January 14, 2013

Comments on the "Alternative Feasibility Study: Final Creek, Settling Ponds, and Spoils Disposal Areas, Badger Army Ammunition Plant"
ESC, LLC

Executive Summary

This Alternative Feasibility Study (AFS) intends to update the previously approved Feasibility Study from 1994 in which the Army proposed, and the state of Wisconsin approved, a remedy involving in-place stabilization of contaminated soils with a cover. The US EPA approved that remedy. Now, the Army proposes to revise the remedial goals for soil clean up and considers three alternative treatment strategies:

1. No action, as required by regulation and law
2. Stabilization and solidification, as per the 1994 remedy
3. Removal of contaminated soils and placement in a landfill on site

The Army recommends alternative 3. The proposed level of cleanup based on this alternative is not protective of all human and ecological receptors based on increases in the proposed remediation goals and elimination of other contaminants of concern. Consideration needs to be made for returning to the lower contaminant concentrations that were initially approved in 1994, as well as including important DNT isomers.

Comments

The Site Background and Site Investigation sections indicate that groundwater and soil sampling have not provided any evidence that the Settling Ponds and related areas are a source of chemical contamination to the underlying groundwater aquifers. The assumption is supported only in those areas where there is the greatest distance between the contaminated soil, i.e. the source, and the underlying groundwater. The distance between the contaminated soil and underlying groundwater ranges from 80 feet to 5 feet.

Section 6.0 briefly discusses the extent of the contamination at the Badger Army Ammunition Plant (BAAP) site and, based on soil and groundwater testing, concludes that groundwater contamination in the Settling Ponds area has come from the Propellant Burning Ground (PBG). These conclusions are found in the *Alternative Feasibility Study, Groundwater Remedial Strategy* (SpecPro/BTS, December 2011), but should be briefly summarized in this report.

Section 7.1 discusses the possible sources of contamination. Lacking is the data to support the AFS supposition that the IRM/MIRM and WWTP backwash water is not a source of contamination. This untreated discharge to Final Creek is ongoing and any pertinent analytical data on the backwash water should be summarized here. A source document and a discussion of these results would be appropriate in this context. Of note is the discharge to Final Creek that still occurs today which “evaporates and infiltrates the soil prior to reaching Settling Pond 1.” Higher contaminant concentrations can be found at the point of discharge in the area adjacent to Settling Pond 1. Also of note: Figure 9 indicates that there have been no soil borings taken along the footprint of the PBG groundwater plume east of Final Creek to rule out interaction between soils in this area and groundwater contamination.

Section 7.3.2 explains that institutional controls will be applied to limit human exposures. First, such efforts have no impact on wildlife or other ecological receptors. Second, such controls are imperfect and serve to reduce, but not eliminate exposures due to the inherent unpredictability of humans. The limitation of institutional controls is that they must be maintained in perpetuity, raising the question of long term viability of deed restrictions, warning signs, fences, etc.

Section 9.2 explains that the AFS considered 60 contaminants of concern for remediation, included only 5 of these in the final list (2,4, DNT, 2,6 DNT, nitroglycerin, chromium, and lead), but failed to include four DNT isomers because of a lack of regulatory standards for soil. The Army elected to not derive site-specific soil standards for the other four DNT isomers, despite the fact that the state of Wisconsin has regulatory values for the summed total concentration of all six DNT isomers for groundwater.

The AFS added total arsenic and total chromium to the list of soil contaminants.

Section 9.3 presents the Risk Analysis with the assumptions for future use and possible exposure scenarios. The AFS assumes that parcels where the wastewater and groundwater treatment facilities are located will remain as such and thus classify these parcels as "industrial use." This assumption is met so long as both facilities continue as used presently. If future conditions change, it is possible that the parcels would no longer be used in an "industrial use" category.

Parcel M1 is the one that is proposed for recreational use, or a non-industrial purpose. The AFS notes correctly that the exposure times for recreational visitors is limited in duration, but fails to account for the different types of exposures that occur in a recreational area vs. other limited time areas (i.e. not residential with 24 hour occupancy).

