2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO DNT IN THE UNITED STATES

Dinitrotoluene (DNTs) are 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 (TDI). DNTs are also used in the production of 2,4,6-TNT, dyes, and polyurethane foams.

When released to the environment, DNTs have 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 DNTs and as a consequence, they are not frequently detected in ambient air. DNTs generally biodegrade slowly under environmental conditions, but are 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.

DNTs are 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. DNTs have 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 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 DNTs for populations residing near hazardous waste sites or munitions facilities is via ingestion of contaminated water or dermal contact with contaminated soil. Inhalation and/or dermal exposure to DNTs could also occur when washing or bathing with DNT-contaminated water or when cooking with DNT-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 DNTs.

2.2 SUMMARY OF HEALTH EFFECTS

Data on health effects of DNTs 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 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 DNTs; however, systemic effects of DNTs 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, especially methemoglobinemia and anemia, have been reported in laboratory animals exposed to oral 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, 3,5-, or Tg-DNT for acute, intermediate, and chronic durations. Hematological effects of DNTs 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, increased reticulocyte count, decreased erythrocyte count, decreased hematocrit, and decreased 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. In acute-duration (14-day) oral studies of rats, this effect was seen after exposure to each of the six isomers of DNT. 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, have also 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 is also identified as a sensitive target for DNT-induced toxicity, with symptoms of neurotoxicity reported in laboratory animals exposed to oral 2,4-, 2,6-, 3,4-, 3,5-, 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 were 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. Demyelinization 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 2,3-, 2,4-, 2,6-, 3,4-, 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 DNTs 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, increased liver weights, hepatocellular hyperplasia and hypertrophy, degeneration of hepatocytes, hepatocellular necrosis, karyocytomegaly, proliferation of bile duct epithelium, and elevated blood levels of hepatic enzymes, have been reported in oral exposure studies of 2,3-, 2,4-, 2,6-, 3,4-, or Tg-DNT in laboratory animals. Hepatic effects occurred after exposure to 2,3-DNT doses ≥275 mg/kg/day, to 2,4-DNT doses ≥10 mg/kg/day, to 2,6-DNT doses ≥7 mg/kg/day, and to 3,4-DNT doses ≥113 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-, 3,5-, 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 testes and epididymides weights, decreased sperm production, testicular atrophy, degeneration of seminiferous tubules, multinucleated giant cell formation, 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 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 DNTs, developmental toxicity from DNTs 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 DNTs. Occupational studies do not include reports of respiratory effects in workers.

The carcinogenic activity of DNTs 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 *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. Three studies of German copper miners exposed to high levels of Tg-DNT from explosives found increases in the incidence of urothelial cancers. An association between renal cancer and duration of Tg-DNT exposure has also been reported in this cohort. The National Toxicology Program (NTP) has not evaluated DNTs. 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.

Heath effects of 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-DNT ingestion in laboratory animals and the dose ranges at which these effects occur are shown in Figures 2-1 through 2-6, respectively. Estimates of oral doses posing minimal risk to humans (MRLs) are also presented in these figures.

2.3 MINIMAL RISK LEVELS (MRLs)

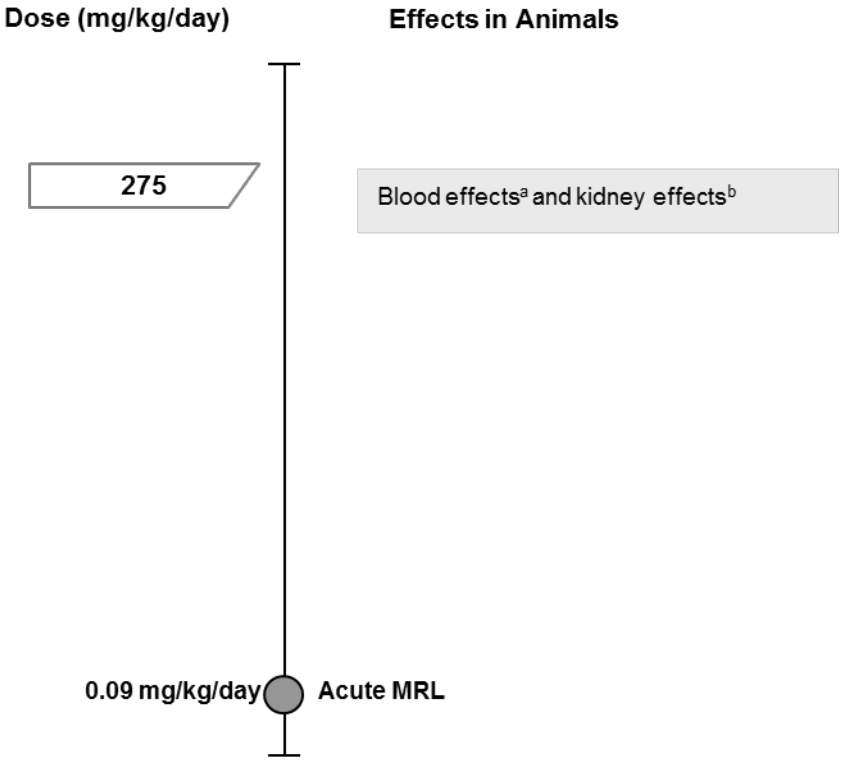
Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-DNT. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified 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

