2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO DNT IN THE UNITED STATES

Dinitrotoluene (DNT) is generally produced as a mixture called technical-grade DNT (Tg-DNT), which contains approximately 76.5% 2,4-DNT and 18.8% 2,6-DNT (with the remainder consisting of other isomers and minor contaminants such as TNT and mononitrotoluenes). It is primarily used as a chemical intermediate for the production of toluene diisocyanate. DNT is also used in the production of 2,4,6-TNT, dyes, and polyurethane foams. 2,4-DNT has reportedly been used in air bags of automobiles.

When released to the environment, DNT has the potential to leach into groundwater or contaminate surface waters through soil runoff and erosion. Volatilization from water bodies or soil surfaces occurs slowly for DNT and as a consequence, it is not frequently detected in ambient air. DNT generally biodegrades slowly under environmental conditions, but it is rapidly degraded by photolysis in natural water. Measured bioconcentration factors in fish suggest that the potential for bioaccumulation is low in fish and other aquatic organisms.

DNT is very infrequently detected in drinking water. There were no detections of 2,4-DNT in 3,251 samples taken from small public water systems and there was only 1 detection out of 30,513 samples obtained from large systems throughout the United States. 2,6-DNT was not detected in any of the 33,765 samples (both large and small systems) for which it was tested. DNT has been detected in surface water and groundwater near source locations such as munitions sites. Concentrations of 2,4- and 2,6-DNT obtained from a small brook and a river adjacent to a former ammunition plant were 0.5–13.0 μg/L and 0.1–7.6 μg/L, respectively. DNT levels as high as 10,000 μg/L were reported in potable groundwater at the Joliet Army Ammunition Plant located in Will County, Illinois. 2,3-, 2,5-, 3,4-, and 3,5-DNT isomers were identified in both monitoring wells and a few private water supply wells near an Army ammunition site in Wisconsin. 2,4-DNT was measured in soil samples obtained from the Joliet Army Ammunition at levels of <0.1 mg/kg (detection limit) to 117 mg/kg. 2,6-DNT was detected on this site at concentrations ranging from <0.1 to 8 mg/kg.

The major route of exposure to DNT for populations residing near hazardous waste sites or munitions facilities is via ingestion of contaminated water or dermal contact with contaminated soil. Dermal exposure to DNT could also occur when washing or bathing with contaminated water. Occupational exposure by inhalation or dermal contact may occur at workplaces where DNT is manufactured or used.
Monitoring data suggest that populations that do not live near source areas are not exposed to significant levels of DNT.

### 2.2 SUMMARY OF HEALTH EFFECTS

Data on health effects of DNT are available from occupational exposure studies and studies in laboratory animals. Most occupational exposure studies evaluate health effects in workers exposed to 2,4-, 2,6-, or technical-grade (Tg)-DNT. However, most of these studies were conducted before 1950 and provide very little information on exposure concentrations. Furthermore, interpretation of study results is limited due to the absence of appropriate control groups, mixed exposures to other chemicals, and small number of workers studied. Nearly all studies in laboratory animals are oral exposure studies of 2,4-, 2,6-, or Tg-DNT, and include data for acute, intermediate, and chronic exposure durations. Very little information is available regarding health effects of inhaled DNT; however, systemic effects of DNT are expected to be similar for all routes of exposure.

Results of occupational exposure studies and studies in laboratory animals identify the hematological (methemoglobinemia, anemia, and compensatory hematopoiesis) and nervous systems (clinical signs of neurotoxicity, ataxia, tremors, leg weakness, and convulsions) as the most sensitive targets of DNT-induced toxicity. In addition to hematological and neurological effects, results of animal studies provide evidence that the liver, respiratory tract, and reproductive system also are targets for DNT-induced toxicity. However, effects to these other organ systems occur at levels that are higher than those producing hematological effects or neurotoxicity under similar exposure conditions.

In occupational exposure studies, findings of anemia and cyanosis in workers exposed to 2,4- or Tg-DNT are consistent with the DNT-induced hematological effects observed in laboratory animals; however, available human data provide only limited evidence, as studies did not include adequate control groups or report exposure concentrations. Adverse hematological effects, specifically methemoglobinemia and anemia, have been reported in laboratory animals exposed to oral 2,4-, 2,6-, or Tg-DNT for acute, intermediate, and chronic durations. Hematological effects of DNT are initiated by methemoglobin production, which occurs when the ferrous iron in complex with the heme groups of hemoglobin is oxidized to ferric iron. Ferric iron does not bind oxygen and, therefore, reduces the oxygen-carrying capacity of the blood. Ferric iron also contributes to the denaturation of hemoglobin and subsequent removal of erythrocytes from the blood, resulting in anemia. Heinz bodies, granules of denatured hemoglobin, form and can be detected in erythrocytes. Increased hematopoiesis is often observed as a
compensatory response to decreased erythrocyte count. Hematological parameters typically affected by DNT exposure include increased blood methemoglobin levels and reticulocyte count, decreased erythrocyte count, hematocrit, and blood hemoglobin levels, and the presence of Heinz bodies; the severity of effects increases with dose. In addition, extramedullary erythropoiesis of the spleen, a compensatory response, may also occur. Results of a single-dose oral exposure study in rats indicate that the onset of hematological effects is rapid, with effects observed 24 hours after administration of 99 mg/kg of 2,4- or 2,6-DNT. For acute exposure durations, the lowest daily oral dose associated with hematological effects is 4 mg/kg/day in dogs exposed to 2,6-DNT for 14 consecutive days. Similar hematological effects have been observed following intermediate-duration exposure, with the development of methemoglobinemia, anemia, and compensatory erythropoiesis in dogs administered daily oral doses of 1.5 mg/kg of 2,4-DNT for 3–9 months. Extramedullary erythropoiesis of the spleen was observed in dogs administered ≥4 mg/kg/day of 2,6-DNT for 4–13 weeks. For chronic-duration exposure, hematological effects were observed following exposure of dogs to 2,4-DNT at 1.5 mg/kg/day for 24 months. Following cessation of exposure, hematological effects showed complete reversal, although time to reversal increased with severity of effects.

