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 30333.
MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 2,4-DNT
CAS Numbers: 121-14-2
Date: October 2012
Profile Status: Final Draft for Pre-Public Comment
Route: [X] Oral
Duration: [X] Acute  [ ] Intermediate  [ ] Chronic
Graph Key: 10
Species: Dog

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


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

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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 (decreased serum albumin and gene expression
changes) and hematological effects (erythrocytosis) in female Sprague-Dawley rats treated at
99 mg/kg/day via gavage and observed for 24 or 48 hours (Deng et al. 2011), 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
MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 2,4-DNT
CAS Numbers: 121-14-2
Date: October 2012
Profile Status: Final Draft for Pre-Public Comment
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [X] Intermediate [ ] Chronic
Graph Key: 27
Species: Dog

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


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

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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-1. 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-1. Hematological Effects in Beagle Dogs Exposed to 2,4-DNT for 9 Months

<table>
<thead>
<tr>
<th>End point</th>
<th>Dose (mg/kg/day)</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.2</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Erythrocyte count</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(x10/mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.51±0.12 (6)</td>
<td>6.23±0.18 (6)</td>
<td>6.36±0.13 (6)</td>
</tr>
<tr>
<td>Heinz bodies (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0±0.0(6)</td>
<td>0.0±0.0(6)</td>
<td>0.0±0.0(6)</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.69±0.15 (6)</td>
<td>0.55±0.09 (6)</td>
<td>0.57±0.11 (6)</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>46.7±1.0 (6)</td>
<td>45.3±1.1 (6)</td>
<td>45.0±0.9 (6)</td>
</tr>
<tr>
<td>Hemoglobin (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16.6±0.3 (6)</td>
<td>15.4±0.4 (6)</td>
<td>15.7±0.3 (6)</td>
</tr>
<tr>
<td>Methemoglobin (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0±0.0 (6)</td>
<td>0.0±0.0 (6)</td>
<td>0.9±0.6 (6)</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.49±0.24 (6)</td>
<td>5.90±0.17 (6)</td>
<td>5.78±0.21 (6)</td>
</tr>
<tr>
<td>Heinz bodies (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0±0.0(6)</td>
<td>0.0±0.0(6)</td>
<td>0.0±0.0(6)</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>0.60±0.08 (6)</td>
<td>0.70±0.17 (6)</td>
<td>0.45±0.10 (6)</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>46.0±1.3 (6)</td>
<td>42.8±1.7 (6)</td>
<td>42.5±1.2 (6)</td>
</tr>
<tr>
<td>Hemoglobin (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16.1±0.5 (6)</td>
<td>14.7±0.6 (6)</td>
<td>14.8±0.5 (6)</td>
</tr>
<tr>
<td>Methemoglobin (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.4±0.4 (6)</td>
<td>0.0±0.0 (6)</td>
<td>1.3±0.6 (6)</td>
</tr>
</tbody>
</table>

aValues are means±standard error (number of animals) [percent change from controls].
bStatistically 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 BMDL1SD 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, (Table A-1). 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% confidence interval 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-2, A-3, and A-4, 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-2. Model Predictions for 2,4-DNT for Increased Reticulocytes (%) in Female Dogs Following 9 Months of Exposure (U.S. Army 1979)