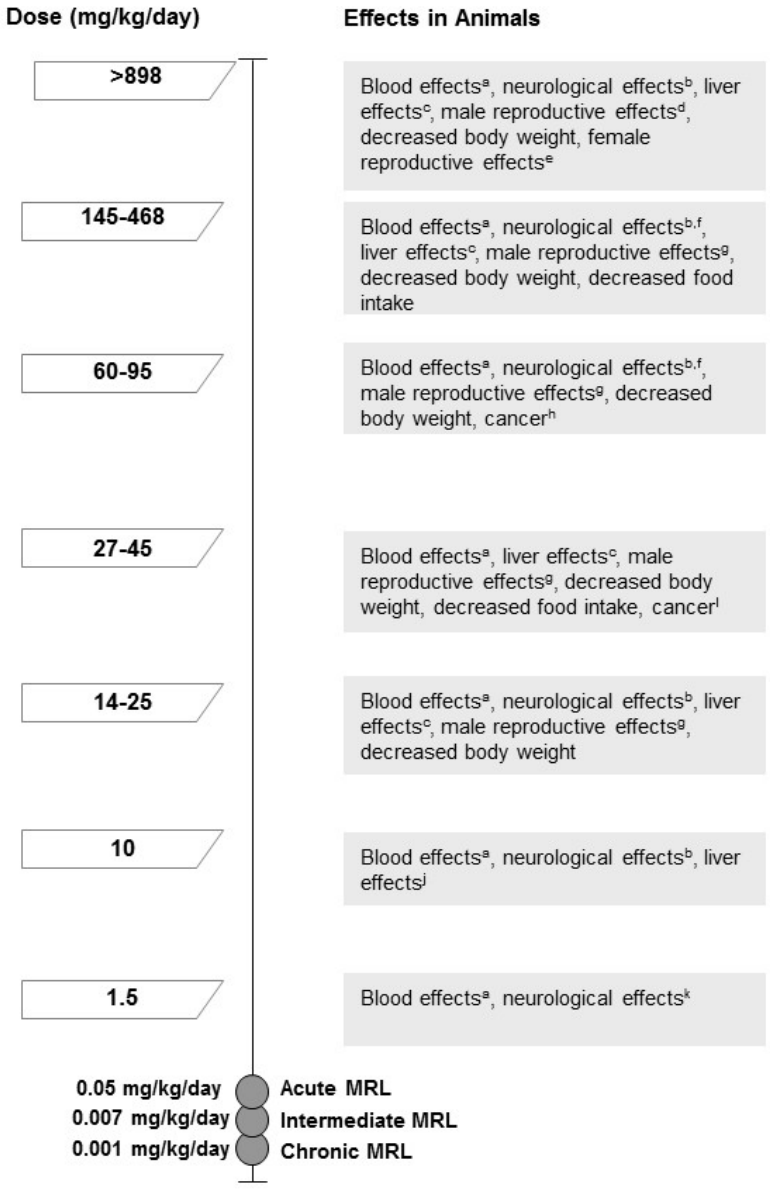
Most of the data on health effects associated with inhalation exposure of humans to DNTs are from studies of workers exposed to 2,4-, 2,6-, or Tg-DNT. However, most of these studies were conducted before 1950 and provide very little information on exposure concentrations. Furthermore, interpretation of study results is limited due to the absence of appropriate control groups, mixed exposures to other

