DEPARTMENT OF THE ARMY
US ARMY CENTER FOR HEALTH PROMOTION AND PREVENTIVE MEDICINE
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MCHB-TS-THE

14 August 2007

MEMORANDUM FOR Badger Army Ammunition Plant, Joan M. Kenney, 2 Badger Road, Baraboo, WI 53913-5000

SUBJECT: Request for Technical Support to Evaluate Interim Health Advisory Level for total dinitrotoluene (DNT) residues in Wisconsin Groundwater

1. References:

a. Letter, State of Wisconsin Department of Natural Resources, Division of Water, Mr. Todd L. Ambs, 4 May 2007, subject: Request for health advisory levels for dinitrotoluene (DNT) isomers: 2,3-DNT; 2,5-DNT; 3,4-DNT & 3,5-DNT.

b. Drinking Water Health Advisory for Dinitrotoluenes, State of Wisconsin Department of Health and Family Services, Bureau of Environmental and Occupational Health, Division of Public Health, Lynda Knobeloch, Ph.D., 14 June 2007.

c. Letter, State of Wisconsin Department of Health and Family Services, Bureau of Environmental and Occupational Health, Division of Public Health, Dr. Henry A. Anderson, 3 July 2007.

d. E-mail, Badger Army Ammunition Plant, Ms. Joan M. Kenney, 18 July 2007, subject: New Wisconsin DNT health advisory.

e. E-mail, U.S. Army Environmental Command, Ms. Lia M. Gaizick, 20 July 2007, subject: New Wisconsin DNT health advisory.

2. Background. At the request of the State of Wisconsin Department of Natural Resources (WDNR) (reference a), the State of Wisconsin Department of Health and Family Services (WDHFS), Division of Public Health (WDPH) developed an interim drinking water health advisory for dinitrotoluenes (DNT) (references b and c). The advisory recommends the summed concentration of all DNT isomers not exceed 0.05 µg/L. The state will not direct the Army's response to the guidance provided by WDPH unless the regulation for state drinking water standard (NR140) is changed. At this time US Army Center for Health Promotion and Preventive Medicine (USACHPPM) has not performed any studies related to drinking water health advisory levels for DNT, but is conducting this literature summary and review of the Wisconsin Drinking Water Health Advisory for Dinitrotoluenes at the request of Ms. Joan M. Kenney, Badger Army Ammunition Plant, (references d and e).

3. Recommendations.

a. Additional toxicology studies are needed to assess the health risks of the minor isomers of DNT. Most studies have been conducted using either technical grade DNT (TG-DNT) or other mixtures of 2,4- and 2,6-DNT, making independent risk assessments for the minor isomers impossible. Chronic tests on 2,6-DNT are also lacking and, as this isomer is reportedly responsible for the carcinogenic effects of TG-DNT, such studies are important for establishing toxicity and drinking water guidelines.

b. The most reliable, currently available assessments of the carcinogenic potential of individual DNT isomers indicates that 2,4-DNT and all of the minor isomers are not responsible for the carcinogenicity reported for TG-DNT. The mutagenic activity reported based on in vitro studies is not a reliable indicator of carcinogenic potential of the minor isomers of DNT. Although all of the isomers of DNT have shown mutagenic activity in at least one short-term assay, many of the assays indicate that none of the isomers are mutagenic. The DNT isomers have not exhibited mutagenic activity in mammalian cell cultures, which has been attributed to the need for entero-hepatic metabolism in the genotoxicity of DNT. In vitro mutagenicity assays have failed to predict the carcinogenic effects of 2,4- and 2,6-DNT and should not be expected to accurately predict the carcinogenic effects of the minor isomers. In vivo hepatic initiation-promotion assays which report 2,4-DNT and the minor isomers to be non-hepatocarcinogenic are likely a more reliable indication of the carcinogenic potential of the minor isomers.

c. A single, carcinogenicity-based health advisory for the summed concentration of all DNT isomers in the absence of carcinogenicity data on each of the isomers may not provide an appropriate level of protection. This interim Health Advisory Level recommended by WDPH is based on the U.S. EPA's evaluation of the carcinogenic potential of DNT as indicated in a study which used a mixture of 2,4- and 2,6-DNT (98% and 2%, respectively). Because the effects of the two isomers could not be separated, the guideline is reported to apply to each of the isomers as well as the mixture of the two. However, in vivo hepatocarcinogenesis studies conducted with each of the six isomers and TG-DNT indicate that the carcinogenic effects of DNT can be attributed to the 2,6-DNT isomer. Therefore, the cancer risk estimates established based on a mixture may not be appropriate for either of the two isomers or TG-DNT.