Section 9.3.2 Exposure Frequency makes specific assumptions for reducing exposure based on unknown and un-referenced conditions, such as limiting the worker exposure frequency to 33% of the total because the AFS does not envision a worker needing to work each day on the parcels. This reduction could underestimate the risk to workers and is inconsistent with the way in which a Reasonable Maximum Exposure is set.

Similarly, the AFS reduces the standard visitation frequency for a recreational visitor from 75 to 55 days because of weather. The duration of each visit is derived from the US EPA and some data from the local park service employees.

Section 9.3.4 Ingestion Factor

The soil ingestion factor is the soil intake from incidental consumption for adults and for children, where the soil intake is higher for children due to differences in behavior, as well as taking into account sensitivity based on height and body weight.

Several soil ingestion rates are appropriate here: workers, adult visitors and child visitors. The more common EPA soil ingestion rates are the 100-200 mg/day for workers, and 100 mg/day for adults and 200 mg/day for children. These values may be reduced for shortened exposure times, and the results yield higher values than the ones in the AFS (page 27). Higher values would be more consistent with the conservative approach taken in developing RME exposure estimates.

Section 9.4 Proposed Soil Remediation Goals

The AFS proposes to increase every soil remediation goal for the chemical contaminants retained in this AFS, and several chemicals are eliminated (disqualified) from consideration for remediation (see Table 1 Approved and Proposed Soil Remediation Goals).

The remedial goals are insufficient for DNT because the AFS does not address the combined mixture of all 6 isomers, which could be conducted using basic assumptions used by the state of Wisconsin in developing groundwater standards for DNT. The WDNR toxicologists with the Wisconsin Division of Health found that the lesser isomers of DNT (2,3-, 3,4-, 3,5-, and 2,5-DNT) are as toxic or more toxic than 2,4- and 2,6-DNT. Wisconsin adopted an enforceable groundwater standard for total DNT (the summed total concentration of all six isomers of DNT) of 0.05 µg/l. The Army needs to determine concentrations of the other four DNT isomers in soils at the Settling Ponds site and use an approach similar to that used by Wisconsin for groundwater (See previous comments by ESC in Appendix A). Remedial goals and standards can be calculated on a site-specific basis to include the total mass of all six isomers.

Many of the remediation goals are increased because the AFS applies industrial, not residential soil standards.

Lead standard of 500 mg/kg is inappropriate for any application, based on information used by both CDC and EPA. The Centers for Disease Control and Prevention (CDC) recently changed the reference point for blood lead levels considered safe, reducing the reference value from 10 µg/dL to 5 µg/dL. EPA is in the process of modifying agency reference values for soil cleanup, based on the lower CDC reference value that EPA has always used as the basis for soil remediation standards. The lower value will mean more soils need to be cleaned up to lower levels than they were previously. The AFS for BAAP needs to account for a lower lead remediation value in order to avoid returning to clean up more soils a second time.

Furthermore, using the industrial standard for soil lead is inappropriate for children and the risks need to be recalculated with higher exposures and the lower blood lead reference value.

The AFS has not demonstrated that the industrial DNT standards for 2,4 DNT and 2,6 DNT are appropriate and protective for recreational exposures for children and expectant mothers.

Table 1. Approved and Proposed Soil Remediation Goals

Contaminant of Concern (COC)	Approved Remediation Goal [1]	Proposed Remediation Goal [2]	Proposed Remediation Goal-Regulatory Reference
Aluminum (Al)	19	DQ/NE	NA
Arsenic (As)	DQ/NE	DQ/NE	NA
Chromium (Cr)	DQ/NE	35.5	BAAAP Background
Lead (Pb)	30	500	NR 720 Table 2 Industrial RCL
Tin (Sn)	10	DQ/NE	NA
Zinc (Zn)	81.3	DQ/NE	NA
2,4-DNT	2.5	24.7	NR 720.19 SSRCL
2,6-DNT	4.29	620	Industrial USEPA RSL
2,4/2,6-DNT	DQ/NE	11.4	NR 720.19 SSRCL
Nitroglycerin (NG)	3.6	62	Industrial USEPA RSL
Diphenylamine (DPA)	3.5	DQ/NE	NA
Diethylphthalate (DEP)	20	DQ/NE	NA
Carcinogenic Polyaromatic Hydrocarbons (CPAH)[3]	0.4	DQ/NE	NA