Figure 2-1. Health Effects for Ingesting 2,3-Dinitrotoluene



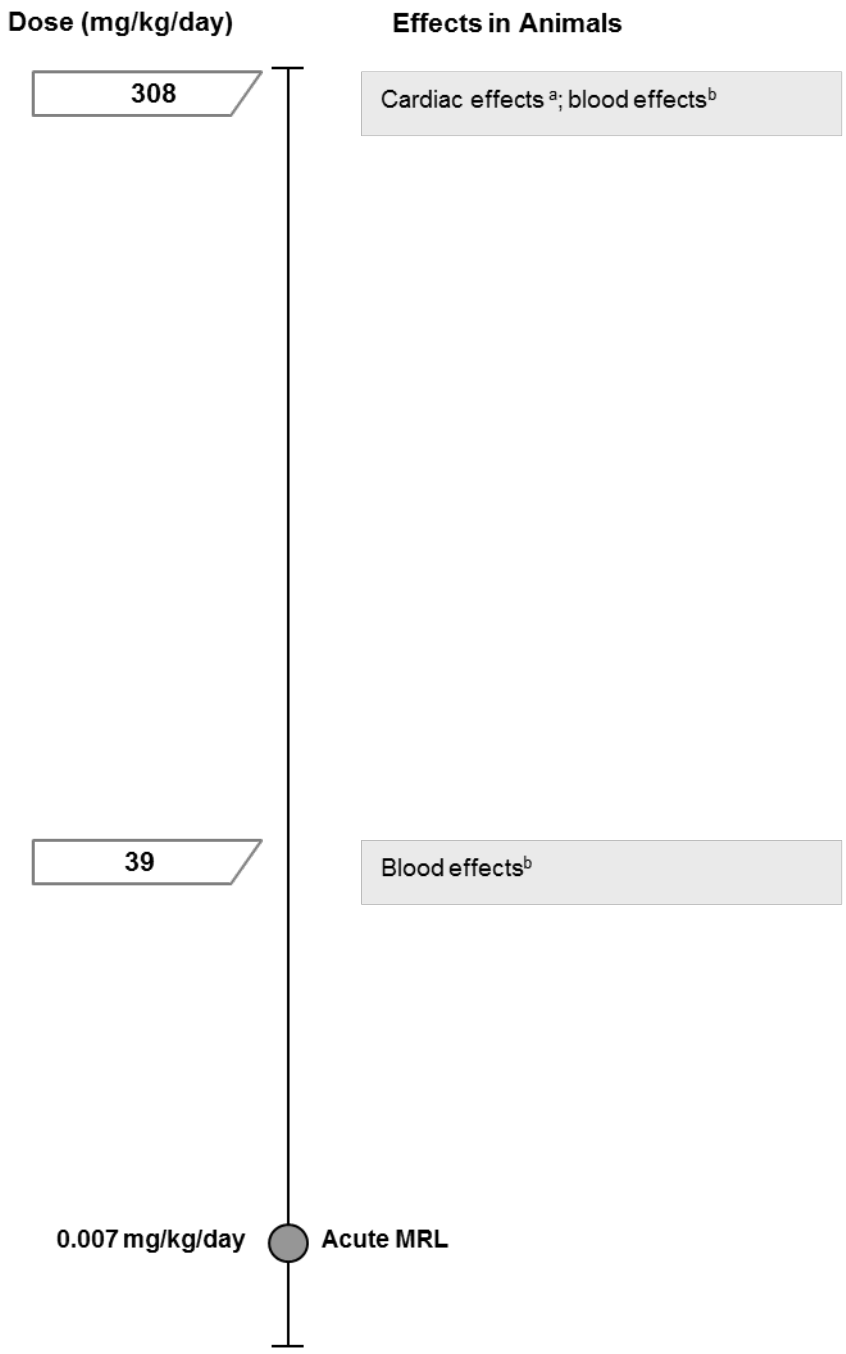
[a]Extramedullary hematopoiesis, lymphoid hyperplasia, and lymphoid depletion of spleen
[b]Trace tubular dilatation and lymphocytic infiltration of the kidney

Figure 2-2. Health Effects for Ingesting 2,4-Dinitrotoluene



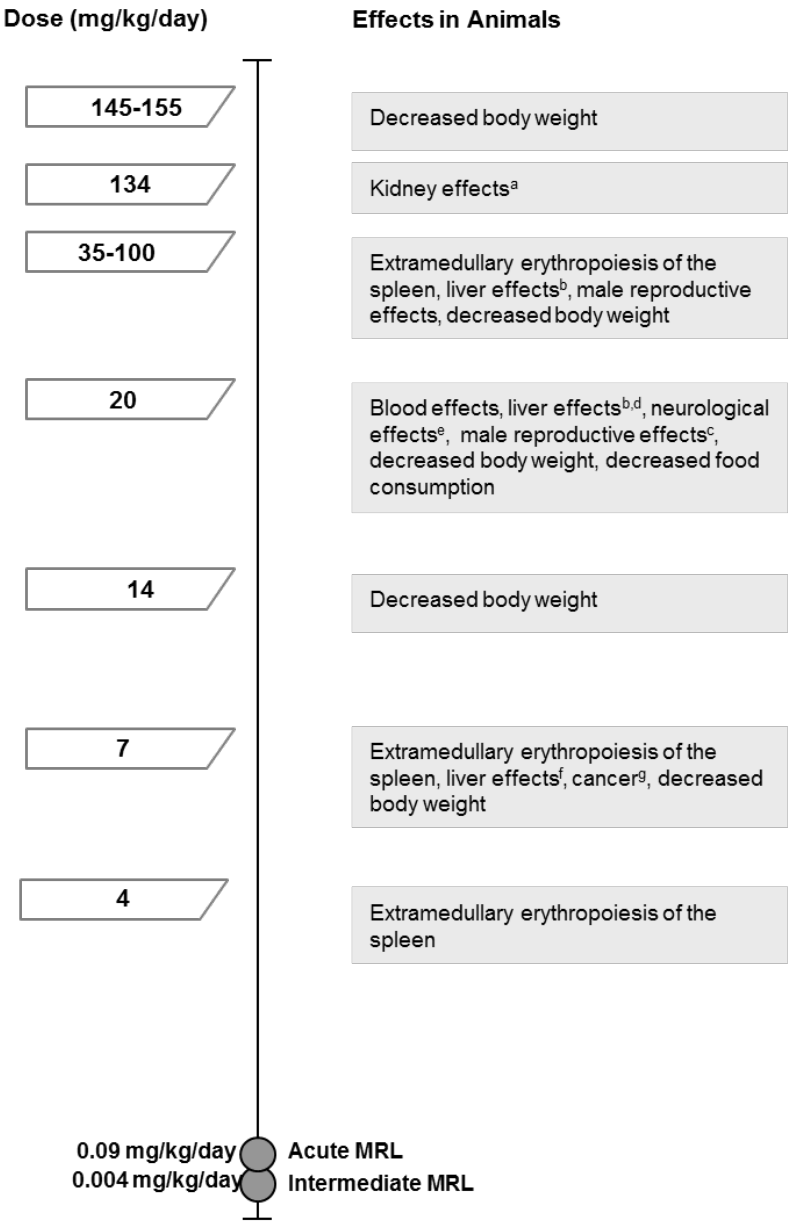
[a]Methemoglobinemia, anemia and compensatory hematopoiesis
[b]Signs of neurotoxicity (e.g., loss of hindquarter control, convulsions, incoordination, stiffness, abnormal gait, paralysis)
[c]Hepatocellular degeneration or dysplasia
[d]Decreased fertility
[e]Ovarian atrophy and nonfunctioning follicles
[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 control and convulsions)

