PEER REVIEW OF ATSDR TOXICOLOGICAL PROFILES FOR DINITROTOLUENES

Reviewer #1

OVERALL:

Overall this is a very well written review of the toxicology and potential health effects of Dinitrotoluenes. It is written in the format of previous toxicological profiles and follows the prescribed standard format very well. The document is well referenced. The profile covers potential exposures and potential health effects based on the available literature from early life exposure to maturity. Some minor comments are made in the document for consideration.

CHILD HEALTH AND DEVELOPMENTAL EFFECTS:

This reviewer is not aware of any additional data relevant to child health and developmental effects that needs to be discussed in the profile.

CHAPTER 1. PUBLIC HEALTH STATEMENT

This chapter is well written and provides the proper tone for the non-technical average citizen. No alternate wording is suggested. Answers to potential questions by the lay public are adequately addressed.

CHAPTER 2. RELEVANCE TO PUBLIC HEALTH

I agree with the effects noted in humans. Of concern is that the single epidemiology study that is relied on for chronic effect (Levine) did not control for lifestyle (smoking, diet) and thus the conclusions that cardiovascular effects are the results of dinitrotoluene exposure needs to be viewed with caution. The animal effects are well described and conclusions are appropriate.

CHAPTER 3 HEALTH EFFECTS

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

Well written no changes suggested. All of the noted target organs for DNT exposure in human and animals have been reported on and discussed. The salient toxicology endpoints for DNT effects have also appropriately referenced and discussed.

3.3 GENOTOXICITY

This section is well written and referenced. An appropriate discussion of the mammalian and non-mammalian genotoxicty studies for DNT is made. The tables are very well done and appropriately referenced. Good review of the genotoxicity.

Toxicity - Quality of Human Studies

This section is well written and covers the available literature on human effects. Study limitations were noted and addressed. The appropriate NOAELs and LOAELs were provided. The appropriate statistics are provided. No additional studies are apparent to this reviewer that needs to be included.

Toxicity - Quality of Animal Studies

As with the human section, This section is well written and covers the available literature on human effects. Study limitations were noted and addressed. The appropriate statistics are indicated. The appropriate NOAELs and LOAELs were provided. No additional studies are apparent to this reviewer that needs to be included.

Levels of Significant Exposure (LSE) Tables and Figures

The tables and figures presented a e appropriate. No changes are suggested. Limitations are adequately and accurately discussed. The appropriate effects and endpoints of the referenced studies have been evaluated and noted. Where available the appropriate dose response data has been noted.

3.4 TOXICOKINETICS

This section provides adequate discussion of absorption, distribution, metabolism, and excretion of dinitrotoluene based on the available literature. The potential routes of exposure of are covered very well. The ADME of these compounds has been appropriately discussed and referenced. Figures 3-3 to 3-5 are particularly very informative.

3.5 MECHANISMS OF ACTION

The known mechanisms of action for toxicity and carcinogenicity are addressed.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

The data or lack thereof for this section are noted and appropriately addressed.

3.7 CHILDREN'S SUSCEPTIBILITY

While there is limited data on children effects, this section does an appropriate job at addressing the child susceptibility issue .

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Based on the available literature, this subject is appropriately discussed in the text. As noted , no biomarkers for DNT have been validated .

3.9 INTERACTIONS WITH OTHER CHEMICALS

The section addresses and cites the available limited literature.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

Text appropriately notes the potential susceptible populations.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

While limited information exists, this sections, provides reference to standard texts for addressing this topic and summarizes standard approaches to modifying absorption and metabolism.

3.12 ADEQUACY OF THE DATABASE

This section adequately addresses the topic and possible data needs.

4. CHEMICAL AND PHYSICAL INFORMATION

This section is very straight forward and adequately addresses the chemical and physical properties of DNTs. The tables are very informative.

5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

This section is straight forward and adequately addresses the topic.

6. POTENTIAL FOR HUMAN EXPOSURE

This section is excellent. It provides a good overview of the releases and environmental fate of DNTs. The addressing of the population and occupational exposure is appropriate. An area of concern that should be enhanced is the possible human exposure when military bases (with high levels in soil) are decommissioned and turn over for public use (parks and recreation) .

7. ANALYTICAL METHODS

This section is straight forward and well done.

8. REGULATIONS, ADVISORIES, AND GUIDELINES

This section is straight forward and well done. No changes are suggested. One concern , not restricted to this document , is how will the CDC/ATSDR address changes in regulations in the document (how to update the document as new regulations come forth)

9. REFERENCES

Complete and well done.

UNPUBLISHED STUDIES (IF APPLICABLE TO REVIEW)

STUDY 1 Mammalian toxicity of Munitions Compounds Phase III: Effects of Life time exposure PART I: 2,4-DINITROTOLUENE This study from a contract , reported in 1979 .

