APPENDIX A. ATSDR MINIMAL RISK LEVELS AND WORKSHEETS

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [42 U.S.C. 9601 et seq.], as amended by the Superfund Amendments and Reauthorization Act (SARA) [Pub. L. 99–499], requires that the Agency for Toxic Substances and Disease Registry (ATSDR) develop jointly with the U.S. Environmental Protection Agency (EPA), in order of priority, a list of hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL); prepare toxicological profiles for each substance included on the priority list of hazardous substances; and assure the initiation of a research program to fill identified data needs associated with the substances.

The toxicological profiles include an examination, summary, and interpretation of available toxicological information and epidemiologic evaluations of a hazardous substance. During the development of toxicological profiles, Minimal Risk Levels (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 for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. MRLs are based on noncancer health effects only and are not based on a consideration of cancer effects. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the no-observed-adverse-effect level/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (365 days and longer) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive chemical-induced end point considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that

are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology and Human Health Sciences, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology and Human Health Sciences, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop F-57, Atlanta, Georgia 30329-4027.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 2,3-DNT
CAS Numbers: 602-01-7
Date: February 2016
Profile Status: Final
Route: [ ] Inhalation [X] Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Graph Key: 4
Species: Rat

Minimal Risk Level: 0.09 [X] mg/kg/day [ ] ppm

References: Lent EM, Crouse CB, Quinn MJ, et al. 2012a. Comparison of the repeated dose toxicity of isomers of dinitrotoluene. Int J Toxicol 31(2):143-157.

U.S. Army Public Health Command (USAPHC). 2011a. Toxicology Study No. 87-XE-08G0A-08, October 2011, Toxicology Portfolio. Fourteen-day oral toxicity and in vivo genotoxicity of 2,3-dinitrotoluene in rats, June-July 2008. USAPHC, Aberdeen Proving Ground, Maryland.

Experimental design: In a comparative toxicity study, 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 14 days. Both untreated and vehicle control groups were included. Evaluations during the study included twice daily observations for morbidity and clinical signs and measurement of body weight and food consumption on days 0, 1, 3, 7, and 14. Prior to sacrifice at the end of exposure, blood was collected for evaluation of hematology (total and differential white blood cell counts; red blood cell count; hemoglobin, hematocrit, MCV, MCH, mean corpuscular hemoglobin concentration [MCHC], red cell distribution width; platelet count; and mean platelet volume) and serum chemistry (albumin, ALP, ALT, AST, BUN, calcium, cholesterol, creatinine, glucose, globulin, LDH, total bilirubin, total protein, sodium, potassium, and chlorine) assessments. All animals received gross necropsy and organ weight determinations (brain, heart, kidney, epididymides, liver, spleen, and testes). Microscopic examination of organs with exposure-related changes in weight was performed for controls and any dose groups exhibiting changes in the corresponding organ weights.

Effects noted in study and corresponding doses: All rats exposed to 550 mg/kg/day died or were sacrificed moribund within the first 2–3 days of dosing. Clinical signs of toxicity were observed only in this dose group. In the surviving dose groups, there were no statistically significant differences from control in body weight or food consumption, although body weight was lower (~9% less than vehicle controls) in the 275 mg/kg/day group. In addition, there were no significant, treatment-related changes in hematology or clinical chemistry parameters. The authors noted 2.3-fold increases in ALP, ALT, and AST in the 138 mg/kg/day group, but the difference from control was not statistically significant, and comparable increases were not seen at in the higher dose group (275 mg/kg/day). Significantly increased relative (to body weight) liver and kidney weights were observed at 275 mg/kg/day (20 and 14% higher than vehicle controls, respectively); lower body weights in this group likely contributed to the changes. Absolute organ weights and organ weights relative to brain weight were not significantly different from controls. There were no accompanying histopathology lesions in the liver, nor were there clinical chemistry changes indicative of liver or kidney injury. In animals surviving to terminal sacrifice, however, renal lesions were observed at 275 mg/kg/day, including trace tubular dilatation in 2/6 rats (compared with 1/6 controls) and trace lymphocytic infiltration in 4/6 rats (compared with 1/6 controls). In addition, extramedullary hematopoiesis (3/6 rats) and lymphoid hyperplasia (1/6) of the spleen, as well as lymphoid depletion (1/6), were observed at 275 mg/kg/day. These lesions were not seen in controls.

Although none of these incidences was statistically significantly increased in pairwise comparisons with controls, the group sizes were very small, and the increases are considered to be biologically significant. Kidneys and spleens of animals in the lower dose groups were not examined microscopically. Results of this study identify an acute-duration LOAEL for kidney and splenic effects (see Table A-1) of 275 mg/kg/day, respectively, in rats. A NOAEL for kidney and splenic effects cannot be determined in the absence of histopathology examinations in lower dose groups.

Table A-1. Renal and Splenic Effects in Rats Exposed to 2,3-DNT for 14 Days

	Dose (mg/kg/day)						
End point	0	17	34	69	138	275	550
Renal tubular dilatation	1/6[a]	ND	ND	ND	ND	2/6	NA
Renal lymphocytic infiltration	1/6	ND	ND	ND	ND	4/6	NA
Splenic extramedullary hematopoiesis	0/6	ND	ND	ND	ND	3/6	NA
Splenic lymphoid hyperplasia	0/6	ND	ND	ND	ND	1/6	NA
Splenic lymphoid depletion	0/6	ND	ND	ND	ND	1/6	NA

[a]Number affected/number examined.

DNT = dinitrotoluene; NA = not applicable; all animals in this group died or were sacrificed moribund; ND = no data; histopathology examinations were not performed on these animals.

Sources: Lent et al. 2012a; USAPHC 2011a

Dose and end point used for MRL derivation:

[] NOAEL [X] LOAEL 275 mg/kg/day was the LOAEL for renal and hematopoietic effects (renal tubular dilatation, renal lymphocytic infiltration, splenic extramedullary hematopoiesis and lymphoid hyperplasia).

The LOAEL value of 275 mg/kg/day for renal and hematopoietic effects in rats was identified as the POD for derivation of the acute-duration oral MRL for 2,3-DNT (Lent et al. 2012a; USAPHC 2011a). A NOAEL was not determined from the study as the target organs (kidney and spleen) were not examined microscopically in lower dose groups. Available data were not suitable for BMD modeling, as incidence data were limited to the 275 mg/kg/day group and the control group. 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, and 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.

Uncertainty Factors used in MRL derivation:

[X] 10 for use of a LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability
[X] 3 for database limitations (the only other data available are from an acute lethality study)

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? No.

Other additional studies or pertinent information which lend support to this MRL: The only other acute-duration oral study of 2,3-DNT was an acute lethality study, in which LD_{50} values of 1,120 and 1,070 mg/kg were identified in rats and mice, respectively (Vernot et al. 1977).

Agency Contact (Chemical Manager): Carolyn Harper

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 2,4-DNT
CAS Numbers: 121-14-2
Date: February 2016
Profile Status: Final
Route: [ ] Inhalation [X] Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Graph Key: 14
Species: Dog

Minimal Risk Level: 0.05 [X] mg/kg/day [ ] ppm

References: Ellis HV, Hong CB, Lee CC, et al. 1985. Subchronic and chronic toxicity studies of 2,4-dinitrotoluene. Part I. Beagle dog. J Am Coll Toxicol 4:233-242.

U.S. Army. 1978b. Mammalian toxicity of munitions compounds. Phase II: Effects of multiple doses. Part II: 2,4-Dinitrotoluene. Progress report no. 3. Fort Detrick, MD: U.S. Army, Medical Bioengineering Research and Development Laboratory. ADA061715.

Experimental design: In a subchronic study, beagle dogs (4/sex/group) were administered 0, 1, 5, or 25 mg/kg/day 2,4-DNT for up to 13 weeks. 2,4-DNT was mixed with lactose and administered as capsules. Dogs were observed daily for behavioral changes and clinical signs of toxicity. Body weights were recorded weekly. Blood was taken before initiation of treatment and at 4, 8, and 13 weeks for evaluation of hematological parameters (erythrocyte, reticulocyte, platelet, and total and differential leukocyte counts; Heinz bodies; hematocrit, hemoglobin, and methemoglobin concentrations; mean cell volume, hemoglobin, and hemoglobin concentration) and clinical chemistry analyses (glucose, urea nitrogen, sodium, potassium, calcium, magnesium, and chloride; and the serum enzyme activity of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase). Animals (1 sex/group) were sacrificed after 4 or 13 weeks of continuous treatment; an additional dog/sex/group was discontinued treatment after 4 or 13 weeks and sacrificed after 4 weeks of recovery. The two high-dose dogs removed from treatment at 4 weeks were not sacrificed until 8 months after cessation of treatment to test the reversibility of effects after a longer recovery period. When animals were sacrificed moribund or at study termination, they were examined for gross lesions. Major organs and tissues (heart, liver, spleen, kidneys, adrenals, and gonads) were weighed; "various" tissues (not specified) were subjected to histopathology. To evaluate the immunologic response to 2,4-DNT, the concentration of IgE in the serum was assessed after treatment for 4, 8, or 13 weeks or after treatment for 4 or 13 weeks followed by recovery for 4 weeks. Bone marrow and kidney cultures were also maintained and cytogenetic analyses (evaluation of chromosome number and morphology) were performed.

Effects noted in study and corresponding doses: Data for acute-duration oral exposure were obtained from daily cageside observations for behavioral changes and clinical signs of toxicity during the first 14 days of treatment. No mortality was observed during the first 14 days of treatment. No behavioral changes or clinical signs of toxicity were observed in dogs treated with 1 or 5 mg/kg/day. Neurotoxicity was observed at 25 mg/kg/day. Evidence of neurotoxicity, identified as loss of hind leg control, was first observed in a female dog on day 12 of treatment. Three additional male dogs showed similar signs on day 14 of treatment. All high-dose dogs showed signs of neurotoxicity after treatment for 12–22 days. The onset and severity of toxic signs reportedly varied among dogs within the same treatment group; some dogs were moribund at the same time that others began experiencing symptoms. In individual dogs, symptom severity varied over time, with no duration-related pattern of severity. Although incidence data were not reported, the study authors noted that the neurotoxic effects most often observed in the 13-week

study were incoordination of the hind legs and stiffness that produced an abnormal hopping gait. Some dogs experienced paralysis of the hind legs. In severe cases, stiffness progressed from the hind legs to the trunk, forelegs, neck, and head. Histopathological assessments conducted at 4 or 13 weeks showed lesions of the central nervous system, including generalized vacuolization, hypertrophy, mitosis of the endothelium and focal gliosis in the cerebellum, and/or perivascular hemorrhage in the cerebellum and brain stem in high-dose animals (2/2 and 3/3 animals evaluated at 4 and 13 weeks, respectively). However, the study authors noted that the most severe of these lesions occurred in dogs that developed toxic signs of neurotoxicity late in the study (time to onset of symptoms not reported). Results of this study identify acute-duration NOAEL and LOAEL values for neurotoxicity in dogs of 5 and 25 mg/kg/day, respectively.

Dose and end point used for MRL derivation:

[X] NOAEL [ ] LOAEL [] 5 mg/kg/day was the NOAEL for neurological effects (loss of hind leg control).

The NOAEL value of 5 mg/kg/day for neurotoxicity in dogs was identified as the POD for derivation of the acute-duration oral MRL for 2,4-DNT (Ellis et al. 1985; U.S. Army 1978b). Neurotoxicity data were not suitable for BMD modeling, since effects were only observed at the highest dose tested. Therefore, the NOAEL value for 5 mg/kg/day was used at the POD. This value 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.

Uncertainty Factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? No.

Other additional studies or pertinent information which lend support to this MRL: No other acute-duration studies were located in which neurotoxicity was reported after oral exposure to 2,4-DNT. Other acute-duration studies in rodents identified hepatic effects (single cell necrosis and glycogen deposition) and hematological effects (splenic extramedullary hematopoesis) in male Sprague-Dawley rats treated at 36 and 71 mg/kg/day (respectively) via gavage for 14 days (Lent et al. 2012a; USAPHC 2011b), slight cyanosis in male Sprague-Dawley rats treated at 60 mg/kg (the lowest tested dose) for 5 days (Lane et al. 1985), and decreased fertility in CD-1 female mice dosed with 250 mg/kg 2,4-DNT for 2 days (Soares and Lock 1980). Neurotoxicity was observed in beagle dogs after subchronic or chronic treatment with 2,4-DNT. Clinical signs of neurotoxicity (including incoordination and paralysis), sometimes accompanied by central nervous system lesions (generalized vacuolization, hypertrophy, mitosis of the endothelium and focal gliosis in the cerebellum, and perivascular hemorrhages of the cerebellum and brain stem) were reported in dogs (4/sex/group) dosed with 25 mg/kg 2,4-DNT for up to 13 weeks (Ellis et al. 1985; U.S. Army 1978b) and in dogs (6 sex/group) treated at 1.5 (one dog) or 10 mg/kg/day (all dogs) for up to 24 months (Ellis et al. 1985; U.S. Army 1979). Dogs appear to be the most sensitive species for 2,4-DNT-induced neurotoxicity; in CD rats and CD-1 mice treated with 2,4-DNT for up to 24 months, neurotoxic effects were absent or occurred at much higher doses in similarly designed studies

(U.S. Army 1978b, 1979). Neuromuscular effects similar to those observed in dogs occurred in rats administered 2,4-DNT at 266 or 145 mg/kg/day (for males and females, respectively) for up to 13 weeks, but not in rats treated with 2,4-DNT at up to 34 or 45 mg/kg/day (for males and females, respectively) for 24 months. Mice treated with 2,4-DNT at 413 or 468 mg/kg/day (for males and females, respectively) for up to 13 weeks or 898 mg/kg/day for 24 months did not show clinical signs of neurotoxicity (U.S. Army 1978b, 1979).

Agency Contact (Chemical Manager): Carolyn Harper

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 2,4-DNT
CAS Numbers: 121-14-2
Date: February 2016
Profile Status: Final
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [X] Intermediate [ ] Chronic
Graph Key: 32
Species: Dog

Minimal Risk Level: 0.007 [X] mg/kg/day [ ] ppm

References: U.S. Army. 1979. Mammalian toxicity of munitions compounds. Phase III: Effects of lifetime exposure. Part I. 2,4-Dinitrotoluene. Final report no. 7. Fort Detrick, MD: U.S. Army and Medical Bioengineering Research Development Laboratory. ADA077692.

Ellis HV, Hong CB, Lee CC, et al. 1985. Subchronic and chronic toxicity studies of 2,4-dinitrotoluene. Part I. Beagle dog. J Am Coll Toxicol 4:233-242.

Experimental design: Young beagle dogs (6 dogs/sex/group; age not specified) were administered 0, 0.2, 1.5, or 10 mg/kg 2,4-DNT in capsules for 24 months. Dogs were observed daily for behavioral changes and clinical signs of toxicity. Body weights were recorded weekly. Feed consumption was measured during 1 week each month starting in month 6. Blood was taken before initiation of treatment and after 3, 6, 9, 12, 18, and 24 months of exposure for assessment of hematological parameters (erythrocyte, reticulocyte, platelet, and total and differential leukocyte counts; Heinz bodies, clotting time, hematocrit, hemoglobin, and methemoglobin concentrations; and mean cell volume, hemoglobin, and hemoglobin concentration) and clinical chemistry (fasting glucose, urea nitrogen, levels of sodium, potassium, calcium, magnesium, chloride, and bilirubin [high-dose dogs with toxic signs], and the serum enzyme activities of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase) analyses. Animals (one male and one female/group) were sacrificed after 12 or 24 months of continuous treatment; an additional dog/sex/group was discontinued from treatment at these time points and were sacrificed after a 4-week recovery period to evaluate the reversibility of effects (including clinical signs, hematology and clinical chemistry, organ weights, and histopathological effects). Animals that were moribund during the study and those that survived to study termination were sacrificed and examined for gross lesions; major organs and tissues (including the brain, heart, liver, spleen, kidneys, adrenals, thyroids, pituitary, and gonads) were weighed, and comprehensive histopathological analyses (35 tissues) were performed.

Effects noted in study and corresponding doses: Intermediate-duration oral exposure of dogs to 2,4-DNT produced methemoglobinemia, anemia, and compensatory hematopoiesis (Ellis et al. 1985; U.S. Army 1979). Hematological effects of 2,4-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, resulting in anemia. Ferric iron also contributes to the denaturation of hemoglobin and subsequent removal of erythrocytes from the blood. Heinz bodies are also detected as granules in erythrocytes resulting from denatured hemoglobin. Increased hematopoiesis is typically observed as a compensatory response to decreased erythrocyte count. Hematological effects consistent with development of methemoglobinemia were observed in dogs administered oral 2,4-DNT 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. Therefore, only data from the 9-month evaluation were considered for derivation of the intermediate-duration oral MRL. The only significant effects

observed at the intermediate-duration time points were changes to hematological parameters. At the mid- and low dose, no clinical signs of toxicity, behavioral changes, or effects on clinical chemistry parameters were observed at the intermediate-duration time points at any of the doses tested. At the high dose, clinical signs of neurotoxicity (decreased muscle control and incoordination), sometimes accompanied by decreased body weight, were observed; these effects contributed to the death of four of six dogs within the first 20 weeks of the study.

Effects on hematological parameters in male and female dogs administered oral 2,4-DNT for 9 months are summarized in Table A-2. Male and female dogs exposed to 2,4-DNT for 9 months at doses of 1.5 and 10 mg/kg/day showed detectable amounts of methemoglobin in the serum (the initiating hematological effect), with changes reaching statistical significance in males and females in the 10 mg/kg/day group. 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. Effects on hematological parameters in male dogs were similar to those in female dogs, although changes did not reach statistical significance in the 10 mg/kg/day group, possibly due to low numbers of male dogs evaluated (hematological data available for only two males in the 10 mg/kg/day group). After treatment for 18 or 24 months, slight or no anemia, near-normal reticulocyte levels, no Heinz bodies, and minimal amounts of methemoglobin were detected, likely reflective of an adaptive response. Recovery from hematological effects also occurred in dogs allowed to recover for 4 weeks after dosing for 12 or 24 months.

