

TIP No. 87-120-0121

EVALUATION OF PEER REVIEWED TOXICITY VALUES AND SUPPORTING DOCUMENTS FOR 2-AMINO-4,6-DINITROTOLUENE AND 4-AMINO-2,6-DINITROTOLUENE

PURPOSE

Evaluate the revised U.S. Environmental Protection Agency (EPA) Provisional Peer Reviewed Toxicity Values (PPRTVs) for oral exposure to 2-amino-4,6-dinitrotoluene (2-ADNT) and 4-amino-2,6-dinitrotoluene (4-ADNT) (EPA 2020a, EPA 2020b). This information paper recommends that these toxicity values be used in qualitative hazard screening but not for quantitative risk assessment. The rationale for this position is that the values were not derived directly from data for ADNTs but from a surrogate chemical used in a limited read-across approach, and there is evidence to suggest that their toxicity is less than that of their surrogate chemical, 4,6-trinitrotoluene (TNT).

REFERENCES

See Appendix A for a list of the references cited in this report.

POINTS OF MAJOR INTEREST AND FACTS

Background

The EPA Office of Research and Development (ORD) Center for Public Health and Environmental Assessment (CPHEA) develops PPRTVs for the EPA Superfund program. PPRTVs are updated every 5 years to incorporate new data or methodologies. In June 2020, the EPA published PPRTV documents for 2-ADNT and 4-ADNT, collectively called ADNTs (EPA 2020a, EPA 2020b). Because there were insufficient data to derive PPRTVs directly, the EPA instead used TNT as a surrogate and published “screening-level PPRTVs.” A review of these values was conducted in order to determine if 1) they were supported by the available data, 2) they should be considered Tier 3 values per U.S. Army Public Health Center (APHC) Technical Guide (TG) 373 standards (APHC 2020) and therefore usable to quantify health risk, and 3) they can be used for Army-site 5-year reviews.

Environmental degradation of TNT is the main source of ADNTs, which can be produced by bacteria found in wet soils or sediments (via microbial reductases) (Johnson 2015). ADNTs may also be produced under anaerobic or aerobic conditions; in both cases, the process is reductive (Esteve-Núñez et al. 2001). ADNTs can irreversibly bind to the organic (humic) fractions of the soil (Johnson and Reddy 2015) which can reduce their bioavailability (Thorn and Kennedy 2002), but studies on the absorption or bioavailability of ADNTs from soil have not been conducted. Certain chemical/physical properties of the ADNTs (e.g., water solubility) differ from those of TNT, implying differences in toxicity. A few animal studies have shown that acute toxicity is lower for ADNTs compared to TNT. Additionally, ecotoxicological bioassays show significant differences in toxicity between TNT and the ADNTs (Neuwoehner et al. 2007).

Provisional Peer Reviewed Toxicity Values (PPRTVs) for 2-ADNT and 4-ADNT

For both 2- and 4-ADNT, the EPA stated “it is inappropriate to derive provisional toxicity values because of a paucity of chemical-specific information” (EPA 2020a, EPA 2020b). The EPA considers these screening PPRTVs as Tier 3 values in a hierarchy that includes Minimum Response Levels (MRLs) published by the Agency for Toxic Substance Disease Registry (ATSDR) and values published by the California EPA (EPA 2013). The overall hierarchy is Integrated Risk Information System (IRIS) (Tier 1), PPRTVs (Tier 2), and ATSDR, CalEPA, screening PPRTVs, and Health Effects Assessment Summary Tables (HEAST) (Tier 3) (EPA 2013).

In the absence of primary studies for ADNTs, the EPA applied a read-across approach to both cancer and non-cancer evaluations, using surrogate chemicals and their toxicity summaries for comparison with ADNTs. This approach relied on a range of structural, metabolic/toxicokinetic, and toxicity similarities to compare a range of preselected chemicals, concluding with a final expert decision on the best surrogate for both ADNTs. TNT was identified as the most appropriate chemical on which to base subchronic and chronic PPRTVs for both 2-ADNT and 4-ADNT. TNT was also used as a surrogate for potential carcinogenic effects of ADNTs. TNT was primarily chosen based on “metabolic similarity, structural similarity, and shared metabolites” (EPA 2020a, EPA 2020b).

Other important data included an Army-funded, single-dose, radiolabeled study that used rats, mice, rabbits, and dogs to examine nitrotoluenes, including TNT and ADNTs (USAMRDC 1980). This study showed that the majority of ingested TNT was excreted within 24 hours, and it also provided acute 50% Lethal Dose (LD₅₀) values for both ADNTs for mice and rats (Table 1). A range of bacterial and mammalian cell genotoxicity studies were also available for review (EPA 2020a, EPA 2020b).