<table>
<thead>
<tr>
<th>Model</th>
<th>Test for significant difference</th>
<th>Scaled residuals</th>
<th>BMD&lt;sub&gt;1SD&lt;/sub&gt;</th>
<th>BMDL&lt;sub&gt;1SD&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p-value&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Variance p-value&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Means p-value&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Dose below BMD</td>
</tr>
<tr>
<td>Constant variance</td>
<td>0.0007</td>
<td>0.12</td>
<td>0.23</td>
<td>-1.32</td>
</tr>
<tr>
<td>Exponential (model 2)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.0007</td>
<td>0.12</td>
<td>0.18</td>
<td>-0.98</td>
</tr>
<tr>
<td>Exponential (model 3)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.0007</td>
<td>0.12</td>
<td>0.06</td>
<td>-1.51</td>
</tr>
<tr>
<td>Exponential (model 4)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.0007</td>
<td>0.12</td>
<td>NA</td>
<td>-0.98</td>
</tr>
<tr>
<td>Exponential (model 5)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.0007</td>
<td>0.12</td>
<td>NA</td>
<td>-0.98</td>
</tr>
<tr>
<td>Hill&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.0007</td>
<td>0.12</td>
<td>0.17</td>
<td>-1.51</td>
</tr>
<tr>
<td>Linear&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.0007</td>
<td>0.12</td>
<td>0.36</td>
<td>-1.06</td>
</tr>
<tr>
<td>Polynomial (2-degree)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.0007</td>
<td>0.12</td>
<td>0.40</td>
<td>-0.99</td>
</tr>
<tr>
<td>Polynomial (3-degree)&lt;sup&gt;e,f&lt;/sup&gt;</td>
<td>0.0007</td>
<td>0.12</td>
<td>0.18</td>
<td>-0.98</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values >0.05 fail to meet conventional goodness-of-fit criteria.
<sup>b</sup>Values <0.10 fail to meet conventional goodness-of-fit criteria.
<sup>c</sup>Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
<sup>d</sup>Power restricted to ≥1.
<sup>e</sup>Coefficients restricted to be positive.
<sup>f</sup>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)

Polynomial Model with 0.95 Confidence Level

Mean Response

Polynomial

BMDL

BMD

Dose

16:04 04/02 2012

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

<table>
<thead>
<tr>
<th>Model</th>
<th>Test for significant difference p-value</th>
<th>Variance p-value</th>
<th>Means p-value</th>
<th>Scaled residuals</th>
<th>Dose below BMD</th>
<th>Dose above BMD</th>
<th>Overall largest residual BMD</th>
<th>BMD1SD (mg/kg-day)</th>
<th>BMDL1SD (mg/kg-day)</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant variance</td>
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<tr>
<td>Exponential (model 2)</td>
<td>0.04</td>
<td>0.92</td>
<td>0.14</td>
<td>-0.40</td>
<td>0.10</td>
<td>1.46</td>
<td>41.47</td>
<td>5.90</td>
<td>3.66</td>
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</tr>
<tr>
<td>Exponential (model 3)</td>
<td>0.04</td>
<td>0.92</td>
<td>0.14</td>
<td>-0.40</td>
<td>0.10</td>
<td>1.46</td>
<td>41.47</td>
<td>5.90</td>
<td>3.66</td>
<td></td>
</tr>
<tr>
<td>Exponential (model 4)</td>
<td>0.04</td>
<td>0.92</td>
<td>0.07</td>
<td>0.29</td>
<td>-0.03</td>
<td>-1.36</td>
<td>42.88</td>
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</tr>
<tr>
<td>Exponential (model 5)</td>
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<td>0.92</td>
<td>0.07</td>
<td>0.29</td>
<td>-0.03</td>
<td>-1.36</td>
<td>42.88</td>
<td>2.43</td>
<td>0.01</td>
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<tr>
<td>Hill</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>Linear</td>
<td>0.04</td>
<td>0.92</td>
<td>0.14</td>
<td>-0.43</td>
<td>0.09</td>
<td>1.48</td>
<td>41.52</td>
<td>6.12</td>
<td>3.94</td>
<td></td>
</tr>
<tr>
<td>Polynomial (2-degree)</td>
<td>0.04</td>
<td>0.92</td>
<td>0.14</td>
<td>-0.43</td>
<td>0.09</td>
<td>1.48</td>
<td>41.52</td>
<td>6.12</td>
<td>3.94</td>
<td></td>
</tr>
<tr>
<td>Polynomial (3-degree)</td>
<td>0.04</td>
<td>0.92</td>
<td>0.14</td>
<td>-0.43</td>
<td>0.09</td>
<td>1.48</td>
<td>41.52</td>
<td>6.12</td>
<td>3.94</td>
<td></td>
</tr>
<tr>
<td>Power</td>
<td>0.04</td>
<td>0.92</td>
<td>0.14</td>
<td>-0.43</td>
<td>0.09</td>
<td>1.48</td>
<td>41.52</td>
<td>6.12</td>
<td>3.94</td>
<td></td>
</tr>
</tbody>
</table>

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)