d. Application of a guideline derived from data for a mixture of 2,4- and 2,6-DNT to the summed concentration of all DNT isomers is not appropriate. It is not known how the presence of the minor isomers alters the toxicity/carcinogenicity of DNT. However, since the minor isomers have not been shown to have initiating or promoting potential, they likely do not contribute to the carcinogenicity of TG-DNT. Additionally, there is some indication that competition for the metabolic pathway that activates the procarcinogen 2,6-DNT reduces the carcinogenicity of the isomer when it occurs in mixtures. Since the minor isomers do not appear

to contribute to the carcinogenicity of DNT mixtures in an additive manner, a guideline for the summed concentration of all isomers is not warranted.

4. Conclusion. The current toxicology database for the minor isomers of DNT is not sufficient to support development of a health advisory for these isomers.

5. Questions pertaining to memorandum can be directed to Emily May LaFiandra, Ph.D. She may be reached at DSN 584-7749, commercial 410-436-7749, or via e-mail at emily.lafiandra@us.army.mil.

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Encl
Summary paper: Response to WDPH

CF:
Mr. Peter Rissell, USAEC

Summary Paper: Reseponse to WDPH Drinking Water Health Advisory for Dinitrotoluenes

The Wisconsin drinking water health advisory for dinitrotoluenes states: *"The six isomers of DNT are structurally and toxicologically similar. In addition, these isomers have a common commercial source and are frequently found together in the environment."*

Response: The isomers of DNT that carry a nitro group on an odd numbered ring position normally make up only a minor portion of DNT mixtures. The reason for this is that nitration of the toluene ring occurs in a predictable step-wise manner. Toluene is a flat, ring-shaped compound with 6 carbons in the ring and one carbon attached to the ring The ring position of the attached carbon is the "1" position. The first nitration during the synthesis of DNT or TNT normally occurs at the 2, 4 or 6 position (next to or directly opposite the #1 position on the ring) and the second nitration also occurs at one of these positions. Thus, in unpurified mixtures of DNTs you have concentrations of 2,4-DNT and 2,6-DNT predominating. It is possible to maximize either the concentration of the 2,4 or 2,6 form by changing reaction conditions, but quantities of DNT that is nitrated either at the 3 or 5 position make up only a minor percentage because the ring carbons at the 2, 4 and 6 positions are much more reactive toward nitration. It may also be possible to increase the portion of isomers that contain isomers on the 3 or the 5 position by varying reaction conditions, but no evidence has been found that synthesis of these compounds was ever a manufacturing goal. Therefore, it is anticipated that isomers carrying the nitro groups on ring positions 3 and 5 will make up only a small portion of DNT environmental pollution.

The Wisconsin drinking water health advisory for dinitrotoluenes states: *"A complete toxicological database is available for technical grade DNT, which is a mixture of all isomers, and for the two major isomers (2,4- and 2,6-DNT). Only limited testing has been conducted with the other 4 isomers making independent risk assessments for them impossible. In 2000, the Chemical Manufacturer's Association petitioned the US EPA to remove individual isomers of DNT from the High Production Challenge Program arguing that none of the minor isomers is produced separately in commerce. In a letter to Charles M. Auer, Director of the USEPA's Chemical Control Division, CMA stated, "Separately evaluating each isomer under the HPV program will not result in a better understanding of the adverse health or safety implications of dinitrotoluene." EPA's approval of this request alleviated a requirement for the manufacturers to provide screening level toxicity and environmental fate data for individual DNT isomers and allowed submission of data for technical grade DNT instead."*

Response: Most studies have been conducted using either the technical grade (TG) mixture or purified 2,4- or 2,6-DNT, making independent risk assessments for the minor isomers impossible. Additionally, chronic tests on 2,6-DNT are lacking and, as this isomer is reportedly responsible for the carcinogenic effects of TG-DNT, such studies are important for establishing toxicity and drinking water guidelines. It is also important to note that some of the studies reporting effects of 2,4-DNT were conducted with test compounds that were contaminated with 2,6-DNT (e.g. Ellis 1979 ~2%). As such, some of the effects attributed to 2,4-DNT may be due to 2,6-DNT or the interaction of the two isomers. Assessment of the toxicity of TG-DNT may be appropriate for determining the health risks associated with current DNT manufacturing

processes; however, these assessments may not be appropriate for military grade DNT which requires highly purified 2,4-DNT flakes or DNT mixtures resulting from the production of TNT. Assessment of the toxicity of the individual isomers may provide a better understanding of the health risks of varied DNT mixtures.