Table A-2. Hematological Effects in Beagle Dogs Exposed to 2,4-DNT for 9 Months

	Dose (mg/kg/day)			
End point	0	0.2	1.5	10
			Males	
Erythrocyte count (x10/mm)	6.51±0.12 (6)[a]	6.23±0.18 (6) [↓4]	6.36±0.13 (6) [↓2]	6.36±0.13 (2) [↓2]
Heinz bodies (%)	0.0±0.0(6)	0.0±0.0(6) [NA]	0.0±0.0(6) [NA]	1.8±0.6(2)[b] [NA]
Reticulocytes (%)	0.69±0.15 (6)	0.55±0.09 (6) [↓20]	0.57±0.11 (6) [↓17]	1.39±0.47 (2) [↑101]
Hematocrit (%)	46.7±1.0 (6)	45.3±1.1 (6) [↓3]	45.0±0.9 (6) [↓4]	42.0±3.0 (2) [↓10]
Hemoglobin (%)	16.6±0.3 (6)	15.4±0.4 (6)	15.7±0.3 (6)	15.7±0.3 (6)
Methemoglobin (%)	0.0±0.0 (6)	0.0±0.0 (6) [NA]	0.9±0.6 (6) [NA]	2.8±0.3 (2)[b] [NA]
			Females	
Erythrocyte count (x10/mm)	6.49±0.24 (6)[a]	5.90±0.17 (6) [↓9]	5.78±0.21 (6) [↓11]	5.05±0.17 (6)[b] [↓22]
Heinz bodies (%)	0.0±0.0(6)	0.0±0.0(6) [NA]	0.0±0.0(6) [NA]	0.84±0.21 (6)[b] [NA]
Reticulocytes (%)	0.60±0.08 (6)	0.70±0.17 (6) [↑17]	0.45±0.10 (6) [↓25]	1.33±0.19 (6)[b] [↑122]
Hematocrit (%)	46.0±1.3 (6)	42.8±1.7 (6) [↓7]	42.5±1.2 (6) [↓8]	37.2±1.6 (6)[b] [↓19]
Hemoglobin (%)	16.1±0.5 (6)	14.7±0.6 (6) [↓9]	14.8±0.5 (6) [↓8]	13.3±0.5 (6)[b] [↓17]
Methemoglobin (%)	0.4±0.4 (6)	0.0±0.0 (6) [↓100]	1.3±0.6 (6) [↑225]	2.8±0.7 (6)[b] [↑600]

[a]Values are means±standard error (number of animals) [percent change from controls].
[b]Statistically significant based on analyses performed by the study authors (Dunnett's multiple comparison procedure).

DNT = dinitrotoluene; NA = not applicable

Sources: Ellis et al. 1985; U.S. Army 1979

Dose and end point used for MRL derivation:

[ ] NOAEL [ ] LOAEL [X] BMDL 0.67 mg/kg/day as a $BMDL_{1SD}$ for hematological effects (decreased hematocrit)

Results of hematology assessments show that intermediate-duration, oral exposure of dogs to 2,4-DNT induced methemoglobinemia, anemia, and compensatory hematopoiesis (Ellis et al. 1985; U.S. Army 1979). Changes in several hematological parameters, including decreased erythrocyte count, hematocrit, and hemoglobin and increased reticulocytes, methemoglobin, and Heinz bodies were observed after treatment with 2,4-DNT for 3, 6, and 9 months. However, hematological effects at 9 months were more pronounced and consistent than those observed at 3 and 6 months; therefore, hematological effects

observed at 9 months were identified as the critical effect for derivation of the intermediate-duration oral MRL. To determine the POD, hematological data from female dogs treated with 2,4-DNT for 9 months were further evaluated by BMD analysis. The following data sets in female dogs were selected for BMD modeling: erythrocyte count, reticulocytes, hematocrit, hemoglobin, and methemoglobin. Data on Heinz bodies in serum were not selected for BMD modeling, since these granules were detected in high-dose animals only (i.e., all-or-nothing response); the absence of changes at lower dose levels suggests that these data would not be suitable for modeling. 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 (for most hematological parameters, data were available for only two dogs).

To determine the POD for derivation of the intermediate-duration oral MRL, all available continuous-variable models in the EPA BMDS (version 2.1) were fit to the data for increased methemoglobin, increased reticulocytes, decreased hemoglobin, decreased erythrocyte count, and decreased hematocrit (Ellis et al. 1985; U.S. Army 1979). The BMD and the 95% lower confidence limit (BMDL) were estimated for doses associated with a change of 1 standard deviation from the controls, and are in units of mg/kg/day. For continuous data, in the absence of a clear criteria as to what level of change should be considered adverse, the BMR is defined as a change equal to 1 standard deviation from the control mean (EPA 2000a). Adequate model fit is judged by three criteria: goodness-of-fit ($p>0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. Among all of the models providing adequate fit to the data, the lowest benchmark dose (BMDL, the lower limit of a one-sided 95% CI on the BMD) is selected as the POD when differences between the BMDLs estimated from these models are >3-fold; otherwise, the BMDL from the model with the lowest AIC is chosen.

Neither the constant nor the non-constant variance model provided an adequate fit to the data for decreased erythrocyte count or increased methemoglobin; therefore, these data were not considered suitable for BMD modeling. BMD model prediction for increased reticulocytes, decreased hemoglobin, and decreased hematocrit are shown in Tables A-3, A-4, and A-5, respectively. Of models meeting adequate fit criteria for each hematological parameter, the lowest $BMDL_{1SD}$ values were 5.64 mg/kg/day for increased reticulocytes (polynomial 3-degree; Figure A-1), 3.66 mg/kg/day for decreased hemoglobin (exponential model 2; Figure A-2), and 0.67 mg/kg/day for decreased hematocrit (exponential model 4; Figure A-3). Of these, the lowest $BMDL_{1SD}$ of 0.67 mg 2,4-DNT/kg/day for decreased hematocrit was selected as the POD for derivation of the intermediate-duration oral MRL for 2,4-DNT. 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.

Table A-3. Model Predictions for 2,4-DNT for Increased Reticulocytes (%) in Female Dogs Following 9 Months of Exposure (U.S. Army 1979)

Model	Test for significant difference p-value[a]	Variance p-value[b]	Means p-value[b]	Scaled residuals[c] Dose below BMD	Scaled residuals[c] Dose above BMD	Scaled residuals[c] Overall largest	AIC	BMD_{1SD} (mg/kg/day)	$BMDL_{1SD}$ (mg/kg/day)
Constant variance									
Exponential (model 2)[d]	0.0007	0.12	0.23	-1.32	0.09	-1.32	-21.60	5.55	4.35
Exponential (model 3)[d]	0.0007	0.12	0.18	-0.98	5.59×10^{-8}	-0.98	-20.75	9.40	4.58
Exponential (model 4)[d]	0.0007	0.12	0.06	-1.51	0.21	-1.50	-18.93	4.61	3.19
Exponential (model 5)[d]	0.0007	0.12	NA	-0.98	8.61×10^{-8}	-0.98	-18.75	9.17	1.59
Hill[d]	0.0007	0.12	NA	-0.98	-9.04×10^{-7}	-0.98	-18.75	9.10	1.60
Linear[e]	0.0007	0.12	0.17	-1.51	0.21	-1.51	-20.93	4.61	3.19
Polynomial (2-degree)[e]	0.0007	0.12	0.36	-1.06	0.02	-1.06	-22.50	6.69	5.60
Polynomial (3-degree)[e,f]	***0.0007***	***0.12***	***0.40***	***-0.99***	***0.003***	***-0.99***	***-22.72***	***7.64***	***5.64***
Power[d]	0.0007	0.12	0.18	-0.98	3.56×10^{-10}	-0.98	-20.75	9.31	3.68

[a]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[d]Power restricted to ≥1.
[e]Coefficients restricted to be positive.
[f]Selected model. Constant variance model provided adequate fit to variance data. With constant variance model applied, all models, except for the Exponential 4 and 5 and Hill models, provided adequate fit to means. BMDLs for models providing adequate fit were considered to be sufficiently close (differed by <2–3-fold), so the model with the lowest AIC was selected.

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); DF = degrees of freedom; DNT = dinitrotoluene; NA = not applicable (BMDL computation failed); SD = standard deviation

Figure A-1. Fit of Polynomial 3-Degree Model to Data on 2,4-DNT for Increased Reticulocytes (%) in Female Dogs Following 9 Months of Exposure (U.S. Army 1979)

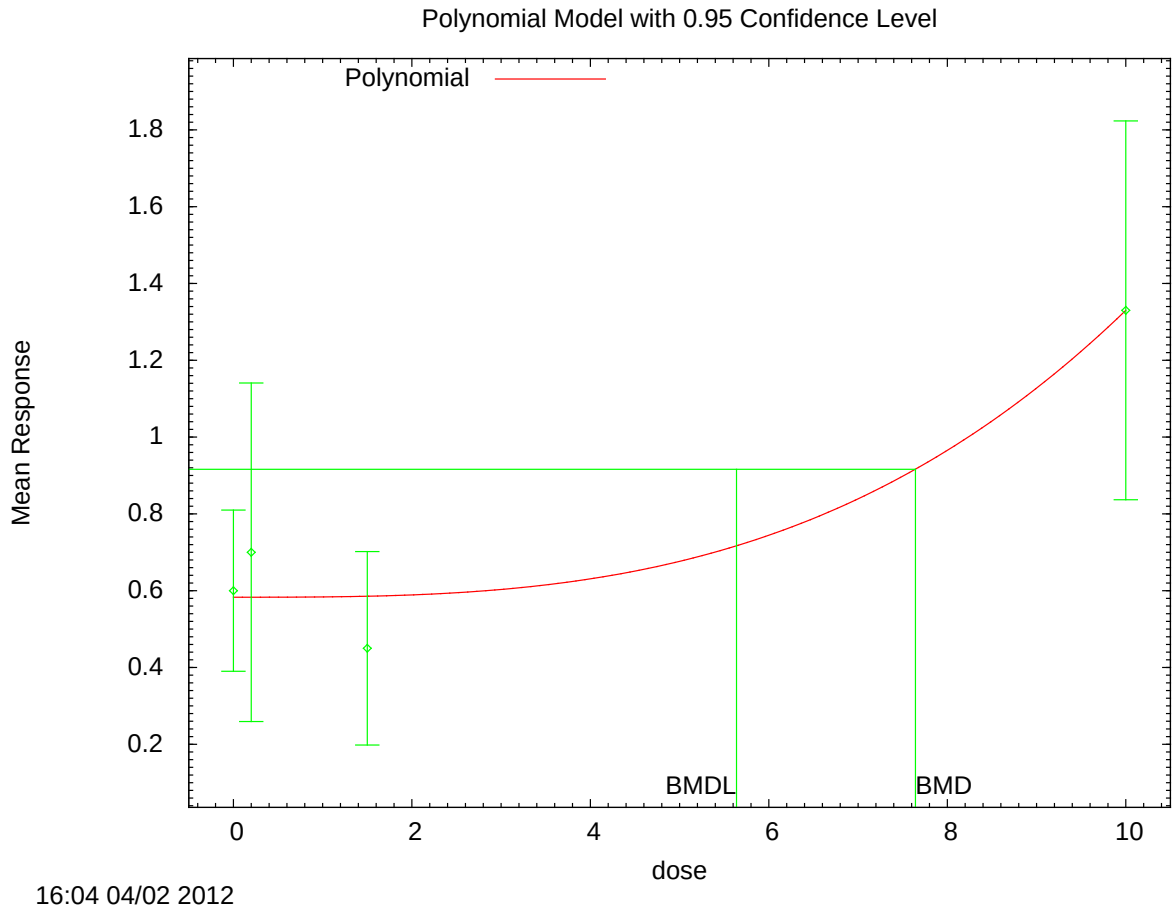


Table A-4. Model Predictions for 2,4-DNT for Decreased Hemoglobin (%) in Female Dogs Following 9 Months of Exposure (U.S. Army 1979)

Model	Test for significant difference p-value[a]	Variance p-value[b]	Means p-value[b]	Scaled residuals[c] Dose below BMD	Scaled residuals[c] Dose above BMD	Scaled residuals[c] Overall largest	AIC	BMD_{1SD} (mg/kg/day)	$BMDL_{1SD}$ (mg/kg/day)
Constant variance									
Exponential (model 2)[d,e]	***0.04***	***0.92***	***0.14***	***-0.40***	***0.10***	***1.46***	***41.47***	***5.90***	***3.66***
Exponential (model 3)[d]	0.04	0.92	0.14	-0.40	0.10	1.46	41.47	5.90	3.66
Exponential (model 4)[d]	0.04	0.92	0.07	0.29	-0.03	-1.36	42.88	2.43	0.01
Exponential (model 5)[d]	0.04	0.92	0.07	0.29	-0.03	-1.36	42.88	2.43	0.01
Hill[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA
Linear[f]	0.04	0.92	0.14	-0.43	0.09	1.48	41.52	6.12	3.94
Polynomial (2-degree)[f]	0.04	0.92	0.14	-0.43	0.09	1.48	41.52	6.12	3.94
Polynomial (3-degree)[f]	0.04	0.92	0.14	-0.43	0.09	1.48	41.52	6.12	3.94
Power[d]	0.04	0.92	0.14	-0.43	0.09	1.48	41.52	6.12	3.94

[a]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[d]Power restricted to ≥1.
[e]Selected model. Constant variance model provided adequate fit to variance data. With constant variance model applied, all models, except for the Exponential 4 and 5 and Hill model (computation failed), provided adequate fit to means. BMDLs for models providing adequate fit were considered to be sufficiently close (differed by <2–3-fold), so the model with the lowest AIC was selected (the Exponential 3 model converged on to the Exponential 2).
[f]Coefficients restricted to be negative.

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); DF = degrees of freedom; DNT = dinitrotoluene; NA = not applicable (BMDL computation failed); SD = standard deviation

Figure A-2. Fit of the Exponential 2 Model to Data on 2,4-DNT for Decreased Hemoglobin (%) in Female Dogs Following 9 Months of Exposure (U.S. Army 1979)

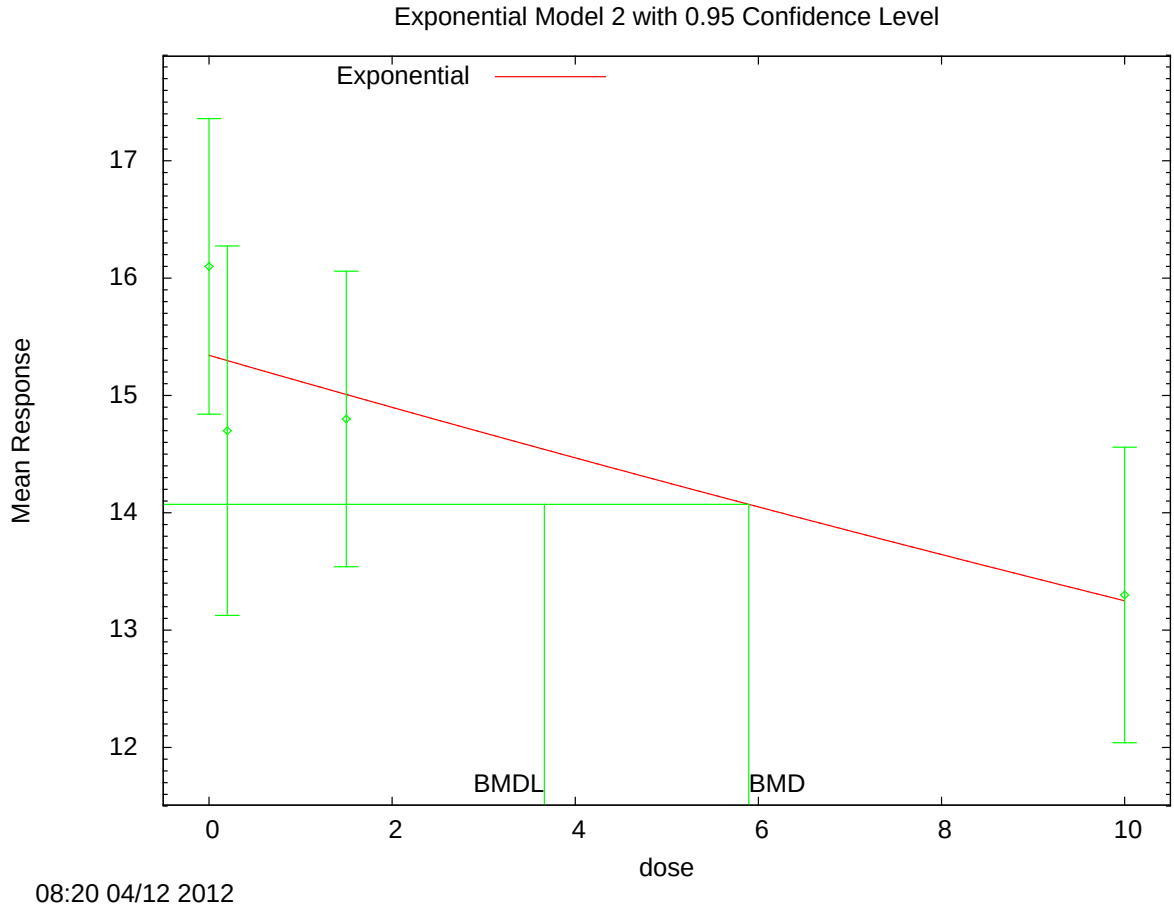


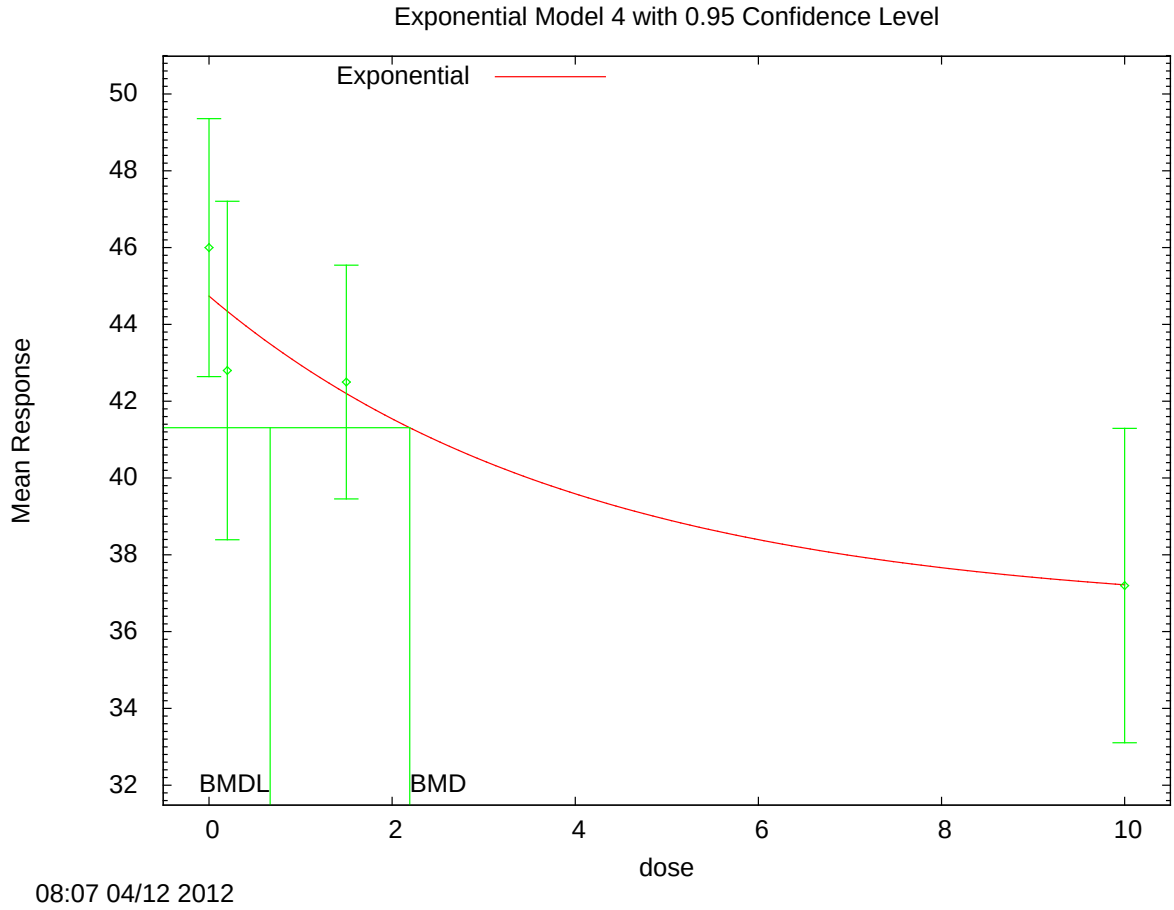
Table A-5. Model Predictions for 2,4-DNT for Decreased Hematocrit (%) in Female Dogs Following 9 Months of Exposure (U.S. Army 1979)