Results expressed in milligrams per kilogram (mg/kg)
[1]Approved Remediation Goal as listed in Feasibility Study/In-Field Conditions Report/Plan Modification
[2]Proposed Remediation Goal was derived from current Regulatory sources: see column 4
[3]CPAH- consists of benz(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, and chrysene
USEPA- United States EPA
RSL- Regional Screening Level
NA- Not Applicable
DQ/NE-Disqualified/None Established
BAAAP- Badger Army Ammunition Plant
RCL- Residual Contaminant Level
NR 720- Chapter NR 720, Wisconsin Administrative Code
DNT- Dinitrotoluene
SSRCL- Site-Specific Residual Contaminant Level

Alternative Feasibility Study Appendix A

This appendix lists the quantitative factors that are described in the text, lists these and provides the equations for using the quantitative factors in quantitative estimates of risk.

The factors in the appendix include the number of days of exposure for a recreational user/visitor at 55 days, a value which is too low for a frequent recreational visitor.

The soil intake rates listed in the Appendix are also low by comparison with the values used in the EPA Exposure Factors Handbook and the Children's Exposure Factors Handbook. The soil intake rates should be 100- 200 mg/day or more.

The exposure time (per day) of 5 hrs/day is not as high as would be anticipated for a reasonable maximum exposure scenario.

Appendix A

Badger Army Ammunition Plant
Breakdown products of 2,4- and 2,6-Dinitrotoluene
Prepared by
Environmental Stewardship Concepts
Henrico, Virginia
June 29, 2009

Executive Summary

According to current research, there are a number of breakdown products associated with dinitrotoluene (DNT) found at the Badger Army Ammunition Plant, several of which the Army tests for in the groundwater monitoring wells and private residential wells. However, the list does not, but should, include 1,3-dinitrobenzene and three forms of nitrotoluene. The potential effects from exposure to any chemical depends on dose, duration, route of exposure, timing of exposure, personal traits and habits, and whether other chemicals are present. The DNT breakdown product 1,3-dinitrotoluene can affect the ability of blood to carry oxygen. Of the three forms of nitrotoluene, the most potent is 2-nitrotoluene, or ortho-nitrotoluene. Ortho-nitrotoluene may affect the liver, blood, testes, and is carcinogenic in experiments on rats and mice. The Army's testing of the wells at Badger should include these breakdown products to protect the health of the community around the ammunition plant.

Research Summary

Based on EPA methods used to determine explosives, the detection of 2,4-dinitrotoluene and 2,6-dinitrotoluene will contribute to the detection and identification of total DNT present in the samples. The Health Consultation by the Agency for Toxic Substances and Disease Registry (ATSDR) mentions that an earlier testing technique also incorporated the specific detection of isomer 2,3-DNT and 3,4-DNT. In this report, the Department of Natural Resources urged the Army to use this earlier method when any DNT is found. EPA approved Method 529 tests municipal drinking water for a number of the chemicals that are identified as DNT breakdown products identified in the earlier ESC report and are shown in Table 1. Method 8330 tests for a number of the DNT breakdown products in ground water, shown in Table 2.

The Health Consultation states that "When BAAP-area groundwater and private well water samples are tested for semi-volatile compounds, the standard laboratory screen looks for 60 different compounds, including certain isomers of dinitrotoluene and several potential DNT degradation products, which are: nitrobenzene; 2-nitroaniline; 3-nitroaniline; and 4-nitroaniline." The identification of these compounds as breakdown products is confirmed by current research. The Health Consultation also states "For the compounds listed in Table [3], all are specifically targeted for in-laboratory analysis of water samples from BAAP, except for 1,3-dinitrobenzene and the three isomers of nitrotoluene." Because 1,3-dinitrobenzene and chemical compounds containing ortho, meta and para nitrotoluene structures are breakdown products of DNT identified in lab research, they should be included in the testing of groundwater at Badger Army Ammunition Plant.