The Wisconsin drinking water health advisory for dinitrotoluenes states: "*Published studies for the minor isomers indicate that their toxic effects are the same as that of TG-DNT and that the minor isomers are as toxic or more toxic than 2,4- and 2,6-DNT.*"

Response: While all of the isomers appear to produce the same types of non-cancerous toxicological effects (hematological effects, liver toxicity, neurological effects, and testicular atrophy), the extent or degree of the effects varies. The effective doses are generally within an order of magnitude, however, 3,5-DNT is approximately an order of magnitude more toxic in rats (Ellis 1978, Lee 1975). 2,3-, 2,4-, and 3,4-DNT are reported to be an order of magnitude more toxic to *Daphnia* and all of the minor isomers are similarly more toxic to fish (*Oryzias latipes* and *Pimephales promelas*) (Burrows et al. 1989).

The Wisconsin drinking water health advisory for dinitrotoluenes states: "*TG-DNT, as well as the purified 2,4- and 2,6-DNT isomers are classified as known animal carcinogens. Minor isomers have not been tested for this effect, but are structurally and toxicologically similar suggesting that they may also have carcinogenic effects.*"

Response: Three two-year bioassays have tested the carcinogenicity of DNT (NCI 1978, CIIT 1982, and Ellis 1979). Two of the studies (Ellis 1979 and CIIT 1982) determined DNT to be hepatocarcinogenic to Sprague-Dawley rats and male F344 rats, respectively. In the third study (NCI 1978), DNT was shown to be nonhepatocarcinogenic in F344 rats. In addition to the strain differences which may explain some of the variance, the differences in carcinogenic effects reported in these studies may be attributed to differences in the composition of the DNT mixture used. The Ellis et al. (1979) and NCI (1978) studies both reportedly tested the carcinogenicity of 2,4-DNT, however, in both cases the compound tested was contaminated to some degree. The 2,4-DNT mixture used by Ellis et al. (1979) contained approximately 2% 2,6-DNT, while the contaminants (~5%) in the 2,4-DNT mixture used by NCI (1978) were not specified (but have been reported by others to be 2,6-DNT). The CIIT (1982) bioassay used a representative TG-DNT containing 76.5% 2,4-, 18.8% 2,6-, 2.43% 3,4-, 1.54% 2,3-, 0.69% 2,5-, and 0.04% 3,5-DNT. Ellis et al. (1979) dosed male rats at 34 mg/kg/day resulting in 33.32 mg/kg/day of 2,4-DNT and 0.68 mg/kg/day 2,6-DNT. The high dose group (0.02% in food) in the NCI study received approximately 14 mg/kg/day DNT or 13.3 mg/kg/day 2,4-DNT and 0.7 mg/kg/day of contaminants, while the low dose group (0.008%) received 5.6 mg/kg/day (5.32 and 0.28 mg/kg/day 2,4-DNT and contaminants, respectively) (per Rickert et al. 1984). Dosing in the CIIT (1982) study at 35 and 14 mg/kg/day resulted in doses of 2,4-DNT of 26.74 and 10.70 mg/kg/day, respectively. Doses of 2,6-DNT in this study were 6.58 mg/kg/day for the high dose, and 2.63 mg/kg/day for the low dose group.

As the high dose of the NCI study and the mid-dose of the CIIT study are approximately equivalent (14 mg/kg/day) and both studies use F344 rats, comparison of the carcinogenic effects reported in these studies is appropriate. The CIIT study reported high incidences of hepatocarcinogenicity (males=95% and females=60%) after two years of treatment with TG-

DNT, whereas the NCI study reported no hepatocarcinogenicity with 95% 2,4-DNT. This may be due to the differences in DNT composition. In the two studies reporting hepatocarcinomas (Ellis et al. 1979 and CIIT 1982), increased incidences were reported at the high dose in the Ellis (1979) study (34 and 45 mg/kg/day for males and females, respectively) and at the mid- and high dose groups (14 and 35 mg/kg/day) in the CIIT (1982) study. Owing to the composition of DNT used, the high dose in the Ellis (1979) study resulted in doses of 2,6-DNT (~0.7 mg/kg/day) that were similar to those in the low dose of the CIIT (1982) study, which was not associated with increased hepatocarcinogenesis. While this may be due to strain differences, the effects of the other minor isomers present in the TG-DNT used in the CIIT (1982) study have not been investigated and should not be disregarded.