Exponential Model 2 with 0.95 Confidence Level

08:20 04/12 2012

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

<table>
<thead>
<tr>
<th>Model</th>
<th>Test for significant difference</th>
<th>Scaled residuals&lt;sup&gt;c&lt;/sup&gt;</th>
<th>BMD&lt;sub&gt;1SD&lt;/sub&gt;</th>
<th>BMDL&lt;sub&gt;1SD&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p-value&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Variance p-value&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Means p-value&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Dose below</td>
</tr>
<tr>
<td>Constant variance</td>
<td>0.01</td>
<td>0.79</td>
<td>0.26</td>
<td>-0.42</td>
</tr>
<tr>
<td>Exponential (model 2)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.01</td>
<td>0.79</td>
<td>0.26</td>
<td>-0.42</td>
</tr>
<tr>
<td>Exponential (model 3)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.01</td>
<td>0.79</td>
<td>0.26</td>
<td>-0.42</td>
</tr>
<tr>
<td><strong>Exponential (model 4)</strong>&lt;sup&gt;d,e&lt;/sup&gt;</td>
<td><strong>0.01</strong></td>
<td><strong>0.79</strong></td>
<td><strong>0.14</strong></td>
<td><strong>0.21</strong></td>
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<tr>
<td>Exponential (model 5)&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>0.79</td>
<td>0.14</td>
<td>0.21</td>
</tr>
<tr>
<td>Hill&lt;sup&gt;d&lt;/sup&gt;</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Linear&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.01</td>
<td>0.79</td>
<td>0.25</td>
<td>-0.45</td>
</tr>
<tr>
<td>Polynomial (2-degree)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.01</td>
<td>0.79</td>
<td>0.25</td>
<td>-0.45</td>
</tr>
<tr>
<td>Polynomial (3-degree)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.01</td>
<td>0.79</td>
<td>0.25</td>
<td>-0.45</td>
</tr>
<tr>
<td>Power&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.01</td>
<td>0.79</td>
<td>0.25</td>
<td>-0.45</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values >0.05 fail to meet conventional goodness-of-fit criteria.

<sup>b</sup>Values <0.10 fail to meet conventional goodness-of-fit criteria.

<sup>c</sup>Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.

<sup>d</sup>Power restricted to ≥1.

<sup>e</sup>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).

<sup>f</sup>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.

***DRAFT FOR PUBLIC COMMENT***
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)

Exponential Model 4 with 0.95 Confidence Level

08:07 04/12 2012

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:** October 2012  
**Profile Status:** Final Draft for Pre-Public Comment  
**Route:** [ ] Inhalation  [X] Oral  
**Duration:** [ ] Acute  [ ] Intermediate  [X] Chronic  
**Graph Key:** 52  
**Species:** Dog  

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

**References:**  


**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 \( \geq 1.5 \text{ mg/kg/day} \) 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-5). 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-5. Hematological Effects in Beagle Dogs Exposed to 2,4-DNT for 12 Months

<table>
<thead>
<tr>
<th>End point</th>
<th>0</th>
<th>0.2</th>
<th>1.5</th>
<th>10</th>
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<tbody>
<tr>
<td></td>
<td>Dose (mg/kg/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrocyte count (x10/mm)</td>
<td>5.96±0.22 (6)a</td>
<td>5.33±0.16 (6)</td>
<td>5.69±0.19 (6)</td>
<td>5.22±0.19 (2)</td>
</tr>
<tr>
<td>Heinz bodies (%)</td>
<td>0.0±0.0(6)</td>
<td>0.0±0.0(6)</td>
<td>0.0±0.0(6)</td>
<td>0.52±0.37 (2)b</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>0.40±0.09 (6)</td>
<td>0.78±0.13 (6)</td>
<td>0.66±0.11 (6)</td>
<td>1.23±0.23 (2)b</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>45.2±0.9 (6)</td>
<td>41.8±1.2 (6)</td>
<td>44.7±0.8 (6)</td>
<td>44.0±4.0 (2)</td>
</tr>
<tr>
<td>Hemoglobin (%)</td>
<td>15.1±0.4 (6)</td>
<td>14.1±0.3 (6)</td>
<td>14.8±0.4 (6)</td>
<td>14.2±1.2 (2)</td>
</tr>
<tr>
<td>Methemoglobin (%)</td>
<td>0.0±0.0 (6)</td>
<td>0.0±0.0 (6)</td>
<td>0.0±0.0 (6)</td>
<td>0.0±0.0 (2)</td>
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<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrocyte count (x10/mm)</td>
<td>5.87±0.20 (6)a</td>
<td>5.54±0.14 (6)</td>
<td>4.69±0.25 (6)b</td>
<td>4.45±0.26 (6)b</td>
</tr>
<tr>
<td>Heinz bodies (%)</td>
<td>0.0±0.0(6)</td>
<td>0.0±0.0(6)</td>
<td>0.0±0.0(6)</td>
<td>0.5±0.2 (6)b</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>0.39±0.04 (6)</td>
<td>0.84±0.07 (6)b</td>
<td>0.45±0.06 (6)</td>
<td>1.59±0.18 (6)b</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>45.8±1.1 (6)</td>
<td>44.7±1.3 (6)</td>
<td>41.8±1.2 (6)b</td>
<td>40.8±0.6 (6)b</td>
</tr>
<tr>
<td>Hemoglobin (%)</td>
<td>15.1±0.4 (6)</td>
<td>15.1±0.4 (6)</td>
<td>13.7±0.8 (6)b</td>
<td>12.9±0.2 (6)b</td>
</tr>
<tr>
<td>Methemoglobin (%)</td>
<td>0.0±0.0 (6)</td>
<td>0.6±0.6 (6)</td>
<td>0.0±0.0 (6)</td>
<td>0.0±0.0 (6)</td>
</tr>
</tbody>
</table>

