mice, no effects of any type were noted in the treated mice. Therefore, it appears that doses were too low, such that the MTD was not achieved, in the mouse study and possibly also the rat study. Consequently, these studies do not rule out the possibility of a tumorigenic effect of 2,6-DAT at doses higher than those tested. Genotoxicity studies indicate a strong potential for 2,6-DAT to produce mutations in bacteria with metabolic activation, and some potential to produce DNA and chromosomal effects in mammalian cells as well, particularly at high doses. Taking into account the too-low doses in the negative cancer bioassays in rodents, and the demonstrated genotoxic potential of the chemical, the available data are considered insufficient to assess the carcinogenic potential of 2,6-DAT in animals or humans.

According to the U.S. EPA Guidelines for Carcinogen Risk Assessment, there is inadequate information to assess the carcinogenic potential of 2,6-DAT (US EPA 2005).

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