Figure 2-3. Health Effects for Ingesting 2,5-Dinitrotoluene



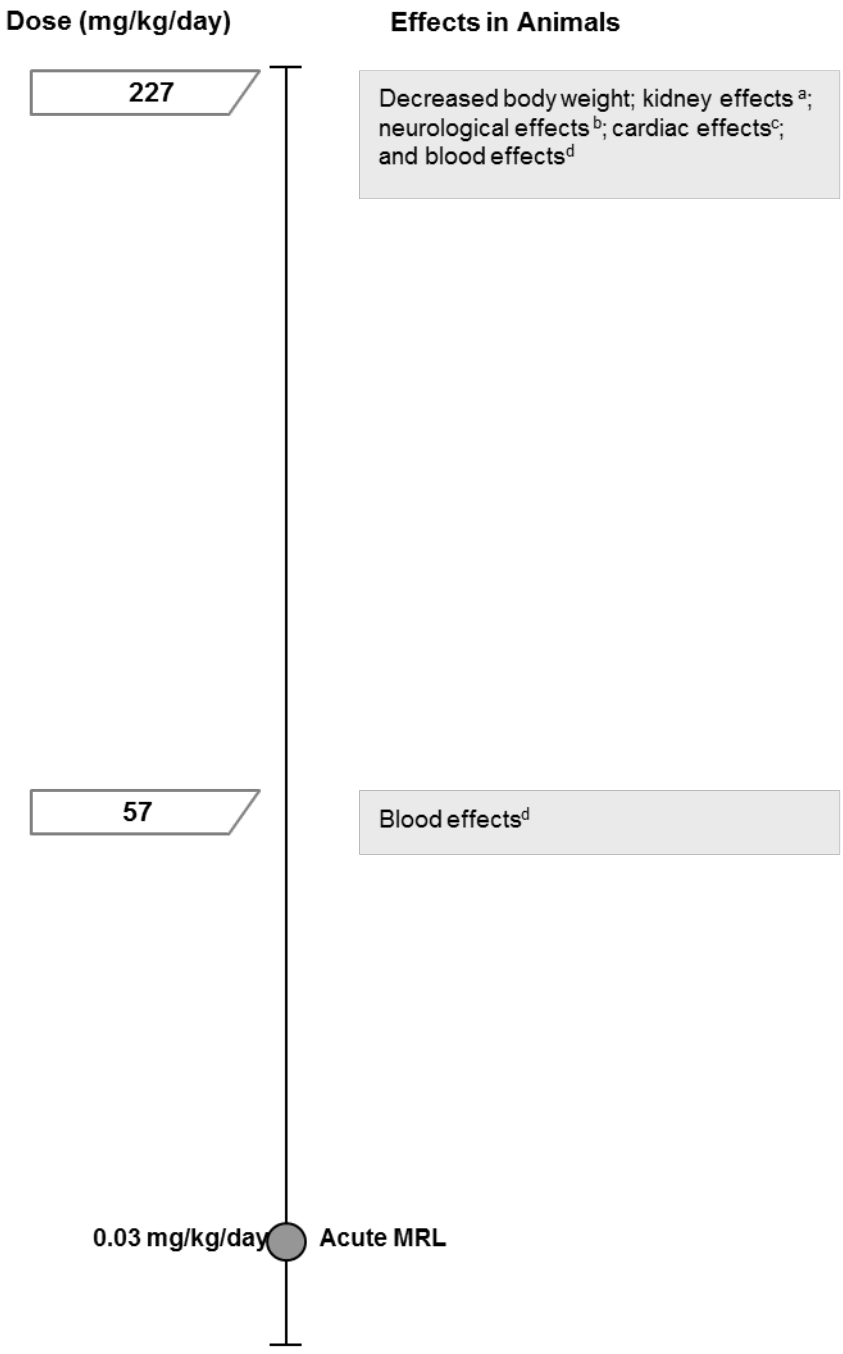
[a]Increased absolute and relative heart weight; mild-moderate fibrosis and trace-moderate inflammation
[b]Dark spleen; mild to moderate extramedullary hematopoiesis