aValues are means±standard error (number of animals) [percent change from controls].
bStatistically 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 hematological 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-3). 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% confidence interval 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 decreased hematocrit and decreased erythrocyte count are shown in Tables A-6 and A-7, 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 intermediate chronic-duration oral MRL of 0.001 mg/kg/day.
Table A-6. Model Predictions for 2,4-DNT for Decreased Hematocrit (%) in Female Dogs Following 12 Months of Exposure (U.S. Army 1979)

<table>
<thead>
<tr>
<th>Model</th>
<th>Test for significant difference p-value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Variance p-value&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Means p-value&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Scaled residuals&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Dose below BMD</th>
<th>Dose above BMD</th>
<th>Overall largest</th>
<th>AIC</th>
<th>BMD&lt;sub&gt;1SD&lt;/sub&gt; (mg/kg-day)</th>
<th>BMDL&lt;sub&gt;1SD&lt;/sub&gt; (mg/kg-day)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Constant variance</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exponential (model 2)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.01</td>
<td>0.34</td>
<td>0.06</td>
<td>-1.82</td>
<td>0.29</td>
<td>-1.82</td>
<td>78.08</td>
<td>6.77</td>
<td>4.09</td>
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</tr>
<tr>
<td>Exponential (model 3)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.01</td>
<td>0.34</td>
<td>0.06</td>
<td>-1.82</td>
<td>0.29</td>
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<td>78.08</td>
<td>6.77</td>
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<tr>
<td>Exponential (model 4)&lt;sup&gt;d,e&lt;/sup&gt;</td>
<td>0.01</td>
<td>0.34</td>
<td>0.92</td>
<td>-0.07</td>
<td>0.04</td>
<td>-0.07</td>
<td>74.50</td>
<td>0.61</td>
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<td>0.34</td>
<td>0.92</td>
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<td>74.50</td>
<td>0.61</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Hill&lt;sup&gt;f&lt;/sup&gt;</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.01</td>
<td>0.34</td>
<td>0.06</td>
<td>-1.83</td>
<td>0.27</td>
<td>-1.83</td>
<td>78.15</td>
<td>6.96</td>
<td>4.31</td>
<td></td>
</tr>
<tr>
<td>Polynomial (2-degree)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.01</td>
<td>0.34</td>
<td>0.06</td>
<td>-1.83</td>
<td>0.27</td>
<td>-1.83</td>
<td>78.15</td>
<td>6.96</td>
<td>4.31</td>
<td></td>
</tr>
<tr>
<td>Polynomial (3-degree)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.01</td>
<td>0.34</td>
<td>0.06</td>
<td>-1.83</td>
<td>0.27</td>
<td>-1.83</td>
<td>78.15</td>
<td>6.96</td>
<td>4.31</td>
<td></td>
</tr>
<tr>
<td>Power&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.01</td>
<td>0.34</td>
<td>0.06</td>
<td>-1.83</td>
<td>0.27</td>
<td>-1.83</td>
<td>78.15</td>
<td>6.96</td>
<td>4.31</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Values >0.05 fail to meet conventional goodness-of-fit criteria.
<sup>b</sup>Values <0.10 fail to meet conventional goodness-of-fit criteria.
<sup>c</sup>Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
<sup>d</sup>Power restricted to ≥1.
<sup>e</sup>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).
<sup>f</sup>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

