Captafol/*o*-Nitrotoluene Expert Panel Report

Part A – Peer Review of the Background Document on *o*-Nitrotoluene

The Report on Carcinogens (RoC) expert panel for Captafol/*o*-Nitrotoluene met at the Sheraton Chapel Hill Hotel on October 16, 2007, to peer review the draft background document on *o*-Nitrotoluene and make a recommendation for its listing status in the 12th Edition of the RoC. Members of the expert panel are as follows:

Lauren Zeise, Ph.D. (Chair)
California Environmental Protection Agency
Office of Environmental Health Hazard Assessment

Michael Elwell, D.V.M., Ph.D.
Covance Laboratories, Inc.
Department of Pathology

Penelope A. Fenner-Crisp, Ph.D., D.A.B.T.
Independent Consultant
(Retired from the International Life Science Institute and the U.S. Environmental Protection Agency)

Gregory L. Kedderis, Ph.D.
Independent Consultant

Steven Markowitz, M.D.
Queens College, City University of New York
Center for the Biology of Natural Systems

Robert C. Millikan, D.V.M., Ph.D.
University of North Carolina,
School of Public Health,
Department of Epidemiology

Shane S. Que Hee, Ph.D.
University of California, Los Angeles
School of Public Health, Department of Environmental Health Sciences

Thomas J. Slaga, Ph.D.
University of Texas Health Science Center
Department of Pharmacology

Alexander W. Teass, Ph.D.
(Retired from the National Institute of Occupational Safety & Health)

One of the charges to this panel was to peer review the draft background document, which includes determining whether the information in the draft background document on captafol is presented in a clear and objective manner, identifying any missing information from the body of knowledge presented in the document, and determining the utility of the body of knowledge in the background document for drawing conclusions about the carcinogenicity of a candidate substance and for applying the RoC criteria for listing. Following the discussion of all sections of the draft background document the expert panel reached a consensus concerning the critique of the draft background document, including its adequacy and any proposed revisions and voted 7 yes/0 no, with 1 absent to accept the draft background document with the proposed changes suggested by the expert panel. Therefore, the expert panel agreed that the background document would be adequate for drawing conclusions about the carcinogenicity of *o*-nitrotoluene and for applying the RoC listing criteria.

The expert panel proposed revisions for each section of the *ortho*-Nitrotoluene background document that are appended.

General comments

Use of foreign language journals. The panel recommended that publications in foreign language journals should be translated when the studies are relevant for applying the RoC listing criteria. English abstracts from these publications can be used as a source for other types of information (such as exposure levels), if the information in the abstracts is presented clearly.

Executive summary

Mechanistic and Genotoxicity data, Pviii, L16-21: Delete “Based on this proposed …various tissues.” Change to “ *o*-Nitrotoluene caused significantly increased incidences of tumors in tissues other than the liver in both rats and mice, including mammary gland, skin, lung, large intestine, and hemangiosarcomas in various tissues. This suggests that there are other mechanisms of activation for *o*-nitrotoluene.”

Section 1: Introduction

The expert panel identified additional synonyms for *o*-nitrotoluene (Table 1-1) and suggested some clarifications for the description of the chemical properties (Table 1-2). The revised tables with the revisions in blue font are below.

Table 1-1. Chemical identification of *o*-nitrotoluene

Characteristic	Information
CAS Registry number	88-72-2
Molecular formula	$C_7H_7NO_2$
Synonyms	1-methyl-2-nitrobenzene 2-methylnitrobenzene 2-methyl-1-nitrobenzene 2-nitrotoluene 2-nitrotoluol benzene, 1-methyl-2-nitro o-mononitrotoluene *o*-nitrotoluol *o*-methylnitrobenzene ONT NSC 9577 RTECs No: XT3150000 DOT/UN No: 1664

Source: ChemIDplus 2007, Scifinder Scholar

Table 1-2. Physical and chemical properties of *o*-nitrotoluene

Property	Information
Molecular weight	137.14
Melting point (°C)	–9.5 (needles); –2.9 (crystals)
Boiling point (°C)	222 at 760 mm Hg
Critical temperature (°C)	NA
Specific gravity	1.162 at 19°C/15°C
Solubility in water (at 30°C)	650 mg/L
Octanol-water partition coefficient (log K_{ow})	2.30
Dissociation constant (pK_a)	NA
Vapor pressure (mm Hg)	0.188 at 25°C
Vapor density relative to air	4.73
Henry's law constant	1.25×10^{-5} atm-cu m/mole @ 25°C