In occupational exposure studies, neurological effects, including headache, dizziness, insomnia, unpleasant taste in the mouth, and pain, numbness, and tingling in the extremities, also have been reported in workers exposed to 2,4- or Tg-DNT; however, available human data provide only limited evidence, as studies did not include adequate control groups or report exposure concentrations. The nervous system also is identified as a sensitive target for DNT-induced toxicity, with symptoms of neurotoxicity reported in laboratory animals exposed to oral 2,4-, 2,6-, and Tg-DNT for acute, intermediate, and chronic durations. However, results of these studies indicate that, in general, the nervous system is a less sensitive target than the hematological system. Symptoms of neurotoxicity observed in laboratory animals include weakness, stiffness, or rigid paralysis of the hind legs, abnormal gait, tremors, ataxia, and convulsions, with severity increasing with dose. For acute- and intermediate-duration exposures, the lowest doses producing symptoms of neurotoxicity are 25 mg/kg/day in dogs exposed to 2,4-DNT for 12 days to 13 weeks and 20 mg/kg/day in dogs exposed to 2,6-DNT for 13 weeks. Demyelination in the cerebellum and brain stem was observed in rats exposed to dietary exposures of 93 mg/kg/day 2,4-DNT for 13 weeks. In chronic-duration exposure studies, the lowest dose producing neurotoxicity was 1.5 mg/kg/day 2,4-DNT in a 2-year study in dogs; however, this effect (loss of hindquarter control) was only observed intermittently in one of six dogs. More severe signs of neurotoxicity and central nervous system lesions (vacuolization, hypertrophy, endothelial mitosis, focal gliosis in the cerebellum, and perivascular hemorrhage in the cerebellum and brain stem) occurred at 10 mg/kg/day.
Occupational exposure studies do not provide conclusive evidence of DNT-induced hepatotoxicity due to lack of appropriate controls. Hepatic effects have been observed in animals exposed to 2,4-, 2,6-, or Tg-DNT in laboratory animals. However, as discussed below, 2,4-, 2,6-, and Tg-DNT have been shown to induce hepatocellular carcinoma following chronic-duration oral exposure. Thus, hepatic effects observed at less-than-chronic exposure durations may represent early stages of progressive development to hepatic cancer. The potential for DNT to induce liver cancer complicates interpretation of study results showing possible effects in the liver, as hepatotoxicity may be a precursor to or a result from the development of hepatic neoplasms. Hepatic effects, including liver discoloration, inflammation, degeneration of hepatocytes, proliferation of bile duct epithelium, and elevated blood levels of hepatic enzymes, have been reported in oral exposure studies of 2,4-, 2,6-, or Tg-DNT in laboratory animals. Hepatic effects occurred at 2,4-DNT doses ≥10 mg/kg/day and at 2,6-DNT doses ≥7 mg/kg/day, with severity related to dose and exposure duration. At higher oral doses (≥50 mg/kg/day), onset of hepatic toxicity appears to be rapid; congested sinusoids with sloughed hepatocytes, infiltration of segmented neutrophils, pyknotic nuclei, microvesiculated cytoplasm, and apoptosis of hepatocytes were observed 48 hours after administration of 2,6-DNT. Following chronic dietary exposure to 2,4-DNT for 1 year, hepatocellular alterations were observed in male rats at a dose of 0.6 mg/kg/day; however, neoplastic nodules of the liver were also observed in these animals at doses ≥0.6 mg/kg/day. Biliary hyperplasia was observed in dogs exposed to 2,4-DNT for 24 months, with no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) values of 1.5 and 10 mg/kg/day, respectively.

Adverse reproductive effects, including effects on male and female reproductive systems and decreased neonatal viability, have been observed in laboratory animals following acute, intermediate, and chronic oral exposure to 2,4-, 2,6-, or Tg-DNT. Severity of reproductive effects is dose- and exposure duration-related, and the male reproductive system is more sensitive than the female reproductive system or neonatal viability. In males, adverse effects include decreased sperm production, testicular atrophy, degeneration of seminiferous tubules, and changes in Sertoli cell morphology. The lowest dose producing effects to the male reproductive system (testicular atrophy and decreased spermatogenesis) was 14 mg/kg/day in mice fed diets containing 2,4-DNT for up to 2 years. Effects on the female reproductive system (ovarian atrophy and nonfunctional follicles) were observed in mice fed 898 mg/kg/day 2,4-DNT. Decreased neonatal viability was observed in rats fed ≥34.5 mg/kg/day 2,4-DNT for up to 6 months in a three-generation reproductive study. Occupational exposure studies do not provide evidence of DNT-induced adverse reproductive effects due to both concomitant exposure of workers to other

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2. RELEVANCE TO PUBLIC HEALTH

chemicals and reporting insufficiencies. Dietary exposure of rats to 2,4-DNT for up 6 months in a three-
generation reproductive study produced delayed eye opening at doses fed ≥35 mg/kg/day, but no other
developmental effects. In neonates exposed to Tg-DNT during gestation, systemic effects similar to those
observed in adult animals, including delayed eye opening and signs of mild neurological effects (cliff
avoidance behavior) and effects on hematological parameters, were observed at maternal doses
≥100 mg/kg/day; recovery from these effects was observed by postpartum day 60. Although no studies
were located regarding developmental effects in humans after oral exposure to DNT, developmental
toxicity from DNT could potentially occur because exposure to any substance that depletes the amount of
oxygen available to developing fetal tissues may cause toxicity to the developing organism.