The EPA approved method for detection of these DNT breakdown products, Method 529 Determination of Explosives and Related Compounds in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography/Mass Spectrometry, involves using known compounds that have similar chemical structures to those of the DNT breakdown products. Each part of a chemical's structure has a definitive signature that is identifiable between all the compounds that contain that same structural part. For instance, a benzene ring acts as a "base" that other "groups", like forms of nitrogen, can attach to and become a new compound. The groups may attach at different points on the base, and this is what differentiates 2-nitrotoluene (ortho) from 3-nitrotoluene (meta) from 4-nitrotoluene (para). If there is a chemical with an additional group attached to 2-nitrotoluene, such as 4-amino-2-nitrotoluene, these will also be identified in the method used to identify compounds with 2-nitrotoluene.
Likewise, if there is a chemical with an additional group attached to 4-nitrotoluene, such as 2-amino-4-nitrotoluene, these will be identified in the sample using the method that is looking for 4-nitrotoluene.

The potential effects from exposure to any chemical are based on dose, duration, how someone is exposed, timing of exposure, personal traits and habits, and whether other chemicals are present. Exposure to 1,3-dinitrobenzene can occur from contaminated water, food, air and soil. The Agency for Toxic Substances and Disease Registry states that high levels of 1,3-dinitrobenzene affect the ability of blood to carry oxygen and cause skin to turn bluish in color. If exposed for a long period of time, there can be a reduction in the number of red blood cells (anemia). Other symptoms may include headache, nausea, and dizziness. This compound is slightly soluble in water and can move into groundwater, but is not likely to build up in fish or people. Affects on humans to small exposures over long periods of time to nitrotoluenes are not as well known. Long term or repeated exposure to 2-nitrotoluene may affect the liver, blood and testes, and is carcinogenic in rats.

Table 1. Chemicals detected in the EPA Method 529: Organic Compounds in Municipal Drinking Water

ANALYTE
2-amino-4,6-dinitrotoluene
4-amino-2,6-dinitrotoluene
3,5-dinitroanaline
1,3-dinitrobenzene
2,4-dinitrotoluene
2,6-dinitrotoluene
hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)
nitrobenzene
2-nitrotoluene
3-nitrotoluene
4-nitrotoluene
1,3,5-trinitrobenzene
2,4,6-trinitrophenylmethylnitramine (Tetryl)
2,4,6-trinitrotoluene (TNT)

Table 2. Chemicals detected in EPA Method 8330: Organic Pollutants in Ground Water

Compound
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
Hexahydro-1,3,5-trinitro-1,3,5-triazine
1,3,5-Trinitrobenzene
1,3-Dinitrobenzene
Methyl-2,4,6-trinitrophenylnitramine
Nitrobenzene
2,4,6-Trinitrotoluene
4-Amino-2,6-dinitrotoluene
2-Amino-4, 6-dinitrotoluene
2,4-Dinitrotoluene
2,6-Dinitrotoluene
2-Nitrotoluene
3-Nitrotoluene
4-Nitrotoluene

Table 3. This table is from the Health Consultation:

Table 2: Health-Based Comparison Values for Dinitrotoluene Isomers and Potential Degradation Compounds

All Concentrations in Micrograms per Liter (μg/L)

Compound	Wisconsin Groundwater Enforcement Standard (NR140)	Other Drinking Water Quality Guidelines
2,4-dinitrotoluene	0.05	70.0[a]
2,6-dinitrotoluene	0.05	37.0[c]
3,4-dinitrotoluene	n/a	n/a
2,3-dinitrotoluene	n/a	n/a
2,5-dinitrotoluene	n/a	n/a
3,5-dinitrotoluene	n/a	n/a
2-nitroaniline	n/a	110.0[d]
3-nitroaniline	n/a	330.0[d]
4-nitroaniline	n/a	330.0[d]
1,3-dinitrobenzene	n/a	1.0[b]
p-nitrotoluene	n/a	0.62[d]
m-nitrotoluene	n/a	120.0[d]
o-nitrotoluene	n/a	0.046[d]
Nitrobenzene	n/a	3.5e