Although hepatic cancer is the most prevalent carcinogenic effect of DNT, the U.S. EPA evaluation of the carcinogenic potential and classification for DNT is based on the incidence of mammary gland tumors (benign and malignant) in female rats in Ellis et al. (1979). The incidence of mammary gland carcinomas was rare in this study and was not treatment related. The combined incidence of benign tumors (fibroadenoma, fibroma, and adenoma-papilloma) and carcinomas was significantly increased in the high treatment group (45.3 mg/kg/day). For the evaluation of carcinogenic potential, the U.S. EPA converted the animal doses to human equivalents and determined that for a benchmark risk level of 0.10, the benchmark dose (BMD) was 0.25 mg/kg/day and benchmark dose level (BMDL) was 0.15 mg/kg/day (U.S. EPA 2006). The concentration of DNT in drinking water at the 10^{-6} risk level was calculated based on a 70 kg adult consuming 2 L of water per day using the human oral slope factor $(0.067 \text{ mg/kg/day})^{-1}$ derived in the BMD model. As the study on which this assessment was based used a mixture of 98% 2,4- and 2% 2,6-DNT, the resulting drinking water level, 0.05 µg/L, is reported to apply to both 2,4- and 2,6-DNT as well as mixtures of the two isomers. The effects of the two isomers on mammary gland tumor incidence can not be distinguished in the Ellis et al. (1979) study, and no subsequent studies have investigated the effects of purified isomers on mammary tumor incidence. However, in vivo hepatocarcinogenesis studies conducted with each of the six isomers and TG-DNT indicate that the carcinogenic effects of DNT can be attributed to the 2,6-DNT isomer (Popp and Leonard 1982, Leonard et al. 1983, Leonard et al. 1986, and Leonard et al. 1987).

Evaluation of the hepatocarcinogenic potential of DNT using an in vivo hepatic initiation-promotion system in male F344 rats indicated that TG-DNT and purified 2,6-DNT has weak hepatocyte initiating activity, whereas the remaining five isomers have no detectable initiating activity (Popp and Leonard 1982). TG-DNT, and purified 2,4- and 2,6-DNT also have demonstrable promoting activity when administered following a diethylnitrosamine initiating regimen (Popp and Leonard 1982). 2,6-DNT is the most active promoter and is approximately ten times more potent than 2,4-DNT (Leonard et al. 1986). It was concluded from these studies that the hepatic neoplasms previously reported following TG-DNT administration likely resulted from the initiating activity of 2,6-DNT followed by the promoting activity of both 2,4- and 2,6-DNT (Popp and Leonard 1982). It has been suggested that these results provide an explanation for the observed differences in hepatocarcinogenesis in the two year bioassays (i.e. hepatocarcinogenesis of TG-DNT in CIIT (1982) study and lack of hepatocarcinogenesis of 2,4-DNT in NCI (1978) study).

The 2,6-DNT isomer was further confirmed to be a complete hepatocarcinogen in a one year feeding study using male F344 rats (Leonard et al. 1987). Hepatocellular carcinomas were found in 100% and 85% of rats fed 14 and 7 mg/kg/day, respectively. 2,4-DNT given at 27

mg/kg/day did not cause hepatic tumors, whereas, treatment with 35 mg/kg/day TG-DNT (i.e. 26.6 mg/kg/day 2,4- and 6.3 mg/kg/day 2,6-DNT) resulted in hepatocellular tumors in 47% of animals. The authors suggested that the co-administration of 2,4- and 2,6-DNT reduces the carcinogenicity of 2,6-DNT by competing for the metabolic pathway required for 2,6-DNT activation (Leonard et al. 1987). Therefore, the cancer risk estimates established based on a mixture (98% 2,4-DNT and 2% 2,6-DNT; Ellis, 1979) may not be appropriate for either of the two isomers or TG-DNT (76% 2,4-DNT, 19% 2,6-DNT, and 5% minor isomers). Although the minor isomers do not have initiating or promoting potential and likely do not contribute to the carcinogenicity of TG-DNT, it is not known whether these isomers could reduce the carcinogenicity of 2,6-DNT by competing, as 2,4-DNT does, for the 2,6-DNT metabolic activation pathway. As such, a single, carcinogenicity-based health advisory for the summed concentration of all DNT isomers in the absence of carcinogenicity data on each of the isomers may not provide an appropriate level of protection.