Respiratory distress, pulmonary congestion, and increased relative lung weights were observed in rats
following a single 6-hour inhalation exposure of rats to near-lethal or lethal concentrations of 2,6-DNT
(vapor or aerosol). No effects on the respiratory system have been observed following acute,
intermediate, or chronic oral exposure of laboratory animals to DNT. Occupational studies do not include
reports of respiratory effects in workers.

The carcinogenic activity of DNT has been extensively studied in typical chronic bioassays and in some
less-than-lifetime studies. Results show the development of renal cancer in mice fed 2,4-DNT at
95 mg/kg/day and hepatocellular carcinoma in male and female rats fed 2,4-DNT at doses of 34.5 and
45.3 mg/kg/day, respectively. Hepatocellular carcinomas were observed in rats administered 2,6-DNT at
doses ≥7 mg/kg/day. 2,4-DNT was a hepatic tumor promoter, but not a tumor initiator, using \textit{in vivo}
hepatic initiation-promotion protocols. Both tumor-initiating and -promoting activities of 2,6- and
Tg-DNT in rat liver were reported. A retrospective cohort mortality study at two army ammunition plants
that used Tg-DNT and/or 2,4-DNT showed no significant increases in mortality from malignant
neoplasms as a whole or from particular cancers (liver, lung, gallbladder, kidney, and connective tissues),
although interpretation of study results is limited by a small cohort size. Two studies of copper miners
exposed to high levels of Tg-DNT from explosives found significant increases in the incidence of
urothelial cancers. An increase in renal cancers was also found; however, the increased incidence did not
appear to be related to Tg-DNT exposure levels. The National Toxicology Program (NTP) has not
evaluated DNT in its 12\textsuperscript{th} Report on Carcinogens. EPA has classified 2,4-DNT/2,6-DNT mixture as a
probable human carcinogen based on sufficient evidence of carcinogenicity in animals. The International
Agency for Research on Cancer (IARC) has classified 2,4- and 2,6-DNT as possibly carcinogenic to
humans, and 3,5-DNT as not classifiable as to its carcinogenicity to humans.

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Heath effects of 2,4- and 2,6-DNT ingestion in laboratory animals and the dose ranges at which these effects occur are shown in Figures 2-1 and 2-2, respectively. Estimates of oral doses posing minimal risk to humans (MRLs) are also presented in these figures.

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for 2,4- and 2,6-DNT. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised as appropriate.

Inhalation MRLs

Most of the data on health effects associated with inhalation exposure of humans to DNT are from studies of workers exposed to 2,4- 2,6-, or 2g-DNT. However, most of these studies were conducted before 1950 and provide very little information on exposure concentrations. Furthermore, interpretation of study results is limited due to the absence of appropriate control groups, mixed exposures to other chemicals, and small number of workers studied. Therefore, while available occupational studies provide supportive information for observations in animals regarding identification of target organs, they do not report dose-response data that can be used to derive MRLs for DNT. Regarding available animal data, one study was located that examined the acute inhalation toxicity of 2,6-DNT in rats (CMA 1991). This study identified mortality and clinical signs of respiratory toxicity (exaggerated respiratory movements) in rats exposed to...
Figure 2-1. Health Effects for Ingesting 2,4-DNT

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
<th>Effects in Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;898</td>
<td>Blood effects(^a), neurological effects(^b), liver effects(^c), male reproductive effects(^d), decreased body weight, female reproductive effects(^e)</td>
</tr>
<tr>
<td>145-468</td>
<td>Blood effects(^a), neurological effects(^b), liver effects(^c), male reproductive effects(^d), decreased body weight, decreased food intake</td>
</tr>
<tr>
<td>60-95</td>
<td>Blood effects(^a), neurological effects(^b), male reproductive effects(^g), decreased body weight, cancer(^h)</td>
</tr>
<tr>
<td>27-45</td>
<td>Blood effects(^a), liver effects(^c), male reproductive effects(^g), decreased body weight, decreased food intake, cancer(^i)</td>
</tr>
<tr>
<td>14-25</td>
<td>Blood effects(^a), neurological effects(^b), liver effects(^c), male reproductive effects(^g), decreased body weight</td>
</tr>
<tr>
<td>10</td>
<td>Blood effects(^a), neurological effects(^b), liver effects(^c)</td>
</tr>
<tr>
<td>1.5</td>
<td>Blood effects(^a), neurological effects(^b)</td>
</tr>
<tr>
<td>0.05 mg/kg/day</td>
<td>Acute MRL</td>
</tr>
<tr>
<td>0.007 mg/kg/day</td>
<td>Intermediate MRL</td>
</tr>
<tr>
<td>0.001 mg/kg/day</td>
<td>Chronic MRL</td>
</tr>
</tbody>
</table>

\(^a\)Methemoglobinemia, anemia and compensatory hematopoiesis  
\(^b\)Signs of neurotoxicity (e.g., loss of hindquarter control, convulsions, incoordination, stiffness, abnormal gait, paralysis)  
\(^c\)Hepatocellular degeneration or dysplasia  
\(^d\)Decreased fertility  
\(^e\)Ovarian atrophy and nonfunctioning follicles  
\(^f\)Histopathologic changes to the central nervous system  
\(^g\)Testicular degeneration or atrophy and decreased or absent spermatogenesis  
\(^h\)Renal carcinoma  
\(^i\)Hepatocellular carcinoma  
\(^j\)Biliary hyperplasia  
\(^k\)Intermittent clinical signs of neurotoxicity (e.g., loss of hindquarter and convulsions)  