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**DRAFT FOR PUBLIC COMMENT***
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)

Exponential Model 4 with 0.95 Confidence Level
### Table A-7. Model Predictions for Decreased Erythrocyte Count in Dogs Treated with 2,4-DNT for 12 Months

<table>
<thead>
<tr>
<th>Model</th>
<th>Test for significant difference p-value</th>
<th>Variance p-value</th>
<th>Means p-value</th>
<th>Scaled residuals$^c$</th>
<th>BMD1SD BMDL1SD (mg/kg-day)</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant variance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exponential (model 2)$^d$</td>
<td>0.0005</td>
<td>0.46</td>
<td>0.005</td>
<td>-2.40</td>
<td>0.43</td>
<td>6.16</td>
</tr>
<tr>
<td>Exponential (model 3)$^d$</td>
<td>0.0005</td>
<td>0.46</td>
<td>0.005</td>
<td>-2.40</td>
<td>0.43</td>
<td>6.16</td>
</tr>
<tr>
<td><strong>Exponential (model 4)$^{d,e}$</strong></td>
<td><strong>0.0005</strong></td>
<td><strong>0.46</strong></td>
<td><strong>0.91</strong></td>
<td><strong>-0.08</strong></td>
<td><strong>0.05</strong></td>
<td><strong>-2.55</strong></td>
</tr>
<tr>
<td>Exponential (model 5)$^d$</td>
<td>0.0005</td>
<td>0.46</td>
<td>0.91</td>
<td>-0.08</td>
<td>0.05</td>
<td>-0.08</td>
</tr>
<tr>
<td>Hill$^d$</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Linear$^f$</td>
<td>0.0005</td>
<td>0.46</td>
<td>0.004</td>
<td>-2.41</td>
<td>0.35</td>
<td>-2.41</td>
</tr>
<tr>
<td>Polynomial (2-degree)$^f$</td>
<td>0.0005</td>
<td>0.46</td>
<td>0.004</td>
<td>-2.41</td>
<td>0.35</td>
<td>-2.41</td>
</tr>
<tr>
<td>Polynomial (3-degree)$^f$</td>
<td>0.0005</td>
<td>0.46</td>
<td>0.004</td>
<td>-2.41</td>
<td>0.35</td>
<td>-2.41</td>
</tr>
<tr>
<td>Power$^d$</td>
<td>0.0005</td>
<td>0.46</td>
<td>0.004</td>
<td>-2.41</td>
<td>0.35</td>
<td>-2.41</td>
</tr>
</tbody>
</table>

---

$^a$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
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
MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 2,6-DNT  
CAS Numbers: 606-20-2  
Date: October 2012  
Profile Status: Final Draft for Pre-Public Comment  
Route: [X] Oral  
Duration: [X] Acute  
Graph Key: 6  
Species: Dog  

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


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 hematopoiisis (U.S. Army 1976) (data summarized in Table A-8). 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 hematopoiisis 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-8. Hematological effects in Beagle Dogs Exposed to 2,6-DNT for 2 Weeks