Section 2: Human Exposure[1]

1. Section 2.2 Production
   - P8, L29, After "(EPA 2007)": Delete "One U.S. . . . SRI (2007)" and replace with "SRI (2007) reported that only one U.S. facility produced *o*-nitrotoluene in 2007, which according to its website has 134 employees, 50 contractors, and 20 retirees (http://www.firstchem.com/).
2. Section 2.3 Environmental occurrence and fate (Page 9)
   - P9, L19: NTP should search the Reporting Center for Hazardous Materials Spills database to see if there is any information on *o*-nitrotoluene spills.
3. Section 2.3.1 Air (Page 9)
   - P9, L23, After "(see…Exposure)": insert the following statements from additional studies identified by the expert panel:
     - "Two ambient air samples collected at Boise Idaho in the winter of 1986-1987 contained 3 and 29 ng/m^3 of *o*-nitrotoluene vapor (Nishioka and Lewtas, 1992)."
     - "Smog in Japan and China has been shown to contain *o*-nitrotoluene (Takahara and Hayakawa, 1984 (Li *et al.* 2005, Wu *et al.* 2006)."
     - "*o*-Nitrotoluene has been found adsorbed to snow (Roth *et al*. 2004)
     - "In a laboratory investigation, Atkinson *et al.* (1989) found minor amounts of *o*-nitrotoluene were formed when toluene vapor and nitrogen oxides were mixed in air: *m*-nitrotoluene was the major product."
   - P9, L28, After "2-methyl-4-nitrophenol": Insert "(Nojima and Kanno 1977)." NTP will confirm HSDB reference for the remainder of sentence.
   - Revise summary to reflect the new information.
4. Section 2.3.2 Water (Pages 10 to 11)
   - P11, L11, After "(Pantex 2006)": Insert " In contrast with the Ogallala aquifer, Spain *et al.* (1999) and Best *et al*. (2000, 2001) studied groundwater highly contaminated with explosives and their impurities and metabolites, including *o*-nitrotoluene at concentrations greater than 2.1 mg/L (Spain *et al.* reported 2.9 mg/L). Both research teams were studying remediation of contaminated groundwater at a Tennessee munitions arsenal." (Additional studies identified by the expert panel).
5. Section 2.3.3 Soil (Page 12)
   - P12, L2: "Delete "No information…however" and replace with the findings of additional studies identified by the expert panel: "A soil contaminated with 39,100 ppm of trinitrotoluene at a historical testing ground in Idaho contained 1.4 ppm of *o*-nitrotoluene (Radtke *et al.* 2002a,b)." Continue with "*o*-Nitrotoluene can result…
   - P12, L17, After "(GAO 2002, 2003)": Insert "A similar situation occurs in other countries where munitions manufacture and ammunition testing have been done historically (Toze *et al.* 1999; Spiegel *et al.* 2005)."
   - P12, L20, After "HSDB 2007)": Insert "Obernosterer *et al*., 2000 used air stripping to remove 20% of *o*-nitrotoluene from quartz sand and loess loam. [This implies that volatility could be an important transport and remediation mechanism.] A review on sorption, mobility, and biodegradation of nitrotoluenes in soil is available (Toze *et al.* 1999)."
6. Section 2.4 General population exposure (Page 12)

[1] Note: Section number, page numbers and line numbers refer to the location in the draft background document.