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2. RELEVANCE TO PUBLIC HEALTH

Figure 2-2. Health Effects for Ingesting 2,6-DNT

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
<th>Effects in Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>145-155</td>
<td>Decreased body weight</td>
</tr>
<tr>
<td>35-55</td>
<td>Extramedullary erythropoiesis of the spleen, liver effects(^a), male reproductive effects, decreased body weight</td>
</tr>
<tr>
<td>20</td>
<td>Blood effects, liver effects(^a),(^c), neurological effects(^d), male reproductive effects(^b), decreased body weight, decreased food consumption</td>
</tr>
<tr>
<td>14</td>
<td>Decreased body weight</td>
</tr>
<tr>
<td>7</td>
<td>Extramedullary erythropoiesis of the spleen, liver effects(^a), cancer(^f), decreased body weight</td>
</tr>
<tr>
<td>4</td>
<td>Extramedullary erythropoiesis of the spleen</td>
</tr>
</tbody>
</table>

\(^a\)Bile duct hyperplasia
\(^b\)Testicular degeneration, decreased spermatogenesis
\(^c\)Inflammatory changes
\(^d\)Incoordination and lack of balance
\(^e\)Hepatocellular degeneration
\(^f\)Hepatocellular carcinoma

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2,6-DNT as an aerosol at concentrations ≥196 mg/m³; rats that died showed increased relative lung weights and evidence of lung congestion. No effects were observed in rats exposed nose-only to 2,6-DNT as a vapor at 26 mg/m³ for 6 hours and observed 14 days after dosing. No studies were located regarding inhalation exposure to 2,3-, 2,5-, or 3,5-DNT in experimental animals. Thus, data were insufficient for the derivation of acute-, intermediate-, and chronic-duration inhalation MRLs for 2,3-, 2,4-, 2,5-, 2,6-, and 3,5-DNT.

**Oral MRLs**

Acute-, intermediate-, and chronic-duration oral MRLs were derived for 2,4-DNT and acute- and intermediate-duration oral MRLs were derived for 2,6-DNT. For 2,4- and 2,6-DNT, studies in humans did not provide sufficient data regarding exposure levels and their correlation with observed effects; thus, animal studies were used for the derivation of these oral MRLs. Separate acute- and intermediate-duration MRLs were derived for 2,4- and 2,6-DNT because very little comparative data for these isomers are available. For 2,3- and 2,5-DNT, the only available oral exposure study is a single-dose study, which reported data on lethality; however, no additional information was reported (Vernot et al. 1977). No studies on the effects of oral exposure to 3,5-DNT in animals were identified. Therefore, data were insufficient for the derivation of acute-, intermediate-, and chronic-duration oral MRLs for 2,3-, 2,5-, and 3,5-DNT.

**Acute-Duration Oral MRL for 2,4-DNT**

- An MRL of 0.05 mg/kg/day has been derived for acute-duration oral exposure (≤14 days) to 2,4-DNT.

No human acute-duration oral toxicity studies on 2,4-DNT were identified. Acute-duration studies in animals identified the nervous system, hematological system, liver, and reproductive system as targets for DNT-induced toxicity, with neurotoxicity as the most sensitive effect. Neurotoxicity was observed following acute-duration oral exposure of beagle dogs, as part of an intermediate-duration study (U.S. Army 1978b). Dogs (4/sex/group) exposed to 2,4-DNT at 0, 1, 5, or 25 mg/kg/day in capsules for 13 weeks were observed for behavioral changes and clinical signs of toxicity during the first 14 days of treatment. Signs of neurotoxicity were evident at 25 mg/kg/day. Onset of neurotoxicity (loss of hind leg control) was observed in one female dog on day 12; three male dogs developed similar effects on day 14. Signs of neurotoxicity were reported in all dogs exposed to 25 mg/kg/day after treatment for 12–22 days. No clinical signs were observed in dogs treated with 1 or 5 mg/kg/day. Results of this study identify
2. RELEVANCE TO PUBLIC HEALTH

Acute-duration NOAEL and LOAEL values for neurotoxicity in dogs of 5 and 25 mg/kg/day, respectively. Although comprehensive end points were not evaluated at 14 days, data obtained following 13 weeks of treatment in this study identify the same NOAEL and LOAEL values of 5 and 25 mg/kg/day, respectively, for neurotoxicity, hematological effects, and reproductive effects. Thus, it is unlikely that hematological or reproductive effects would occur at lower doses than neurotoxicity for acute-duration exposure.

Acute-duration studies in rodents identified the hematological system, liver, and reproductive system as targets of acute-duration exposure to 2,4-DNT. The most sensitive hematological effect (slight cyanosis) was observed in male Sprague-Dawley rats exposed to 2,4-DNT at 60 mg/kg/day (lowest tested dose) for 5 days (Lane et al. 1985). The lowest LOAELs for hepatotoxicity (increased cholesterol accompanied by increased serum alanine aminotransferase levels in males) were reported in Sprague-Dawley rats treated at 78 and 82 mg/kg/day (for males and females, respectively) for 14 days; NOAELs were not identified (McGown et al. 1983). The lowest LOAEL for reproductive effects (decreased thickness of spermatogenic cell layers) was 78 mg/kg/day for male Sprague-Dawley rats administered 2,4-DNT for 14 days (McGown et al. 1983). Lane et al. (1985) identified a NOAEL of 60 mg/kg/day, with a LOAEL of 180 mg/kg/day, for decreased fertility in male rats treated with 2,4-DNT for 5 days.