Model	Test for significant difference p-value[a]	Variance p-value[b]	Means p-value[b]	Scaled residuals[c] Dose below BMD	Dose above BMD	Overall largest	AIC	BMD_{1SD} (mg/kg/day)	$BMDL_{1SD}$ (mg/kg/day)
Constant variance									
Exponential (model 2)[d]	0.01	0.79	0.26	-0.42	0.09	1.24	89.65	4.60	3.04
Exponential (model 3)[d]	0.01	0.79	0.26	-0.42	0.09	1.24	89.65	4.60	3.04
Exponential (model 4)[d,e]	***0.01***	***0.79***	***0.14***	***0.21***	***-0.01***	***-1.10***	***91.12***	***2.19***	***0.67***
Exponential (model 5)[d]	0.01	0.79	0.14	0.21	-0.01	-1.10	91.12	2.19	0.67
Hill[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA
Linear[f]	0.01	0.79	0.25	-0.45	0.09	1.26	89.71	4.86	3.32
Polynomial (2-degree)[f]	0.01	0.79	0.25	-0.45	0.09	1.26	89.71	4.86	3.32
Polynomial (3-degree)[f]	0.01	0.79	0.25	-0.45	0.09	1.26	89.71	4.86	3.32
Power[d]	0.01	0.79	0.25	-0.45	0.09	1.26	89.71	4.86	3.32

[a]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[d]Power restricted to ≥1.
[e]Selected model. Constant variance model provided adequate fit to variance data. With constant variance model applied, all models, except for the Hill model (computation failed), provided adequate fit to means. BMDLs for models providing adequate fit were not considered to be sufficiently close (differed by >2–3-fold), so the model with the lowest BMDL was selected (the Exponential 5 model converged on to the Exponential 4).
[f]Coefficients restricted to be negative.

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); DF = degrees of freedom; DNT = dinitrotoluene; NA = not applicable (BMDL computation failed); SD = standard deviation

Figure A-3. Fit of the Exponential 4 Model to Data on 2,4-DNT for Decreased Hematocrit (%) in Female Dogs Following 9 Months of Exposure (U.S. Army 1979)



Uncertainty Factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? No.

Other additional studies or pertinent information which lend support to this MRL: The hematological effects observed in this study are consistent with well-characterized effects observed after exposure to aromatic amines and with effects observed at higher doses in other studies of intermediate duration (Hong et al. 1985; Lee et al. 1985; Kozuka et al. 1979; U.S. Army 1978b). Similar hematological effects (anemia, accompanied by the presence of Heinz bodies) were observed in beagle dogs treated at 25 mg/kg/day (but not 5 mg/kg/day) for up to 13 weeks (Ellis et al. 1985; U.S. Army 1978b). Dogs

appear to be the most sensitive species. In Wistar rats, methemoglobin was increased substantially after treatment with 2,4-DNT at 347 mg/kg/day for 6 months (Kozuka et al. 1979). Milder hematological effects (mild reticulocytosis and hemosiderosis of the spleen) were also observed in CD rats treated at 93 or 108 mg/kg/day (for males or females, respectively) for up to 13 weeks (Lee et al. 1985; U.S. Army 1978b). Mice treated with 2,4-DNT for up to 13 weeks showed evidence of hematological effects (mild anemia, characterized by increased reticulocytes and decreased hematocrit and hemoglobin) only at the highest tested dose (413 mg/kg/day for males or 468 mg/kg/day for females) (Ellis et al. 1985; U.S. Army 1978b). Hematological effects were also observed in 2-year studies in beagle dogs, CD rats, and CD-1 mice, with dogs being the most sensitive species. Female dogs treated with 2,4-DNT at 1.5 mg/kg/day showed decreased erythrocyte count, hematocrit, and hemoglobin after 12 months; similar effects were observed in dogs of both sexes at 10 mg/kg/day (U.S. Army 1979). Anemia occurred at higher doses in 2-year studies in rats (≥3.9 mg/kg/day) and mice (898 mg/kg/day).

Agency Contact (Chemical Manager): Carolyn Harper

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 2,4-DNT
CAS Numbers: 121-14-2
Date: February 2016
Profile Status: Final
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [ ] Intermediate [X] Chronic
Graph Key: 57
Species: Dog

Minimal Risk Level: 0.001 [X] mg/kg/day [ ] ppm

References: U.S. Army. 1979. Mammalian toxicity of munitions compounds. Phase III: Effects of lifetime exposure. Part I. 2,4-Dinitrotoluene. Final report no. 7. Fort Detrick, MD: U.S. Army and Medical Bioengineering Research Development Laboratory. ADA077692.

Ellis HV, Hong CB, Lee CC, et al. 1985. Subchronic and chronic toxicity studies of 2,4-dinitrotoluene. Part I. Beagle dog. J Am Coll Toxicol 4:233-242.

Experimental design: Young beagle dogs (6 dogs/sex/group; age not specified) were administered 0, 0.2, 1.5, or 10 mg/kg/day 2,4-DNT in capsules for 24 months. Dogs were observed daily for behavioral changes and clinical signs of toxicity. Body weights were recorded weekly. Feed consumption was measured during 1 week each month starting in month 6. Blood was taken before initiation of treatment and after 3, 6, 9, 12, 18, and 24 months of exposure for assessment of hematological parameters (erythrocyte, reticulocyte, platelet, and total and differential leukocyte counts; Heinz bodies; clotting time, hematocrit, hemoglobin, and methemoglobin concentrations; and mean cell volume, hemoglobin, and hemoglobin concentration) and clinical chemistry (fasting glucose, urea nitrogen, sodium, potassium, calcium, magnesium, chloride, and bilirubin [high-dose dogs with toxic signs], and the serum enzyme activities of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase) analyses. Animals (one male and one female/group) were sacrificed after 12 or 24 months of continuous treatment; an additional dog/sex/group was discontinued from treatment at these time points and were sacrificed after a 4-week recovery period to evaluate the reversibility of effects (including clinical signs, hematology and clinical chemistry, organ weights, and histopathological analyses). Animals that were moribund during the study and those that survived to study termination were sacrificed and examined for gross lesions; major organs and tissues (including the brain, heart, liver, spleen, kidneys, adrenals, thyroids, pituitary, and gonads) were weighed, and comprehensive histopathological analyses (35 tissues) were performed.

Effects noted in study and corresponding doses: Chronic-duration oral exposure of dogs to 2,4-DNT at ≥1.5 mg/kg/day produced anemia and compensatory hematopoiesis (Ellis et al. 1985; U.S. Army 1979). Hematological effects of 2,4-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, resulting in anemia. Ferric iron also contributes to the denaturation of hemoglobin and subsequent removal of erythrocytes from the blood. Heinz bodies (granules of denatured hemoglobin) are also detected within erythrocytes. Increased hematopoiesis is typically observed as a compensatory response to decreased erythrocyte count.

Hematological effects consistent with the development of methemoglobin-induced anemia and compensatory hematopoiesis were observed after dosing for 12 months in dogs administered 1.5 and 10 mg/kg/day (Table A-6). 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 after treatment. At 10 mg/kg/day, more pronounced changes in these hematological parameters were observed, with statistically significant reductions in erythrocyte count, hematocrit, and hemoglobin and a statistically significant increase in reticulocyte count. In the low-dose group, similar hematological effects (decreased erythrocyte count and decreased hematocrit) were observed in female dogs, but these changes were not statistically significant. Effects on hematological parameters in male dogs were similar to those seen in female dogs, although many changes (with the exception of reticulocytes) did not reach statistical significance in the 10 mg/kg/day group, possibly due to low numbers of male dogs evaluated (hematological data available for only two males in the 10 mg/kg/day group). After treatment for 18 or 24 months in both males and females, only slight or no anemia, near normal reticulocyte levels, no Heinz bodies, and minimal amounts of methemoglobin were detected, likely reflective of an adaptive response. Therefore, only data from the 12-month evaluation were considered for derivation of the chronic-duration oral MRL.

No clinical signs of toxicity, behavioral changes, or effects on hematological or clinical chemistry parameters were observed in dogs administered 2,4-DNT at 0.2 mg/kg/day. Four high-dose dogs (three males and one female) exhibited severe signs of neurotoxicity (characterized by decreased muscle control and incoordination), sometimes accompanied by a reduction in body weight. These effects contributed to the death of three of six high-dose dogs (all males) prior to study termination (study weeks 8–20). Clinical signs of neurotoxicity were also noted intermittently in one male dog administered 2,4-DNT at 1.5 mg/kg/day. Although biliary hyperplasia was noted at necropsy in male and female dogs administered 2,4-DNT, the frequency of the response did not exhibit dose-dependence.

Table A-6. Hematological Effects in Beagle Dogs Exposed to 2,4-DNT for 12 Months

	Dose (mg/kg/day)			
End point	0	0.2	1.5	10
			Males	
Erythrocyte count (x10/mm)	5.96±0.22 (6)[a]	5.33±0.16 (6) [↓11]	5.69±0.19 (6) [↓5]	5.22±0.19 (2) [↓12]
Heinz bodies (%)	0.0±0.0(6)	0.0±0.0(6) [NA]	0.0±0.0(6) [NA]	0.52±0.37 (2)[b] [NA]
Reticulocytes (%)	0.40±0.09 (6)	0.78±0.13 (6) [↑95]	0.66±0.11 (6) [↑65]	1.23±0.23 (2)[b] [↑208]
Hematocrit (%)	45.2±0.9 (6)	41.8±1.2 (6) [↓8]	44.7±0.8 (6) [↓1]	44.0±4.0 (2) [↓3]
Hemoglobin (%)	15.1±0.4 (6)	14.1±0.3 (6) [↓7]	14.8±0.4 (6) [↓2]	14.2±1.2 (2) [↓6]
Methemoglobin (%)	0.0±0.0 (6)	0.0±0.0 (6) [NA]	0.0±0.0 (6) [NA]	0.0±0.0 (2) [NA]
			Females	
Erythrocyte count (x10/mm)	5.87±0.20 (6)[a]	5.54±0.14 (6) [↓6]	4.69±0.25 (6)[b] [↓20]	4.45±0.26 (6)[b] [↓24]
Heinz bodies (%)	0.0±0.0(6)	0.0±0.0(6) [NA]	0.0±0.0(6) [NA]	0.5±0.2 (6)[b] [NA]
Reticulocytes (%)	0.39±0.04 (6)	0.84±0.07 (6)[b] [↑115]	0.45±0.06 (6) [↑15]	1.59±0.18 (6)[b] [↑308]
Hematocrit (%)	45.8±1.1 (6)	44.7±1.3 (6) [↓2]	41.8±1.2 (6)[b] [↓9]	40.8±0.6 (6)[b] [↓11]
Hemoglobin (%)	15.1±0.4 (6)	15.1±0.4 (6) [0]	13.7±0.8 (6)[b] [↓9]	12.9±0.2 (6)[b] [↓15]
Methemoglobin (%)	0.0±0.0 (6)	0.6±0.6 (6) [NA]	0.0±0.0 (6) [NA]	0.0±0.0 (6) [NA]

[a]Values are means±standard error (number of animals) [percent change from controls].
[b]Statistically significant based on analyses performed by the study authors (Dunnett's multiple comparison procedure).

DNT = dinitrotoluene; NA = not applicable

Sources: Ellis et al. 1985; U.S. Army 1979

Dose and end point used for MRL derivation:

[ ] NOAEL [ ] LOAEL [X] BMDL 0.12 mg/kg was the $BMDL_{1SD}$ for hematological effects (decreased erythrocyte count).

Results of hematology assessments show that chronic-duration, oral exposure of dogs to 2,4-DNT induced anemia and compensatory hematopoiesis (Ellis et al. 1985; U.S. Army 1979). Changes in several hematological parameters, including decreased erythrocyte count, hematocrit, and hemoglobin were observed after treatment with 2,4-DNT at 1.5 mg/kg/day for 12 months (Table A-6). 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 for derivation of the chronic-duration oral MRL for 2,4-DNT, hematological data from female dogs treated with 2,4-DNT for 12 months were further evaluated by BMD analysis. The following data sets in female dogs were selected for BMD modeling: erythrocyte count, hematocrit, and hemoglobin. 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). All available continuous-variable models in the EPA BMDS (version 2.1) were fit to the data. The BMD and the 95% lower confidence limit (BMDL) were estimated for doses associated with a change of 1 standard deviation from the controls, and are in units of mg/kg/day. For continuous data, in the absence of a clear criteria as to what level of change should be considered adverse, the BMR is defined as a change equal to 1 standard deviation from the control mean (EPA 2000a). Adequate model fit is judged by three criteria: goodness-of-fit ($p>0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. Among all of the models providing adequate fit to the data, the lowest benchmark dose (BMDL, the lower limit of a one-sided 95% CI on the BMD) is selected as the POD when differences between the BMDLs estimated from these models are >3-fold; otherwise, the BMDL from the model with the lowest AIC is chosen.

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. BMD model prediction for hematocrit and erythrocyte count are shown in Tables A-7 and A-8, respectively. Of models meeting adequate fit criteria, the lowest $BMDL_{1SD}$ values for each hematological end point were 0.13 mg/kg/day for decreased hematocrit (exponential 4 model; Figure A-4) and 0.12 mg/kg/day for decreased erythrocyte count (exponential 4 model; Figure A-5). Of these, the lowest $BMDL_{1SD}$ of 0.12 mg/kg/day for decreased erythrocyte count was selected as the POD for derivation of the intermediate-duration oral MRL for 2,4-DNT. 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.

Table A-7. Model Predictions for 2,4-DNT for Decreased Hematocrit (%) in Female Dogs Following 12 Months of Exposure (U.S. Army 1979)

Model	Test for significant difference p-value[a]	Variance p-value[b]	Means p-value[b]	Scaled residuals[c] Dose below BMD	Scaled residuals[c] Dose above BMD	Scaled residuals[c] Overall largest	AIC	BMD_{1SD} (mg/kg/day)	$BMDL_{1SD}$ (mg/kg/day)
Constant variance									
Exponential (model 2)[d]	0.01	0.34	0.06	-1.82	0.29	-1.82	78.08	6.77	4.09
Exponential (model 3)[d]	0.01	0.34	0.06	-1.82	0.29	-1.82	78.08	6.77	4.09
Exponential (model 4)[d,e]	***0.01***	***0.34***	***0.92***	***-0.07***	***0.04***	***-0.07***	***74.50***	***0.61***	***0.13***
Exponential (model 5)[d]	0.01	0.34	0.92	-0.07	0.04	-0.07	74.50	0.61	0.13
Hill[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA
Linear[f]	0.01	0.34	0.06	-1.83	0.27	-1.83	78.15	6.96	4.31
Polynomial (2-degree)[f]	0.01	0.34	0.06	-1.83	0.27	-1.83	78.15	6.96	4.31
Polynomial (3-degree)[f]	0.01	0.34	0.06	-1.83	0.27	-1.83	78.15	6.96	4.31
Power[d]	0.01	0.34	0.06	-1.83	0.27	-1.83	78.15	6.96	4.31

[a]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[d]Power restricted to ≥1.
[e]Selected model. Constant variance model provided adequate fit to variance data. With constant variance model applied, the only models that provided adequate fit to the means were the Exponential 4 and 5 models (the Exponential 5 converged on to the Exponential 4).
[f]Coefficients restricted to be negative.