Figure 2-4. Health Effects for Ingesting 2,6-Dinitrotoluene



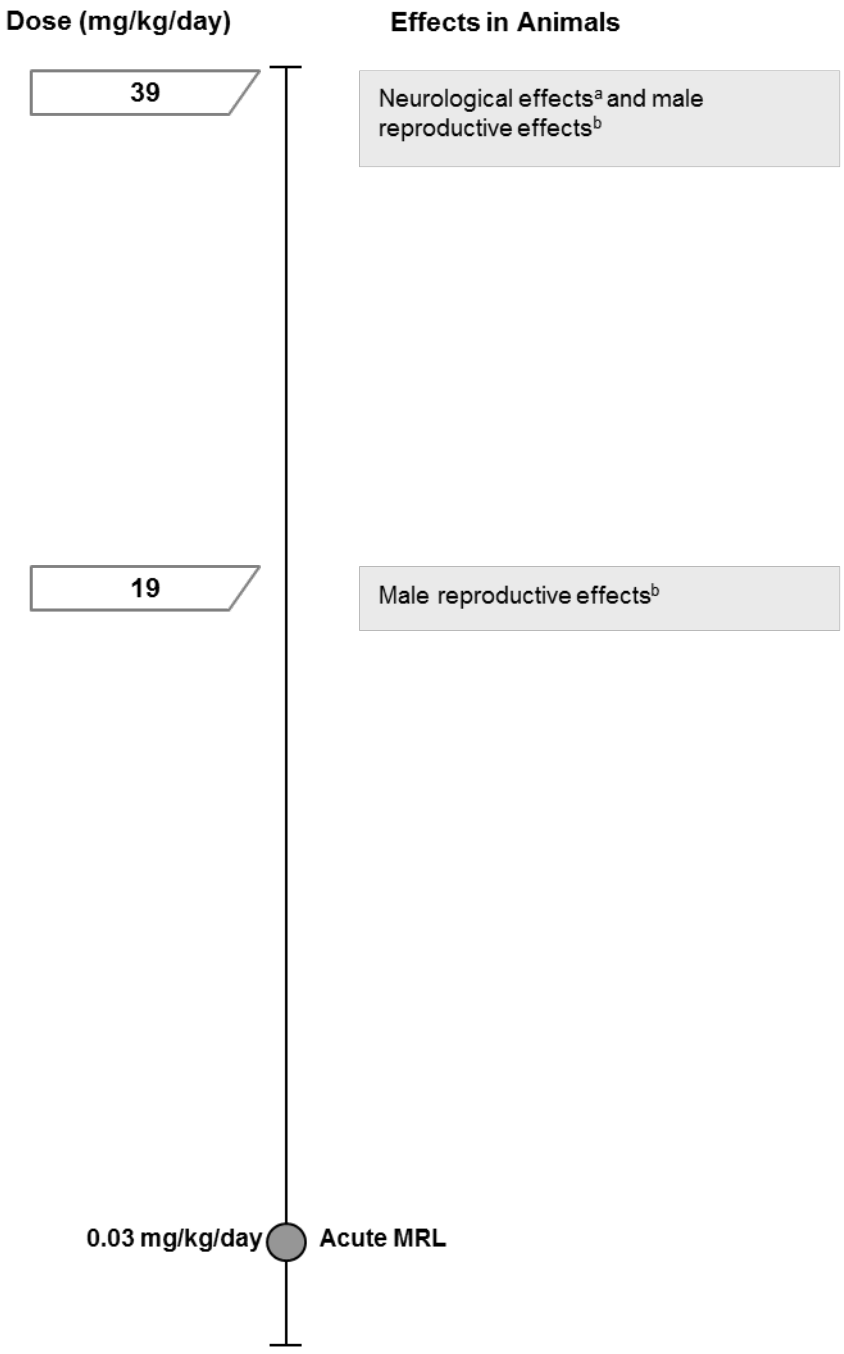
[a]Proximal tubule degeneration and renal tubule basophilia
[b]Bile duct hyperplasia, hepatocellular hypertrophy, single cell necrosis, and focal hepatocellular vacuolation
[c]Testicular degeneration, decreased spermatogenesis
[d]Inflammatory changes
[e]Incoordination and lack of balance
[f]Hepatocellular degeneration
[g]Hepatocellular carcinoma

Figure 2-5. Health Effects for Ingesting 3,4-Dinitrotoluene



[a]Proximal tubule degeneration, renal tubule basophilia, and lymphocytic infiltration
[b]Facial twitching, hypoactivity, staring
[c]Myocardial fibrosis, inflammation, and necrosis
[d]Extramedullary hematopoiesis and lymphoid hyperplasia

Figure 2-6. Health Effects for Ingesting 3,5-Dinitrotoluene



[a]Facial twitching and paralysis; inflammatory infiltrates in the brain
[b]Small testes; significantly reduced testes weight; tubular degeneration and multinucleated giant cell formation in the testes