This report is very straight forward report of studies performed under contract. The design, results and conclusions are appropriate. This reviewer agrees with the conclusions.

STUDY 2 Mammalian toxicity of Munitions Compounds Phase II: Effects of Multiple Doses
PART III: 2,6- DINITROTOLUENE Progress Report NO. 4 July 1976

This report is very straight forward report of studies performed under contract. The design, results and conclusions are appropriate. This reviewer agrees with the conclusions.

Study 3 Subchronic and Chronic toxicity of 2,4 DNT in Beagle dogs Part 1, 1985

This is a peer reviewed published study in the J of American College of Tox. This report is very straight forward report of studies performed under contract. The design, results and conclusions are appropriate. This reviewer agrees with the conclusions

Dinitrotoluene
Reviewer #3

Background Statement and Questions

1.) I compared all submitted documents and the current toxicological profile to the former version that was released in 1998 by ATSDR and detected the improvements and differences in the text. Furthermore, I checked PubMED database for the current literature.
Additionally, I read the addendum to the Toxicological Profile written in October 2009. I was surprised to note that the following publications mentioned in the addendum to the

Toxicological Profile written in October 2009 have not been referred to.

Albert KJ, Myrick ML, Brown SB, et al. 2001. Field-deployable sniffer for 2,4-dinitrotoluene de-tection. Environ Sci Technol 35(15):3193-200.
Albert KJ, Walt DR. 2000. High-speed fluorescence detection of explosives-like vapors. Anal Chem 72(9):1947-55.
Brüning T, Chronz C, Thier R, et al. 1999. Occurrence of urinary tract tumors in miners highly exposed to dinitrotoluene. J Occup Environ Med 41(3):144-9.
Campbell S, Ogoshi R, Uehara G, et al. 2003. Trace analysis of explosives in soil: pressurized fluid extraction and gas and liquid chromatography-mass spectrometry. J Chromatogr Sci 41(6):284-8.
Content S, Trogler WC, Sailor MJ. 2000. Detection of nitrobenzene, DNT, and TNT vapors by quenching of porous silicon photoluminescence. Chemistry 6(12):2205-13.
George SE, Allison JC, Brooks LR, et al. 1998. Modulation of 2,6-dinitrotoluene genotoxicity by alachlor treatment of Fischer 344 rats. Environ Mol Mutagen 31(3):274-81.
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.
Jones CR, Sepai O, Liu YY, et al. 2005b. Urinary metabolites of workers exposed to nitrotol-uenes. Biomarkers 10(1):10-28.
Maeda T, Nakamura R, Kadokami K, et al. 2007. Relationship between mutagenicity and reactiv-ity or biodegradability for nitroaromatic compounds. Environ Toxicol Chem 26(2):237-41.
Neuwoehner J, Schofer A, Erlenkaemper B, et al. 2007. Toxicological characterization of 2,4,6-trinitrotoluene, its transformation products, and two nitramine explosives. Environ Toxicol Chem 26(6):1090-9.
Ozturk K, Durusoy M. 1999. The detection and comparison of the genotoxic effects of some nitro aromatic compounds by the umu and SOS chromotest systems. Toxicol Lett 108(1):63-8.
Padda RS, Wang C, Hughes JB, et al. 2003. Mutagenicity of nitroaromatic degradation com-pounds. Environ Toxicol Chem 22(10):2293-7.

abbioni G, Jones CR, Sepai O, et al. 2006. Biomarkers of exposure, effect, and susceptibility in workers exposed to nitrotoluenes. Cancer Epidemiol Biomarkers Prev 15(3):559-66.
Sayama M, Mori M, Shoji M, et al. 1998. Mutagenicities of 2,4- and 2,6-dinitrotoluenes and their reduced products in Salmonella typhimurium nitroreductase- and O-acetyltransferase-overproducing Ames test strains. Mutat Res 420(1-3):27-32.
Smirnova IA, Dian C, Leonard GA, et al. 2004. Development of a bacterial biosensor for nitrotol-uenes: the crystal structure of the transcriptional regulator DntR. J Mol Biol 340(3):405-18.
Yang H, Halasz A, Zhao JS, et al. 2008. Experimental evidence for in situ natural attenuation of 2,4- and 2,6-dinitrotoluene in marine sediment. Chemosphere 70(5):791-9.
Zhang HX, Cao AM, Hu JS, et al. 2006. Electrochemical sensor for detecting ultratrace nitroar-omatic compounds using mesoporous SiO2-modified electrode. Anal Chem 78(6):1967-71.