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); DF = degrees of freedom; DNT = dinitrotoluene; NA = not applicable (BMDL computation failed); SD = standard deviation

Figure A-4. Fit of Exponential 4 Model to Data on 2,4-DNT for Decreased Hematocrit (%) in Female Dogs Following 12 Months of Exposure (U.S. Army 1979)

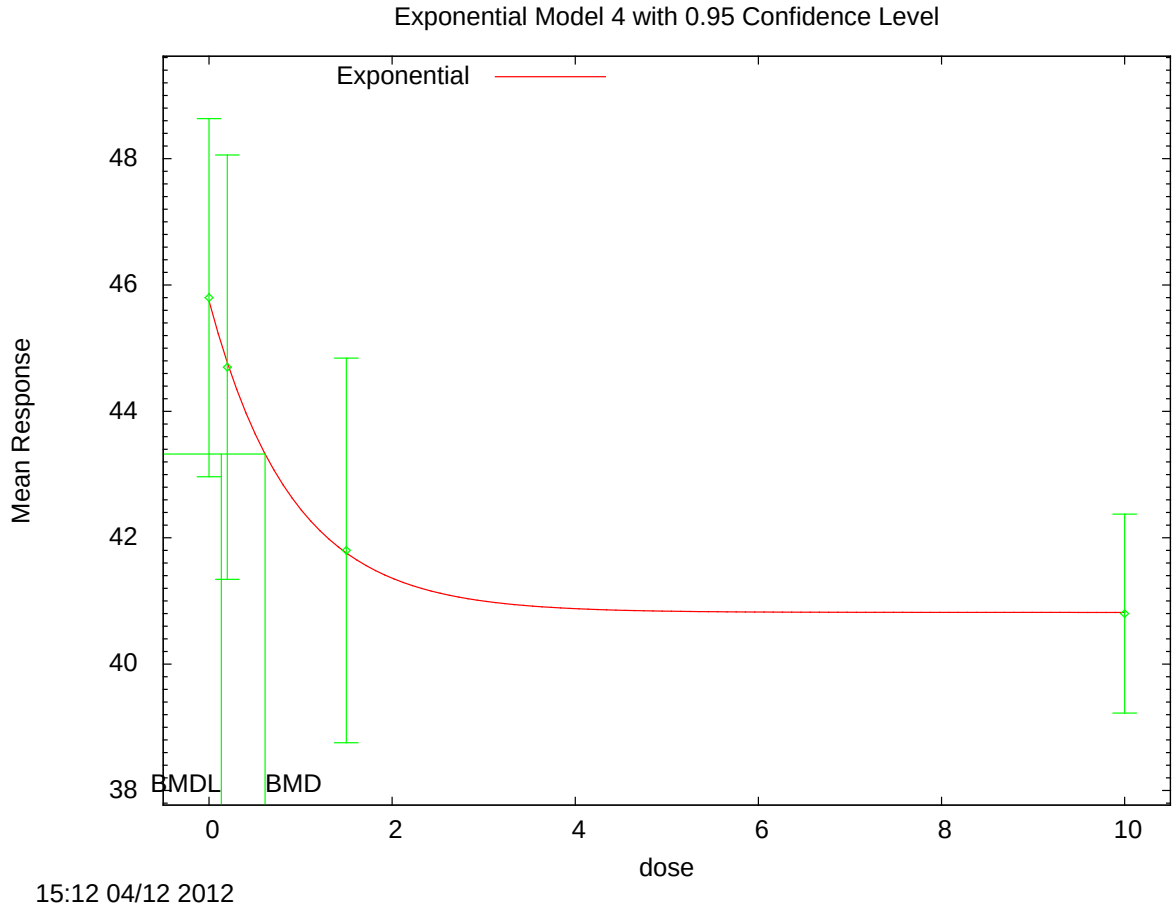


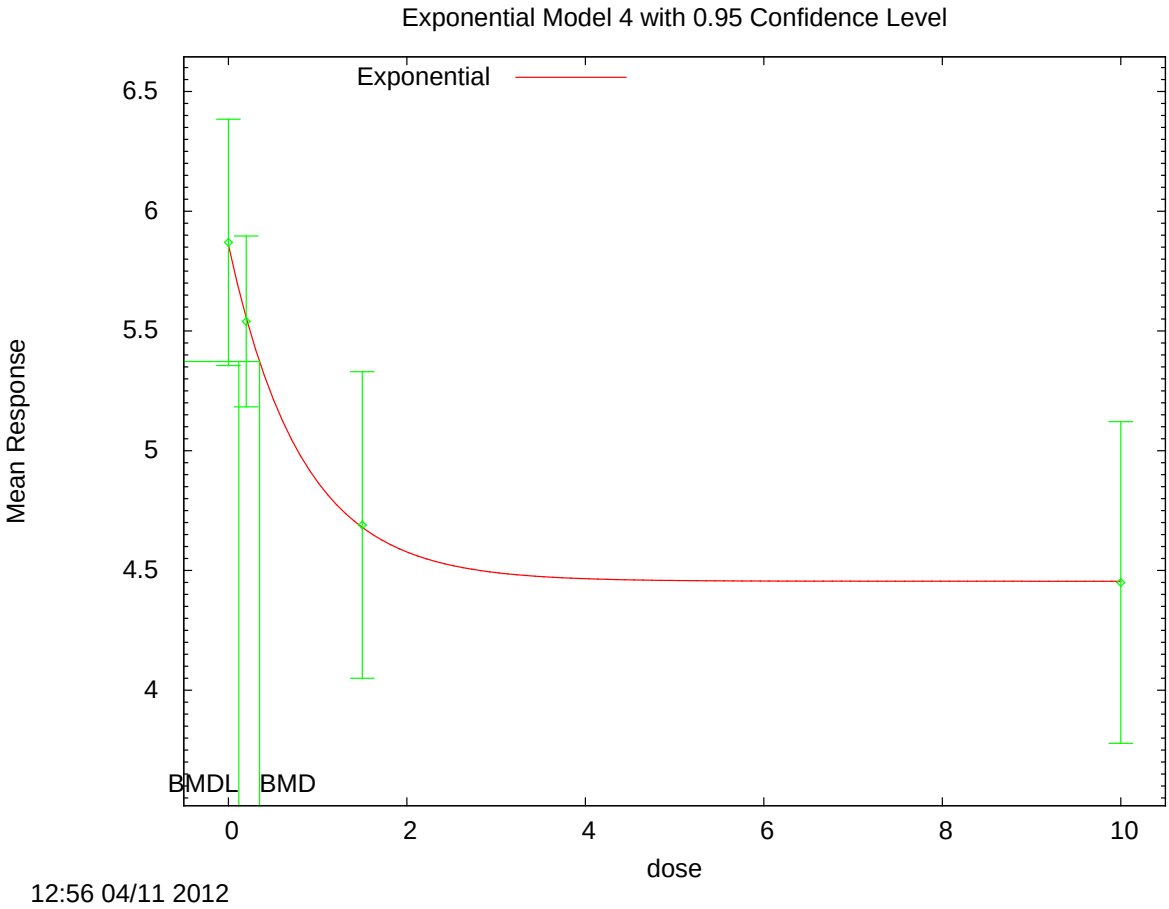
Table A-8. Model Predictions for Decreased Erythrocyte Count in Dogs Treated with 2,4-DNT for 12 Months

Model	Test for significant difference p-value[a]	Variance p-value[b]	Means p-value[b]	Scaled residuals[c] Dose below BMD	Scaled residuals[c] Dose above BMD	Scaled residuals[c] Overall largest	AIC	BMD_{1SD} (mg/kg/day)	$BMDL_{1SD}$ (mg/kg/day)
Constant variance									
Exponential (model 2)[d]	0.0005	0.46	0.005	-2.40	0.43	-2.40	6.16	5.11	3.21
Exponential (model 3)[d]	0.0005	0.46	0.005	-2.40	0.43	-2.40	6.16	5.11	3.21
Exponential (model 4)[d,e]	***0.0005***	***0.46***	***0.91***	***-0.08***	***0.05***	***-0.08***	***-2.55***	***0.35***	***0.12***
Exponential (model 5)[d]	0.0005	0.46	0.91	-0.08	0.05	-0.08	-2.55	0.35	0.12
Hill[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA
Linear[f]	0.0005	0.46	0.004	-2.41	0.35	-2.41	6.47	5.58	3.69
Polynomial (2-degree)[f]	0.0005	0.46	0.004	-2.41	0.35	-2.41	6.47	5.58	3.69
Polynomial (3-degree)[f]	0.0005	0.46	0.004	-2.41	0.35	-2.41	6.47	5.58	3.69
Power[d]	0.0005	0.46	0.004	-2.41	0.35	-2.41	6.47	5.58	3.69

[a]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[d]Power restricted to ≥1.
[e]Selected model. Constant variance model provided adequate fit to variance data. With constant variance model applied, the only models that provided adequate fit to the means were the Exponential 4 and 5 models (the Exponential 5 converged on to the Exponential 4).
[f]Coefficients restricted to be negative.

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); DF = degrees of freedom; DNT = dinitrotoluene; NA = not applicable (BMDL computation failed); SD = standard deviation

Figure A-5. Fit of Exponential Model 4 to Data on Decreased Erythrocyte Count in Female Dogs Treated with 2,4-DNT for 12 Months



Uncertainty Factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? No.

Other additional studies or pertinent information which lend support to this MRL: The hematological effects observed in this study are consistent with effects observed at higher doses in studies of intermediate duration (Hong et al. 1985; Lee et al. 1985; Kozuka et al. 1979; U.S. Army 1978b). Dogs were the most sensitive species in studies of chronic duration. In a 2-year study, male CD rats administered 2,4-DNT at 3.9 mg/kg/day showed decreased erythrocyte count after treatment for 12 months. Additional evidence for anemia, including further reductions in red blood cell count,

decreased hematocrit, decreased hemoglobin, and a compensatory increase in reticulocytes, was observed in male and female rats administered high-dose 2,4-DNT (34 and 45 mg/kg/day for males and females, respectively) for 12 or 18 months (Lee et al. 1985; U.S. Army 1979). CD-1 mice administered 2,4-DNT for 2 years showed no evidence of methemoglobin-induced anemia or compensatory reticulocytosis, except for decreased erythrocyte count and hemoglobin, and increased numbers of reticulocytes at the highest tested dose (898 mg/kg/day) (Lee et al. 1985; U.S. Army 1979).

Agency Contact (Chemical Manager): Carolyn Harper

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 2,5-DNT
CAS Numbers: 619-15-8
Date: February 2016
Profile Status: Final
Route: [ ] Inhalation [X] Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Graph Key: 4
Species: Rat

Minimal Risk Level: 0.007 [X] mg/kg/day [ ] ppm

References: Lent EM, Crouse CB, Quinn MJ, et al. 2012a. Comparison of the repeated dose toxicity of isomers of dinitrotoluene. Int J Toxicol 31(2):143-157.

U.S. Army Public Health Command (USAPHC). 2011c. Toxicology Study No. 87-XE-08G0E-10, October 2011, Toxicology Portfolio. Fourteen-day oral toxicity and in vivo genotoxicity of 2,5-dinitrotoluene in rats, January 2009-March 2010. USAPHC, Aberdeen Proving Ground, Maryland.

Experimental design: In a comparative toxicity study, 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. Both untreated and vehicle control groups were included. Evaluations during the study included twice daily observations for morbidity and clinical signs and measurement of body weight and food consumption on days 0, 1, 3, 7, and 14. Prior to sacrifice at the end of exposure, blood was collected for evaluation of hematology (total and differential white blood cell counts; red blood cell count; hemoglobin, hematocrit, MCV, MCH, MCHC, red cell distribution width; platelet count; and mean platelet volume) and serum chemistry (albumin, ALP, ALT, AST, BUN, calcium, cholesterol, creatinine, glucose, globulin, LDH, total bilirubin, total protein, sodium, potassium, and chlorine) assessments. All animals received gross necropsy and organ weight determinations (brain, heart, kidney, epididymides, liver, spleen, and testes). Microscopic examination of organs with exposure-related changes in weight was performed for controls and any dose groups exhibiting changes in the corresponding organ weights.

Effects noted in study and corresponding doses: One rat exposed to 308 mg/kg/day died prematurely; the timing of death was not reported. Clinical signs of toxicity consisting of cyanosis, squinting, prostrate posture, rapid or labored breathing, hunched posture, dark urine, red discharge around the nose, and discolored or soft feces were observed at ≥154 mg/kg/day. There were no differences from control in body weight or food consumption of exposed groups. Dose-related hematology changes were noted, including decreased red blood cells at ≥77 mg/kg/day; increased MCV and MCH at ≥154 mg/kg/day; and increased red blood cell distribution width (at 77 and 154 mg/kg/day, but not at 308 mg/kg/day). Total white blood cell count was increased at 308 mg/kg/day, resulting from a nonsignificant increase in lymphocytes; in addition, the percent of eosinophils was decreased at this dose. There were no clinically significant, dose-related changes in serum chemistry parameters. At gross necropsy, dark and enlarged spleens were observed in 6/6 rats in the 154 mg/kg/day group and in 4/5 surviving rats exposed to 308 mg/kg/day. In addition, dark spleens were noted in 3/6 and 1/6 rats of the 77 and 39 mg/kg/day dose groups. Absolute and relative organ weight increases occurred in the heart at the highest dose (~70% higher than controls). Marked, statistically significant increases (≥90%) in absolute and relative spleen weight were observed at doses ≥77 mg/kg/day. In addition, nonsignificant, but dose-related, increases of 20% or more were seen at ≥19 mg/kg/day. No other organ weight changes were observed. Histopathology findings in the spleen consisted of mild-to-moderate extramedullary hematopoiesis in 3/6 rats exposed to 39 mg/kg/day and all rats exposed to higher doses; one vehicle control exhibited trace

extramedullary hematopoiesis. Table A-9 shows the splenic effects. The authors also reported mild-to-moderate fibrosis (4/5 rats given 308 mg/kg/day) and trace-to-moderate inflammation (2/5 rats given 308 mg/kg/day) in the heart. Results of this study identify an acute-duration LOAEL of 39 mg/kg/day for hematopoietic system effects 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.

Table A-9. Splenic Effects in Rats Exposed to 2,5-DNT for 14 Days

	Dose (mg/kg/day)						
End point	0	10	19	39	77	154	308
Mild-to-moderate extramedullary hematopoiesis (number exposed/ number examined)	0/6	ND	ND	3/6	5/5	6/6	5/5
Absolute spleen weight (g)	0.6533± 0.07016 (6)[a]	0.7185± 0.0.1107 (6) [↑10]	0.7733± 0.010433 (6) [↑18]	0.9203± 0.12979 (6) [↑41]	1.2428± 016262[b] (6) [↑90]	0.1.8722± 0.22994[b] (6) [↑187]	2.2824± 0.46711[b] (5) [↑249]
Spleen weight relative to body weight	0.1851± 0.01497 (6)	0.2159± 0.03228 (6) [↑17]	0.2223± 0.03484 (6) [↑20]	0.259± 0.04213 (6) [↑40]	0.3591± 0.03192[b] (6) [↑94]	0.546± 0.0586[b] (6) [↑195]	0.6721± 0.10433[b] (5) [↑263]
Spleen weight relative to brain weight	39.4777± 13.39556 (6)	45.1837± 14.36118 (6) [↑14]	39.8441± 5.59536 (6) [↑1]	46.0439± 6.72756 (6) [↑17]	62.8775± 8.01491[b] (6) [↑59]	95.9326± 14.5203[b] (6) [↑143]	122.6007± 27.29763[b] (5) [↑211]

[a]Values are means±standard deviation (number of animals) [percent change from controls].
[b]Statistically significant based on analyses performed by the study authors (Tukey's or Dunnett's multiple comparison test).

DNT = dinitrotoluene; ND = no data; histopathology examination was not performed for these animals

Sources: Lent et al. 2012a; USAPHC 2011c

Dose and end point used for MRL derivation:

[] NOAEL [ ] LOAEL [X] BMDL 2.05 mg/kg/day was the $BMDL_{10}$ for hematopoietic effects (mild-to-moderate extramedullary hematopoiesis).

Hematopoietic system toxicity was identified as the most sensitive effect 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). To determine the POD, the incidence of extramedullary hematopoiesis and the absolute and relative spleen weights (relative to body weight and relative to brain weight) were subjected to BMD modeling. For quantal data (incidence of extramedullary hematopoiesis), the BMD and the 95% lower confidence limit (BMDL) were estimated for doses associated with a 10% increase in incidence over the control incidence, and are in units of mg/kg/day. For continuous data (spleen weights), the BMD and the 95% lower confidence limit (BMDL) were estimated for doses associated with a 1 standard deviation increase over the control value, and are also in units of mg/kg/day. Adequate model fit is judged by three criteria: goodness-of-fit ($p>0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. Among all of the models providing adequate fit to the data, the model with the lowest benchmark dose (BMDL, the lower limit of a one-sided 95% CI on the BMD) is selected when differences between the BMDLs estimated from these models are >3-fold; otherwise, the model with the lowest AIC is chosen.

No model fit was achieved with the data on spleen weight relative to brain weight, so this end point was not considered further. BMD model predictions for incidence of extramedullary hematopoiesis and increases in absolute and relative spleen weights are shown in Tables A-10, A-11 and A-12, respectively. The BMDLs from the best-fitting models for each parameter were 2.05 mg/kg/day ($BMDL_{10}$) for increased incidence of extramedullary hematopoiesis (multistage one-degree; Figure A-6); 7.71 mg/kg/day ($BMDL_{1SD}$) for increased absolute spleen weight (Exponential Model 5; Figure A-7) and 8.26 mg/kg/day ($BMDL_{1SD}$) for increased relative spleen weight (Exponential model 5; Figure A-8). The lowest BMDL of 2.05 mg/kg/day was selected as the POD. The POD of 2.05 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 2,5-DNT of 0.007 mg/kg/day.

Table A-10. Incidence of Mild-to-Moderate Extramedullary Hematopoiesis in the Spleen of Male Sprague-Dawley Rats Administered 2,5-DNT via Gavage for 14 Days

				Scaled residuals[b]					
Model	DF	χ^2	χ^2 Goodness-of-fit p-value[a]	Dose below BMD	Dose above BMD	Overall largest	AIC	BMD_{10} (mg/kg)	$BMDL_{10}$ (mg/kg)
Gamma[c]	4	0.00	1.00	0.00	-0.01	0.06	10.32	28.28	2.77
Logistic	3	0.00	1.00	0.00	0.00	0.00	12.32	34.60	9.23
LogLogistic[d]	4	0.00	1.00	0.00	0.00	0.00	10.32	34.52	7.43
LogProbit[d]	3	0.00	1.00	0.00	0.00	0.00	12.32	33.45	6.14
Multistage (1-degree)[e,f]	*4*	*1.50*	*0.83*	*0.00*	*-0.90*	*-0.90*	*12.40*	*3.69*	*2.05*
Multistage (2-degree)[e]	4	0.32	0.99	0.00	-0.35	0.44	10.82	13.76	2.52
Multistage (3-degree)[e]	4	0.02	1.00	0.00	-0.06	0.14	10.36	20.56	2.74
Multistage (4-degree)[e]	3	0.01	1.00	0.00	-0.02	0.08	12.33	21.54	2.71
Probit	3	0.00	1.00	0.00	0.00	0.00	12.32	30.40	8.31
Weibull[c]	3	0.00	1.00	0.00	0.00	0.00	12.32	25.68	2.77

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the benchmark dose; also the largest residual at any dose.
[c]Power restricted to ≥1.
[d]Slope restricted to ≥1.
[e]Betas restricted to ≥0.
[f]Selected model. All models provided adequate fit to the data. BMDLs for models providing adequate fit were not sufficiently close (differed by >3-fold), so the model with the lowest BMDL was selected (Multistage 1-degree).