<table>
<thead>
<tr>
<th>End point</th>
<th>0</th>
<th>4</th>
<th>20</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte count (x10/mm³)</td>
<td>5.62±0.16 (8) a</td>
<td>5.06±0.10 (8)</td>
<td>4.73±0.20 (8) b</td>
<td>1.85±0.28 (7) b</td>
</tr>
<tr>
<td></td>
<td>[↓10]</td>
<td>[↓16]</td>
<td>[↓67]</td>
<td></td>
</tr>
<tr>
<td>Heinz bodies (%)</td>
<td>0.00±0.00 (8)</td>
<td>0.00±0.00 (8)</td>
<td>0.00±0.00 (8)</td>
<td>0.00±0.00 (7) [NA]</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>0.76±0.07 (8)</td>
<td>1.10±0.13 (8)</td>
<td>1.43±0.32 (8)</td>
<td>16.99±3.33 (7) b</td>
</tr>
<tr>
<td></td>
<td>[145]</td>
<td>[188]</td>
<td>[12,136]</td>
<td></td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>42.1±1.7 (8)</td>
<td>38.9±0.6 (8)</td>
<td>39.3±1.2 (8)</td>
<td>22.9±2.8 (7) b</td>
</tr>
<tr>
<td></td>
<td>[↓8]</td>
<td>[↓7]</td>
<td>[↓46]</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (%)</td>
<td>14.8±0.5 (8)</td>
<td>13.3±0.2 (8)</td>
<td>13.0±0.4 (8)</td>
<td>6.3±0.9 (7) b</td>
</tr>
<tr>
<td></td>
<td>[145]</td>
<td>[122]</td>
<td>[57]</td>
<td></td>
</tr>
<tr>
<td>Methemoglobin (%)</td>
<td>0.0±0.0 (8)</td>
<td>0.0±0.0 (8)</td>
<td>0.0±0.0 (8)</td>
<td>0.0±0.0 (7)</td>
</tr>
<tr>
<td>Mean cell hemoglobin (micro µg)</td>
<td>26.3±0.2 (8)</td>
<td>26.3±0.3 (8)</td>
<td>27.7±0.3 (8) b</td>
<td>34.4±1.2 (7) b</td>
</tr>
</tbody>
</table>

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 conducted 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 spermatic cells).

Dose and end point used for MRL derivation:

[ ] NOAEL  [ ] LOAEL  [X] BMDL 9.31 mg/kg was the BMDL1SD 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-8). 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% confidence interval 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 increased 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 decreased erythrocyte count (Table A-9). The polynomial and power models converged to the linear model. The figure shown from the linear model (Figure A-6) 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-9. Model Predictions for Decreased Erythrocyte Count in Dogs Treated with 2,6-DNT for 2 Weeks

<table>
<thead>
<tr>
<th>Model</th>
<th>Test for significant difference p-value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Variance p-value&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Mean p-value&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Scaled residuals&lt;sup&gt;c&lt;/sup&gt;</th>
<th>BMD&lt;sub&gt;1SD&lt;/sub&gt; (mg/kg-day)</th>
<th>BMDL&lt;sub&gt;1SD&lt;/sub&gt; (mg/kg-day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant variance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear&lt;sup&gt;d&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
<td>0.08</td>
<td>0.25</td>
<td>-1.22</td>
<td>0.12</td>
<td>-1.22</td>
</tr>
<tr>
<td>Non Constant variance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exponential (model 2)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
<td>0.18</td>
<td>0.09</td>
<td>-1.55</td>
<td>1.26</td>
<td>-1.55</td>
</tr>
<tr>
<td>Exponential (model 3)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
<td>0.18</td>
<td>0.04</td>
<td>-1.60</td>
<td>0.64</td>
<td>-1.60</td>
</tr>
<tr>
<td>Exponential (model 4)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
<td>0.18</td>
<td>0.09</td>
<td>-1.55</td>
<td>1.26</td>
<td>-1.55</td>
</tr>
<tr>
<td>Exponential (model 5)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
<td>0.18</td>
<td>0.04</td>
<td>-1.60</td>
<td>0.64</td>
<td>-1.60</td>
</tr>
<tr>
<td>Hill&lt;sup&gt;f&lt;/sup&gt;</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Linear&lt;sup&gt;d,f&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
<td>0.18</td>
<td>0.16</td>
<td>-1.42</td>
<td>0.13</td>
<td>-1.42</td>
</tr>
<tr>
<td>Polynomial (2-degree)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
<td>0.18</td>
<td>0.16</td>
<td>-1.42</td>
<td>0.13</td>
<td>-1.42</td>
</tr>
<tr>
<td>Polynomial (3-degree)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
<td>0.18</td>
<td>0.16</td>
<td>-1.42</td>
<td>0.13</td>
<td>-1.42</td>
</tr>
<tr>
<td>Power&lt;sup)e&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
<td>0.18</td>
<td>0.16</td>
<td>-1.42</td>
<td>0.13</td>
<td>-1.42</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values >0.05 fail to meet conventional goodness-of-fit criteria.<br>
<sup>b</sup>Values <0.10 fail to meet conventional goodness-of-fit criteria.<br>
<sup>c</sup>Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.<br>
<sup>d</sup>Coefficients restricted to be negative.<br>
<sup>e</sup>Power restricted to ≥1.<br>
<sup>f</sup>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-6. 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 SD 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). 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) was 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
MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 2,6-DNT
CAS Numbers: 606-20-2
Date: October 2012
Profile Status: Final Draft for Pre-Public Comment
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [X] Intermediate [ ] Chronic
Graph Key: 12
Species: Dog