Neurotoxicity (loss of hind limb control) was identified as the most sensitive effect of acute-duration oral exposure to 2,4-DNT, based on the NOAEL value of 5 mg/kg/day in dogs administered 2,4-DNT via capsules for 12–14 days (U.S. Army 1978b). Based on the available data, neurotoxicity was selected as the critical effect for the basis of the acute-duration oral MRL. The NOAEL value of 5 mg/kg/day was used as the point of departure (POD). Neurotoxicity data were not suitable for benchmark dose (BMD) modeling, since effects were observed only at the highest dose tested. The POD of 5 mg/kg/day was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability), resulting in an acute-duration oral MRL for 2,4-DNT of 0.05 mg/kg/day.

**Intermediate-Duration Oral MRL for 2,4-DNT**

- An MRL of 0.007 mg/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to 2,4-DNT.

Information on effects of intermediate-duration oral exposure of laboratory animals is available from studies treating animals with 2,4-DNT for intermediate durations (15–364 days) and from chronic-duration studies evaluating end points at intermediate-duration time points. Results of studies in dogs,
rats, and mice show the development hematological, neurological, and reproductive effects following intermediate-duration exposure to 2,4-DNT, with the hematological effects as the most sensitive end point. Adverse hematological effects were observed in studies in dogs, rats, and mice, with the lowest NOAEL and LOAEL values of 1.5 and 10 mg/kg/day, respectively, observed following intermediate-duration oral exposure of dogs to 2,4-DNT (Ellis et al. 1985; U.S. Army 1979). As part of this 2-year study in dogs, hematological end points were evaluated in dogs administered 2,4-DNT at 3, 6, and 9 months as part of a 2-year study in dogs administered 0, 0.2, 1.5, or 10 mg/kg/day. Hematological effects consistent with development of methemoglobinemia were observed at doses of 1.5 and 10 mg/kg/day at all intermediate-duration time points (3, 6, and 9 months). Although effects at all time points were qualitatively similar, hematological changes observed after 9 months of exposure were more consistent and pronounced than those observed at the 3- and 6-month time periods (data are presented in Table A-1 of Appendix A). Male and female dogs exposed for 9 months at doses of 1.5 and 10 mg/kg/day showed detectable amounts of methemoglobin in the serum, with changes reaching statistical significance in males and females at 10 mg/kg/day. In female dogs administered 10 mg/kg/day, statistically significant decreases in erythrocyte count, hematocrit, and hemoglobin, a statistically significant increase in reticulocyte count, and the presence of Heinz bodies in serum were observed. Similar hematological effects were observed in female dogs administered 0.2 and 1.5 mg/kg/day, although effects did not reach statistical significance, most likely because the power of the study to detect statistically significant changes was compromised by the small number of dogs per treatment group. However, based on a clinically significant increase in methemoglobin levels of 225% in female dogs administered 1.5 mg/kg/day, the NOAEL and LOAEL values for hematological effects in this study are 0.2 and 1.5 mg/kg/day, respectively. Similar effects were observed in male dogs, although changes did not reach statistical significance in the 10 mg/kg/day group, possibly due to the small number of male dogs evaluated. Comprehensive end points were not evaluated at intermediate-duration time points in this study. However, the chronic-duration portion of this study, in which comprehensive end points were examined, identified hematological effects as the most sensitive effect of chronic-duration exposure. Thus, it is unlikely that effects to other target organs would occur at lower doses than the hematological effects for intermediate exposure durations (see overview of chronic-duration oral data).

Intermediate-duration studies in animals administered higher daily doses show the development of similar hematological effects, along with neurotoxicity and toxicity to the reproductive system. Beagle dogs administered 2,4-DNT for up to 13 weeks developed hematological effects (methemoglobinemia, anemia, and compensatory hematopoiesis), neurological effects (incoordination, abnormal gait, and paralysis), and reproductive effects (testicular degeneration and decreased spermatogenesis) at 25 mg/kg/day, with a
NOAEL for these effects of 5 mg/kg/day (Lee et al. 1985; U.S. Army 1979). Hematological effects similar to those observed in dogs were reported in rats treated with 2,4-DNT at 93–371 mg/kg/day (Kozuka et al. 1979; U.S. Army 1978b) and in mice treated with 2,4-DNT at 413 and 468 mg/kg/day (for males and females, respectively) for up to 13 weeks (Hong et al. 1985; U.S. Army 1978b). The lowest LOAEL for neurological effects (clinical signs of neurotoxicity and demyelination of the cerebellum and brain stem) in rodents was 93 mg/kg/day for male CD rats administered 2,4-DNT for 4 or 13 weeks; the NOAEL for neurological effects in males was 34 mg/kg/day (Lee et al. 1985; U.S. Army 1978b). In rodents, respective NOAEL and LOAEL values for reproductive effects (testicular atrophy and decrease or absent spermatogenesis) were 9–34 and 35–371 mg/kg/day in rats and 137–295 and 413–1,032 mg/kg/day in mice treated for 13 weeks (Bloch et al. 1988; Hong et al. 1985; Kozuka et al. 1979; Lee et al. 1985; U.S. Army 1978b, 1979).

Based on the available data, changes to hematological parameters in dogs exposed to oral 2,4-DNT for 9 months (Ellis et al. 1985; U.S. Army 1979) were evaluated as possible PODs for the intermediate-duration oral MRL for 2,4-DNT. To determine the POD, all available continuous-variable models in the EPA BMD Software (BMDS, version 2.1) were fit to the data for increased methemoglobin and reticulocytes and decreased hemoglobin, erythrocyte count, and hematocrit (see Appendix A for detailed description of BMD modeling). The BMD and the 95% lower confidence limit (BMDL) were estimated for doses associated with a change of 1 standard deviation from the controls. Neither the constant nor the non-constant variance model provided an adequate fit to the data for decreased erythrocytes or increased methemoglobin; therefore, these data were not considered suitable for BMD modeling. Models provided an adequate fit to data for decreased hematocrit and hemoglobin and increased reticulocytes. Among all of the models providing adequate fit to the data for these parameters, the lowest BMDL$_{1SD}$ values were 0.67 mg/kg/day for hematocrit (exponential model 4), 3.66 mg/kg/day for hemoglobin (the exponential model 2), and 5.64 mg/kg/day for reticulocytes (polynomial 3-degree model); of these, the lowest BMDL$_{1SD}$ of 0.67 mg 2,4-DNT/kg/day for decreased hematocrit was selected as the POD. This value was divided by an uncertainty factor of 100 (10 for animals to human extrapolation and 10 for human variability) resulting in an intermediate-duration oral MRL of 0.007 mg/kg/day.