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the dose associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{10}$ = dose associated with 10% extra risk); DF= degrees of freedom; DNT = dinitrotoluene

Figure A-6. Fit of Multistage (1-Degree) Model to Data on 2,5-DNT, Incidence of Mild-to-Moderate Extramedullary Hematopoiesis in the Spleen of Rats Exposed for 14 Days (Lent et al. 2012a; USAPHC 2011c)

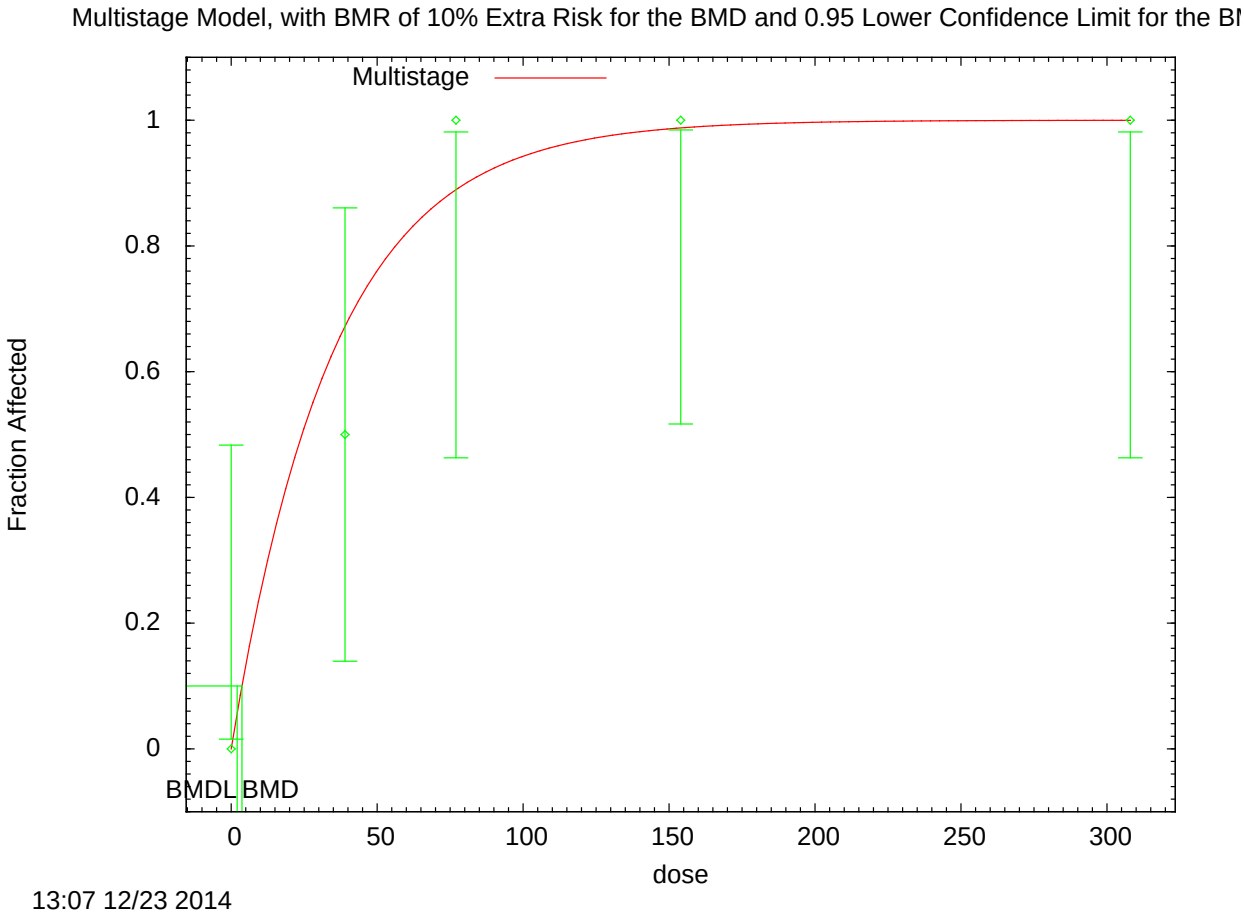


Table A-11. Model Predictions for Increased Absolute Spleen Weight in Male Sprague-Dawley Rats Administered 2,5-DNT via Gavage for 14 Days

Model	Test for significant difference p-value[a]	Variance p-value[b]	Means p-value[b]	Scaled residuals[c] Dose below BMD	Scaled residuals[c] Dose above BMD	Scaled residuals[c] Overall largest	AIC	BMD_{1SD} (mg/kg)	$BMDL_{1SD}$ (mg/kg)
All doses									
Constant variance									
Linear[d]	<0.0001	<0.0001	0.005	-0.24	0.88	2.92	-72.15	41.35	34.02
Non-constant variance									
Exponential (model 2)[e]	<0.0001	0.87	<0.0001	-0.53	-0.14	-2.47	-87.77	18.74	13.56
Exponential (model 3)[e]	<0.0001	0.87	<0.0001	-0.53	-0.14	-2.47	-87.77	18.74	13.56
Exponential (model 4)[e]	<0.0001	0.87	0.51	0.62	-0.07	1.39	-108.51	8.50	6.15
Exponential (model 5)[e,f]	*<0.0001*	*0.87*	*0.88*	*0.32*	*0.13*	*0.52*	*-109.15*	*15.01*	*7.78*
Hill[e]	<0.0001	0.87	0.78	0.36	0.16	0.72	-108.73	15.46	NA
Linear[d]	<0.0001	0.87	0.21	-0.15	-0.32	-1.90	-106.70	10.58	NA
Polynomial (2-degree)[d]	<0.0001	0.87	0.21	-0.15	-0.32	-1.90	-106.70	10.58	7.81
Polynomial (3-degree)[d]	<0.0001	0.87	0.21	-0.15	-0.32	-1.90	-106.70	10.58	7.81
Polynomial (4-degree)[d]	<0.0001	0.87	0.21	-0.15	-0.32	-1.90	-106.70	10.58	7.81
Polynomial (5-degree)[d]	<0.0001	0.87	0.21	-0.15	-0.32	-1.90	-106.70	10.58	7.81
Polynomial (6-degree)[d]	<0.0001	0.87	0.21	-0.15	-0.32	-1.90	-106.70	10.58	7.81
Power[e]	<0.0001	0.87	0.21	-0.15	-0.32	-1.90	-106.70	10.58	7.81

[a]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and above the benchmark dose; also the largest residual at any dose.
[d]Coefficients restricted to be positive.
[e]Power restricted to ≥1
[f]Selected model. Constant variance model did not fit the variance data, but non-constant variance model did. With non-constant variance model applied, all models, except for the Exponential 2 and 3, the Hill and the Linear models, provided adequate to the data (BMDL computation failed for the Hill and the Linear). BMDLs for models providing adequate fit were sufficiently close (differed by <2–3-fold), so the model with the lowest AIC was selected (exponential model 5).

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); DF = degrees of freedom; DNT = dinitrotoluene; NA = not applicable (BMDL computation failed or the BMD was higher than the highest dose tested); SD = standard deviation

Figure A-7. Fit of Exponential Model 5 to Data on 2,5-DNT for Increased Absolute Spleen Weight in Rats Exposed for 14 Days (Lent et al. 2012a; USAPHC 2011c)

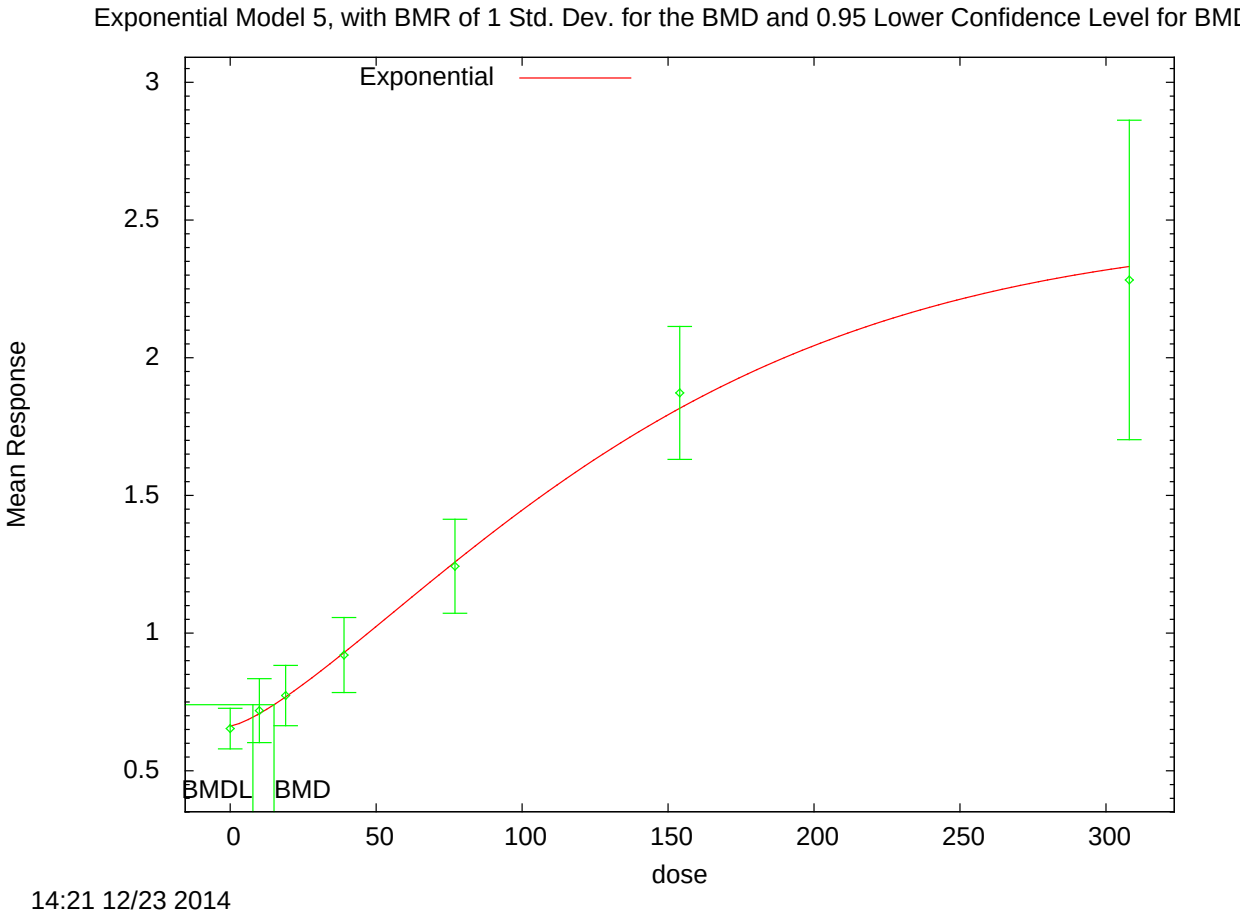


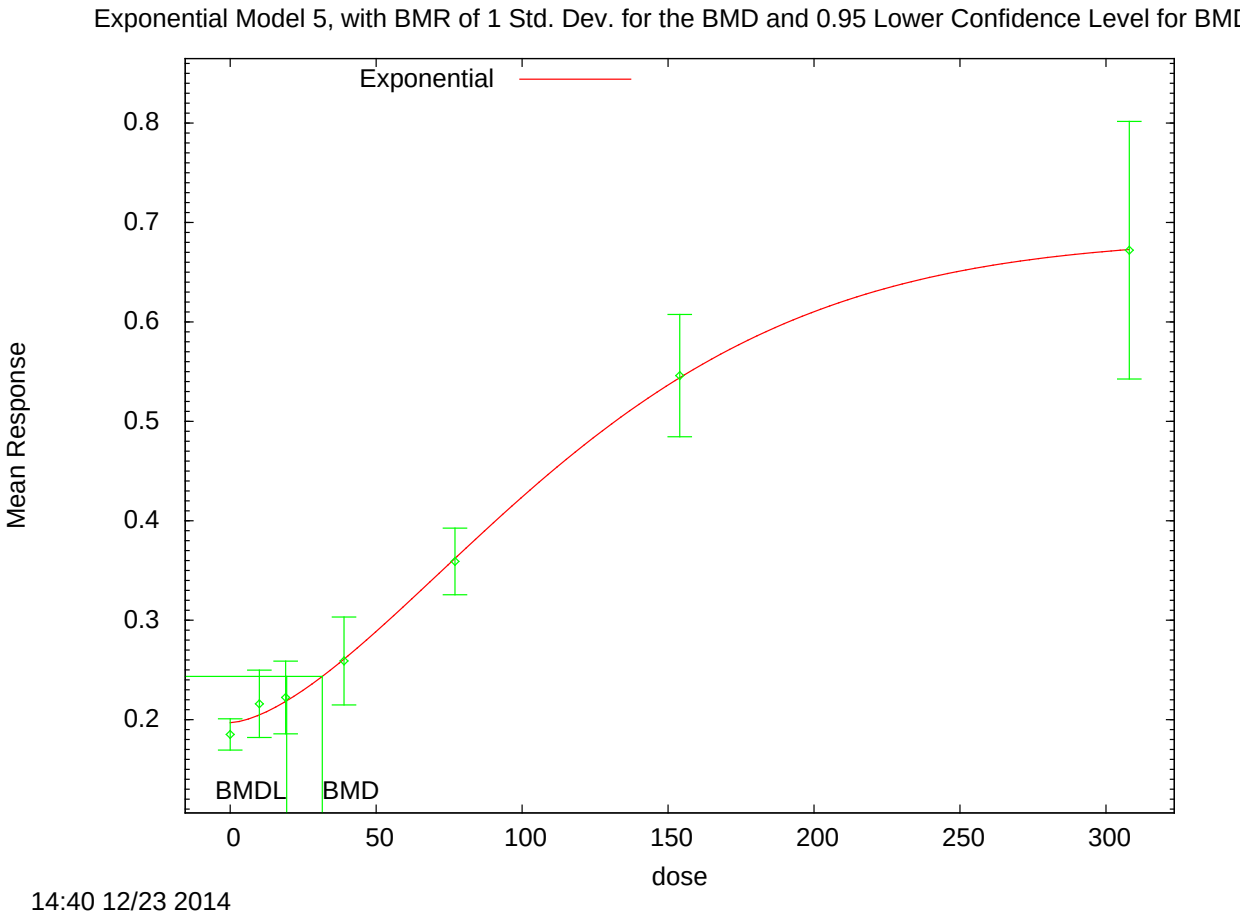
Table A-12. Model Predictions for Model Predictions for Increased Relative Spleen Weight in Male Sprague-Dawley Rats Administered 2,5-DNT via Gavage for 14 Days

Model	Test for significant difference p-value[a]	Variance p-value[b]	Means p-value[b]	Scaled residuals[c] Dose below BMD	Scaled residuals[c] Dose above BMD	Scaled residuals[c] Overall largest	AIC	BMD_{1SD} (mg/kg)	$BMDL_{1SD}$ (mg/kg)
All doses									
Constant variance									
Linear[d]	<0.0001	0.0007	0.0005	-0.68	-0.55	3.28	-183.44	35.89	29.69
Non-constant variance									
Exponential (model 2)[e]	<0.0001	0.37	<0.0001	-0.25	0.38	-2.52	-190.27	19.37	14.06
Exponential (model 3)[e]	<0.0001	0.37	<0.0001	-0.25	0.38	-2.52	-190.27	19.37	14.06
Exponential (model 4)[e]	<0.0001	0.37	0.22	0.34	0.78	1.60	-213.48	8.74	6.32
Exponential (model 5)[e,f]	*<0.0001*	*0.37*	*0.39*	*1.07*	*0.14*	*1.07*	*-214.23*	*17.64*	*8.26*
Hill[e]	<0.0001	0.37	0.29	1.11	0.18	-0.83	-213.46	18.31	NA
Linear[d]	<0.0001	0.37	0.06	0.66	-0.42	-1.98	-210.46	11.04	NA
Polynomial (2-degree)[d]	<0.0001	0.37	0.06	0.66	-0.42	-1.98	-210.46	11.04	8.13
Polynomial (3-degree)[d]	<0.0001	0.37	0.06	0.66	-0.42	-1.98	-210.46	11.04	8.13
Polynomial (4-degree)[d]	<0.0001	0.37	0.06	0.66	-0.42	-1.98	-210.46	11.04	8.13
Polynomial (5-degree)[d]	<0.0001	0.37	0.06	0.66	-0.42	-1.98	-210.46	11.04	8.13
Polynomial (6-degree)[d]	<0.0001	0.37	0.06	0.66	-0.42	-1.98	-210.46	11.04	8.13
Power[e]	<0.0001	0.37	0.06	0.66	-0.42	-1.98	-210.46	11.04	8.13

[a]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and above the benchmark dose; also the largest residual at any dose.
[d]Coefficients restricted to be positive.
[e]Power restricted to ≥1
[f]Selected model. Constant variance model did not fit the variance data, but non-constant variance model did. With non-constant variance model applied, the Exponential 4 and 5 models provided adequate to the data (BMDL computation failed for the Hill and the Linear models). BMDLs for models providing adequate fit were sufficiently close (differed by <3-fold), so the model with the lowest AIC was selected (Exponential model 5).

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); DF = degrees of freedom; DNT = dinitrotoluene; NA = not applicable (BMDL computation failed or the BMD was higher than the highest dose tested); SD = standard deviation

Figure A-8. Fit of Exponential Model 5 to Data on 2,5-DNT for Increased Relative Spleen Weight in Rats Exposed for 14 Days (Lent et al. 2012a; USAPHC 2011c)



Uncertainty Factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability
[X] 3 for database limitations (the only other data available are from an acute lethality study)

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? No.

Other additional studies or pertinent information which lend support to this MRL: LD_{50} values of 710 and 1,230 mg/kg/day, respectively, were reported in male Sprague-Dawley rats and male CF-1 mice exposed to 2,5-DNT (Vernot et al. 1977); no additional information was provided. No other acute-duration oral studies of 2,5-DNT were located, nor were there any studies of longer durations.

Agency Contact (Chemical Manager): Carolyn Harper

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 2,6-DNT
CAS Numbers: 606-20-2
Date: February 2016
Profile Status: Final
Route: [ ] Inhalation [X] Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Graph Key: 8
Species: Dog

Minimal Risk Level: 0.09 [X] mg/kg/day [ ] ppm

References: U.S. Army. 1976. Mammalian toxicity of munitions compounds. Phase II: Effects of multiple doses. Part III: 2,6-Dinitrotoluene. Progress report no. 4. Fort Detrick, MD: U.S. Army, Medical Bioengineering Research and Development Laboratory. ADA062015.

Experimental design: Young beagle dogs (4 dogs/sex/group; age not specified) were administered 0, 4, 20, or 100 mg/kg 2,6-DNT in capsules for 13 weeks (U.S. Army 1976). Dogs were observed daily for behavioral changes and clinical signs of toxicity. Body weights were recorded weekly. Feed consumption was measured daily. Blood was taken before initiation of treatment and at 2, 4, 8, and 13 and/or 17 weeks (4-week post-treatment recovery period) for hematological (erythrocyte, reticulocyte, platelet, and total and differential leukocyte counts; Heinz bodies; hematocrit, hemoglobin, and methemoglobin concentrations; and mean cell volume, hemoglobin, and hemoglobin concentration) and clinical chemistry (glucose, urea nitrogen, levels of sodium, potassium, calcium, magnesium, and chloride; and serum enzyme activities of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase) analyses. No additional assessments were conducted for acute-duration exposure. Assessments conducted for intermediate-duration exposure (4–13 weeks and 4-week post-treatment recovery period) included hematology and clinical chemistry; gross pathological examination, organ weights (heart, liver, spleen, kidneys, adrenals, and gonads), and microscopic examination of tissues ("various" tissues, not specified) were assessed in animals dying before the end of treatment, and at the end of the 13-week treatment period and the 4-week post-treatment recovery period. Bone marrow and kidney cultures were also maintained and cytogenetic analyses (of chromosome number and morphology) were performed.

Effects noted in study and corresponding doses: Acute-duration oral exposure of dogs to 2,6-DNT at ≥20 mg/kg/day show the development of anemia and compensatory hematopoiesis (U.S. Army 1976) (data summarized in Table A-13). Hematological effects of 2,6-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, resulting in anemia. Ferric iron also contributes to the denaturation of hemoglobin and subsequent removal of erythrocytes from the blood. Heinz bodies (granules of denatured hemoglobin) are also detected within erythrocytes. Increased hematopoiesis is typically observed as a compensatory response to decreased erythrocyte count. Since immature erythrocytes are typically larger, mean cell volume and mean cell hemoglobin tend to be increased. Dogs treated at 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 low-dose group, similar hematological effects (decreased erythrocyte count, hemoglobin, and hematocrit, and increased reticulocytes) were observed, but these changes did not achieve statistical significance. Mid- and high-dose dogs (but not low-dose dogs) continued to show signs of anemia and compensatory

hematopoiesis (decreased hematocrit and hemoglobin, and increased numbers of reticulocytes) for the duration of the 13-week study. Dogs treated at 20 mg/kg/day for 4 or 13 weeks and then removed from treatment showed recovery from hematological effects after 4 weeks; dogs treated at 100 mg/kg/day for 4 weeks did not show complete recovery until 19 weeks after cessation of treatment.