Table 1. Comparison between TNT and ADNTs

Compound	Rat LD ₅₀ (mg/kg)	Mouse LD ₅₀ (mg/kg)	In vivo carcinogenicity	Slope Factor (mg/kg-d) ⁻¹
TNT	607	660	Yes (C)*	3.0 x 10 ⁻²
4-ADNT	959	1318	NA	No
2-ADNT	1394	1522	NA	No

*Classification C = possible human carcinogen

Legend:

mg/kg = milligrams per kilogram

mg/kg-d = milligrams per kilogram per day

Oral Non-Cancer Toxicity Values (pRfD_o)

As defined by the EPA, a chronic provisional reference dose (pRfD), representing a PPRTV, is an estimate of a daily oral exposure to a chemical that is not likely to cause adverse health effects during a lifetime of exposure (EPA 1993). Most pRfD estimates are based on animal data extrapolated to humans and are considered accurate to within an order of magnitude. A

pRfD is typically conservative and is intended to be protective of the general population, including sensitive subgroups (EPA 1993). Table 2 lists the non-cancer screening PPRTV values for 2- and 4-ADNT (EPA 2020a, EPA 2020b).

Table 2. Non-Cancer Screening PPRTV Values for 2-ADNT and 4-ADNT

Type	Effect	POD (mg/kg-d)	UF _c	pRfDo* (mg/kg-d)	Previous Value (mg/kg-d)
Screening <u>sub-chronic</u> provisional reference dose (p-RfDo)	Mild hepatocyte swelling	0.3 (surrogate TNT in dogs)	1000	3.0 x 10 ⁻⁴	NA
Screening <u>chronic</u> Provisional reference dose (p-RfDo)	Mild hepatocyte swelling	0.3 (surrogate TNT in dogs)	3000	1.0 x 10 ⁻⁴	2.0 x 10 ⁻³

*values are the same for 2-ADNT and 4-ADNT

Legend:

POD = point of departure

UF = uncertainty factor

Alternative approaches are used in the development of PPRTVs when available toxicity data do not meet the criteria for deriving pRfDs or no data are available. The results of such alternative approaches are not included in the main body of the PPRTV document but are attached as an appendix, indicating increased uncertainty and lower confidence in the derived numbers, subsequently termed “screening PPRTVs” and labeled “(X)” to distinguish them from full PPRTVs “(P)” in the Regional Screening Level tables (EPA 2020c). The values are assessed in the Evaluation section that follows.

Carcinogenicity

Since no human or animal cancer studies were available, the EPA chose “Inadequate Information to Assess Carcinogenic Potential” as the cancer descriptor. The EPA further evaluated the genotoxicity data using selected compounds for comparison, including TNT, and added a second descriptor, “concern for potential carcinogenicity,” to the cancer assessment in Appendix C of the PPRTVs. Whether this decision was arbitrary or based on CPHEA policy is unclear. Urinary bladder papilloma and carcinoma have been observed in female Fischer 344 rats exposed to TNT (EPA 1988).

Genotoxicity studies show that while bacterial assays generally show positive results for both ADNTs, two (of two) mammalian cell genotoxicity assays were negative for 2-ADNT and one (of two) was positive for 4-ADNT (EPA 2020a, EPA 2020b). On closer examination, the single positive result for 4-ADNT stated that “clear dose-response relationships could not be

established for the mutagenic response of these compounds. They are considered as very weak mutagens in this mammalian test system” (Kennel et al. 2000).

EVALUATION

Screening Subchronic Provisional Reference Dose: In the read-across approach for derivation of the revised reference dose, TNT was selected as the most appropriate surrogate. For 2-ADNT, the critical effect came from a TNT subchronic-duration dog feeding study where trace to mild hepatocyte swelling was observed; the lowest-observed-adverse-effect level (LOAEL) of 0.5 mg/kg-day was used as the point of departure (POD) and a dosimetric adjustment factor (DAF) of 0.64 was applied to attain a POD of 0.3 mg/kg-day. Uncertainty factors (UFs) of 10 for UF_H of 10, UF_D of 10, UF_A of 3, and UF_L of 3 formed a composite UF of 1000 with a resulting screening p-RfDo of 3.0×10^{-4} mg/kg-day. For 4-ADNT, again using TNT with the same critical effect and UFs, an identical p-RfDo of 3.0×10^{-4} mg/kg-day was derived.

Screening Chronic Provisional Reference Dose: The EPA again selected TNT as the appropriate surrogate, concluding that methemoglobinemia (and its downstream hepatic and splenic effects) was the critical effect. However, the incidence of methemoglobinemia did not increase in occurrence or severity with chronic exposures for TNT. For this reason, a 3-fold UF was used for subchronic to chronic extrapolation. Using the same POD of 0.3 mg/kg-day and a composite uncertainty factor of 3000, the screening chronic p-RfDo dose was 1.0×10^{-4} mg/kg-day.