**Chronic-Duration Oral MRL for 2,4-DNT**

- An MRL of 0.001 mg/kg/day has been derived for chronic-duration oral exposure (≥1 year) to 2,4-DNT.
Information on effects of chronic-duration oral exposure is available from studies in dogs, rats, and mice. Results of these studies indicate that hematological, neurological, hepatic, and reproductive effects are associated with chronic-duration exposure to 2,4-DNT, with hematological effects and neurotoxicity as the most sensitive effects. Adverse hematological effects were observed in studies in dogs, rats, and mice, with the lowest NOAEL and LOAEL values of 0.2 and 1.5 mg/kg/day, respectively, observed following chronic oral exposure of dogs to 2,4-DNT (Ellis et al. 1985; U.S. Army 1979). Beagle dogs treated with 2,4-DNT at 1.5 and 10 mg/kg/day after 12 months developed hematological effects consistent with methemoglobinemia, anemia, and compensatory hematopoiesis (data are presented in Table A-3 of Appendix A). Female dogs administered 2,4-DNT at 1.5 mg/kg/day for 12 months showed statistically significant reductions in erythrocyte count, hematocrit, and hemoglobin concentration. At 10 mg/kg/day, more pronounced changes in these hematological parameters were observed, including statistically significant reductions in erythrocyte count, hematocrit, and hemoglobin and a statistically significant increase in reticulocyte count. Similar hematological effects (decreased erythrocytes and decreased hematocrit) were observed in dogs treated with 0.2 mg/kg/day, but changes did not reach statistical significance. Although not statistically significant, effects on hematological parameters in male dogs were also similar to those seen in female dogs. In rodents, the lowest LOAEL for hematological effects was 3.9 mg/kg/day for decreased erythrocyte count (with no changes in methemoglobin or Heinz bodies) in male CD rats after treatment with 2,4-DNT for 12 months; the NOAEL for this effect was 0.6 mg/kg/day (Lee et al. 1985; U.S. Army 1978b, 1979).

Neurotoxicity was also observed in dogs, rats, and mice orally exposed to 2,4-DNT for chronic durations. In chronic-duration exposure studies, the lowest dose producing neurotoxicity was 1.5 mg/kg/day 2,4-DNT in a 2-year study in dogs; however, this effect (loss of hindquarter control) was only observed intermittently in one of six dogs. More severe signs of neurotoxicity and central nervous system lesions (vacuolization, hypertrophy, endothelial mitosis, focal gliosis in the cerebellum, and perivascular hemorrhage in the cerebellum and brain stem) occurred at 10 mg/kg/day (Ellis et al. 1985; U.S. Army 1979). In rodents, the lowest LOAEL values for neurotoxicity were 35 and 45 mg/kg/day for male and female CD rats, respectively, treated with 2,4-DNT for 1–2 years (Lee et al. 1985; U.S. Army 1978b, 1979). In mice, neurotoxicity was reported at 898 mg/kg/day (but not at 95 mg/kg/day) (Hong et al. 1985; U.S. Army 1979).

Hepatic effects have also been observed in laboratory animals following chronic-duration oral exposure to DNT. However, these effects are often observed in conjunction with the development of hepatocellular carcinoma and may represent precancerous changes. For example, dietary exposure of male rats to
2. RELEVANCE TO PUBLIC HEALTH

0.6 mg/kg/day 2,4-DNT induced “hepatocellular” alterations; however, this exposure level also induced neoplastic nodules (Lee et al. 1985). Thus, interpretation of data on hepatic toxicity is complicated due to the potential of 2,4-DNT to induce hepatocellular carcinoma. Biliary hyperplasia was observed in dogs exposed to 2,4-DNT for 24 months, with NOAEL and LOAEL values of 1.5 and 10 mg/kg/day, respectively (U.S. Army 1979). The lowest LOAEL value for hepatic effects in mice was 14 mg/kg/day for hepatocellular dysplasia following exposure to 2,4-DNT for 24 months (Hong et al. 1985; U.S. Army 1979). Hepatocellular degeneration, vacuolization, and altered hepatocellular foci were also observed in male F344 rats treated at 27 mg/kg/day for 52 weeks (Leonard et al. 1987).

Chronic-duration studies in laboratory animals have also demonstrated both male and female reproductive effects. The lowest LOAEL reported for reproductive effects is 14 mg/kg/day for atrophy of the testes and decreased spermatogenesis for in male CD-1 mice exposed to dietary 2,4-DNT for 12 months (Hong et al. 1985). Female mice fed 898 mg/kg/day 2,4-DNT had ovarian atrophy with non-functioning follicles, with no effects observed at 95 mg/kg/day (Hong et al. 1985). Male CD rats that received 34 mg/kg/day 2,4-DNT in the diet for 12 month showed an increased incidence of seminiferous tubule atrophy compared to controls (100% affected at the high-dose versus 0% of controls) (Lee et al. 1985; U.S. Army 1978b, 1979). No adverse reproductive effects were found in dogs fed 10 mg/kg/day 2,4-DNT for 24 months (Ellis et al. 1985; U.S. Army 1979).