Table A-13. Hematological effects in Beagle Dogs Exposed to 2,6-DNT for 2 Weeks

	Dose (mg/kg/day)			
End point	0	4	20	100
Erythrocyte count (x10/mm)	5.62±0.16 (8)[a]	5.06±0.10 (8) [↓10]	4.73±0.20 (8)[b] [↓16]	1.85±0.28 (7)[b] [↓67]
Heinz bodies (%)	0.00±0.00 (8)	0.00±0.00 (8) [NA]	0.00±0.00 (8) [NA]	0.00±0.00 (7) [NA]
Reticulocytes (%)	0.76±0.07 (8)	1.10±0.13 (8) [↑45]	1.43±0.32 (8) [↑88]	16.99±3.33 (7)[b] [↑2,136]
Hematocrit (%)	42.1±1.7 (8)	38.9±0.6 (8) [↓8]	39.3±1.2 (8) [↓7]	22.9±2.8 (7)[b] [↓46]
Hemoglobin (%)	14.8±0.5 (8)	13.3±0.2 (8) [↓10]	13.0±0.4 (8) [↓12]	6.3±0.9 (7)[b] [↓57]
Methemoglobin (%)	0.0±0.0 (8)	0.0±0.0 (8) [NA]	0.0±0.0 (8) [NA]	0.0±0.0 (7) [NA]
Mean cell hemoglobin (micro µg)	26.3±0.2 (8)	26.3±0.3 (8) [0]	27.7±0.3 (8)[b] [↑5]	34.4±1.2 (7)[b] [↑31]

[a]Values are means±standard error (number of animals) [percent change from controls].
[b]Statistically significant based on analyses performed by the study authors (Dunnett's multiple comparison procedure).

DNT = dinitrotoluene; NA = not applicable

Source: U.S. Army 1976

Although incidence data were not reported, the study authors noted that at least three dogs (sex not specified) administered 2,6-DNT at 100 mg/kg/day showed clinical signs of toxicity (listlessness, incoordination, lack of balance, pale gums, dark urine, and weakness, particularly of the hind limbs) within the first 2 weeks of the study,. One dogs (a male) died during week 2. Similar (but milder) symptoms were reported in mid-dose dogs starting in week 4. No clinical signs of toxicity were observed in dogs administered the low dose of 2,6-DNT.

Histopathological assessments of tissues were not conduced in animals exposed for only 2 weeks. However, after 13-week of treatment, mild splenic hematopoiesis was noted in low-dose animals. Numerous lesions were detected in mid- and high-dose dogs; the number and severity of these lesions was increased at the high-dose. Affected organs included the liver (bile duct hyperplasia, degeneration, inflammation, and/or extramedullary hematopoiesis), kidney (degeneration and inflammation, dilated tubules), spleen (extramedullary hematopoiesis and lymphoid depletion), and testes (degeneration and atrophy of spermatogenic cells.

Dose and end point used for MRL derivation:

[ ] NOAEL [ ] LOAEL [X] BMDL 9.31 mg/kg was the $BMDL_{1SD}$ for hematological effects (decreased erythrocyte count).

Results of hematology assessments show that acute-duration, oral exposure of dogs to 2,6-DNT induced anemia and compensatory hematopoiesis (U.S. Army 1976). Statistically significant changes in hematological parameters, including decreased erythrocyte count and increased mean cell hemoglobin, were observed after treatment with 2,6-DNT at 20 mg/kg/day for 2 weeks (Table A-13). 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, erythrocyte count and mean cell hemoglobin further evaluated by BMD analysis. All available continuous-variable models in the EPA BMDS (version 2.1) were fit to the data. The BMD and the 95% lower confidence limit (BMDL) were estimated for doses associated with a change of 1 standard deviation from the controls, and are in units of mg/kg/day. For continuous data, in the absence of a clear criteria as to what level of change should be considered adverse, the BMR is defined as a change equal to 1 standard deviation from the control mean (EPA 2000a). Adequate model fit is judged by three criteria: goodness-of-fit ($p>0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. Among all of the models providing adequate fit to the data, the lowest benchmark dose (BMDL, the lower limit of a one-sided 95% CI on the BMD) is selected as the POD when differences between the BMDLs estimated from these models are >3-fold; otherwise, the BMDL from the model with the lowest AIC is chosen.

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 non-constant variance model applied, the linear, polynomial, and power models provided an adequate fit to the data for erythrocyte count (Table A-14). The polynomial and power models converged to the linear model. The figure shown from the linear model (Figure A-9) is representative of figures from the polynomial and power models (not shown). 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.

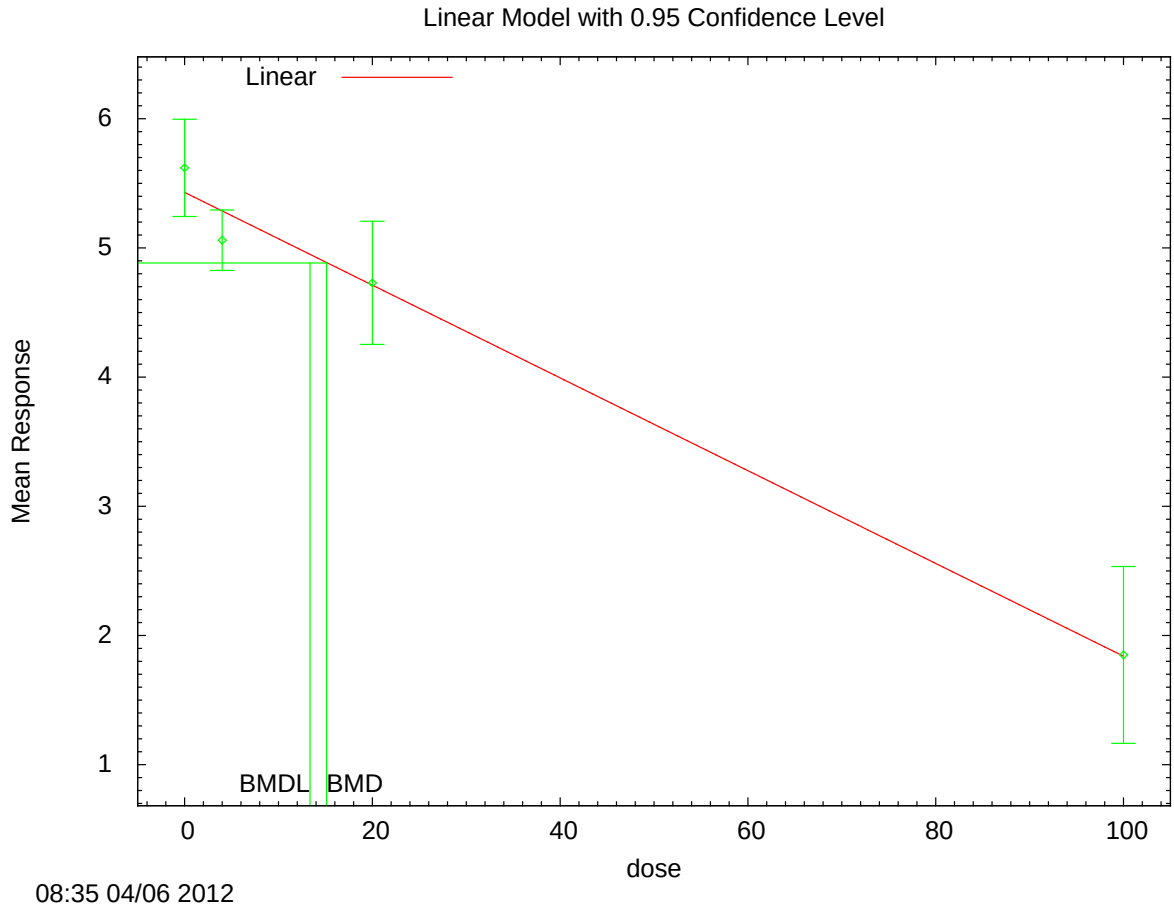
Table A-14. Model Predictions for Decreased Erythrocyte Count in Dogs Treated with 2,6-DNT for 2 Weeks

Model	Test for significant difference p-value[a]	Variance p-value[b]	Means p-value[b]	Scaled residuals[c] Dose below BMD	Scaled residuals[c] Dose above BMD	Scaled residuals[c] Overall largest	AIC	BMD_{1SD} (mg/kg/day)	$BMDL_{1SD}$ (mg/kg/day)
Constant variance									
Linear[d]	<0.0001	0.08	0.25	-1.22	0.12	-1.22	-4.12	14.39	11.60
Non Constant variance									
Exponential (model 2)[e]	<0.0001	0.18	0.09	-1.55	1.26	-1.55	-3.38	8.15	6.00
Exponential (model 3)[e]	<0.0001	0.18	0.04	-1.60	0.64	-1.60	-1.90	11.15	6.17
Exponential (model 4)[e]	<0.0001	0.18	0.09	-1.55	1.26	-1.55	-3.38	8.15	5.84
Exponential (model 5)[e]	<0.0001	0.18	0.04	-1.60	0.64	-1.60	-1.90	11.15	6.07
Hill[e]	NA	NA	NA	NA	NA	NA	NA	NA	NA
Linear[d,f]	***<0.0001***	***0.18***	***0.16***	***-1.42***	***0.13***	***-1.42***	***-4.47***	***12.21***	***9.31***
Polynomial (2-degree)[d]	<0.0001	0.18	0.16	-1.42	0.13	-1.42	-4.47	12.21	9.31
Polynomial (3-degree)[d]	<0.0001	0.18	0.16	-1.42	0.13	-1.42	-4.47	12.21	9.31
Power[e]	<0.0001	0.18	0.16	-1.42	0.13	-1.42	-4.47	12.21	9.31

[a]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[d]Coefficients restricted to be negative.
[e]Power restricted to ≥1.
[f]Selected model. Constant variance model did not provide adequate fit to variance data, but non-homogenous variance model did. With non-constant variance model applied, all models, except for the Exponential (means <0.1) and Hill models (computation failed), provided adequate fit to the means. The polynomial and power models all converged to the linear model.

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); DF = degrees of freedom; DNT = dinitrotoluene; NA = not applicable (BMDL computation failed); SD = standard deviation

Figure A-9. Fit of Linear Model to Data on Decreased Erythrocyte Count in Dogs Treated with 2,6-DNT for 2 Weeks



BMDs and BMDLs indicated are associated with a change of 1 standard deviation from the control, and are in units of mg/kg/day.

Source: U.S. Army 1976

Uncertainty Factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? No.

Other additional studies or pertinent information which lend support to this MRL: Hematological effects consistent with methemoglobinemia-induced anemia and compensatory hematopoiesis have been observed in laboratory animals orally exposed to 2,6-DNT for acute and intermediate durations. No chronic-duration studies were identified that evaluated hematological effects after exposure to 2,6-DNT. Hematological effects (increased hemoglobin, hematocrit, and increased erythrocyte count, granulocyte, and reticulocyte counts) were observed in female Sprague-Dawley rats administered 2,6-DNT at 199 mg/kg/day via gavage for 48 hours (Deng et al. 2011) and in male Sprague-Dawley rats exposed to 134 mg/kg/day via gavage for 14 days (Lent et al. 2012a; USAPHC 2011d). In intermediate-duration studies, dogs appear to more sensitive than rats or mice. In dogs orally exposed to 2,6-DNT at 4 mg/kg/day for 4 or 13 weeks, extramedullary erythropoiesis in the spleen secondary to methemoglobinemia and anemia was observed (U.S. Army 1976). Changes in hematological parameters associated with anemia and compensatory hematopoiesis (including decreased hematocrit and hemoglobin, and increased numbers of reticulocytes) occurred at 20 and 100 mg/kg/day. The incidence and severity of these effects were more pronounced at 100 mg/kg/day. Similar effects were observed in rats (U.S. Army 1976). In CD rats administered 2,6-DNT at ≥7 mg/kg/day and sacrificed after treatment for 4 or 13 weeks, increased incidences of extramedullary hematopoiesis and/or splenic hemosiderosis (increased iron accumulation) were observed. However, changes in hematological parameters (measured at 4, 8, and 13 weeks) indicative of anemia and compensatory hematopoiesis (including significant decreases in erythrocyte count, hematocrit, hemoglobin, and increased reticulocytes) were observed only at the highest tested dose (145 and 155 mg/kg/day for male and female rats, respectively); these effects were most pronounced after treatment for 4 weeks. Although histopathological effects (extramedullary hematopoiesis) were observed in CD-1 mice administered 2,6-DNT at ≥51 mg/kg/day (but not 11 mg/kg/day) for 4 or 13 weeks, no statistically significant changes in hematological parameters were seen. The study authors indicated that some blood samples clotted, making hematological analyses impossible to perform. The small number of animals evaluated likely contributed to the identification of histopathological findings of the spleen in the apparent absence of 2,6-DNT-induced hematological effects. The results of intermediate-duration studies indicate that hematological effects (or histopathological effects secondary to methemoglobinemia and anemia) are the most sensitive effects after exposure to 2,6-DNT; additional effects observed in intermediate-duration studies occurred at higher doses than hematological effects.

Agency Contact (Chemical Manager): Carolyn Harper

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 2,6-DNT
CAS Numbers: 606-20-2
Date: February 2016
Profile Status: Final
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [X] Intermediate [ ] Chronic
Graph Key: 18
Species: Dog

Minimal Risk Level: 0.004 [X] mg/kg/day [ ] ppm

Reference: U.S. Army. 1976. Mammalian toxicity of munitions compounds. Phase II: Effects of multiple doses. Part III: 2,6-Dinitrotoluene. Progress report no. 4. Fort Detrick, MD: U.S. Army, Medical Bioengineering Research and Development Laboratory. ADA062015

Experimental design: Young beagle dogs (4 dogs/sex/group; age not specified) were administered 0, 4, 20, or 100 mg/kg 2,6-DNT in capsules for 13 weeks (U.S. Army 1976). Dogs were observed daily for behavioral changes and clinical signs of toxicity. Body weights were recorded weekly. Feed consumption was measured daily. Blood was taken before initiation of treatment and at 2, 4, 8, and 13 and/or 17 weeks (4-week post-treatment recovery period) for hematological (erythrocyte, reticulocyte, platelet, and total and differential leukocyte counts; Heinz bodies; hematocrit, hemoglobin, and methemoglobin concentrations; and mean cell volume, hemoglobin, and hemoglobin concentration) and clinical chemistry (glucose, urea nitrogen, levels of sodium, potassium, calcium, magnesium, and chloride; and serum enzyme activities of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase) analyses. Animals (1 sex/group) were sacrificed after 4 or 13 weeks of continuous treatment; an additional dog/sex/group was discontinued treatment after 4 or 13 weeks and sacrificed after 4 weeks of recovery (week 8 or 17) to evaluate the reversibility of effects. The two high-dose dogs removed from treatment at 4 weeks were not sacrificed until 19 weeks after cessation of treatment to test the reversibility of effects after a longer recovery period. When animals were moribund or at study termination, they were examined for gross lesions; major organs and tissues were weighed (heart, liver, spleen, kidneys, adrenals, and gonads); and "various" tissues (number not specified) were subjected to histopathological examinations. Bone marrow and kidney cultures were also maintained and cytogenetic analyses (of chromosome number and morphology) were performed.

Effects noted in study and corresponding doses: Intermediate-duration oral exposure of dogs to 2,6-DNT at 4 mg/kg/day produced extramedullary erythropoiesis (formation of erythrocytes outside of the bone marrow) in the spleen secondary to methemoglobinemia and anemia (U.S. Army 1976). Hematological effects and compensatory erythropoiesis induced by 2,6-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, resulting in anemia. Ferric iron also contributes to the denaturation of hemoglobin and subsequent removal of erythrocytes from the blood. Increased erythropoiesis is typically observed as a compensatory response to decreased erythrocyte count.

Mortality occurred in dogs administered 20 and 100 mg/kg/day. Two mid-dose female dogs died in week 9; all high-dogs died by week 8. Effects observed in dogs treated at 20 and 100 mg/kg/day were clinical signs of neurotoxicity (listlessness, incoordination, and lack of balance), decreased feed consumption and subsequent reductions in body weight, and changes in hematological parameters associated with anemia and compensatory hematopoiesis (including decreased hematocrit and hemoglobin, and increased numbers of reticulocytes). The incidence and severity of these effects were

more pronounced at 100 mg/kg/day relative to 20 mg/kg/day. Dogs administered 2,6-DNT at 4 mg/kg/day showed no clinical signs of toxicity, and although similar hematological effects occurred, these changes were not statistically significant. No significant effects on clinical chemistry end points were observed in any 2,6-DNT treatment group. In general, dogs treated at 20 mg/kg/day for 4 or 13 weeks and then removed from treatment showed recovery from neurotoxicity and hematological effects after 4 weeks; dogs treated at 100 mg/kg/day for 4 weeks did not show complete recovery until 19 weeks after cessation of treatment.

Histopathological evaluation of the spleen showed an increased incidence of extramedullary erythropoiesis, an adaptive response to 2,6-DNT-induced methemoglobinemia and anemia, in dogs administered ≥4 mg/kg/day for 4 or 13 weeks (Table A-15). The incidence and severity of this lesion was dose-related. Additional histopathological changes observed dogs administered 2,6-DNT at 20 or 100 mg/kg/day for 4 or 13 weeks included effects on the thymus (involution), liver (extramedullary hematopoiesis, bile duct hyperplasia, degeneration, and inflammation), kidneys (degeneration, inflammation, and diluted tubules), and testes (degeneration and/or decreased spermatogenesis). High-dose dogs also showed evidence of lymphoid depletion in the spleen and lymph nodes. No other treatment-related histopathological changes were observed in dogs dosed with 2,6-DNT at 4 mg/kg/day for 13 weeks.

Table A-15. Extramedullary Erythropoiesis of the Spleen in Beagle Dogs Exposed to 2,6-DNT for 4 or 13 Weeks

Timepoint (weeks)	Dose (mg/kg/day)			
	0	4	20	100
4	0/2[a]	1/2 (mild)	2/2 (moderate)	2/2 (1 mild; 1 markedly severe)
13	0/2	2/2 (1 minimal; 1 mild)	3/3 (1 minimal, 1 mild, 1 moderate)	4/4 (2 marked, 2 markedly severe)

[a]Number examined/number affected (severity of lesion).