The Wisconsin drinking water health advisory for dinitrotoluenes states: "*All isomers of DNT have shown mutagenic activity in short-term studies.*"

Response: Although all of the isomers of DNT have shown mutagenic activity in at least one short-term assay, the results of mutagenicity assays are mixed, with many of the assays indicating that none of the isomers are mutagenic. TG-DNT and the six isomers of DNT were determined to be weakly mutagenic in three assays using *Salmonella typhimurium* (TA 98, TA1538, and TM677), with the 3,5-DNT isomer being the most mutagenic (Couch et al. 1981). The 2,4-DNT isomer also demonstrated weak mutagenicity in the TA98 and TA100 strains (Mori et al. 1982 and Couch et al. 1987). In contrast, similar tests showed that 2,4- and 2,6-DNT were not mutagenic in TA98 and only weakly active in TA100 (Spanggord et al. 1982). Whereas Chiu et al. (1978) reported that DNT was not mutagenic in either TA98 or TA100. 2,4- and 2,6-DNT showed no genotoxic potential in three bacterial test systems (umu test in TA1535, NM2009 test, and SOS Chromotest in *E. coli*) at concentrations up to 27.4 and 36.3 mg/L, respectively (Neuwoehner et al. 2007).

DNT isomers have failed to exhibit mutagenic activity in mammalian cell cultures. Four isomers (2,4-, 2,6-, 2,3- and 3,4-DNT) and TG-DNT did not induce an increase in morphological transformation of Syrian hamster embryo (SHE) cells (Holen et al. 1990). In similar tests 2,4- and 2,6-DNT produced negative results in the Chinese hamster ovary (CHO) mutagenesis assay (Lee et al. 1976 and 1978). In pH 6.7 SHE cell transformation assays, however, 2,4-DNT induced transformations in a dose dependent manner (no other isomers tested) (Kerckaert et al. 1998). 2,4- and 2,6-DNT also failed to exhibit mutagenic activity in the in vitro human hepatocyte unscheduled DNA synthesis (UDS) assay (Bermudez et al. 1979). These results are in contrast to the known hepatocarcinogenicity of 2,6-DNT. In contrast, in vivo assessment of the genotoxicity of DNT using the in vivo-in vitro hepatocyte DNA repair assay indicated that TG-DNT is a potent genotoxic agent, with 2,6-DNT being primarily responsible for this effect (Mirsalis et al. 1982a, Mirsalis and Butterworth 1982, and Mirsalis et al. 1989), results that are consistent with the carcinogenicity bioassays. These results were interpreted as indicating that 2,6-DNT activation in vivo requires processes other than hepatic metabolism. Metabolism by gut flora has been shown to be necessary for the formation of carcinogenic metabolites of DNT (Rickert et al. 1981 and Mirsalis et al. 1982b).

As in vitro mutagenicity assays have failed to predict the carcinogenic effects of 2,4- and 2,6-DNT and in vivo assays have demonstrated that entero-hepatic metabolism is a necessary step in the genotoxicity of DNT, the mutagenic activity reported in short-term studies are likely not reliable indicators of carcinogenic potential of the minor isomers of DNT. Rather, the in vivo hepatic initiation-promotion assays which reported 2,4-DNT and the minor isomers to be non-hepatocarcinogenic (Popp and Leonard 1982, Leonard et al. 1983 and Leonard et al. 1986) are likely the most reliable, currently available, indication of the carcinogenic potential of the minor isomers.