Hematological effects and neurotoxicity were identified as the most sensitive effects of chronic-duration exposure to 2,4-DNT, with NOAEL and LOAEL values of 0.2 and 1.5 mg/kg/day, respectively, in dogs exposed to 2,4-DNT for 12 months (Ellis et al. 1985; U.S. Army 1979). For derivation of the chronic-duration oral MRL, hematological effects were selected as the critical effect rather than neurotoxicity, which was observed only intermittently in one of six dogs exposed to 1.5 mg/kg/day. Hematological data are expressed as group means; therefore, these data are considered more robust than observations of intermittent neurotoxicity in a single animal. To determine the POD, all available continuous-variable models in the EPA BMDS (version 2.1) were fit to the data for decreased erythrocyte count, hematocrit, and hemoglobin in female dogs. Hematological data from male dogs were not considered for additional BMD analyses due to the low number of dogs evaluated in the 10 mg/kg/day group (data were available for only two dogs). The BMD and the 95% lower confidence limit (BMDL) were estimated for doses associated with a change of 1 standard deviation from the controls. A detailed description of BMD modeling is provided in Appendix A. Neither the constant nor the non-constant variance model provided an adequate fit to the data for decreased hemoglobin; therefore, these data were not considered suitable for BMD modeling. Models provided an adequate fit to data for decreased erythrocyte count and
decreased hematocrit. Among all of the models providing adequate fit to the data for these parameters, the lowest BMDL\textsubscript{1SD} values were 0.12 mg/kg/day for hematocrit (exponential model 4) and 0.13 mg/kg/day for hemoglobin (exponential model 4); of these, the lowest BMDL\textsubscript{1SD} of 0.12 mg/kg/day for decreased erythrocyte count was selected as the POD. This value was divided by an uncertainty factor of 100 (10 for animals to human extrapolation and 10 for human variability) resulting in a chronic-duration oral MRL of 0.001 mg/kg/day.

**Acute-Duration Oral MRL for 2,6-DNT**

- An MRL of 0.09 mg/kg/day has been derived for acute-duration oral exposure (≤14 days) to 2,6-DNT.

Information on effects of acute-duration oral exposure of laboratory animals to 2,6-DNT are available from a single-dose lethality studies in rats (Deng et al. 2011; Lee et al. 1975; U.S. Army 1978a; Vernot et al. 1977) and from an intermediate-duration study in dogs that evaluated clinical signs of toxicity, hematology, and clinical chemistry after 14 days of exposure (U.S. Army 1976). Results of these studies show that acute exposure to 2,6-DNT induces hematological effects, neurotoxicity, and hepatotoxicity, with hematological effects (methemoglobin-induced anemia and compensatory hematopoiesis) as the most sensitive effect. Results of acute lethality studies provide very little information on 2,6-DNT-induced toxicity at nonlethal levels.

Administration of 2,6-DNT to dogs at 20 and 100 mg/kg/day (but not 4 mg/kg/day) for 2 weeks produced hematological effects consistent with the development of methemoglobin-induced anemia and compensatory hematopoiesis (U.S. Army 1976). Dogs treated with 20 mg/kg/day showed a statistically significant decrease in erythrocyte count and a significant increase in mean cell hemoglobin. At 100 mg/kg/day, more pronounced changes in these hematological parameters were observed; dogs showed statistically significant reductions in erythrocyte count, hematocrit, and hemoglobin and a statistically significant increase in reticulocyte count. In the 4 mg/kg/day group, similar hematological effects (decreased erythrocyte count, hemoglobin, and hematocrit, and increased reticulocytes) were observed, but these changes did not achieve statistical significance. Data are presented in Table A-5 of Appendix A. No effects on clinical chemistry parameters were observed at any dose. Results of this study identify a NOAEL and a LOAEL of 4 and 20 mg/kg/day, respectively, for hematological effects in dogs treated with 2,6-DNT for 2 weeks. Although comprehensive end points were not evaluated after 2 weeks, the identification of hematological effects as the most sensitive effect after acute-duration
exposure is consistent with the most sensitive effect identified after intermediate-duration exposure to 2,6-DNT.

In addition to hematological effects, clinical signs of neurotoxicity were observed in dogs administered 2,6-DNT at 100 mg/kg/day (U.S. Army 1976). The study authors noted that at least three dogs (gender not specified) developed neurotoxicity (listlessness, incoordination, lack of balance, and weakness, particularly of the hind limbs) and other clinical signs of toxicity (pale gums, dark urine) within the first 2 weeks of the exposure (incidence data were not reported). At this dose, one male dog died during the second week of exposure. No clinical signs of toxicity were observed at doses up to 20 mg/kg/day during the first 14 days of exposure, although similar (but milder) symptoms were reported at this dose following 4 weeks of treatment.

Hepatotoxicity, including increased alanine aminotransferase (ALT) activity and histopathological changes to the liver (congested sinusoids with sloughed hepatocytes and segmented neutrophils, disorganized midzonal regions characterized by infiltration of erythrocytes and hepatocytes with pyknotic nuclei and microvesiculated cytoplasm, and apoptotic hepatocytes) were reported in rats administered doses of 2,6-DNT (Deng et al. 2011). Results of this study identify acute-duration NOAEL and LOAEL values for hepatotoxicity for 2,6-DNT of 25 and 50 mg/kg/day, respectively.