Notes: n/a - no available health-based guidance value

Source: a - ATSDR Chronic Environmental Media Evaluation Guideline
b - EPA Drinking Water Lifetime Health Advisory
c - Withdrawn EPA Reference Dose Concentration or Cancer Risk Evaluation Guideline
d - EPA Region III Provisional Health Value
e - EPA Reference Dose Value for Drinking Water

Review of
"Dinitrotoluene in Deer Tissues, Final Report"
A report by L. R. Shugart from the Oak Ridge National Laboratory
To Badger Army Ammunition Plant

Review prepared by Environmental Stewardship Concepts
Henrico, Virginia
March 18, 2009

The Study of Dinitrotoluene in Deer Tissue at Badger Army Ammunition Plant was conducted to determine whether the deer on the Badger Army Ammunition Plant (BAAP) site contained 2,4- or 2,6-DNT in their tissues at concentrations that would be unsuitable for human consumption. As a former army ammunition plant, the Badger site was exposed to 2,4 and 2,6 Dinitrotoluene, precursors in the production of TNT and explosives; other forms of DNT are also present on the site but were not considered in this study. The study focused on examining the presence of DNT in the liver, muscle and heart tissues of the deer. The entire investigation was conducted using tissue samples from deer that had been shot by hunters during the fall hunting season of 1990. The study assumed the BAAP deer population is resident and held on site within a security-fenced area surrounding the facility. However, the report had no information to confirm that the deer are resident and did not cite the height of the fence or mark the deer to confirm site residence.

Neither of the two forms of DNT were found in the tissue samples that were collected from deer shot on BAAP. The study failed to look at the presence of DNT in brain tissue, kidney, blood, or reproductive organs, all of which can be affected by DNT (see DNT study summaries below). The study lacks laboratory experimentation or references to such a study that would indicate the choice of tissues analyzed. There is no exposure control or knowledge of site utilization by the deer. The report does not comment on the location of the deer when killed. Experimental data with varying doses in live deer would indicate the spread and intensity of DNT in the body. The study analyzed the results (no measurable DNT) with statistical analyses which are sound and the statistical results are reliable. However, the problem is that the design provides results of limited use and applicability. This investigation was not able to confirm or deny whether it is safe to eat the deer from the BAAP site.

Dinitrotoluene in Deer Tissues study summary

Dinitrotoluene, $C_6H_3(CH_3)(NO_2)_2$, is an explosive with six possible isomers. It is the precursor to trinitrotoluene (TNT) in the three step nitration of toluene that produces TNT. This study was conducted to determine whether the deer on the Badger Army Ammunition Plant (BAAP) site contained 2,4- or 2,6-DNT in their tissues at concentrations that would be unsuitable for human consumption. The 12 randomly sampled deer had two 2 gram samples each of liver, muscle and heart tissue and were tested using high performance liquid chromatography (HPLC). The sensitivity of the HPLC was set to 0.04 at 254nm. The detection limit for the isomers of DNT was set to 0.01 ppm, or 100ng of either compound in 1gram of deer tissue. Recovery data from five non-munition-contaminated deer tissue spiked with the isomers of DNT yielded successful percentage recoveries of the contaminants after measurement with the HPLC method.

There were 103 separate analyses conducted and none were observed with an individual compound concentration greater than the detection limit. No DNT was detected in samples from any of the tissues. The analysis consisted of statistical interpretation based on the sample size and population size. The upper 95% confidence limit on the proportion of the population that exceeds the detection limit was 0.22, or 93 out of the estimated 430 whitetail deer populating the Badger Army Ammunitions Plant site. No more than 0.04% of the 430 deer exceeded the criterion limit of 1.0 ppm, or less than one deer out of 430. The criterion limit, or the toxicologically significant level that would preclude human consumption, has not been determined for DNT in deer tissue. However, based on previous studies of TNT, the criterion limit of 1.0 ppm of DNT in animal tissue "appears reasonable."

