1,3,5-Trinitrobenzene; CASRN 99-35-4

Human health assessment information on a chemical substance is included in the IRIS database only after a comprehensive review of toxicity data, as outlined in the IRIS assessment development process. Sections I (Health Hazard Assessments for Noncarcinogenic Effects) and II (Carcinogenicity Assessment for Lifetime Exposure) present the conclusions that were reached during the assessment development process. Supporting information and explanations of the methods used to derive the values given in IRIS are provided in the guidance documents located on the IRIS website.

STATUS OF DATA FOR 1,3,5-Trinitrobenzene

File First On-Line 09/07/1988

Category (section)	Assessment Available?	Last Revised
Oral RfD (I.A.)	yes	10/01/1997
Inhalation RfC (I.B.)	not evaluated	
Carcinogenicity Assessment (II.)	not evaluated	

I. Chronic Health Hazard Assessments for Noncarcinogenic Effects

I.A. Reference Dose for Chronic Oral Exposure (RfD)

Substance Name — 1,3,5-Trinitrobenzene
CASRN — 99-35-4
Last Revised — 10/01/1997

The oral Reference Dose (RfD) is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Please refer to the Background Document for an elaboration of these concepts. RfDs can also be derived for the noncarcinogenic health effects of substances that are also carcinogens. Therefore, it is

essential to refer to other sources of information concerning the carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

I.A.1. Oral RfD Summary

Critical Dose -- 2.68 mg/kg-day
UF -- 100
MF -- 1
RfD -- 3E-2 mg/kg-day

*****Critical Study *****

Critical Effect -- Methemoglobinemia and spleen-erythroid cell hyperplasia

Study Type -- Rat 2-Year Dietary Study

Reference -- Reddy et al., 1996, 1997

NOAEL -- 2.68 mg/kg-day

LOAEL -- 13.31 mg/kg-day

Conversion Factors and Assumptions -- Based on food consumption data, the authors calculated the intake of TNB from dietary concentrations of 0, 5, 60 and 300 ppm: 0, 0.23, 2.68 and 13.31 mg/kg/day (females) and 0, 0.22, 2.64 and 13.44 mg/kg/day (males).

I.A.2. Principal and Supporting Studies (Oral RfD)

Reddy, T.V., F.B. Daniel, G.R. Olson, B. Wiechman and G. Reddy. 1996. Chronic toxicity studies of 1,3,5-trinitrobenzene in Fischer 344 rats. U.S. Army, Fort Detrick, MD. (Final Report)

Reddy, G., T.V. Reddy, H. Choudhury, F.B. Daniel and G. Leach. 1997. Assessments of environmental Hazards of 1,3,5-trinitrobenzene (TNB). J. Toxicol. Environ. Health 52:101-114.

Chronic toxic effects of 1,3,5-TNB in male and female Fisher 344 rats were evaluated by feeding powdered certified laboratory chow diet supplemented with varied concentrations of

TNB for 2 years. Based on food consumption, the average TNB intake was calculated for both males and females.

The study was conducted in accordance with the U.S. EPA guidelines for chronic toxicity studies as required by the GLP standards. One of the unique features of this study is that 10 animals/sex were sacrificed at the end of 90 days, 6 months and 1 year, and 25 or more rats were sacrificed at 2 years; complete toxicological evaluations were performed during these periods.

High-dose animals showed decreased body weight gains associated with decreased food consumption. Relative organ weight changes for the brain (increase), spleen (increase), liver (increase) and testes (decrease in 90- and 180-day periods) were reported for all treated animals dosed with TNB at levels higher than 3 mg/kg/day; adverse hematological findings (decreased hematocrit and hemoglobin) and increased methemoglobulin) were consistently reported in all animals treated at these levels. Histopathological findings in the 1-year study revealed extramedullary hematopoiesis in rats treated with TNB at doses of 3 mg/kg-day or higher. In the 2-year study, these effects were seen only in rats dosed with TNB at the high dosage level (13.23 mg/kg/day). The adverse effects, such as increased methemoglobin, erythroid cell hyperplasia, and increased relative organ weights, observed during interim sacrifices in rats receiving 60 ppm TNB did not persist and were not detected in rats fed 60 ppm TNB for 2 years, suggesting that an adaptive mechanism has taken place in order to compensate adverse effects observed during interim sacrifices.

Results of this study exhibited clear evidence of toxicity of the hematopoietic system as has been reported for other nitroaroniatics such as, dinitrobenzene and trinitrotoluene. The NOAEL for this study is 2.68 mg/kg/day and the LOAEL for hematological effects is 13.31 mg/kg/day.

I.A.3. Uncertainty and Modifying Factors (Oral RfD)

UF — 100. Ten for inter- and 10 for intra-species extrapolation. The previous RfD developed in 1988 was based on analogy to dinitrobenzene with a UF of 10,000. Subsequently, several subchronic, chronic, reproductive and developmental toxicity studies were conducted in rats and mice through the collaborative efforts of the U.S. EPA and the U.S. Army; thus reducing uncertainties in the studies and database for TNB. The data in these studies provided adequate evidence that rats were more sensitive than mice in terms of both systemic and developmental toxicity. The RfD, based on 2-year oral toxicity study, is supported by additional subchronic studies in mice and rats, reproductive, developmental and single-generation studies where adverse effects were observed at doses higher than that reported in the 2-year study; thus precluding uncertainties in database.