The addendum cited crucial publications concerning the following chapters:
2. HEALTH EFFECTS
3. CHEMICAL AND PHYSICAL INFORMATION
4. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL
5. POTENTIAL FOR HUMAN EXPOSURE
6. ANALYTICAL METHODS
7. REGULATIONS AND ADVISORIES
8. REFERENCES

I am aware of the fact that the purpose of this addendum is to provide to the public and other federal, state, and local agencies a non-peer reviewed sup-plement of the scientific data that were published in the open peer-reviewed literature since the release of the profile in 1998. The addendum should be used in conjunction with the profile.

Questions:

Didn't these publications meet the criteria for the current ATSDR-profile?

Is the addendum still valid or not valid from the date of publication of the "new Profile"?

2.) Currently, I am involved in the publication of the results of the Mansfeld study. The case-cohort study is an extended follow-up of the pilot study of Brüning et al. (1999, 2001) and comprises now 16.441 workers of the copper mining industry (closed in 1990 after the German reunification), in order to

further elucidate a relationship between Tg-DNT and urothelial as well as kidney cancer.

3.) Additionally, I made some text suggestions (marked in correction mode) directly in the manuscript (see pdf in the attachment).

Remarks and comments on pages:

xi, 4, 13, 16, 29, 30, 34, 35, 40, 45, 54, 98, 99, 122, 123, 143, 167, 176.

CHILDREN‘S HEALTH

-Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be?

No.

-Are there any general issues relevant to child health that have not been dis-cussed in the profile and should be?

No.

-If you answer yes to either of the above questions, please provide any relevant references.

Not applicable.

CHAPTER 1. PUBLIC HEALTH STATEMENT

-Does the chapter present the important information in a non-technical style suit-able for the average citizen?

Yes.

-Major headings are stated as a question. In your opinion, do the answers to the questions adequately address the concerns of the lay public?

Yes.

- Are these summary statements consistent, and are they supported by the tech-nical discussion in the remainder of the text?

Yes.
-Are scientific terms used that are too technical or that require additional explana-tion?

No.

CHAPTER 2. RELEVANCE TO PUBLIC HEALTH

-Do you agree with those effects known to occur in humans as reported in the text? If not, provide a copy of additional references you would cite and indicate where (in the text) these references should be included.

Yes, I agree with the effects as reported in the text.

I missed some conclusions of the publications mentioned in the addendum to the Toxicological Profile written in October 2009.

-Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

Yes, indeed. The effects are of concern to humans as mentioned in the pro-file.

-Have exposure conditions been adequately described?

The exposure conditions have been adequately described.

CHAPTER 3. HEALTH EFFECTS

Section 3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE Review Toxicological

Toxicity - Quality of Human Studies

-Were adequately designed human studies identified in the text (i.e., good expo-sure data, sufficiently long period of exposure to account for observed health ef-fects, adequate control for confounding factors)? If not, were the major limitations of the studies sufficiently described in the text without providing detailed discus-sions. If study limitations were not adequately addressed, please suggest appro-priate changes.

The major limitations of the cited studies are adequately addressed in the text. The quality of exposure data in the current literature is unfortunately limited.

-Were the conclusions drawn by the authors of the studies appropriate and accu-rately reflected in the profile? If not, did the text provide adequate justification for including the study (e.g., citing study limitations)? Please suggest appropriate changes.

Yes, conclusions are appropriate and accurate.

-Were all appropriate NOAELs and/or LOAELs identified for each study? If not, did the text provide adequate justification for excluding NOAELs/LOAELs includ-ing, but not limited to, citing study limitations?
Please suggest appropriate changes.

Yes, all appropriate NOAELs and/or LOAELs were identified. The uncertainty factors were adequately chosen.

-Were the appropriate statistical tests used in the studies? Would other statistical tests have been more appropriate? Were statistical test results of study data evaluated properly? NOTE: As a rule, statistical values are not reported in the text, but proper statistical analyses contribute to the reliability of the data.

Yes, all statistical tests were appropriate and results were evaluated proper-ly.

-Are you aware of other studies which may be important in evaluating the toxicity of the substance? Please provide a copy of each study and indicate where in the text each study should be included.

Brüning et al. (1999) cited in the "Addendum to the Toxicological Profile for 2,4- and 2,6 Dinitrotoluene" (see my background statement).
Toxicity - Quality of Animal Studies

In the addendum (2009), the study of Reifenrath et al. (2002) was presented about dermal absorption.
Reifenrath WG, Kammen HO, Palmer WG, Major MM, Leach GJ. Percutaneous absorption of explosives and related compounds: an empirical model of bioavailability of organic nitro compounds from soil. Toxicol Appl Pharmacol. 2002 Jul 15;182(2):160-8.

-Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.

Yes, all criteria were met.

-Were the animal species appropriate for the most significant toxicological end-point of the study? If not, which animal species would be more appropriate and why?

Yes, all species were appropriate.

