Review of
"Dinitrotoluene in Deer Tissues, Final Report"

By Environmental Stewardship Concepts
Henrico, Virginia

Prepared for CSWAB
March 18, 2009

Executive Summary

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. 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 Overview

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.

References (Other 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/Aug 2005, 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.

About the Author:

Dr. Peter L. deFur is president of Environmental Stewardship Concepts, an independent private consultant, serving as a technical advisor to citizen organizations and government agencies. He is an Affiliate Associate Professor in the Center for Environmental Studies at Virginia Commonwealth University where he conducts research on environmental health and ecological risk assessment. Dr. deFur is President of the Association for Science in the Public Interest and on the board of the Virginia Conservation Network.

For More Information:

Citizens for Safe Water Around Badger was organized in 1990 when rural families near Wisconsin's Badger Army Ammunition Plant learned private drinking water wells were polluted with high levels of cancer-causing chemicals. CSWAB is working to build a safe and healthy future for their community and these lands by protecting and restoring the integrity of soil, water, air, and biological diversity.

Citizens for Safe Water Around Badger (CSWAB)
E12629 Weigand's Bay South, Merrimac, WI 53561
Telephone: (608) 643-3124
Email: info@cswab.org
Website: www.cswab.org