The Wisconsin drinking water health advisory for dinitrotoluenes states: "*The recommended Interim Health Advisory Level for total dinitrotoluene residues in Wisconsin groundwater is 0.05 μg/L.*"

Response: This advisory level is based on the U.S. EPA's evaluation of the carcinogenic potential of DNT as indicated in a study which used a mixture of 98% 2,4- and 2% 2,6-DNT (Ellis et al. 1979). The EPA states that because the effects of the two isomers could not be separated, the guideline would apply to each of the isomers as well as the mixture. As the DNT mixture used in the Ellis et al. (1979) study did not include any of the minor isomers, the guidelines derived from this study are not applicable to the minor isomers. It is not known how the presence of the minor isomers might alter the toxicity/carcinogenicity of DNT; however, there is some indication that competition for the metabolic pathway that activates the procarcinogen 2,6-DNT reduces the carcinogenicity of the isomer when it occurs in mixtures. In addition, in vivo initiation-promotion assays indicate that the minor isomers are not mutagenic and would likely not add to the carcinogenic potential of DNT mixtures. As such, the application of a guideline derived for 2,4-/2,6-DNT to the summed concentration of all DNT isomers is not appropriate.

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References cited

Bermudez, E., D. Tillery, B.E. Butterworth. 1979. The effect of 2,4-dinitrotoluene and isomers of dinitrotoluene on unscheduled DNA synthesis in primary rat hepatocytes. Environmental Mutagen 1(4): 391-398.

Burrows, E.P., D.H. Rosenblatt, W.R. Mitchell, D.L. Parmer. 1989. Organic explosives and related compounds: environmental and health considerations. Technical Report 8901. U.S. Army Biomedical Research and Development Laboratory, Fort Detrick, Frederick, MD.

Chiu, W.C., H.L. Langfong, C.Y. Wang, G.T. Bryan. 1978. Mutagenicity of some commercially available nitro compounds for Salmonella typhimurium. Mutation Research/Genetic Toxicology 58(1): 11-22.

CIIT. 1982. Chemical Industry Institute of Toxicology: 104-Week Chronic Toxicity Study in Rats - Dinitrotoluene. Final Report, Volume I of II. CIIT Docket No. 12362, Research Triangle Institute, Research Triangle Park, North Carolina.

Couch, D.B., P.F. Allen, D.J. Abernethy. 1981. The mutagenicity of dinitrotoluenes in Salmonella typhimurium. Mutation Research 90(4):373-83.

Couch, D.B., D.J. Abernethy, P.F. Allen. 1987. The effect of biotransformation of 2,4-dinitrotoluene on its mutagenic potential. Mutagenesis 2(6): 415-418.

Ellis, III, H.V., J.R. Hodgson, S.W. Hwang, L.M. Halpap, D.O. Helton, B.S. Anderson, D.L. VanGoethem and C.-C. Lee. 1978. Mammalian toxicity of munitions compounds phase I: acute oral toxicity, primary skin and eye irritation, dermal sensitization, disposition and metabolism, and Ames tests of additional compounds. Midwest Research Institute, Kansas City, MO. Supported by U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD.

Ellis III, H.V., J.H. Hagensen, J.R. Hodgson, J.L. Minor, C.-B. Hong, E.R. Ellis, J.D. Girvin, D.O. Helton, B.L. Herndon and C.-C. Lee. 1979. Mammalian toxicity of munitions compounds phase III: effects of lifetime exposure part I: 2,4-dinitrotoluene. Midwest Research Institute, Kansas City, MO. Supported by U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD.

Holen, I., S.O. Mikalsen, T. Sanner. 1990. Effects of dinitrotoluenes on morphological cell transformation and intercellular communication in Syrian hamster embryo cells. Journal of Toxicology and Environmental Health 29(1): 89-98.

Kerckaert, G.A., Leboeuf, R.A., Isfort, R.J. 1998. Assessing the predictiveness of the Syrian hamster embryo cell transformation assay for determining the rodent carcinogenic potential of single ring aromatic/nitroaromatic amine compounds. Toxicological Sciences 41(2): 189-197.

Lee, C.-C., J.V. Dilley, J.R. Hodgson, D.O. Helton and W.J. Wiegand. 1975. Mammalian toxicity of munitions compounds phase I: acute oral toxicity, primary skin and eye irritation, dermal sensitization, and disposition and metabolism. Midwest Research Institute, Kansas City, MO. Prepared for Environmental Protection Research Division, U.S. Army Medical Research and Development Command, Washington, DC.

Lee, C.-C., H.V. Ellis III, J.J. Kowalski, J.R. Hodgson, R.D. Short, J.C. Bhandari, T.W. Reddig and J.L. Minor. 1976. Mammalian toxicity of munitions compounds Phase II: effects of multiple doses part III: 2,6-dinitrotoluene. ADA062015. Midwest Research Institute, Kansas City, MO. Supported by U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD.