-Were the conclusions drawn by the authors of the studies appropriate and accu-rately reflected in the text? If not, did the text provide adequate justification for including the study (e.g., citing study limitations)?

Yes, the conclusions drawn by the authors of the studies were appropriate and accurately reflected in the text.

-Were all appropriate NOAELs and LOAELs identified for each study? Were all appropriate toxicological effects identified for the studies? If not, please explain.

Yes, all appropriate NOAELs and/or LOAELs were identified. Review Toxicological Profile

-If appropriate, is there a discussion of the toxicities of the various forms of the substance? If not, please give examples of toxicological effects that might be im-portant for forms of the substance.

Yes, there is sufficient discussion about the toxicities of the various forms of the substance.

-Were the appropriate statistical tests used in the interpretation of the studies? If not, which statistical tests would have been more appropriate? Were statistical test results of study data evaluated properly? NOTE: As a rule, statistical values are not reported in the text, but proper statistical analyses contribute to the relia-bility of the data.

Yes, all statistical tests were appropriate and results were evaluated proper-ly.

-Are you aware of other studies that may be important in evaluating the toxicity of the substance? If you are citing a new reference, please provide a copy and indi-cate where (in the text) it should be included.

No, currently I am not aware of any other study which is of interest.

Levels of Significant Exposure (LSE) Tables and Figures

-Are the LSE tables and figures complete and self-explanatory? Does the "Users Guide" explain clearly how to use them? Are exposure levels (units, dose) accu-rately presented for the route of exposure? Please offer suggestions to improve the effectiveness of the LSE tables and figures and the "User's Guide."

I have no suggestion to improve the effectiveness of the LSE tables and fig-ures. They are complete and self-explanatory.

-Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables?

Yes, I completely agree.

-If MRLs have been derived, are the values justifiable? If no MRLs have been derived, do you agree that the data do not support such a derivation?

Yes, the derived MRLs values are justifiable.

Evaluation of Text

-Have the major limitations of the studies been adequately and accurately dis-cussed? How might discussions be changed to improve or more accurately reflect the proper interpretation of the studies?

Yes, they have been adequately and accurately discussed.

-Has the effect, or key endpoint, been critically evaluated for its relevance in both humans and animals?

Yes, they have been critically evaluated and discussed.

-Have "bottom-line" statements been made regarding the relevance of the endpoint for human health?

Yes, in an appropriate way.

-Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.

Yes, the conclusions given the overall database are appropriate.
-Has adequate attention been paid to dose-response relationships for both human and animal data? Please explain.

As far as possible, adequate attention has been paid to dose-response rela-tionships.
-Has the animal data been used to draw support for any known human effects? If so, critique the validity of the support.

Yes, they have. The used extrapolations are adequately chosen.
Section 3.4 TOXICOKINETICS

In the addendum, the following study of Jones et al. (2005) on excreted me-tabolites was presented additionally:
Jones CR, Sepai O, Liu YY, et al. 2005b. Urinary metabolites of workers exposed to nitrotoluenes. Biomarkers 10(1):10-28.

-Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.

Yes.

-Have the major organs, tissues, etc. in which the substance is stored been identified? If not, suggest ways to improve the text.

Yes.

-Have all applicable metabolic parameters been presented? Have all available pharmacokinetic/pharmacodynamic models and supporting data been presented? If not, please explain.

The models and supporting data have been presented adequately.
-Is there adequate discussion of the differences in toxicokinetics between humans and animals? What other observations should be made?

The differences in toxicokinetics between humans and animals were ade-quately discussed and respected.

-Is there an adequate discussion of the relevance of animal toxicokinetic infor-mation for humans? If not, please explain.

Yes.

Section 3.8 BIOMARKERS OF EXPOSURE AND EFFECT
In the addendum, the study by Sabbioni et al. (2006) was discussed where workers with SULT1A1, SULT1A2, NAT1, GSTT1, GSTP1 genotypes may be more susceptible to chromosome aberrations resulting from nitrotoluene exposure.
Sabbioni G, Jones CR, Sepai O, et al. 2006. Biomarkers of exposure, effect, and susceptibility in workers exposed to nitrotoluenes. Cancer Epidemiol Biomarkers Prev 15(3):559-66.

This section begins with standard language (in bold).

-Are the biomarkers of exposure specific for the substance or are they for a class of substances? If they are not specific, how would you change the text?

The mentioned biomarkers are both class- and substance-specific.

-Are there valid tests to measure the biomarker of exposure? Is this consistent with statements made in other sections of the text? If not, please indicate where inconsistencies exist.

The tests are valid as mentioned in the text.
-Are the biomarkers of effect specific for the substance or are they for a class of substances? If they are not specific, how would you change the text?

The biomarkers of effect are adequately presented and discussed.