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 DNTs. 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 2,6-DNT as an aerosol at concentrations ≥196 mg/m^3; 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/m^3 for 6 hours and observed 14 days after dosing. No studies were located regarding inhalation exposure to 2,3-, 2,5-, 3,4-, 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-, 3,4-, 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; acute-duration oral MRLs were derived for 2,3-, 2,5-, 3,4-, and 3,5-DNT. For all isomers, 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. Data were insufficient for the derivation of intermediate- and chronic-duration oral MRLs for 2,3-, 2,5-, 3,4-, and 3,5-DNT and for the derivation of a chronic oral MRL for 2,6-DNT. Table 2-1 summarizes the oral MRLs for the DNT isomers.

Acute-Duration Oral MRL for 2,3-DNT

- An MRL of 0.09 mg/kg/day has been derived for acute-duration oral exposure (≤14 days) to 2,3-DNT.

No human acute-duration oral toxicity studies on 2,3-DNT were identified. For 2,3-DNT, the only available oral exposure studies are a 14-day gavage study in rats, in which a number of toxicological end points were assessed (Lent et al. 2012a; USAPHC 2011a), and a single-dose gavage study, which reported data on lethality only (Vernot et al. 1977). Vernot et al. (1977) reported LD_{50} values of 1,120 and 1,070 mg/kg in rats and mice, respectively. The 14-day study in rats (Lent et al. 2012a; USAPHC 2011a) identified effects on survival and the kidney and hematopoietic system. Groups of six male Sprague-Dawley rats were given 2,3-DNT via gavage doses of 0, 17, 34, 69, 138, 275, or 550 mg/kg/day for

Table 2-1. Summary of Oral MRLs (mg/kg/day) for Dinitrotoluenes

Isomer	Acute	Intermediate	Chronic
2,3-DNT	0.09	NA	NA
2,4-DNT	0.05	0.007	0.001
2,5-DNT	0.007	NA	NA
2,6-DNT	0.09	0.004	NA
3,4-DNT	0.03	NA	NA
3,5-DNT	0.03	NA	NA

DNT = dinitrotoluene; NA = not applicable; data are not adequate to derive MRL

14 days; evaluations included morbidity, clinical observations, hematology, serum chemistry, gross necropsy, organ weights, and histopathology. At the highest dose (550 mg/kg/day), all rats died. Rats exposed to 275 mg/kg/day 2,3-DNT exhibited renal tubular dilatation (2/6) and lymphocytic infiltration (4/6), as well as trace extramedullary hematopoiesis (3/6 rats), lymphoid hyperplasia (1/6), and lymphoid depletion (1/6) in the spleen. Histopathology examinations of the kidney and spleen were not performed on lower dose groups. Although none of these incidences was statistically significantly increased in pairwise comparisons with controls, the lack of statistical significance likely reflects the very small group sizes rather than a lack of treatment-related effect, and the increases are considered to be biologically significant. Results of this study identify an acute-duration LOAEL value for renal and splenic effects of 275 mg/kg/day in rats. A NOAEL cannot be determined in the absence of histopathology examinations in lower dose groups.

Effects on the kidney and spleen were identified as the most sensitive effects of acute-duration oral exposure to 2,3-DNT, based on the LOAEL value of 275 mg/kg/day in rats administered 2,3-DNT via gavage for 14 days (Lent et al. 2012a; USAPHC 2011a). The LOAEL value of 275 mg/kg/day was used as the point of departure (POD). Available data were not suitable for benchmark dose (BMD) modeling, as spleen and kidney histopathology examinations were limited to the 275 mg/kg/day group and the control groups. The POD of 275 mg/kg/day was divided by an uncertainty factor of 3,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, 10 for human variability, and 3 for database limitations), resulting in an acute-duration oral MRL for 2,3-DNT of 0.09 mg/kg/day.

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.

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 a female dog on day 12; three additional male dogs showed 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 acute-duration no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (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 the 13-week portion of 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 in the acute-duration portion of the study.

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 LOAEL for hepatotoxicity (increased incidences of single cell necrosis and glycogen deposition) was reported in male Sprague-Dawley rats treated at 36 mg/kg/day for 14 days; the NOAEL was 18 mg/kg/day (Lent et al. 2012a; USAPHC 2011b). 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 for 5 mg/kg/day was used as POD. Neurotoxicity data were not suitable for 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 showed the development of 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 LOAEL values of 1.5 and 10 mg/kg/day in females and males, 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. 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 selected hematological values (methemoglobin, reticulocyte count, 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 erythrocyte count or methemoglobin; therefore, these data were not considered suitable for BMD modeling. Models provided an adequate fit to data for hematocrit, hemoglobin, and reticulocyte count. 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 reticulocyte count (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. 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

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 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 hematological data for 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 hemoglobin; therefore, these data were not considered suitable for BMD modeling. Models provided an adequate fit to data for erythrocyte count and hematocrit. Among all of

the models providing adequate fit to the data for these parameters, the lowest $BMDL_{1SD}$ values were 0.12 mg/kg/day for decreased hematocrit (exponential model 4) and 0.13 mg/kg/day for decreased hemoglobin (exponential model 4); of these, the lowest $BMDL_{1SD}$ 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 2,5-DNT

- An MRL of 0.007 mg/kg/day has been derived for acute-duration oral exposure (≤14 days) to 2,5-DNT.