- P12, L25, After "(HSDB 2007)": Insert "[as well as via skin contact with contaminated surfaces and via oral ingestion of contaminated food, water, or dust.]." NTP will confirm the accuracy of this information.
- P12, L28-29: "No exposure to *o*-nitrotoluene was documented" contradicts the finding of *o*-nitrotoluene detected in 19.1% of air samples. NTP will clarify this statement.
- P12, L29, After (Krotosynski *et al*. 1979)": Insert "Since *o*-nitrotoluene has a fish log BCF (bioconcentration factor) of 1.30 (Guo *et al*. 2004), [human exposure via fish residues should not be very important as a source.] Accumulation and depletion of *o*-nitrotoluene in carp (cyprinus carpio) has been modeled (Hou *et al*. 1997)." (Additional studies identified by the expert panel).
- P12, L 29, insert after (Krotoszynski *et al.* 1979), information from P10, L19-21 "At a former production site in Wisconsin between1999 and 2002, *o*-nitrotoluene was detected in offsite, private well-water at a maximum concentration of 0.095 μg/L"

7. Section 2.6 Biological indices of exposure (Pages 13 and 14)
   - P13, L12, After "alcohol": Insert "[probably present as *o*-nitrobenzyl glucuronide]"
   - P14, L3-5: The units of measure should be "nmol/g creatinine."
   - P14, L7, After "metabolites": Insert "The importance of skin exposure was unknown."
   - P14, L13, After "bioactivation-system": Insert "; however, work in other animal species such as rabbit and rat has shown that *o*-nitrotoluene has low methemoglobin-activating potency (Akahori 1954, Vasilenko 1976, Mlynarczyk and Sadowski, 2001). [Where exposures are to technical grade nitrotoluene, most of the methemoglobin activity will be related to the concentration of the *para*- and *meta*-nitro- isomers rather than the *ortho*- nitro- isomer." (Additional studies identified by the expert panel).
8. Regulations (Pages 14 and 15)
   - P14, L21, After "transporting.": Insert "Safety measures after spills or leaks are prescribed in accordance with *o*-nitrotoluene being a combustible toxic hazardous material."
   - P15, L5: After "skin]": Insert "Biological Exposure Index (BEI): 1.5% of methemoglobin during or at the end of the shift"
9. Summary (Page 15)
   - P15, L19, After "Netherlands": Delete "and Germany" and replace with "Germany, and the United States."
   - P19, L24, After "grounds": Insert "*o*-Nitrotoluene has been detected in workplace and ambient air, surface water, ground water, fish, and soils."
   - Modify summary to reflect other changes listed above.

Section 3: Human Cancer Studies[2]

The human exposure study subgroup reviewed the description of human cancer studies reported in Sections 3 (magenta manufacturing workers who were or may have been exposed to *o*-nitrotoluene) and in section 5 (workers exposed to dinitrotoluenes or *ortho*-toluidine).

1. General Comments
   - Create a new Section 3.2: Studies of workers exposed to dinitrotoluenes or *ortho*-toluidine. Move the description of studies on workers exposed to dinitrotoluenes (Section 5.5.2) and *ortho*-toluidine (Section 5.5.3) to this new Section (3.2) and summarize these studies in the discussion and summary. (The discussion and summary will become Section 3.3).
2. Section 3.1 Studies of magenta manufacturing workers
   - P17 L25, After "deaths)": Insert "Larynx, esophageal and lung cancer also showed statistically significant elevations in the entire cohort, but the small sample in the magenta/safranine sub-cohort did not permit evaluation of these other cancer types."
   - P17 to 18: Incorporate the following text in the description of the study reported by Rubino *et al.*: "The study had an excellent follow-up rate (96%). However, like all occupational cohort SMR-based studies, the effect estimates utilize an external comparison group (to determine expected disease rates). Rubino *et al.* used expected death rates in all Italy, in order to give more precise estimates, but the workers were males from Northern Italy. A strength of the study is the fact that the authors studied a group of workers who were specifically exposed to *o*-nitrotoluene, although also exposed to *o*-toluidine and 4,4'-methylene bis(2-methylaniline) (but not to naphthylamines or benzidines)."
   - Page 18, L11, Before "mortality": Insert "bladder cancer"
   - P18 L11, After "study." Insert "The authors observed an earlier age at diagnosis, as well as an excess of bladder cancer mortality, in the cohort compared to the general population."
   - P18, L17, Before "case-control study": Insert "hospital-based" before
   - P18, L21, After "literature.": insert "Vineis and Magnani (1985) looked at 74 chemicals as part of a job exposure matrix. No adjustments were made for multiple comparisons"
3. Section 5.5.2 Dinitrotoluenes
   - P72, L21: Replace "450" workers with "457" workers
   - P72, L23: Replace "observed" with "expected.", then insert "The numbers of observed deaths for specific cancer sites were small. However, the authors did observe a non-significant excess of colon cancer (SMR = 186)."
   - P72, L25: Replace "(some of which…)" with "(< 10% of whom ….")
   - P73, L1, After "this": Insert " Potential exposure to carcinogens other than dinitrotoluenes was a concern."
   - P73, L3, After "dinitrotoluenes": Insert " through inhalation and direct skin contact"
   - P73, L4, After "dinitrotoluenes": Insert "(75% 2,4 DNT and 20% 2,6 DNT)"
   - P73, L5, After "renal-cell cancer": Insert "Confidence intervals were not provided."
   - P73, L6, After "humans': Insert "Harth (2006) reported a cluster of 3 urothelial cancers among 60 workers exposed to 2,4 dinitrotoluene, with a calculated SIR = 15.9 (confidence intervals were not provided)". (Harth is a study identified by the expert panel)
4. Section 5.5.3 *o*-Toluidine hydrochloride