-Are there valid tests to measure the biomarker of effect? Is this consistent with statements made in other sections of the text? If not, please indicate where inconsistencies exist.

The tests are valid as mentioned in the text.

Section 3.9 INTERACTIONS WITH OTHER CHEMICALS

Discuss the influence of other substances on the toxicity of the substance.

-Is there adequate discussion of the interactive effects with other substances?

Yes, there is adequate discussion of the interactive effects with other sub-stances.

-Does the discussion concentrate on those effects that might occur at hazardous waste sites? If not, please clarify and add additional references.

The discussion concentrates on both the general effects and the effects that might occur at hazardous waste sites.

-If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? If not, please clarify and provide any appro-priate references.

Yes.

Section 3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section begins with standard language (in bold) and identifies known or potential unusually-susceptible populations.

-Is there a discussion of populations at higher risk because of biological differ-ences which make them more susceptible? Do you agree with the choices of populations? Why or why not? Are you aware of additional studies in this area?

Yes, and I agree with the choices of populations. Brüning et al. (1999) pub-lished data about susceptibility genes in workers of the Mansfeld cohort (see also addendum 2009). The genotyping indicated that the persons with urothelial cancer were all "slow acetylators."

Section 3.11 METHODS FOR REDUCING TOXIC EFFECTS

Peak absorption:

-Is the management and treatment specific for the substance, or is it general for a class of substances?

The management and treatment is also used for other chemical substances.

-Is there any controversy associated with the treatment? Is it a "well accepted" treatment?

To my knowledge, the treatment is well accepted and part of guidelines.
-Are there any hazards associated with the treatment of populations that are unusually susceptible to the substance (e.g., infants, children)?

The hazards and contraindications are mentioned in the text.
Enhance the elimination:

-Are treatments available to prevent the specific substance from reaching the tar-get organ(s), or are the actions general for a class of substances?

The treatment is also used for other chemical substances.

-Is there any controversy associated with the treatment? Is it a "well-accepted" treatment? If the discussion concerns an experimental method, do you agree with the conceptual approach of the method?

To my knowledge, the treatment is well accepted and part of guidelines.

-Are there any hazards associated with the treatment of populations that are unu-sually susceptible to the substance (e.g., infants, children)?

The hazards and contraindications are mentioned in the text.

-Are there treatments to prevent adverse effects as the substance is being elimi-nated from the major organs/tissues where it has been stored (e.g., as a sub-stance is eliminated from adipose tissue, can we prevent adverse effects from occurring in the target organ[s])?

A comprehensive range of treatments has been discussed in the text.

Clinical or experimental methods:

-Are treatments available to prevent the specific substance from reaching the tar-get organ(s), or are the treatment's actions general for a class of substances?

A comprehensive range of treatments has been discussed in the text.
The treatment is also used for intoxications by other chemical substances.

-Is there any controversy associated with the treatment? Is it a "well accepted" treatment? If the discussion concerns an experimental method, do you agree with the conceptual approach of the method?

To my knowledge, the treatment is well accepted and part of guidelines. I agree with the conceptual approach.

-Are there any hazards associated with the treatment of populations that are unusually susceptible to the substance (e.g., infants, children)?

The hazards and contraindications are mentioned in the text.

Section 3.12 ADEQUACY OF THE DATABASE

-Do you know of other studies that may fill a data gap? If so, please provide the reference.

See Background Statement. Seidler et al. (Mansfeld)
Unfortunately, the paper is currently under review and not yet published.
Identification of Data Needs

Carefully consider the data needs because they will serve as the basis for estab-lishing a substance-specific research agenda. Data needs are discussed in Sec-tions 6.8.1, 6.8.2 and 7.3.1 as well. The following questions also pertain to both of those sections.
-Are the data needs presented in a neutral, non-judgmental fashion? Please note where the text shows bias.

Yes, the data needs are presented in a neutral, non-judgmental fashion.

-Do you agree with the identified data needs? If not, please explain your response and support your conclusions with appropriate references.

Yes, I agree. Review Toxicological Profile

-Does the text indicate whether any information on the data need exists?

No, the text does not indicate such data.

-Does the text adequately justify why further development of the data need would be desirable; or, conversely, justify the "inappropriateness" of developing the data need at present? If not, how can this justification be improved.

Yes, the text does.

CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

-Are you aware of any information or values that are wrong or missing in the chemical and physical properties tables? Please provide appropriate references for your additions or changes.

No, I am not aware of such data.

CHAPTER 5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

-Are you aware of any information that is wrong or missing? If so, please provide copies of the references and indicate where (in the text) the references should be included.

No, I am not aware of any information that it wrong or missing. It would be desirable if further information could be provided on the imported quantity of DNT in the U.S.A. The citation (EPA 1996) might be replaced by actual da-ta.