DNT = dinitrotoluene

Source: U.S. Army 1976

Dose and end point used for MRL derivation:

[ ] NOAEL [X] LOAEL 4 mg/kg/day for mild extramedullary erythropoiesis in the spleen.

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 acute-duration oral MRL for 2,6-DNT of 0.004 mg/kg/day.

Uncertainty Factors used in MRL derivation:

[X] 10 for use of a LOAEL
[X] 10 for extrapolation from animals to humans

[X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? No.

Other additional studies or pertinent information which lend support to this MRL: Similar histopathological effects (extramedullary hematopoiesis and/or splenic hemosiderosis), indicative of an adaptive response to anemia and compensatory erythropoiesis, were observed in CD rats administered 2,6-DNT at ≥7 mg/kg/day and in CD-1 mice administered 2,6-DNT at ≥51 mg/kg/day (but not 11 mg/kg/day) for 4 or 13 weeks (U.S. Army 1976). Hematological effects were also identified in intermediate-duration studies. In these studies, dogs appear to be the most sensitive species. Dogs treated at 20 mg/kg/day showed a statistically significant decrease in erythrocyte count and a significant increase in mean cell hemoglobin at 2 weeks. At 100 mg/kg/day, more pronounced changes in these hematological parameters were observed; statistically significant reductions in erythrocyte count, hematocrit, and hemoglobin and a statistically significant increase in reticulocyte count were observed. In CD rats, changes in hematological parameters indicative of anemia (significant decreases in erythrocyte count, hematocrit, and hemoglobin) and compensatory hematopoiesis (increased reticulocytes) were observed only at the highest tested dose (145 and 155 mg/kg/day for male and female rats, respectively). Although histopathological effects (extramedullary hematopoiesis) was observed in CD-1 mice administered 2,6-DNT at 51 mg/kg/day (but not 11 mg/kg/day), no statistically significant changes in hematological parameters were seen after treatment for 4 or 13 weeks. The study authors indicated that some blood samples clotted, making hematological analyses impossible to perform. The small number of animals evaluated likely contributed to the identification of histopathological findings of the spleen in the apparent absence of 2,6-DNT-induced hematological effects.

Agency Contact (Chemical Manager): Carolyn Harper

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 3,4-DNT
CAS Numbers: 610-39-9
Date: February 2016
Profile Status: Final
Route: [ ] Inhalation [X] Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Graph Key: 1
Species: Rat

Minimal Risk Level: 0.03 [X] mg/kg/day [ ] ppm

References: Lent EM, Crouse CB, Quinn MJ, et al. 2012a. Comparison of the repeated dose toxicity of isomers of dinitrotoluene. Int J Toxicol 31(2):143-157.

U.S. Army Public Health Command (USAPHC). 2011e. Toxicology Study No. 87-XE-08G0B-08, October 2011, Toxicology Portfolio. Fourteen-day oral toxicity and in vivo genotoxicity of 3,4-dinitrotoluene in rats, June-July 2008. USAPHC, Aberdeen Proving Ground, Maryland.

Experimental design: In a comparative toxicity study, 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. Both untreated and vehicle control groups were included. Evaluations during the study included twice daily observations for morbidity and clinical signs and measurement of body weight and food consumption on days 0, 1, 3, 7, and 14. Prior to sacrifice at the end of exposure, blood was collected for evaluation of hematology (total and differential white blood cell counts; red blood cell count; hemoglobin, hematocrit, MCV, MCH, MCHC, red cell distribution width; platelet count; and mean platelet volume) and serum chemistry (albumin, ALP, ALT, AST, BUN, calcium, cholesterol, creatinine, glucose, globulin, LDH, total bilirubin, total protein, sodium, potassium, and chlorine) assessments. All animals received gross necropsy and organ weight determinations (brain, heart, kidney, epididymides, liver, spleen, and testes). Microscopic examination of organs with exposure-related changes in weight was performed for controls and any dose groups exhibiting changes in the corresponding organ weights.

Effects noted in study and corresponding doses: All rats in the study of 3,4-DNT survived until scheduled sacrifice. Rats in the highest dose group (227 mg/kg/day) exhibited clinical signs of toxicity including cyanosis, lethargy, dark urine, facial twitching, and hypoactivity or staring behavior; one rat exposed to 113 mg/kg/day exhibited dark urine. High-dose rats had lower body weights than controls throughout the study, and a biologically significant (but not statistically significant) 10% reduction in terminal body weight was observed at this dose. Food intake was also decreased at this dose, albeit not at a level that reached statistical significance. Both hemoglobin and hematocrit were lower than controls at all doses of 3,4-DNT, with statistically significant reductions at doses of 14, 28, and 113 mg/kg/day. The authors indicated that both parameters remained within reference ranges at all doses. White blood cell counts were not affected by treatment, but the percent of neutrophils was increased and percent of lymphocytes was decreased at 14, 28, and 227 mg/kg/day; a clear dose-response relationship was not evident. Other hematology parameters were not affected by treatment, and none of the serum chemistry parameters exhibited a dose-related change. There were no gross necropsy findings of note. The only treatment-related organ weight change was an increase (≥13% compared with vehicle controls) in relative liver-to-body weight at the two highest doses (≥113 mg/kg/day); absolute liver weight was not affected, and there were no treatment-related histopathology findings in the liver. Relative liver weights may have been altered as a consequence of the lower body weights seen at the highest doses. Microscopic lesions were noted in the heart, spleen, and kidney. One rat in the highest dose group exhibited mild myocardial

fibrosis, inflammation, and necrosis. Splenic lesions, of trace-to mild severity, were observed at doses ≥57 mg/kg/day, including extramedullary hematopoiesis (2/6, 2/5, and 3/6 rats at the three highest doses) and lymphoid hyperplasia (3/6, 2/5, 2/6 at 57, 113, and 227 mg/kg/day respectively) (see Table A-16). Spleens were not examined in lower dose animals. Microscopic lesions of the kidney occurred in the high dose group, and included trace-to-moderate proximal tubule degeneration (1/6 rats), renal tubular basophilia (4/6), and lymphocytic infiltration (5/6); kidneys from animals in lower dose groups were not examined. 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.

Table A-16. Splenic Lesions in Rats Exposed to 3,4-DNT for 14 Days

	Dose (mg/kg/day)						
End point	0	7	14	28	57	113	227
Extramedullary hematopoiesis	0/6[a]	ND	ND	ND	2/6	2/5	3/6
Lymphoid hyperplasia	0/6	ND	ND	ND	3/6	2/5	2/6

[a]Number affected/number examined

DNT = dinitrotoluene; ND = no data; histopathology examination was not performed for these animals.

Sources: Lent et al. 2012a; USAPHC 2011e

Dose and end point used for MRL derivation:

[] NOAEL [ ] LOAEL [X] BMDL 8.05 mg/kg/day was the $BMDL_{10}$ for hematopoietic effects (trace-to-mild extramedullary hematopoiesis and lymphoid hyperplasia).

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). To select a POD, the following data sets were subjected to BMD modeling: incidences of extramedullary hematopoiesis and lymphoid hyperplasia of the spleen. The BMD and the 95% lower confidence limit (BMDL) were estimated for doses associated with a 10% increase in incidence over the control incidence, and are in units of mg/kg/day. Adequate model fit is judged by three criteria: goodness-of-fit ($p>0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. Among all of the models providing adequate fit to the data, the model with the lowest benchmark dose (BMDL, the lower limit of a one-sided 95% CI on the BMD) is selected when differences between the BMDLs estimated from these models are >3-fold; otherwise, the model with the lowest AIC is chosen.

BMD model predictions for incidences of extramedullary hematopoiesis and lymphoid hyperplasia of the spleen are shown in Tables A-17 and A-18, respectively. The $BMDL_{10}$ values from the best-fitting models for were the same (8.05 mg/kg/day) for both increased incidence of extramedullary hematopoiesis (log-logistic; Figure A-10) and increased incidence of lymphoid hyperplasia of the spleen (log-logistic; Figure A-11). Thus, the $BMDL_{10}$ of 8.05 mg/kg/day 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.

Table A-17. Model Predictions for Incidence of Extramedullary Hematopoiesis in the Spleen of Male Sprague-Dawley Rats Administered 3,4-DNT via Gavage for 14 Days

Model	DF	χ^2	χ^2 Goodness-of-fit p-value[a]	Scaled residuals[b] Dose below BMD	Dose above BMD	Overall largest	AIC	BMD_{10} (mg/kg)	$BMDL_{10}$ (mg/kg)
Gamma[c]	3	0.86	0.83	0.00	0.74	0.74	25.48	25.51	14.32
Logistic	2	1.80	0.41	0.76	0.44	-0.96	29.20	64.08	37.29
LogLogistic[d,e]	*3*	*0.34*	*0.95*	*0.00*	*0.44*	*0.44*	*25.01*	*18.56*	*8.05*
LogProbit[d]	3	1.86	0.60	0.00	1.02	1.02	26.32	40.34	24.07
Multistage (1-degree)[f]	3	0.86	0.83	0.00	0.74	0.74	25.48	25.51	14.32
Multistage (2-degree)[f]	3	0.86	0.83	0.00	0.74	0.74	25.48	25.51	14.32
Multistage (3-degree)[f]	3	0.86	0.83	0.00	0.74	0.74	25.48	25.51	14.32
Probit	2	1.77	0.41	0.79	0.43	-0.91	29.10	60.21	36.19
Weibull[c]	3	0.86	0.83	0.00	0.74	0.74	25.48	25.51	14.32

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the benchmark dose; also the largest residual at any dose.
[c]Power restricted to ≥1.
[d]Slope restricted to ≥1.
[e]Selected model. All models provided adequate fit to the data. BMDLs for models providing adequate fit were not sufficiently close (differed by >3-fold), so the model with the lowest BMDL was selected (LogLogistic; also has the lowest AIC).
[f]Betas restricted to ≥0.

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the dose associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{10}$ = dose associated with 10% extra risk); DF = degrees of freedom; DNT = dinitrotoluene

Figure A-10. Fit of Log-logistic Model to Data on Incidence of Extramedullary Hematopoiesis in Rats Treated with 3,4-DNT for 14 Days

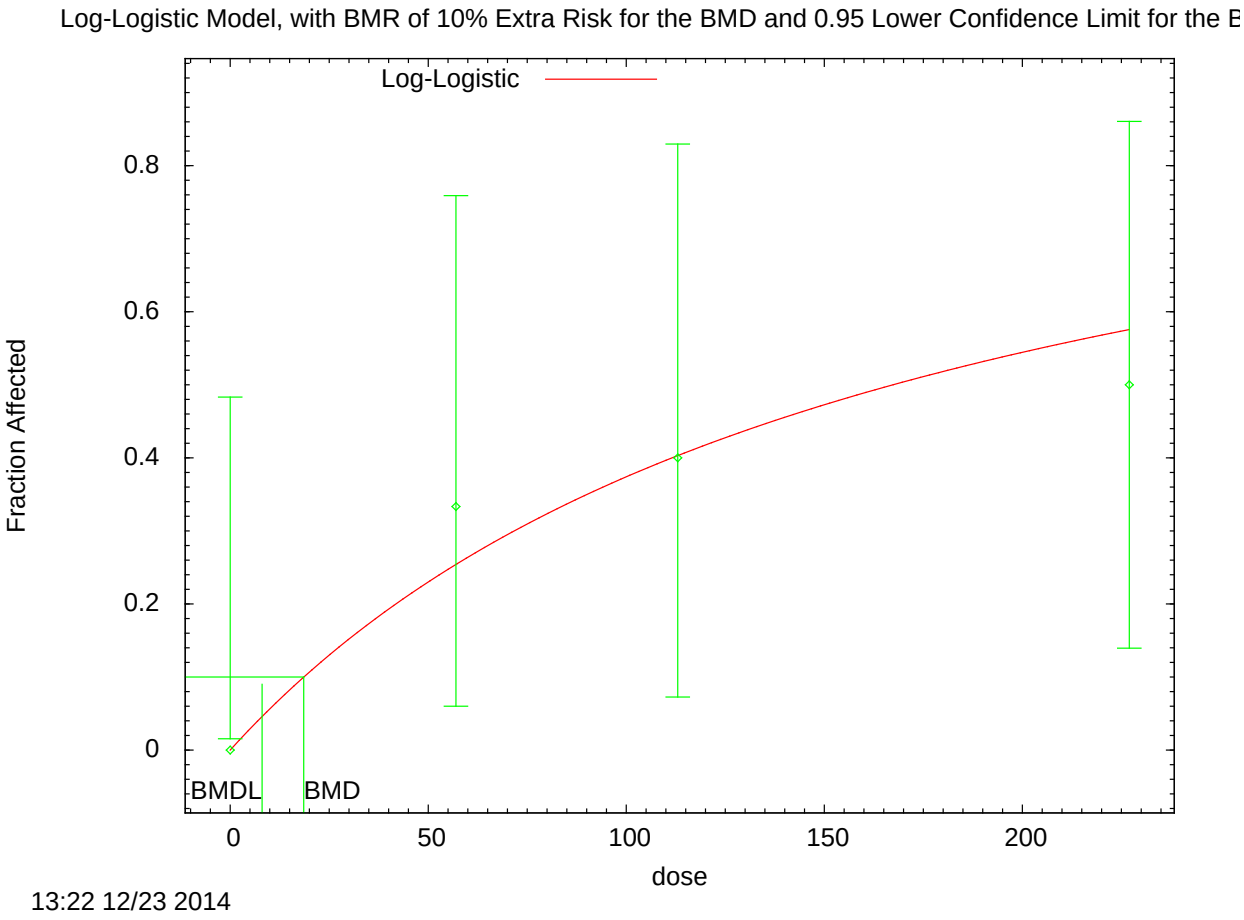


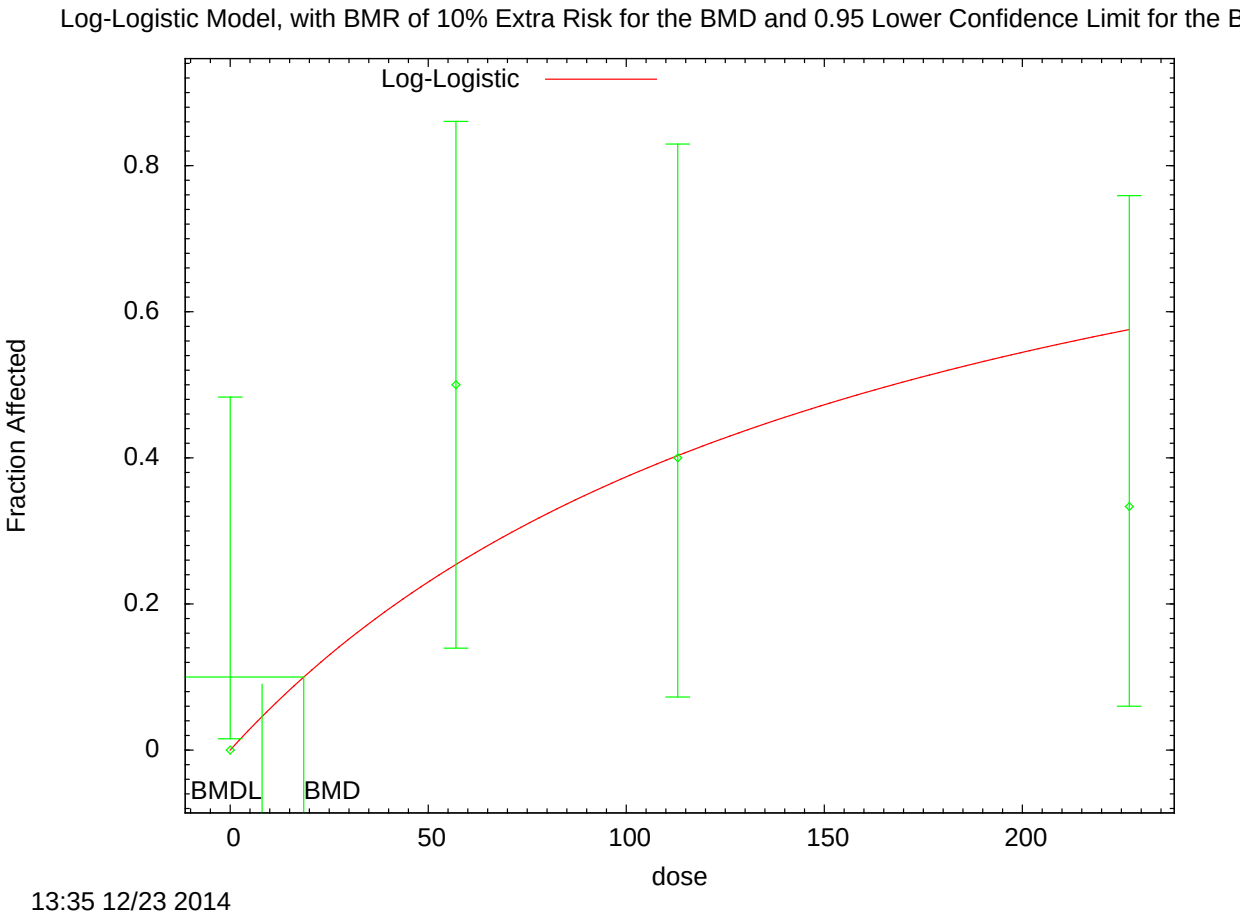
Table A-18. Model Predictions for Incidence of Lymphoid Hyperplasia in the Spleen of Male Sprague-Dawley Rats Administered 3,4-DNT via Gavage for 14 Days

Model	DF	χ^2	χ^2 Goodness-of-fit p-value[a]	Scaled residuals[b] Dose below BMD	Dose above BMD	Overall largest	AIC	BMD_{10} (mg/kg)	$BMDL_{10}$ (mg/kg)
Gamma[c]	2	0.86	0.83	0.00	0.74	0.74	25.48	25.51	14.32
Logistic	2	1.80	0.41	0.76	0.44	-0.96	29.20	64.08	37.29
LogLogistic[d,e]	3	*0.34*	*0.95*	*0.00*	*0.44*	*0.44*	*25.01*	*18.56*	*8.05*
LogProbit[d]	2	1.86	0.60	0.00	1.02	1.02	26.32	40.34	24.07
Multistage (1-degree)[f]	2	0.86	0.83	0.00	0.74	0.74	25.48	25.51	14.32
Multistage (2-degree)[f]	2	0.86	0.83	0.00	0.74	0.74	25.48	25.51	14.32
Multistage (3-degree)[f]	2	0.86	0.83	0.00	0.74	0.74	25.48	25.51	14.32
Probit	2	1.77	0.41	0.79	0.43	-0.91	29.10	60.21	36.19
Weibull[c]	2	0.86	0.83	0.00	0.74	0.74	25.48	25.51	14.32

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the benchmark dose; also the largest residual at any dose.
[c]Power restricted to ≥1.
[d]Slope restricted to ≥1.
[e]Selected model. All models provided adequate fit to the data. BMDLs for models providing adequate fit were not sufficiently close (differed by >3-fold), so the model with the lowest BMDL was selected (LogLogistic).
[f]Betas restricted to ≥0.