No human acute-duration oral toxicity studies on 2,5-DNT were identified. For 2,5-DNT, the only available oral exposure studies are a 14-day repeated dose gavage study in rats, in which a number of toxicological end points were assessed (Lent et al. 2012a; USAPHC 2011c), and a single-dose gavage study, which reported data on lethality only (Vernot et al. 1977). Vernot et al. (1977) reported oral LD_{50} values of 710 and 1,230 mg/kg in rats and mice, respectively. The 14-day study in rats (Lent et al. 2012a; USAPHC 2011c) identified effects on survival, the hematopoietic system, and the heart; the hematopoietic system was the most sensitive. Groups of six male Sprague-Dawley rats were given 2,5-DNT via gavage doses of 0, 10, 19, 39, 77, 154, or 308 mg/kg/day for 14 days; evaluations included morbidity, clinical observations, hematology, serum chemistry, gross necropsy, organ weights, and histopathology. At the highest dose (308 mg/kg/day), one rat died. Of six rats exposed to 39 mg/kg/day 2,5-DNT, three exhibited mild-to-moderate extramedullary hematopoiesis, and all rats in higher dose groups exhibited this lesion; histopathology examinations were not performed in animals of lower dose groups. Marked increases (≥90%) in absolute and relative spleen weight were observed at doses ≥77 mg/kg/day. In addition, nonsignificant, but dose-related, increases (≥20%) in absolute and relative spleen weight were seen at doses ≥19 mg/kg-day. Results of this study identify an acute-duration LOAEL value for hematopoietic system effects (extramedullary hematopoiesis and increased absolute and relative spleen weight) of 39 mg/kg/day in rats. A NOAEL cannot be defined in the absence of spleen histopathology at lower doses, particularly in light of the spleen weight increases at lower doses.

The hematopoietic effects were identified as the most sensitive effects of acute-duration oral exposure to 2,5-DNT, based on the LOAEL value of 39 mg/kg/day in rats administered 2,5-DNT via gavage for 14 days (Lent et al. 2012a; USAPHC 2011c). Data on splenic lesions and absolute and relative spleen weights were subjected to BMD modeling to identify a POD for derivation of the acute oral MRL.

BMDLs for the best-fitting models were the $BMDL_{10}$ of 2.05 for incidence of mild-to-moderate extramedullary hematopoiesis, the $BMDL_{1SD}$ of 7.78 mg/kg/day for absolute spleen weight, and the $BMDL_{1SD}$ of 8.26 mg/kg/day for spleen weight relative to body weight. Neither the homogenous nor non-constant variance models within the EPA BMDS provided adequate fit to the data on spleen weight relative to brain weight. The lowest BMDL value ($BMDL_{10}$ of 2.05 mg/kg/day for extramedullary hematopoiesis) was selected as the POD for derivation of the acute-duration oral MRL. The POD of 2.1 mg/kg/day was divided by an uncertainty factor of 300 (10 for extrapolation from animals to humans, 10 for human variability, and 3 for database limitations), resulting in an acute-duration oral MRL for 2,5-DNT of 0.007 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), a 14-day gavage study in Sprague-Dawley rats (Lent et al. 2012a; USAPHC 2011d), 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.

When groups of six male rats were given 14 gavage doses of 2,6-DNT (0, 4, 7, 14, 35, 68, or 134 mg/kg/day), reductions in body weight of at least 10% were seen in rats exposed to doses ≥35 mg/kg/day, and statistically significant, dose-related reductions in hemoglobin and hematocrit were seen at 134 mg/kg/day. Significant alterations in the leukocyte differential occurred primarily at the highest dose, including increased neutrophils, increased monocytes, and increased percentages of neutrophils and lymphocytes. Gross necropsy showed small testes in all rats receiving 134 mg/kg/day; this was accompanied by marked decreases in absolute and relative testes weight at this dose. At the highest dose, absolute and relative (to brain weight) epididymides weights were decreased by ~30%, and absolute and relative (to brain weight) spleen weights were increased by ~50%. Animals treated with 2,6-DNT exhibited microscopic lesions in the testes (at doses ≥68 mg/kg/day), liver (doses ≥35 mg/kg/day), kidney (at 134 mg/kg/day), and spleen (≥35 mg/kg/day). A LOAEL of 35 mg/kg/day

was identified for this study based on liver (hepatocellular hyperplasia, oval cell hyperplasia, and hepatocellular hypertrophy) and spleen (lymphoid hyperplasia) lesions. The NOAEL was 14 mg/kg/day.