CHAPTER 6. POTENTIAL FOR HUMAN EXPOSURE
This chapter includes general statements describing the ways in which substance releases are modified by time and environmental fate processes and the potential for human exposure to the substance via the different pathways.

In the addendum, a study of Padda et al. 2003 was presented about 2,4- and 2,6-DNT and their intermediates. The results showed that both 2,4- and 2,6-DNT were stable up to 2,000 min. 4-hydroxylamino-2-nitrotoluene was the most stable of the metabolites while 2-hydroxylamino-4-nitrotoluene (a mi-nor intermediate of 2,4-DNT) and 2-hydroxylamino-6-nitrotoluene (the only intermediate of 2,6-DNT) were less stable. Both 2,4 and 2,6-

dihydroxylaminotoluene could not be tested adequately due to their lability in the presence of oxygen.
Padda RS, Wang C, Hughes JB, et al. 2003. Mutagenicity of nitroaromatic degradation compounds. Environ Toxicol Chem 22(10):2293-7.

-Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide suffi-cient and technically sound information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.

Yes, the text traced it properly. As far as I know, no other relevant data has been published yet.

-Does the text cover pertinent information relative to transport, partitioning, trans-formation, and degradation of the substance in all media? Do you know of other relevant information? Please provide references for added information.

Yes, the text covers all information about the transport, partitioning, trans-formation, and degradation of the substance in all media.

-Does the text provide information on levels monitored or estimated in the envi-ronment, including background levels? Are proper units used for each medium? Does the information include the form of the substance measured? Is there an adequate discussion of the quality of the information? Do you know of other rele-vant information? Please provide references for added information.

Yes, the text provides this information.

-Does the text describe sources and pathways of exposure for the general popu-lation and occupations involved in the handling of the substance, as well as popu-lations with potentially high exposures? Do you agree with the selection of these populations? If not, why? Which additional populations should be included in this section?

Yes, the text describes all relevant sources and pathways.

CHAPTER 7. ANALYTICAL METHODS
In the addendum 2009, several analytical methods were presented (see cited literature in my Background Statement above).

-Are you aware of additional methods that can be added to the tables? If so, please provide copies of appropriate references.

No additional methods can be added except those which are suitable out of the addendum 2009.

-Have methods been included for measuring key metabolites mentioned previous-ly in the text?

Yes, they have been included.

-If unique issues related to sampling for the substance exist, have they been ad-equately addressed in the text? What other discussion should be provided?

All issues have been adequately addressed.

CHAPTER 8. REGULATIONS AND ADVISORIES

-Are you aware of other regulations or guidelines that may be appropriate for the table? If so, please provide a copy of the reference.

No, I am not aware of any other regulations or guidelines.

CHAPTER 9. REFERENCES

The intent of this section is to provide a reasonably complete list of references, whether cited in the text or not. Every reference cited in the text should appear with an asterisk in the bibliography.

-Are there additional references that provide new data or are there better studies than those already in the text? If so, please provide a copy of each additional ref-erence.

Please find the inserted citations in the manuscript (pp. 167 and 176).
UNPUBLISHED STUDIES (IF APPLICABLE TO REVIEW)

See previously stated criteria for evaluating the quality of human and animal stud-ies.
Currently, I am involved in the publication of the results of the Mansfeld study. The case-cohort study comprised 16.441 workers of the copper min-ing industry (closed in 1990 after the German reunification), in order to fur-ther elucidate a relationship between Tg-DNT and urothelial as well as kid-ney cancer.

-For each of the unpublished studies included with the profile, prepare a brief evaluation that includes your assessment of the:

The SIR analysis of workers is submitted to Int Arch Occup Environ Health (IAOEH) and currently under review.

-Provide a summary of your conclusions? Do you agree or disagree with those of the author? If not please explain why.

The paper does not allow firm conclusions because of a large percentage of workers not exposed to DNT, incompleteness of cancer registration in the early 1990s, potential healthy worker effect, and relatively young age. A subsequent case-cohort analysis will provide further insight into a potential etiologic role of DNT in renal or urothelial cancer (this further publication is planned for the beginning of 2013).

Toxicological Profile Review for 2,4-Dinitrotolune and 2,6-Dinitrotoluene

1. Public Health Statement

This section of the Toxicology Profile is written in the format as has been done with other toxicological profiles. It is easy to read and presents relevant information in a factual manner. The answers to questions presented to the reader are clear and will adequately address likely concerns of the lay public. There are no scientific terms that need to be explained in this section.

2. Relevance to Public Health

This chapter addresses reported effects of dinitrotoluenes (DNTs) in humans as well as animals. This chapter also addresses exposure conditions, which may be of concern to humans. The effects which have only observed in animals may be of importance to humans under certain circumstances in which dose, duration of exposure, route of exposure, etc. are significant. In addition, effects seen in animals are commonly used to help to identify likely mechanisms that may exist in humans. With respect to exposure conditions, as described, they appear to cover a broad spectrum and appear to be adequate.