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the dose associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{10}$ = dose associated with 10% extra risk); DF = degrees of freedom; DNT = dinitrotoluene

Figure A-11. Fit of Log-logistic Model to Data on Incidence of Lymphoid Hyperplasia in Rats Treated with 3,4-DNT for 14 Days



Uncertainty Factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability
[X] 3 for database limitations (no other toxicological studies of this isomer are available)

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? No.

Other additional studies or pertinent information which lend support to this MRL: No other acute-duration oral studies of 3,4-DNT were located, nor were there any studies of longer durations.

Agency Contact (Chemical Manager): Carolyn Harper

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 3,5-DNT
CAS Numbers: 618-85-9
Date: February 2015
Profile Status: Final
Route: [ ] Inhalation [X] Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Graph Key: 4
Species: Rat

Minimal Risk Level: 0.03 [X] mg/kg/day [ ] ppm

References: Lent EM, Crouse CB, Quinn MJ, et al. 2012a. Comparison of the repeated dose toxicity of isomers of dinitrotoluene Int J Toxicol 31(2):143-157.

U.S. Army Public Health Command (USAPHC). 2011f. Toxicology Study No. 87-XE-08G0F-10, October 2011, Toxicology Portfolio. Fourteen-day oral toxicity and in vivo genotoxicity of 3,5-dinitrotoluene in rats, January 2009-March 2010. USAPHC, Aberdeen Proving Ground, Maryland.

Experimental design: In a comparative toxicity study, groups of six male Sprague-Dawley rats were given 3,5-DNT via gavage doses of 0, 5, 10, 19, 39, 77, or 155 mg/kg/day for 14 days. Both untreated and vehicle control groups were included. Evaluations during the study included twice daily observations for morbidity and clinical signs and measurement of body weight and food consumption on days 0, 1, 3, 7, and 14. Prior to sacrifice at the end of exposure, blood was collected for evaluation of hematology (total and differential red blood cell counts; red blood cell count; hemoglobin, hematocrit, MCV, MCH, MCHC, red cell distribution width; platelet count; and mean platelet volume) and serum chemistry (albumin, ALP, ALT, AST, BUN, calcium, cholesterol, creatinine, glucose, globulin, LDH, total bilirubin, total protein, sodium, potassium, and chlorine) assessments. All animals received gross necropsy and organ weight determinations (brain, heart, kidney, epididymides, liver, spleen, and testes). Microscopic examination of organs with exposure-related changes in weight was performed for controls and any dose groups exhibiting changes in the corresponding organ weights.

Effects noted in study and corresponding doses: All rats exposed to 77 and 155 mg/kg/day 3,5-DNT died or were sacrificed moribund within the first 8 days on study, as did one rat in the 39 mg/kg/day group. Clinical signs of toxicity were observed at the lethal doses; in addition, animals exposed to ≥39 mg/kg/day were reported to exhibit neurological signs progressing from facial twitching to paralysis of the forelimbs. It is not clear whether these signs were seen only in the animals that were moribund or if some neurological signs were seen in animals that survived treatment. Mean terminal body weight was decreased by 13% at 39 mg/kg/day; treatment did not affect food intake. The only hematology or clinical chemistry changes possibly attributable to treatment were increased albumin, total protein, and total bilirubin at 39 mg/kg/day. Gross necropsy findings among animals surviving to termination included small testis size in three rats given 19 mg/kg/day and in five rats given 39 mg/kg/day. These findings were confirmed by organ weight measurements indicating significant reductions (≥40%) in absolute and relative testes weights at these doses. Brain weight relative to body weight was increased by 30% (relative to vehicle controls) in the 39 mg/kg/day group; no other organ weight changes were reported. Testicular histopathology changes were observed in all rats exposed to doses ≥19 mg/kg/day (see Table A-19); these included mild-to-severe tubular degeneration and trace-to-severe multinucleated giant cell formation. Trace lymphoid depletion was observed in the spleens of 1/6 rats in each of the groups exposed to 10 and 19 mg/kg/day; no lesions were seen at 39 mg/kg/day. Finally, mild or moderate

inflammatory infiltrates were observed in the brains of 3/6 rats exposed to 39 mg/kg/day. Results of this study identify acute-duration NOAEL and LOAEL values for testicular lesions of 10 and 19 mg/kg/day, respectively, in rats.

Table A-19. Testes Effects in Rats Exposed to 3,5-DNT for 14 Days

	Dose (mg/kg/day)						
End point	0	5	10	19	39[a]	77	155
Tubular degeneration	0/6[b]	0/6	0/6	6/6[c]	6/6[c]	NA	NA
Multinucleated giant cell formation	0/6	0/6	0/6	6/6[c]	5/6[c]	NA	NA

[a]Includes one animal that died or was sacrificed moribund before the end of the study.
[b]Number affected/number examined.
[c]Statistically significant at $p<0.05$ based on Fisher's exact test performed for this review.

DNT = dinitrotoluene; NA = not applicable; all rats died or were sacrificed moribund at these doses

Sources: Lent et al. 2012a; USAPHC 2011f

Dose and end point used for MRL derivation:

[X] NOAEL [ ] LOAEL [ ] BMDL 10 mg/kg/day was the NOAEL for testicular effects (mild-to-severe tubular degeneration and trace-to-severe multinucleated giant cell formation).

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.

Uncertainty Factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability
[X] 3 for database limitations (no other toxicological studies of this isomer are available)

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? No.

Other additional studies or pertinent information which lend support to this MRL: No other acute-duration oral studies of 3,5-DNT were located, nor were there any studies of longer durations.

Agency Contact (Chemical Manager): Carolyn Harper

APPENDIX B. USER'S GUIDE

Chapter 1

Public Health Statement

This chapter of the profile is a health effects summary written in non-technical language. Its intended audience is the general public, especially people living in the vicinity of a hazardous waste site or chemical release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the chemical.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

Chapter 2

Relevance to Public Health

This chapter provides a health effects summary based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health end points by addressing the following questions:

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The chapter covers end points in the same order that they appear within the Discussion of Health Effects by Route of Exposure section, by route (inhalation, oral, and dermal) and within route by effect. Human data are presented first, then animal data. Both are organized by duration (acute, intermediate, chronic). *In vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this chapter.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotoxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. Minimal Risk Levels (MRLs) for noncancer end points (if derived) and the end points from which they were derived are indicated and discussed.

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Chapter 3 Data Needs section.

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, ATSDR has derived MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a chemical emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Chapter 2, "Relevance to Public Health," contains basic information known about the substance. Other sections such as Chapter 3 Section 3.9, "Interactions with Other Substances," and Section 3.10, "Populations that are Unusually Susceptible" provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive end point which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen end point are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor (UF) of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables.

Chapter 3

Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species, MRLs to humans for noncancer end points, and EPA's estimated range associated with an upper- bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. Use the LSE tables and figures for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 3-1 and Figure 3-1 are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

LEGEND

See Sample LSE Table 3-1 (page B-6)

(1) Route of Exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Tables 3-1, 3-2, and 3-3, respectively). LSE figures are limited to the inhalation (LSE Figure 3-1) and oral (LSE Figure 3-2) routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures.

(2) Exposure Period. Three exposure periods—acute (less than 15 days), intermediate (15–364 days), and chronic (365 days or more)—are presented within each relevant route of exposure. In this example, an inhalation study of intermediate exposure duration is reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Health Effect. The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table (see key number 18).

(4) Key to Figure. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to derive a NOAEL and a Less Serious LOAEL (also see the two "18r" data points in sample Figure 3-1).

(5) Species. The test species, whether animal or human, are identified in this column. Chapter 2, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 3.4, "Toxicokinetics," contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(6) Exposure Frequency/Duration. The duration of the study and the weekly and daily exposure regimens are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to “Chemical x” via inhalation for 6 hours/day, 5 days/week, for 13 weeks. For a more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Nitschke et al. 1981).

(7) System. This column further defines the systemic effects. These systems include respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, one systemic effect (respiratory) was investigated.

(8) NOAEL. A NOAEL is the highest exposure level at which no harmful effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system, which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote "b").

(9) LOAEL. A LOAEL is the lowest dose used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific end point used to quantify the adverse effect accompanies the LOAEL. The respiratory effect reported in key number 18 (hyperplasia) is a Less Serious LOAEL of 10 ppm. MRLs are not derived from Serious LOAELs.

(10) Reference. The complete reference citation is given in Chapter 9 of the profile.

(11) CEL. A CEL is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases.

(12) Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. Footnote "b" indicates that the NOAEL of 3 ppm in key number 18 was used to derive an MRL of 0.005 ppm.

LEGEND

See Sample Figure 3-1 (page B-7)

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure Period. The same exposure periods appear as in the LSE table. In this example, health effects observed within the acute and intermediate exposure periods are illustrated.

(14) Health Effect. These are the categories of health effects for which reliable quantitative data exists. The same health effects appear in the LSE table.

(15) Levels of Exposure. Concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) NOAEL. In this example, the open circle designated 18r identifies a NOAEL critical end point in the rat upon which an intermediate inhalation exposure MRL is based. The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).

(17) CEL. Key number 38m is one of three studies for which CELs were derived. The diamond symbol refers to a CEL for the test species-mouse. The number 38 corresponds to the entry in the LSE table.

(18) Estimated Upper-Bound Human Cancer Risk Levels. This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. These risk levels are derived from the EPA's Human Health Assessment Group's upper-bound estimates of the slope of the cancer dose response curve at low dose levels (q_1*).

(19) Key to LSE Figure. The Key explains the abbreviations and symbols used in the figure.

SAMPLE

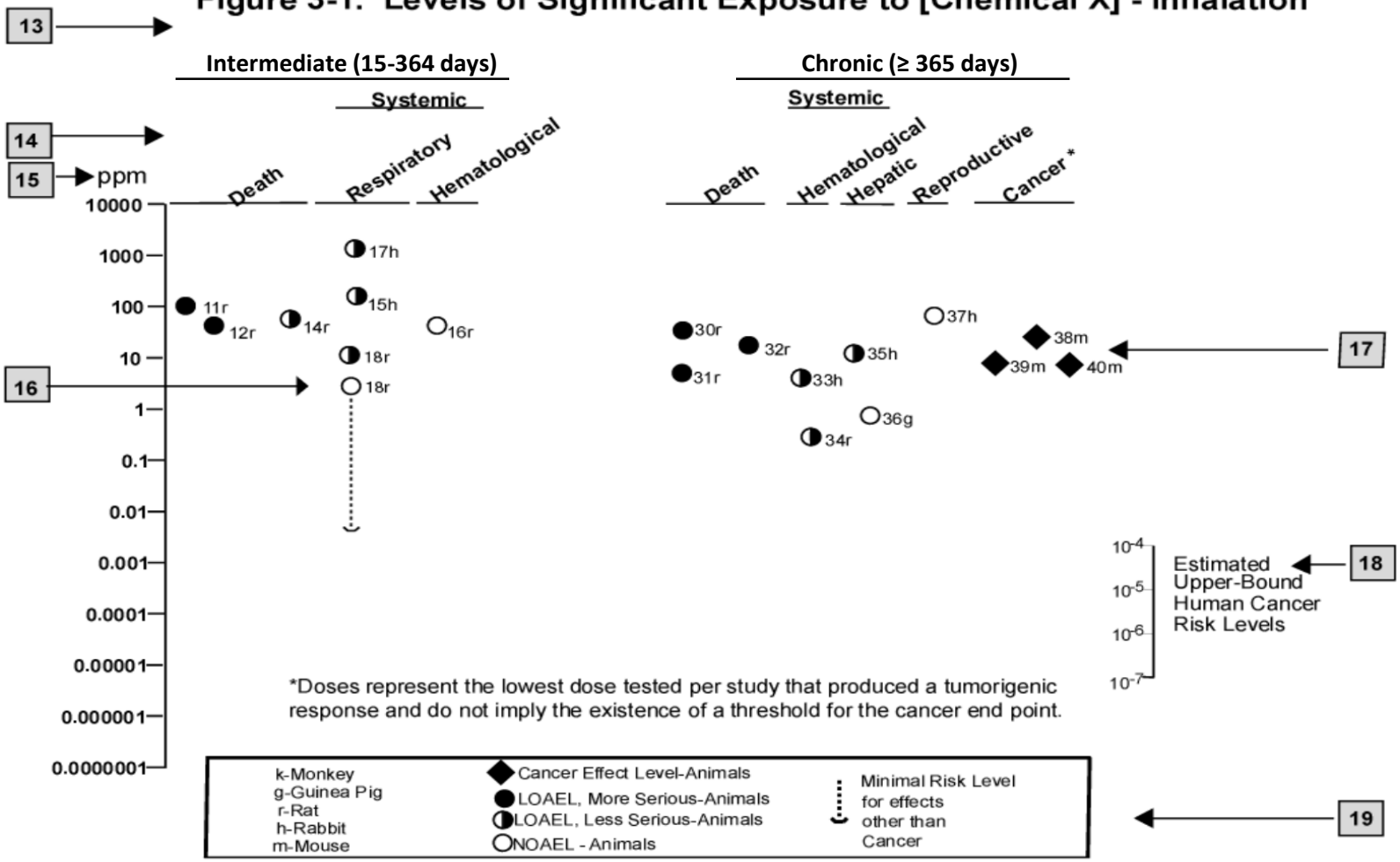
1 → **Table 3-1. Levels of Significant Exposure to [Chemical x] – Inhalation**

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	Serious (ppm)	Reference
2 → INTERMEDIATE EXPOSURE							
	5	6	7	8	9		10
3 → Systemic	↓	↓	↓	↓	↓		↓
4 → 18	Rat	13 wk 5 d/wk 6 hr/d	Resp	3[b]	10 (hyperplasia)		Nitschke et al. 1981
CHRONIC EXPOSURE							
Cancer					11		
					↓		
38	Rat	18 mo 5 d/wk 7 hr/d			20	(CEL, multiple organs)	Wong et al. 1982
39	Rat	89–104 wk 5 d/wk 6 hr/d			10	(CEL, lung tumors, nasal tumors)	NTP 1982
40	Mouse	79–103 wk 5 d/wk 6 hr/d			10	(CEL, lung tumors, hemangiosarcomas)	NTP 1982

12 → [a] The number corresponds to entries in Figure 3-1.
[b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of $5x10^{-3}$ ppm; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).

SAMPLE

Figure 3-1. Levels of Significant Exposure to [Chemical X] - Inhalation



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APPENDIX C. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ACOEM	American College of Occupational and Environmental Medicine
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AED	atomic emission detection
AFID	alkali flame ionization detector
AFOSH	Air Force Office of Safety and Health
ALT	alanine aminotransferase
AML	acute myeloid leukemia
AOAC	Association of Official Analytical Chemists
AOEC	Association of Occupational and Environmental Clinics
AP	alkaline phosphatase
APHA	American Public Health Association
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BAT	best available technology
BCF	bioconcentration factor
BEI	Biological Exposure Index
BMD/C	benchmark dose or benchmark concentration
BMD_X	dose that produces a X% change in response rate of an adverse effect
$BMDL_X$	95% lower confidence limit on the BMD_X
BMDS	Benchmark Dose Software
BMR	benchmark response
BSC	Board of Scientific Counselors
C	centigrade
CAA	Clean Air Act
CAG	Cancer Assessment Group of the U.S. Environmental Protection Agency
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CELDS	Computer-Environmental Legislative Data System
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
CL	ceiling limit value
CLP	Contract Laboratory Program
cm	centimeter
CML	chronic myeloid leukemia
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DOL	Department of Labor

APPENDIX C

DOT	Department of Transportation
DOT/UN/	Department of Transportation/United Nations/
NA/IMDG	North America/Intergovernmental Maritime Dangerous Goods Code
DWEL	drinking water exposure level
ECD	electron capture detection
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EEGL	Emergency Exposure Guidance Level
EPA	Environmental Protection Agency
F	Fahrenheit
F_1	first-filial generation
FAO	Food and Agricultural Organization of the United Nations
FDA	Food and Drug Administration
FEMA	Federal Emergency Management Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FPD	flame photometric detection
fpm	feet per minute
FR	Federal Register
FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GLC	gas liquid chromatography
GPC	gel permeation chromatography
HPLC	high-performance liquid chromatography
HRGC	high resolution gas chromatography
HSDB	Hazardous Substance Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
ILO	International Labor Organization
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
kkg	metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{50}	lethal concentration, 50% kill
LC_{Lo}	lethal concentration, low
LD_{50}	lethal dose, 50% kill
LD_{Lo}	lethal dose, low
LDH	lactic dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
LT_{50}	lethal time, 50% kill
m	meter
MA	*trans*,*trans*-muconic acid
MAL	maximum allowable level
mCi	millicurie

MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
MFO	mixed function oxidase
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
mppcf	millions of particles per cubic foot
MRL	Minimal Risk Level
MS	mass spectrometry
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NATICH	National Air Toxics Information Clearinghouse
NATO	North Atlantic Treaty Organization
NCE	normochromatic erythrocytes
NCEH	National Center for Environmental Health
NCI	National Cancer Institute
ND	not detected
NFPA	National Fire Protection Association
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIOSHTIC	NIOSH's Computerized Information Retrieval System
NLM	National Library of Medicine
nm	nanometer
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NOES	National Occupational Exposure Survey
NOHS	National Occupational Hazard Survey
NPD	nitrogen phosphorus detection
NPDES	National Pollutant Discharge Elimination System
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NSPS	New Source Performance Standards
NTIS	National Technical Information Service
NTP	National Toxicology Program
ODW	Office of Drinking Water, EPA
OERR	Office of Emergency and Remedial Response, EPA
OHM/TADS	Oil and Hazardous Materials/Technical Assistance Data System
OPP	Office of Pesticide Programs, EPA
OPPT	Office of Pollution Prevention and Toxics, EPA
OPPTS	Office of Prevention, Pesticides and Toxic Substances, EPA
OR	odds ratio
OSHA	Occupational Safety and Health Administration
OSW	Office of Solid Waste, EPA
OTS	Office of Toxic Substances

OW	Office of Water
OWRS	Office of Water Regulations and Standards, EPA
PAH	polycyclic aromatic hydrocarbon
PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocytes
PEL	permissible exposure limit
pg	picogram
PHS	Public Health Service
PID	photo ionization detector
pmol	picomole
PMR	proportionate mortality ratio
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PSNS	pretreatment standards for new sources
RBC	red blood cell
REL	recommended exposure level/limit
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
RQ	reportable quantity
RTECS	Registry of Toxic Effects of Chemical Substances
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SIC	standard industrial classification
SIM	selected ion monitoring
SMCL	secondary maximum contaminant level
SMR	standardized mortality ratio
SNARL	suggested no adverse response level
SPEGL	Short-Term Public Emergency Guidance Level
STEL	short term exposure limit
STORET	Storage and Retrieval
TD_{50}	toxic dose, 50% specific toxic effect
TLV	threshold limit value
TOC	total organic carbon
TPQ	threshold planning quantity
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization

>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
μm	micrometer
μg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result