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. 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. The LOAEL from this study (20 mg/kg/day; U.S. Army 1976) was lower than the LOAEL from the rat study (35 mg/kg/day; Lent et al. 2012a, USAPHC 2011d). Although comprehensive end points were not evaluated after 2 weeks in the dog study, 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 mean cell hemoglobin; therefore, these data were not considered suitable for BMD modeling. With the nonconstant variance model applied, the linear, polynomial, and power models provided an adequate fit to the data for erythrocyte count. The polynomial and power models converged to the linear model. The $BMDL_{1SD}$ 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. 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 as 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.

Acute-Duration Oral MRL for 3,4-DNT

- An MRL of 0.03 mg/kg/day has been derived for acute-duration oral exposure (≤14 days) to 3,4-DNT.

No human acute-duration oral toxicity studies on 3,4-DNT were identified. For 3,4-DNT, the only available oral exposure study is a 14-day gavage study in rats in which a number of toxicological end points were assessed (Lent et al. 2012a; USAPHC 2011e). Lent et al. (2012a; USAPHC 2011e) identified effects on body weight, the hematopoietic system, the kidneys, and the neurological system; the hematopoietic system was affected at lower doses than the other target systems. Groups of six male Sprague-Dawley rats were given 3,4-DNT via gavage doses of 0, 7, 14, 28, 57, 113, or 227 mg/kg/day for 14 days; evaluations included morbidity, clinical observations, hematology, serum chemistry, gross necropsy, organ weights, and histopathology. All rats survived. Clinical signs in the highest dose animals included cyanosis, lethargy, facial twitching, and hypoactivity or staring behavior. A 10% reduction in body weight was seen at the highest dose (227 mg/kg/day); at this dose, trace-to-mild renal lesions (proximal tubular degeneration, tubular basophilia, and lymphocytic infiltration) were seen, as were mild fibrosis, inflammation, and necrosis in the heart of one rat. Splenic lesions, including trace-to-mild extramedullary hematopoiesis and lymphoid hyperplasia, were observed at doses ≥57 mg/kg/day. Spleens were not examined in lower dose groups. Results of this study identify an acute-duration LOAEL value for hematopoietic system effects of 57 mg/kg/day in rats. A NOAEL for hematopoietic system effects cannot be determined in the absence of histopathology examinations in lower dose groups.

Hematopoietic system toxicity was identified as the most sensitive effect of acute-duration oral exposure to 3,4-DNT, based on the LOAEL value of 57 mg/kg/day in rats administered 3,4-DNT via gavage for 14 days (Lent et al. 2012a; USAPHC 2011e). Incidences of trace-to-mild extramedullary hematopoiesis

and lymphoid hyperplasia were subjected to BMD modeling. The best-fitting model for both end points yielded the same $BMDL_{10}$ of 8.05 mg/kg/day; this value was selected as the POD. The POD of 8.05 mg/kg/day was divided by an uncertainty factor of 300 (10 for extrapolation from animals to humans, 10 for human variability, and 3 for database limitations), resulting in an acute-duration oral MRL for 3,4-DNT of 0.03 mg/kg/day.

Acute-Duration Oral MRL for 3,5-DNT

- An MRL of 0.03 mg/kg/day has been derived for acute-duration oral exposure (≤14 days) to 3,5-DNT.

No human acute-duration oral toxicity studies on 3,5-DNT were identified. For 3,5-DNT, the only available oral exposure study is a 14-day gavage study in rats, in which a number of toxicological end points were assessed (Lent et al. 2012a; USAPHC 2011f). Lent et al. (2012a; USAPHC 2011f) identified effects on survival, the reproductive tissues, and neurological system; effects on the reproductive tissues were the most sensitive. Groups of six male Sprague-Dawley rats were given 3,5-DNT via gavage doses of 0, 5, 10, 19, 39, 77, and 155 mg/kg/day for 14 days; evaluations included morbidity, clinical observations, hematology, serum chemistry, gross necropsy, organ weights, and histopathology. At doses ≥77 mg/kg/day, all rats died; in addition, one rat exposed to 39 mg/kg/day died. Animals exposed to 39 mg/kg/day exhibited neurological signs progressing from facial twitching to paralysis of the lower forelimbs. Rats exposed to ≥19 mg/kg/day 3,5-DNT exhibited testicular lesions including mild-to-severe tubular degeneration and trace-to-severe multinucleated giant cell formation. Results of this study identify acute-duration NOAEL and LOAEL values for testicular lesions of 10 and 19 mg/kg/day, respectively, in rats.

Testicular toxicity was identified as the most sensitive effect of acute-duration oral exposure to 3,5-DNT, based on the LOAEL value of 19 mg/kg/day in rats administered 3,5-DNT via gavage for 14 days (Lent et al. 2012a; USAPHC 2011f). The NOAEL value of 10 mg/kg/day was used as the POD. Available data were not suitable for BMD modeling, as the incidences of testicular lesions increased from 0/6 in the control group to 6/6 at the LOAEL. The POD of 10 mg/kg/day was divided by an uncertainty factor of 300 (10 for extrapolation from animals to humans, 10 for human variability, and 3 for database limitations), resulting in an acute-duration oral MRL for 3,5-DNT of 0.03 mg/kg/day.