[2] Note: Section number, page numbers and line numbers refer to the location in the draft background document.

- P73, L 29: After “(CI=2.13 to 5.73)’: Insert “The SIR for bladder cancer among the definitely exposed was 6.48 (95% CI: 3.0 to 12.2), which increased to 27.2 (95% CI: 11.8-53.7) for workers who were employed in the department with *o*-toluidine and aniline exposure for $\geq$ 10 years.”
- P74, L2, After “used”: Insert “The authors collected smoking history on a 5% sample of workers using company medical records, and used modeling techniques to estimate that only a small proportion of the elevated SIR (1.05) could be attributed to higher smoking in the cohort versus the general population. This study has the advantage of calculating SIRs (using incidence rates) rather than SMRs (using death certificates). The authors concluded that *o*-toluidine was more likely than aniline to be responsible for the bladder cancer excess, in view of the greater carcinogenic potency of *o*-toluidine than aniline in animals. Stasik (1988) found excess bladder cancer incidence (SIR=72.7, 95% CI: 31.4 to 153.3) among workers exposed to 4-chloro-*o*-toluidine, *o*-toluidine and two other monocyclic amines.” (Stasik is a study identified by the expert panel).

5. Section 3.2 Discussion and Summary
   - P19, L3, After “Workers”: Insert "Most of the aforementioned studies did not address other potential confounders such as cigarette smoking or genetic acetylator phenotype (e.g. NAT1, NAT2 genotypes.) But the relative risks associated with smoking and genetic susceptibility as well as other risk factors are small relative (RR= 2 to 4) to those associated with the dye manufacture."
   - P19, L20, New paragraph: Insert “Limited epidemiologic studies on dinitrotoluenes are available and suggest elevations in hepatobiliary, urothelial, renal, and possibly colon cancer. Two studies of *o*-toluidine reported excess bladder cancer incidence.”

Section 4: Studies of Cancer in Experimental Animals[3]