3. Health Effects

As indicated, the intended audience for this chapter includes community-level public health officials, physicians and concerned citizens. Although it is not intended to be a data review for toxicologists, this section provides certain basic information which will be of value to such researchers.

Section 3.1 Introduction

No changes are needed.

Section 3.2 Discussion of Health Effects by Route of Exposure

Toxicological effects are organized according to route of exposure, duration of exposure and exposure levels. Human studies are basically observed health effects in individuals who work at facilities where these compounds of interest are found. There are obviously some limitations to these kind of studies. However, with respect to assessment of human health effects, risk, etc., such studies have significant value.

Specific Points Adequacy of Human Studies

Studies concerning human health effects of DNTs are obviously more limited with respect to animal studies than is the case with other compounds. Thus, assessing a starting point for evaluation of causal relationship or mechanisms of effects in human becomes more difficult.

Nonetheless, the significant number of animal studies prevents a sound basis on which to approach health effects in man. On the other hand, there are few current studies, as opposed to old studies in animals, as well as occupation exposure assessments would be desirable.

With respect to regulations and/or exposure standards, oral MRLs have been derived for acute, intermittent and chronic exposures. In addition, NOAEL, LOAEL, PeI, TLV and RfD standards are included in the profile. In addressing human studies on DNTs, I have found no major studies which I believe have been excluded in this profile. In addressing the quality of animal studies many of the earlier studies in the literature have lacked the benefit of more current day techniques/methods. Even so, the use of animals such as rats and mice for acute and chronic studies have served as appropriate species.

Levels of Significant Exposure (LSE) Tables and Figures

NOAELS/LOAELS may not have been employed/for each study referred to in the Profile. However, a substantial number of studies indicate exposure levels that allow for interpretation employing these standards.

The tables/figures used to summarize health effects of DNTs, based on specific health effects, as well as routes and durations of exposure, and exposure levels, etc., These data are adequate and provide a convenient reference point for assessing causal relationships.

Obviously, all studies identified in the text are not included in the tables and/or figures describing significant exposure situation. This is understandable. To this point, the tables and figures addressing such exposures appear to be self explanatory and essentially complete.

With respect to classification of the terms "less serious" as opposed to "serious", such separation of effects is reasonable and therefore needed.

Where MRLs have been derived, data have been available to justify the calculations.

Evaluation of the Text

With respect to evaluation of the text, it is difficult to completely critique all studies in terms of adequate discussion. However, in most instances, key end points and "bottom line" conclusions relevant to human health issues are made and appear to be appropriate. And, wherever data have been available, adequate attention has been directed to dose response relationships, especially for animal data.

Section 3.4 Toxicokinetics

This DNT toxicological profile includes the most current major studies concerning absorption, distribution and excretion of DNTs. Studies concerning the major pathways (known as well as proposed) of metabolism of 2,4-DNT, 2,6-DNT and Tg DNT are presented in tables as well as in text form.

PBPK and PDPD models are recognized as tools that are used in risk assessment to identify safe levels of compounds for human exposure. However, in this specific case, there does not appear to be a PBPK model that has been developed for 2,4-DNT and 2,6-DNT. This chapter concerning 2,4-DNT, 2,6-DNT and Tg DNT addresses mechanisms of action, mechanisms of toxicity, as well as target organ toxicity and are discussed providing information which are helpful in toxicity assessment.

Section 3.5 Mechanisms of Action

The biological disposition component for DNTs, including their absorption, distribution and metabolism employing studies in animals are presented and appear to be adequate. Of course, not all metabolic parameters have been included, although major metabolic parameters, models and supporting data are presented.

In terms of adequacy of discussion concerning differences in toxicokinetics between humans and animals, it is well understood that differences, as well as similarities exist. Although such differences and similarities do exist, the utilization of animal toxicokinetics data for assessment of plausible untoward effects in humans is commonly employed and applicable.

Absorption processes for chemical compounds, in general, are known to involve passive and active processes. The absorption distribution and excretion of 2,4-DNT and 2,6-DNT have been studied and reported in a number of animal studies.

Section 3.5.2 Mechanisms of Toxicity

Metabolic pathways describing biotransformation products which results in both inactive as well as active intermediates are discussed in detail. Target organ toxicity as well as lack of toxic effects with respect to various organs are described.

With respect to carcinogenic effects, although mechanisms of DNT induced carcinogenicity have not been described, genotoxic studies have been conducted which suggest the potential for DNA damage. Other included studies indicate that DNTs have tumor promoting activity in liver.