Lee, C.-C., H.V. Ellis III, J.J. Kowalski, J.R. Hodgson, S.W. Hwang, R.D. Short, J.C. Bhandari, J.L. Sanyer, T.W. Reddig and J.L. Minor. 1978. Mammalian toxicity of munitions compounds Phase II: effects of multiple doses part II 2,4-dinitrotoluene. ADA061715. Midwest Research Institute, Kansas City, MO. Supported by U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD.

Lee, C.-C., C.B. Hong, H.V. Ellis III, J.C. Dacre and J.P. Glennon. 1985. Subchronic and chronic toxicity studies of 2,4-dinitrotoluene. Part II. CD rats. J. Am. Coll. Toxicol. 4: 243-256.

Leonard, T.B., O. Lyght, J.A. Popp. 1983. Dinitrotoluene structure-dependent initiation of hepatocytes *in vivo*. Carcinogenesis 4(8): 1059-1061.

Leonard, T.B., T. Adams, J.A. Popps. 1986. Dinitrotoluene isomer-specific enhancement of the gene expression of diethylnitrosamine-initiated hepatocyte foci. Carcinogenesis 7(11): 1797-803.

Leonard, T.B., M.E. Graichen, J.A. Popp. 1987. Dinitrotoluene isomer-specific hepatocarcinogenesis in F344 rats. Journal of the National Cancer Institute 79(6):1313-1319.

Mirsalis, J.C., B.E. Butterworth. 1982a. Induction of unscheduled DNA synthesis in rat hepatocytes following in vivo treatment with dinitrotoluene. Carcinogenesis 3(3):241-5.

Mirsalis, J.C., T.E. Hamm, Jr., J.M. Sherrill, B.E. Butterworth. 1982b. Role of gut flora in the genotoxicity of dinitrotoluene. Nature 295: 322-323.

Mirsalis, J.C., C.K. Tyson, B.E. Butterworth. 1982. Detection of genotoxic carcinogens in the in vivo-in vitro hepatocyte DNA repair assay. Environmental Mutagen 4(5):553-62.

Mirsalis, J.C., C.K. Tyson, K.L. Steinmetz, E.K. Loh, C.M. Hamilton, J.P. Bakke, J.W. Spalding. 1989. Measurement of unscheduled DNA synthesis and S-phase synthesis in rodent hepatocytes following in vivo treatment: testing of 24 compounds. Environmental and Molecular Mutagenesis 14(3): 155-64.

Mori, M.A., T. Miyahara, K. Taniguchi, K. Hasegawa, H. Kozuka, M. Miyagoshi, T. Nagayama. 1982. Mutagenicity of 2,4-dinitrotoluene and its metabolites in Salmonella typhimurium. Toxicology Letters 13(1-2): 1-5.

National Cancer Institute. 1977. Bioassay of 2,4-dintrotoluene for possible carcinogenicity. Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institute of Health, Bethesda, MD.

Neuwoehner, J., A. Schofer, B. Erlenkaemper, K. Steinbach, K. Hund-Rinke, A. Eisentraeger. 2007. Toxicological characterization of 2,4,6-trinitrotoluene, its transformation products, and two nitramine explosives. Environmental Toxicology and Chemistry 26(6): 1090-1099.

Popp, J.A., T.B. Leonard. 1982. The use of in vivo hepatic initiation-promotion systems in understanding the hepatocarcinogenicity of technical grade dinitrotoluene. Toxicological Pathology 10 (2): 190-196.

Rickert, D.E., R.M. Long, S. Krakowka, J.G. Dent. 1981. Metabolism and excretion of 2,4-(^{14}C) dinitrotoluene in conventional and axenic Fischer-344 rats. Toxicology and Applied Pharmacology 59(3):574-579.

Spanggord, R.J., K.E. Mortelmans, A.F. Griffin, V.F. Simmon. 1982. Mutagenicity in Salmonella typhimurium and structure-activity relationships of wastewater components emanating from the manufacture of trinitrotoluene. Environmental Mutagen 4(2): 163-179.

U.S. EPA. 2006. Drinking water health advisory for 2,4-dinitrotoluene and 2,6-dinitrotoluene. Proposal Draft. EPA-822-R-06-017.