The investigation cannot comment on collection methods or tissues that were not sampled. The collection methods relied on normal hunting practices that were not described. Hunting can result in deer that do not expire immediately, but are able to run for some time/distance. Deer that run after being shot will exhibit completely altered physiological functions that can alter blood and body chemistry and may change the tissue levels of contaminants.

Laboratory research summarized below indicates that multiple systems are affected by DNT and therefore these target tissues should have been sampled. Research on dogs and rats indicate that the male reproductive system is a target for DNT and therefore should have been sampled. The investigation on dogs provides sufficient evidence for neurological and blood effects that sampling should have addressed these tissues as well, hard though it may have been to collect blood from freshly killed deer under the unknown circumstances of the hunting.

DNT study summaries

1. Subchronic and chronic toxicity studies of 2.4-dinitrotoluene. Part I. Beagle dogs. Ellis. H. V., C. B. Hong, C. C. Ue, J. C. Dacre, and J. P. Glennon. 1985. *J. Am. Coll. Toxicol.* 4:233-241.

"Subchronic and chronic toxicities of 2,4-dinitrotoluene (2,4-DNT) were studied in beagle dogs. The major adverse effect of 2,4-DNT in dogs was a neuropathy, characterized by incoordination and paralysis. There were vacuolation, endothelial proliferation, and gliosis of the cerebellums of some affected dogs. These effects were seen in 1 dog given 1.5 mg/kg per day for 2 years, in all dogs given 10 mg/kg per day within 6 months, and in all dogs given 25 mg/kg per day within 2 months. There was great variation between individuals in onset and severity of adverse effects. Some dogs progressed to a complete paralysis, leading to death. Methemoglobin and its sequelae were common, but not life threatening. Heinz bodies were a useful indicator of this effect. Less important adverse effects seen included testicular degeneration and biliary tract hyperplasia. No changes were found in tumor incidence, immunoglobin E and cytogenetic assays, and other routine hematologic and clinical laboratory tests." – Abstract, article not accessible through VCU

2. Reproductive toxicity of 2,4-dinitrotoluene in the rat**.** Eric Bloch, Bernard Gondos, Michael Gatz, Santosh K. Varma and Benjamin Thysen**.** *Toxicology and Applied Pharmacology*. Volume 94, Issue 3, July 1988, Pages 466-472

After three weeks of 0.2% DNT exposure, male rats demonstrated a definitive change in Sertoli cell morphology. Sertoli cells function as 'aides' to developing sperm cells and secrete substances that are integral to male rats' development. Animals treated with DNT exhibited swollen endoplasmic reticula and mitochondria, as evidenced by distinct sizes of vesicles in cell samples. "Circulating levels of follicle stimulating hormone and luteinizing hormone were increased in DNT-treated animals. Reduced weights of the epididymides and decreased epididymal sperm reserves were observed in DNT-treated animals." The results of the study imply that DNT exposure in mammals causes "testicular injury, of directly or indirectly disturbing pituitary function, and of exerting a toxic effect at the late stages of spermatogenesis." The altered morphology of the Sertoli cell indicates that DNT concentrates on that specific location, which causes "inhibition of spermatogenesis and changes in testicular-pituitary endocrine activity."

3. Acute toxicity of 2,4,6-trinitrotoluene, 2,4-dinitrotoluene, and 2,6-dinitrotoluene in the adult bullfrog (Lithobates catesbeiana). Norka E. Paden, Ernest E. Smith, Ronald J. Kendall. *Bulletin of Environmental Contamination and Toxicology.*

The study, published in June 2008, was conducted to determine the effects of 2,4-DNT, 2,6-DNT, and TNT on adult male bullfrogs.