Section 3.6 Toxicities Mediated Through The Neuroendocrine Axis

This section/discussion on Toxicities Mediated Though the Neuroendocrine Axis describes endocrine disruptors which are still controversial with respect to their significance on human health. The effects observed in controlled studies employing environmental compounds have been described by certain investigators as being insignificant because of required doses, magnitude of effects reversibility etc More discussion should be included in this section to address this controversy. There are current publications in the literature which address the

controversial significance of environmental endocrine disruption. Some of these studies should be considered for potential inclusion in the reference list:

Beronius, A. Ruden, C., Hakansson, H., and Hanberg,
Reproductive Toxicology
29: 132-146 (2010)

Rajender, S., Avery, K. and Agarwal, A.
Mutation Research
727:67-71 (2011)

Arnich, N, Canivenc-Lavier, M.C., et al.
International Journal of Hygiene and Environmental Health
214: 271-275 (2011)

3.8 Biomarkers

Biomarkers of exposure to 2,4-DNT and 2,6-DNT are described and include DNTs, their metabolites and certain biological markers such as methemoglobin.

4. Chemical and Physical Information

Chemical names, structures, formulas and physico-chemical properties are present in tabular form. These data, as presented in table form, appear to be complete and appropriate.

5. Production, Import/Export and Disposal

Locations in states that have manufactured or processed 2,4-DNT and 2,6-DNT are provided in several tables and have been derived from the Toxics Release Inventory (TRI10/2012). I am not aware of any wrong or missing information concerning Production, Import, Export and Disposal.

6. Potential for Human Exposure

Available data provide a complex although incomplete view of the overall potential for human exposure to DNTs. And, it is recognized that the extent of exposure pathways is not known

Thus, with respect to Potential for Human Exposure, 2,4-dinitrotoluene and 2,6-dinitrotoluene have been identified in a number of hazardous waste facilities. However, there are insufficient data addressing overall potential for human exposure from these sites with respect to exposure pathway, susceptible populations and environmental levels.

7. Analytical Methods

Standard analytical methods utilized to monitor the presence of DNTs in tissues and environmental specimen are adequately described. Methods used for detection, analysis and monitoring of DNTs and their metabolites, as well as biomarkers of exposure are described in this section. The requirement for appropriate biological specimens obtained for assessments following occupational and/or environmental exposures is discussed. Analytical methods required for monitoring levels of DNTs and their metabolites are identified and referenced. Procedures for collection and processing of environmental specimens are identified and referenced. Data tables have been included in this section, which identify levels of detection in various biological tissues.

8. Regulations and Advisories

This section is essentially complete with most available information describing MRLs, cancer classification, RfD, IDLH, PEL and ERPGs. Minimum Risk Levels have been derived for acute, intermediate and chronic duration exposures addressing various biological effects. Classification information from derived by the International Agency for Research on Cancer are included in this chapter. Workplace as well as environmental exposure standards/regulations as promulgated by OSHA, EPA and ACGIH are provided in tabular form. Addition national and international standards are also provided with respect to air/water quality and carcinogen classification

9. References

The list of references as included in this toxicological profile is very comprehensive. Most if not all of the studies, I would refer to from my personally obtained files have already been included in the reference list. One additional reference which I would like to see added is an early review describing the health effects of DNTs, and is as follows:

Tchounwou, P.B., Newsome, C., Glass, K., Centeno, J.A., Leszcynski, J., Bryant, J., Okoh, J. Ishaque, A. and Brower, M.
Review on Environmental Health
18:203-229 (2003).

Summary

This toxicological profile for 2,4-dinitrotolune and 2,6-dinitrotoluene is for the most part very well organized, comprehensive and presents a wealth of information concerning chemistry, physical properties, health effects/risk for humans as a result of exposure via inhalation, dermal and oral routes. Other information including potential for exposure, environmental distribution and animal toxicity studies are presented in a format that may be useful to health care

professionals, public health officials and researchers as well as by the general public. This is so even though the number of studies concerning effects in humans is limited.
Overall, this document has addressed a broad spectrum of human health considerations, including cancer, hepato-, respiratory, hematological, renal, dermal, gastrointestinal, ocular, cardiovascular and neurological effects.

And, descriptions of such potential health effects as a result of exposure via inhalation, oral or dermal routes are described, based on their absence or presence following acute, intermediate and chronic exposures. The presentation/interpretation of relationships between exposure to DNTs and the above considered health effects appears to be carefully presented and should provide a data base for individuals who could rely on updated accurate data base concerning toxicity of DNTs.

The Summary Tables for Toxicity Studies as presented in the Supplemental Document for DNTs allows an easy/convenient way to quickly review the content of each of the reported studies. This supplemental document might be better if there was a cross reference index that would allow the user to focus on those studies which address the specific points of interest such as acute exposures, chronic studies, type of exposure, health outcome/observed effect, etc.