Based on the available data, toxicity to the hematological system (anemia and compensatory hematopoiesis) was identified as the most sensitive effect of acute-duration oral exposure to 2,6-DNT. In dogs exposed for 14 days, a statistically significant decrease in erythrocyte count and a statistically significant increase in mean cell hemoglobin were observed after treatment with 2,6-DNT at 20 mg/kg/day for 2 weeks (U.S. Army 1976). Changes to other hematological parameters only reached statistical significance at 100 mg/kg/day. Therefore, the most sensitive hematological parameters were erythrocyte count and mean cell hemoglobin. To determine the POD for derivation of the acute-duration oral MRL for 2,6-DNT, all available continuous-variable models in the EPA BMDS (version 2.1) were fit to the data for erythrocyte count and mean cell hemoglobin. The BMD and the 95% lower confidence limit (BMDL) were estimated for doses associated with a change of 1 standard deviation from the controls. A detailed description of BMD modeling is provided in Appendix A. Neither the constant nor the non-constant variance model provided an adequate fit to the data for increased mean cell hemoglobin; therefore, these data were not considered suitable for BMD modeling. With the non-constant variance model applied, the linear, polynomial, and power models provided an adequate fit to the data for decreased erythrocyte count. The polynomial and power models converged to the linear model. The
BMDL_{15D} value of 9.31 mg/kg/day derived from this model was selected as the POD. This value was divided by an uncertainty factor of 100 (10 for animals to human extrapolation and 10 for human variability) resulting in an acute-duration oral MRL of 0.09 mg/kg/day.

**Intermediate-Duration Oral MRL for 2,6-DNT**

- An MRL of 0.004 mg/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to 2,6-DNT.

Information on effects of intermediate-duration oral exposure of laboratory animals is available from a 13-week study in beagle dogs, CD rats, and CD-1 mice (U.S. Army 1976). Results of this study show that 2,6-DNT produces hematological, neurological, reproductive, and hepatic toxicity, with histopathological changes of the spleen (extramedullary erythropoiesis) that are likely secondary to methemoglobinemia and anemia.

Intermediate-duration oral exposure of beagle dogs to 2,6-DNT at 4 mg/kg/day (lowest tested dose) produced extramedullary erythropoiesis (formation of erythrocytes outside of the bone marrow) in the spleen secondary to methemoglobinemia and anemia (U.S. Army 1976). The incidence and severity of this lesion was dose-related. Data are presented in Table A-7 of Appendix A. Changes in hematological parameters associated with anemia and compensatory hematopoiesis, including decreased hematocrit and hemoglobin and increased numbers of reticulocytes, were observed at 20 and 100 mg/kg/day. However, mortality also occurred in dogs exposed to these doses. Results of this study identify a LOAEL of 4 mg/kg/day for extramedullary hematopoiesis secondary to hematological effects in dogs treated with 2,6-DNT for 13 weeks (U.S. Army 1976). In rodents exposed for 13 weeks, the lowest LOAELs for hematological effects (extramedullary erythropoiesis) were 7 mg/kg/day for rats and 51 mg/kg/day for mice (U.S. Army 1976).

Hepatic, neurological, and reproductive effects were also observed following a 13-week exposure of dogs, rats, and mice to 2,6-DNT (U.S. Army 1976); however, these effects occurred at doses higher than those producing extramedullary erythropoiesis (4 mg/kg/day) in dogs. Note that in dogs, lethality also occurred at doses of 20 and 100 mg/kg/day. The lowest NOAEL and LOAEL values for hepatic effects (bile duct hyperplasia and degeneration and inflammatory changes to the liver) were 4 and 20 mg/kg/day, respectively, in dogs treated with 2,6-DNT for 13 weeks (U.S. Army 1976). Bile duct hyperplasia was observed at higher doses in rats (35 mg/kg/day) and mice (51 mg/kg/day) fed 2,6-DNT for 13 weeks (U.S. Army 1976). Neurotoxic effects (listlessness, incoordination, and lack of balance) were observed in dogs...
treated with 20 and 100 mg/kg/day. No signs of neurotoxicity were observed in rats at doses up to 145 and 155 mg/kg/day (for males and females, respectively) or in mice at doses up to 289 and 299 mg/kg/day (for males or females, respectively) for 13 weeks (U.S. Army 1976). For reproductive effects following 13-week exposure to 2,6-DNT, the lowest NOAEL and LOAEL values of 4 and 20 mg/kg/day, respectively, were reported in dogs for testicular degeneration (U.S. Army 1976). NOAEL and LOAEL values for reproductive effects were 11 and 51 mg/kg/day, respectively, in male mice for decreased spermatogenesis and 7 and 35 mg/kg/day, respectively, in male mice for testicular atrophy (U.S. Army 1976).

The LOAEL value of 4 mg/kg/day for an increased incidence of extramedullary erythropoiesis in the spleens of dogs was identified as the POD for derivation of the intermediate-duration oral MRL for 2,6-DNT (U.S. Army 1976). Histopathology data were not suitable for BMD modeling, since the number of animals evaluated at each dose and time was small (n=2 animals). Therefore, the LOAEL value for 4 mg/kg/day was used at the POD. This value was divided by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability), resulting in an intermediate-duration oral MRL for 2,6-DNT of 0.004 mg/kg/day.

**Chronic-Duration Oral MRL for 2,6-DNT**

The only chronic exposure study identified for 2,6-DNT is a 1-year study in F344 rats (Leonard et al. 1987). In this study, oral exposure to 7 and 14 mg/kg/day 2,6-DNT produced hepatocellular carcinoma, cellular alterations to hepatocytes (vacuolization accompanied by acidophilic and basophilic foci), and increased liver weights and serum hepatic enzyme activity. Hepatocellular carcinoma was observed in 85 and 100% of rats treated with 7 and 14 mg/kg/day, respectively. No other effects of 2,6-DNT were reported in this study. Data from this study are not appropriate for derivation of a chronic-duration oral MRL for 2,6-DNT, as no noncancer effects were reported.