Carcinogenicity: Given the lack of available studies, the EPA issued the cancer descriptor “inadequate information to assess carcinogenic potential” for 2-ADNT and 4-ADNT. In addition, the EPA performed a screening evaluation of carcinogenicity (EPA 2020a, EPA 2020b) by comparing analogous chemicals including TNT, 2-methyl-5-nitroaniline (urinary metabolite of 2,4-DNT), and 2,6-DNT and 2,4-DNT. Automated tools were used to compare these surrogates in a read-across approach found in Appendix B of each ADNT document. The conclusion for both ADNTs was “Concern for Potential Carcinogenicity” based on the bladder or liver cancer of the surrogates in animal studies. However, there was no common mode of action between surrogates. It is not known how this conclusion should be interpreted or if it indicates a higher alert level than the standard cancer descriptor.

Genotoxicity: The EPA used computational tools to identify genotoxicity and carcinogenicity for ADNTs and their analogue compounds, with heat maps to examine consistency across compounds for a range of outcomes (structural alerts, SAR) (see Table C4 in ADNT documents). For both ADNTs and their analogues, the EPA concludes that there is “substantial evidence of genotoxicity” even though there is a lack of mode and/or mechanism of action (MOA) for any of the analogue compounds. However, while the bacterial assays are largely positive, the mammalian cell assays are mostly negative, and the one positive finding is possibly a false positive. The overall EPA cancer assessment is that there are “limited and inconsistent” findings for mutagenicity in mammalian cells (EPA 2020a, EPA 2020b).

TNT Comparison: While TNT can be reduced to 2-ADNT and 4-ADNT, effects observed after exposure to TNT cannot be directly attributed to 2-ADNT or 4-ADNT. For example, whereas the

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octanol/water partition coefficient of the ADNTs is similar to that of TNT, the water solubility of TNT differs from that of ADNTs by an order of magnitude, which could explain why median LD₅₀ values are consistently lower for TNT (more toxic) than ADNTs (EPA 2020, Table A3). Further, ecotoxicity bioassays suggest significant lower toxicity for ADNTs versus TNT (Frische and Höper 2002, Neuwoehner et al. 2007).

Read-Across: For the read-across approach, the EPA relied on similarity of effects with respect to chemical structure, metabolism, and toxicity (Wang et al. 2012). More recently, expansive criteria that also include bioavailability, solubility, and other chemical properties that impact the toxicology have been recommended (Schultz et al. 2015). With respect to ADNTs, other factors involved in toxicokinetics (i.e., absorption, distribution, and possibly excretion) are likely to differ and play a significant role in affecting the dose that causes toxicity.

Tier 3 Values: Based on the criteria published in TG 373 (APHC 2020), the "screening PPRTVs" for both ADNTs should not be considered as Tier 3 values. This guidance is in contrast to the EPA (2013), which considers such values as Tier 3 based on different criteria.

Prior Screening Values: The previous PPRTV for both 2-ADNT and 4-ADNT was 2.0×10^{-3} mg/kg-day (see Table 2). Since no new information was available to develop revised toxicity values, the new screening PPRTVs were the result of the application of new methodologies such as the read-across approach, the selection of TNT as a surrogate for development of non-cancer values, and the comparative heat-map approach used to validate computational approaches across surrogate chemicals in the cancer assessment.

CONCLUSIONS

There is an absence of direct observational or experimental evidence to estimate the repeated-dose oral toxicity of 2-ADNT and 4-ADNT. Therefore, the EPA relied on the toxic effects of surrogate chemicals to reassess the toxicity values for 2-ADNT and 4-ADNT. The revised values represent new analysis methodologies rather than new data and are considered "screening PPRTVs." There is an order of magnitude difference between the new, lower values and the old values.

The toxicity of ADNTs was imputed from TNT. There is little information on the absorption, distribution, metabolism, and excretion of ADNTs. Considerable differences exist in the water solubility of ADNTs compared to TNT, reflected in the significant differences in the magnitude of median lethal doses in laboratory rodents. Therefore, consistent with this information is the conclusion that TNT is likely more toxic than ADNTs.

The qualitative descriptor of "Insufficient Data" for cancer assessment included an additional statement in Appendix C that indicated "concern for potential carcinogenicity" based on surrogate comparisons. No clear explanation was given for this novel statement, how it relates to the EPA cancer guidelines, or how it should be used in qualitative risk assessments.

Based on the above evaluation, the PPRTV screening values for ADNTs are not considered by APHC to be Tier 3 values usable for quantitative risk assessment. Per TG 373 (APHC 2020), in

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lieu of the availability of properly derived values for quantifying risk, project risk assessment teams can use the PPRTV screening values to qualitatively describe—but not quantify—the risk during the risk characterization phase of the health risk assessment.

POINT OF CONTACT FOR FURTHER INFORMATION

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APPENDIX A

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