Twenty-four frogs were administered suspensions of each toxin via oral galvage and were acclimatized through analogous feeding patterns and habitat conditions. Following the recently approved EPA Up-and-down method, the animals were dosed one at a time. The TNT dose began at 400mg/kg BW, with the subsequent doses increasing by a factor of 2 on the basis of estimated LD50 of 800mg/kg BW established in other studies. The doses for 2,4-DNT and 2,6-DNT both began at 175 mg/kg BW, increasing incrementally by 3.2 as per the UPD method. If the first dose did not prove lethal to the first animal, the following animal would receive an increased dose. In the first hours under direct observation, changes in respiratory rhythm, decrease in motor activities (somnolence, loss of righting reflex, prostration, tremors, tonic and clonic convulsion), salivation, muscle tone changes, GI changes, skin color changes, and ocular signs (relaxation of the nictitating membrane). Testing continued over 14 days, on the basis of UPD guidelines, until: "Three consecutive animals survive at the upper bound; five reversals occur in any six consecutive animals tested; or at least four animals have followed the first reversal and specified likelihood-ratios exceed the critical values" (488).

Results: Animals exposed to 2,6-DNT died approximately 8 hours quicker than frogs exposed to the same dose (2000mg/kg BW) of 2,4-DNT. "Necropsy of animals exposed to TNT and DNT isomers revealed gross morphological changes including liver and kidney necrosis, and heart failure in the case of 2,6-DNT exposed animals" (490). All animals that received 2000mg/kg DNT isomers were found to have enlarged livers with high rates of tissue decay. Three animals also displayed enlarged spleens, as much as twice the typical size. Coagulated blood was also discovered in the body cavities of animals exposed to 2,6-DNT. "All compounds tested caused alterations of the Central Nervous System. Changes in the respiratory and circulatory systems were also detected" (491)

4. Influence of Oral 2,4-Dinitrotoluene Exposure to the Northern Bobwhite (Colinus virginianus). Mark S. Johnson, Michie, Mark W., Bazar, Matthew A., Gogal Jr, Robert M. *International Journal of Toxicology*; Jul/Aug2005, Vol. 24 Issue 4, p265-274, 10p

With the understanding that military training and munitions manufacture have led to soil contamination by DNT, particularly isomers 2,4 and 2-6. This study explored the effects of DNT exposure on bobwhites, using a controlled dosage regime. The birds were dosed with 2,4-DNT and the LD50 was determined to be 55mg/kg. Both sexes demonstrated toxic symptoms following the first exposure, including: weight loss, diarrhea, and lethargy. At higher doses, the experiment revealed changes in egg production and the masses of the ovaries, kidneys, and brain. Feed consumption did not change. "Changes in kidney mass and histological observations suggest accumulation of nitrogenous waste may be the cause of morbidity."

Using three groups of birds, the researchers tested for acute toxicity, subacute toxicity and subchronic toxicity. In the acute group, the lowest tolerable dosage was 17.5mg/kg. Three of the four dosed at 55mg/kg died, and both birds who received 175mg.kg died. These birds exhibited watery stools, lethargy, and 'a single case of excessive drinking.' The birds in the subacute toxicity received lower doses of 25 and 35 mg/kg; all of them died within 72 hours, following excessive weight loss. The birds' kidney/bw ratios were higher than the control group; liver/bw ratios were also higher. "Trends in electrolyte and triglycerides levels corroborated with the mass/bw changes are suggestive of adverse kidney and liver effects." In the subchronic toxicity group, females dosed at 25mg/kg laid fewer eggs per day than all other groups. For both the 15 and 25 mg/kg-day groups, brain/bw ratios increased in both male and female specimens. Male liver/bw increased in the 15 and 25mg/kg-day groups, but female remained the same. Female kidney/bw ratios, however, were increased in the 5mg/kg-day group, and kidney/bw ratios increased in both sexes in the 15 and 25 mg/kg-day groups. Other marked changes between genders include increased red blood cell counts in females, and lower hemoglobin concentrations in females. Females had also developed gout tophi on their kidneys.

The study also cites similar experiments conducted on mammals, specifically rats and mice. According to a study several studies in the '70s and '80s, oral dosages of 2,4-DNT resulted in production of methemoglobin, anemia, peripheral neuropathy, jaundice (hepatotoxicity), tremors, and sensitization.