Section 4: Studies in Experimental Animals

1. General
   - The background document should indicate that the NTP studies represent a high quality data set for making conclusions about potential carcinogenicity of *o*-nitrotoluene.
   - Treatment-related hemangiosarcomas in mice were listed in various sites, which were in the subcutis/skeletal muscle and mesentery. The specific sites should be noted in the document, preferably in a table, with associated statistics.
   - 'The description of each study should (1) include the age of the animals at start of the study; (2) include the purity of the substance; (3) include more details (in the footnotes to the tables or noted in the text) on characteristics, such as laboratory, chronological time window, that the historical control share with the concurrent controls; (4) report significance values for trend tests in the tables, and (5) distinguish *P* values that are highly significant (e.g., *P* <.001 from those that are *P* < 0.05 or < 0.01). Tumor effects are quite striking and the current report does not reflect that.
2. Section 4.1 Rats
   - P 21-22 Section 4.1.1: Move the discussion of the toxic effects observed in the 13-week study to the toxicity section (Section 5.6). Delete all entries except that for the epididymis-mesothelium findings from Table 4-1.
   - P24 L12-14 "slightly greater mean body weight than those in the 26-week-exposure group, but still weighted less than the controls": Were these weight differences statistically different? If yes say so; if not, delete. Magnitude of the effect not defined in paper. It can be noted that the rats in both the stop exposure and the 26 week study weighed more that the control
   - P25, L9: Emphasize the consistencies of the mesothelomia findings in the altered and non-altered fluora study.
   - P 26, L28: If the low survival of the high-dose and/or mid-dose animals is due to malignancy it should be noted.
   - P 27, L 8-23: Delete parenthetical for malignant mesothelioma ("mainly large, papillary or solid areas of pleomorphic mesothelial cells") and replace with site-specific information, e.g. tunica vaginalis of the testis epididymis or surface of abdominal wall. Also, delete the detailed description of tumors for the lung and liver lesions (i.e.,at line 18 and 19 – "which were usually papillary and distorted the alveolar architecture" and the sentence at line 20-23 "The hepatocellular adenomas consisted… three or more cells thick.") .
   - P 29, Table 4-3a: Delete heading, "various tissues" from the heading for Malignant Mesotheliomas.
   - P 30 Table 4-3b: Add adenoma/carcinoma to column heading for the lung tumors
3. Section 4.1 Mice
   - P31, Section 4.2.1: Move the 13-week mouse study (subchronic toxicity) to Section 5.6.
   - P33, Table 4-5: Add the following footnote for the incidence of large-intestine carcinoma (0%) in the 5000-ppm male mice. "Dunnick *et al.* (2003; NTP 2002b) suggested that the 5000-ppm male mice did not survive long enough for tumors to develop in the large intestine."

[3] Note: Section number, page numbers and line numbers refer to the location in the draft background document.

4. Section 4.3 Summary, Table 4-6 (page 34)
    - Change “Mesothelium” to “Malignant Mesothelium” to be consistent with the text.
    - Insert a row under Liver for “Hepatocholangiocarcinoma”, Insert new footnote (b) “Only 2 tumors were observed but they are very rare and the NTP used them as part of their call.”
    - Notation (+=) for colon tumors in female mice, After “different”: Insert “from concurrent controls but highly significant (p>0.001) compared to historical controls”.
    - Footnote “a”: Change “Only adenomas (no carcinomas) were found in female F344/N rats” to “Based on adenomas found in female F344N rats.”

Section 5: Other Relevant Data[4]

1. Section 5.1.1 ADME: Human data (Pages 35-36)
   - P35, L27-29, After "alcohol: Insert "[probably present as *o*-nitrobenzyl glucuronide],"
   - P35, L25: Delete "indirect" before "evidence"
2. Section 5.1.2 ADME: Rodent data (page 36-37)
   - P37 L12, Before " NTP 2002b": Insert the following new references: "Sabbioni 1994; Sabbioni and Sepai 1995;".
   - P37 L13, After "tissues": Insert findings from a new reference, "The hemoglobin binding index for *o*-nitrotoluene in female Wistar rats was measured to be 0.72±0.19 mmol compound/mol Hb)/(mmol compound/kg body weight (Sabbioni 1994)
3. Section 5.1.3 ADME: *in vitro* data (Page 37 to 38)
   - P38, L11: Insert new paragraph describing findings of Kedderis and Rickert 1985. (Additional study identified by the expert panel.)

     Rat liver microsomal cytochromes P450 oxidized the isomeric aminobenzyl alcohols, metabolites of the nitrotoluenes, to hydroxylamine and/or aminophenol metabolites (Kedderis and Rickert, 1985). The metabolites were extractable into ethyl acetate and capable of reducing ferric iron, consistent with the formation of hydroxylamines and/or aminophenols. The oxidation rates of the aminobenzyl alcohols were five times lower than those of the aminonitrobenzyl alcohols (metabolites of dinitrotoluenes). The rate of aminobenzyl alcohol oxidation varied as 2- > 3- > 4-. Thus the 2-aminobenzyl alcohol metabolite of *o*-nitrotoluene (0.5 mM) was oxidized at a greater rate (0.29 ± 0.01 nmol formed/min/mg protein) than the aminobenzyl alcohol metabolites of *m*-nitrotoluene (0.18 ± 0.00) or *p*-nitrotoluene (0.04 ± 0.00). These results are consistent with the toxic potency of the mononitrotoluene isomers.