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


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

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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-10). 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-10. Extramedullary Erythropoiesis of the Spleen in Beagle Dogs Exposed to 2,6-DNT for 4 or 13 Weeks

<table>
<thead>
<tr>
<th>Timepoint (weeks)</th>
<th>Dose (mg/kg/day)</th>
<th>0/2 (mild)</th>
<th>1/2 (moderate)</th>
<th>2/2 (1 mild; 1 markedly severe)</th>
<th>4/4 (2 marked, 2 markedly severe)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>20</td>
<td>1/2</td>
<td>2/2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>100</td>
<td>0/2</td>
<td>2/2</td>
<td>3/3</td>
<td></td>
</tr>
</tbody>
</table>

*aNumber 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 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 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").
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.

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

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.

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.

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.

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

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/m³ or ppm and oral exposure is reported in mg/kg/day.

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).

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

<table>
<thead>
<tr>
<th>Key to figure&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Species</th>
<th>Exposure frequency/duration</th>
<th>System</th>
<th>NOAEL (ppm)</th>
<th>LOAEL (effect)</th>
<th>Less serious (ppm)</th>
<th>Serious (ppm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>INTERMEDIATE EXPOSURE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Systemic</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Rat</td>
<td>13 wk</td>
<td>Resp</td>
<td>3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10 (hyperplasia)</td>
<td></td>
<td></td>
<td>Nitschke et al. 1981</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 d/wk</td>
<td>6 hr/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>18</td>
<td>Cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>18 mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Wong et al. 1982</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 d/wk</td>
<td>7 hr/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>38</td>
<td>Rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NTP 1982</td>
</tr>
<tr>
<td></td>
<td></td>
<td>89–104 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 d/wk</td>
<td>6 hr/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>39</td>
<td>Mouse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NTP 1982</td>
</tr>
<tr>
<td></td>
<td></td>
<td>79–103 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The number corresponds to entries in Figure 3-1.

<sup>b</sup> Used to derive an intermediate inhalation Minimal Risk Level (MRL) of $5 \times 10^{-3}$ 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).
Figure 3-1. Levels of Significant Exposure to [Chemical X] - Inhalation

Acute (≤14 days)

- Systemic

- Death
- Respiratory
- Hematological

Intermediate (15-364 days)

- Systemic

- Death
- Hematological
- Hepatic
- Reproductive
- Cancer

11r
12r
14r
15h
16r
17h
30r
32r
34r
36g
33h
35h
37h
39m
38m
40m

* Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.
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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
BMDX dose that produces a X% change in response rate of an adverse effect
BMDLX 95% lower confidence limit on the BMDX
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
CELDLS 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

***DRAFT FOR PUBLIC COMMENT***
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
F1 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
Koc organic carbon partition coefficient
Kow octanol-water partition coefficient
L liter
LC liquid chromatography
LC₅₀ lethal concentration, 50% kill
LC₇₀ lethal concentration, low
LD₅₀ lethal dose, 50% kill
LD₇₀ lethal dose, low
LDH lactic dehydrogenase
LH luteinizing hormone
LOAEL lowest-observed-adverse-effect level
LSE Levels of Significant Exposure
LT₅₀ lethal time, 50% kill
m meter
MA trans,trans-muconic acid
MAL maximum allowable level
mCi millicurie

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

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\textsubscript{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

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>                      greater than
\geq                    greater than or equal to
=                      equal to
<                      less than
\leq                    less than or equal to
%                      percent
\alpha                  alpha
\beta                   beta
\gamma                  gamma
\delta                  delta
\mu m                   micrometer
\mu g                   microgram
q_i                     cancer slope factor
–                       negative
+                       positive
(+)                     weakly positive result
(−)                     weakly negative result
APPENDIX D. INDEX

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