4. Section 5.1.4 ADME: *In vivo* metabolism of *o*-nitrotoluene in rodents (Pages 39 to 43)
   - P41, 1st Paragraph: Add a sentence to note that minor metabolites can be converted into major metabolites.
   - P41, L14-16: Replace "non-pretreated" with "untreated".
   - P41, L22-25: Delete sentence beginning with "Presumably."
5. Section 5.2 (pages 49 to 52)
   - Page 52, L18-20: Delete "Although hemoglobin… to rats and" since this has nothing to do with DNA adduct formation. Begin new sentence with "DNA adducts are formed…"
   - Page 52 L26-29: Delete sentence beginning with "Jones, *et al. (*2005a)…concentrations."
3. Section 5.3.2: Genetic damage and related effects: Mammalian systems (Pages 53 to 55)
   - P54, L6, After "(CHO)": Insert "and lung (CHL/IU)"
   - P55: Add to text and Table 5-5, results from the following new studies identified by the expert panel:
     - Matsushima *et al.* (1999): *o*-nitrotoluene (2 to 50 μg/ml) caused micronuclei in Chinese hamster lung cells.
     - Ishidate *et al.* 1988: *o*-nitrotoluene caused chromosomal aberrations in CHL
     - Huang 1996: *o*-nitrotoluene caused chromosomal aberrations in human peripheral lymphocyte

[4] Note: Section number, page numbers and line numbers refer to the location in the draft background document.

5. Section 5.3.3: Genetic damage and related effects: Gene expression studies
   - P59--68: Consolidate and integrated this text with the text on page 5.4.2. Add findings from Iida *et al.* 2007, that *o*-nitrotoluene did not down regulate TSC -22.
6. Section 5.4.1: Genetic damage and related effects Mechanistic studies and Considerations: bioactivation of *o*-NT
   - P 62, L4-9: Delete text from "However, there appears…*o*-nitrotoluene administration.". (which states that female rats should be more resistant to the hepatocarcinogenic effects of o-nitrotoluene if only the bioactivation factors were considered). Begin last sentence with "The significantly…."
6. Section 5.5 Carcinogenicity and genotoxicity of o-nitrotoluene analogues and metabolites
   - P68, L10, After "male rats"; Insert text stating that the bioactivation of 2,4 dinitrotoluene is similar to that of *o*-nitrotoluene
7. Section 5.5.2 Dinitrotoluenes (Pages 70-73)
   - P71, L7-23: Correct mistakes in defining the isomers.
     - L8, "2,4-" should be "2,6-"
     - Li 9, "2,6-" should be "2,4-"
     - Li13, "2,6-" should be "2,4-" and "2,4'" should be "2,6-"
   - P 72, L9, Insert new paragraph describing findings in Kedderis et al. 1984 (Additional study identified by the expert panel)

     These studies showed that sulfotransferase inhibition *in vivo* decreased the covalent binding of the hepatocarcinogen 2,6-dinitrotoluene to hepatic DNA by >95%. These results indicate that sulfation is involved in the bioactivation of 2,6-dinitrotoluene. These studies, along with the *in vitro* studies of N-oxidation cited above, helped clarify the complex bioactivation mechanisms of the dinitrotoluenes (Kedderis *et al.* 1984).

   - P72 L9, After Kedderis *et al.* 1984, summarizes the findings of Kedderis et al. 1985 mentioned above (5.1.3)
   - P72 L19 to p73 L6: Move description of human epidemiological studies to the new Section 3.2 (See comments on Section 3: Human Cancer Studies, above)
8. Section 5.5.3 *o*-Toluidine hydrochloride (Pages 73-74)
   - P73, L25-29 to P74, L1-4); Move description of human epidemiological studies to the new Section 3.2 (See comments on Section 3: Human Cancer Studies, above)
9. Summary (Pages 74-77)
   - Section 5.1, P74, L18, Delete "indirect" before evidence.
   - P75, After 17: Add a summary of the i*n vivo* studies summarized in Table 5-6
   - P75, L 28-29 to P76, L 1-2: Delete text related to the statement that female rats should be more resistant to the hepatocarcinogenic effects of *o*-nitrotoluene if only the bioactivation factors were considered. Remove "In addition" on line 2.

Report Approved _____ Date _____
Lauren Zeise, Ph.D. (Chair)

References

1. Akahori W. 1954. Poisoning by aromatic amino- and nitro-compounds. VII. The mechanism of methemoglobin formation. *Folia Pharmacol Japon* 50: 422-432.
2. Atkinson R, Aschmann SM, Arey J, Carter WPL. 1989. Formation of ring-retaining products from the hydroxyl radical-initiated reactions of benzene and toluene. *Int J Chem Kin* 21(9): 801-827.
3. Best EPH, Miller JL, Larson SL. 2000. Explosives removal from groundwater at the Volunteer Army Ammunition Plant, TN, in small-scale wetland modules. In *Wetlands and Remediation, an International Conference, Salt Lake City, UT, November 16-17, 1999*. Means JL, Hinchee RE, eds. Columbus, OH: Battelle Press. pp. 365-373.
4. Best EP, Miller JL, Larson SL. 2001. Tolerance towards explosives, and explosives removal from groundwater in treatment wetland mesocosms. *Water Sci Technol* 44(11-12): 515-21.
5. FCC. 2007. First Chemical Corporation. http://firstchem.com.
6. Guo R, Liang X, Chen J, Zhang Q, Wu W, Kettrup A. 2004. Using HPLC retention parameters to estimate fish bioconcetration factors of organic compounds. *J Liq Chromat Rel Tech* 27(12): 1861-1873.
7. Harth V, Bolt HM, Bruning T. 2005. Cancer of the urinary bladder in highly exposed workers in the production of dinitrotoluenes: a case report. *Int Arch Occup Environ Health* 78(8): 677-80.
8. Hou L, Hu C, Zhao X, Zhao Y, Lang P, Liu Z. 1997. Simulating study on bio-accumulation and elimination or organic pollutants in river cyprinus carpio. *Huanjing Kexue* 18(2): 34-36, 39.
9. Huang Q, Liu Y, Wang L, Han S, Yang J. 1996. Genotoxicity of substituted nitrobenzenes and the quantitative structure-activity relationship. *J Environ Sci (China)* 8(1): 103-109.
10. Iida M, Anna CH, Gaskin ND, Walker NJ, Devereux TR. 2007. The putative tumor suppressor Tsc-22 is downregulated early in chemically induced hepatocarcinogenesis and may be a suppressor of Gadd45b. *Toxicol Sci* 99(1): 43-50.
11. Ishidate M, Jr., Harnois MC, Sofuni T. 1988. A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. *Mutat Res* 195(2): 151-213.
12. Jones CR, Sepai O, Liu YY, Yan H, Sabbioni G. 2005a. Hemoglobin adducts in workers exposed to nitrotoluenes. *Carcinogenesis* 26(1): 133-143. (Supported by the European Commission. Author affiliations: Institute of Environmental and Occupational Toxicology, Switzerland; University of Newcastle upon Tyne, UK; Ludwig-Maximilians-Universität München, Germany; Chinese Academy of Preventative Medicine, China.)
13. Kedderis GL, Dyroff MC, Rickert DE. 1984. Hepatic macromolecular covalent binding of the hepatocarcinogen 2,6-dinitrotoluene and its 2,4-isomer in vivo: modulation by the

14. Kedderis GL, Rickert DE. 1985. Characterization of the oxidation of amine metabolites of nitrotoluenes by rat hepatic microsomes. N- and C-hydroxylation. *Mol Pharmacol* 28(2): 207-14.

15. Li Y, Bai Y, Wang M, Liu C. 2005. GC determination of nitrotoluene in air. *Gongye Weisheng Yu Zhiyebing* 31(2): 127-128.

16. Matsushima T, Hayashi M, Matsuoka A, Ishidate M, Jr., Miura KF, Shimizu H*, et al.* 1999. Validation study of the in vitro micronucleus test in a Chinese hamster lung cell line (CHL/IU). *Mutagenesis* 14(6): 569-80.

17. Mlynarczyk W, Sadowski C. 2001. Toxicometric indexes of mononitrotoluenes. Part V. Methemoglobinemics. *Bromatologia i Chemia Toksykologiczna* 34(4): 321-324.

18. Nishioka MG, Lewtas J. 1992. Quantification of nitro- and hydroxylated nitro-aromatic/polycyclic aromatic hydrocarbons in selected ambient air daytime winter sample. *Atmos Environ* 26A(11): 2077-2087.

19. Nojima K, Kanno S. 1977. Studies on phytochemistry of aromatic hydrocarbons. IV. Mechanism of formation of nitrophenols by the phytochemical reaction of benzene and toluene with nitrogen oxides in air. *Chemosphere* 6(6): 371-376.

20. Obernosterer I, Mies B-M, Bauer H-J, Nienhaus U. 2000. Disposal of contaminated soils of former explosive production sites. *Altlasten Spektrum* 9(5): 261-263, 265-269.

21. Radtke CW, Gianotto D, Roberto FF. 2002a. Effects of particulate explosives on estimating contamination at a historical explosives testing area. *Chemosphere* 46(1): 3-9.

22. Radtke CW, Smith DM, Owen GS, Roberto FF. 2002b. Field demonstration of acetone pretreatment and composting of particulate-TNT-contaminated soil. *Bioremediation J* 6(2): 191-204.

23. Roth CM, Goss KU, Schwarzenbach RP. 2004. Sorption of diverse organic vapors to snow. *Environ Sci Technol* 38(15): 4078-84.

24. Sabbioni G. 1994. Hemoglobin binding of nitroarenes and quantitative structure-activity relationships. *Chem Res Toxicol* 7(2): 267-274.

25. Sabbioni G, Sepai O. 1995. Comparison of hemoglobin binding, mutagenicity, and carcinogenicity of arylamines and nitroarenes. *Chimia* 49(10): 374-380.

26. SciFinderScholar. 2007. *Database available at Duke University*.

27. Spain JC, Nishino SF, Greene MR, Forbort JE, Nogalski NA, Unterman R*, et al.* 1999. Field demonstration of FBR for treatment of nitrotoluenes in groundwater. In *Fifth International In Situ and On-Site Bioremediation Symposium, San Diego, April 19-20, 1999*. Alleman BC, Leeson A, eds. Columbus, OH: Battelle Press. pp. 365-373.

28. Spiegel K, Headly JV, Peru KM, Haidar N, Gurprasard NP. 2005. Residues of explosives in groundwater leached from soils at a military site in Eastern Germany. *Comm Soil Sci Plant Anal* 36(1-3): 133-153.

29. Stasik MJ. 1988. Carcinomas of the urinary bladder in a 4-chloro-o-toluidine cohort. *Int Arch Occup Environ Health* 60(1): 21-4.

30. Takahara Y, Hayakawa T. 1984. Gas chromatographic measurement of aromatic nitro compounds in ambent air with Tenax GC [gas chromatography] glass tube. *Gifu-ken Kogai Kenkyusho Nenpo* 12: 41-46.

31. Tchounwou PB, Newsome C, Glass K, Centeno JA, Leszczynski J, Bryant J, Okoh J, Ishaque A, Brower M. 2003. Environmental toxicology and health effects associated with dinitrotoluene exposure. *Rev Environ Health* 18(3): 203-29.

32. Toze S, Patterson B, Zappia L, Power T, Davis GB. 1999. The effect of sorption and biodegradation on the migration of munition compounds in groundwater and soil environments. In *Contaminated Site Remediation: Challenges Posted by Urban and Industrial Contaminants, Proceedings of the Contaminated Site Remediation Conference, Fremantle, Australia, March 21-25, 1999*. Johnston CD, ed. Wembley, Australia: Centre for Groundwater Studies. p. 375-381.

33. Vasilenko NM. 1976. Specific and nonspecific indexes in blood changes during poisoning by aromatic nitro amino compounds of the benzene series. *Trudy Khar'kovskogo Meditsinskogo Instituta* 124: 34-38.

34. Wu Y-F, Li L-R, Yang J-F, Shi T-R. 2006. Detection of aromatic nitrocompounds in air by gas chromatography/mass spectrometry. *Zhongguo Weisheng Jianyan Zazhi* 16(7): 786-787, 838.