MF — None.

I.A.4. Additional Studies/Comments (Oral RfD)

Male and female Fisher 344 rats were evaluated by feeding certified laboratory chow diets containing different doses of TNB for 90 days. The estimated doses of TNB were 0, 4, 23 and 44 mg/kg/day for males and 0, 4, 25 and 49 mg/kg/day for females (Reddy et al., 1994a,b).

Toxicological evaluation of data indicated the following adverse effects: (a) increased relative spleen and brain weights and decreased testis weight in rats dosed with 23-49 mg/kg/day TNB. Liver weights were increased in the high-dose animals; (b) decreased RBC, hematocrit, and serum alkaline phosphatase and increased methemoglobulin levels in the mid- and high-dose groups: (c) Histopathological examinations showed extramedullary hematopoiesis and testicular degeneration in the mid- and high-dose groups. The data indicated a NOAEL of 4 mg/kg/day and a LOAEL of 23 mg/kg/day for hematopoietic effects (methemoglobulinemia) and testicular degeneration. Hyaline droplets were observed in all dosed male animals but the clinical significance of this lesion cannot be ascertained in terms of human renal effects.

Kinkead et al. (1994, 1995) in their 90-day reproductive toxicity study dosed male and female Sprague-Dawley rats for 14 days prior to mating, and continued through a total of a 90-day dosing period. One group of male rats was treated with the high dose for 90 days followed by a 60-day recovery period to ascertain the reversibility of the testicular toxicity. The dosing regimen for females included premating, gestation and lactation and 4 weeks post-weaning; thus achieving a 90-day total exposure period. The consumed doses of TNB were 2, 9 and 19 mg/kg/day for males and 3, 14 and 29 mg/kg/day for females.

Toxicological evaluation of data indicated adverse effects on the spleen and testis. The high dose males showed testicular degeneration during the 90-day exposure, which continued through the recovery period. Increased methemoglobin levels and hemosiderosis were observed in all rats exposed to 10 mg/kg/day or higher doses of TNB. This study indicated 3 mg/kg/day as the NOAEL and 10 mg/kg/day as the LOAEL for hematological (spleen) effects.

In the single generation study (Kinkead et al., 1994), male and female Sprague-Dawley rats were dosed with TNB during the 14-day premating period and continuing through the end of lactation. The target administered doses were 0, 2, 23 and 51 mg/kg/day for males and 0, 3, 30 and 60 mg/kg/day for females.

In this study, both mid- and high-dose males showed testicular degeneration; high-dose females showed CNS toxicity; and neonatal mortality were observed during lactation period in pups born to high-dose animals.

This study indicated a NOAEL of 3 mg/kg/day for females and a LOAEL of 23 mg/kg/day for testicular degeneration.

In the rat developmental toxicity study (Cooper and Caldwell, 1995) pregnant female Sprague-Dawley rats were gavaged daily with 0, 11.25, 22.50, 45.0 and 90.0 mg/kg-day TNB during 20 days of their gestation period. Complete morphological and microscopic examinations of fetuses on gestation day 20 was performed; material toxicological evaluations were also conducted. This study indicated maternal toxicity and skeletal abnormalities and decreased fetal body weight in the high dosage group, resulting in a NOAEL of 45 mg/kg/day and LOAEL of 90 mg/kg/day.

A subchronic toxicity study was conducted in the White-footed mouse dosed with 0, 150, 375 and 750 mg/kg diet. The calculated doses were 0, 20, 65 and 108 mg/kg/day (females) and 0, 23.5, 67.4 and 113.50 mg/kg/day (males) of TNB during a 90-day period.

Results of this study indicated erythroid cell hyperplasia (spleen), increased reticulocytes, increased relative spleen weight, and testicular degeneration in mice exposed to high dose TNB; no other toxicological effects were observed in this study; thus the White-footed mouse was considered to be less sensitive to TNB exposure (Reddy et al., 1995).

I.A.5. Confidence in the Oral RfD

Study — High
Database — Medium
RfD — Medium

The RfD is based on a well-conducted 2-year study that includes interim sacrifices at 3, 6 and 12 months and is supported by subchronic reproductive and developmental toxicity data in rats and subchronic data in mice. High confidence is recommended for the study. The database contains adequate subchronic studies in rats and mice, reproductive, developmental and chronic studies in rat and lacks additional developmental studies in other species. Medium confidence is therefore recommended for the database and the RfD.

I.A.6. EPA Documentation and Review of the Oral RfD

Source Document — U.S. EPA, 1997

This assessment was peer reviewed by external scientists. These comments have been evaluated and incorporated in the finalization of this IRIS Summary. A record of these comments is included as an appendix to U.S. EPA, 1997.

Other EPA Documentation — U.S. EPA, 1989

Agency Work Group Review — 03/24/1988, 04/20/1988, 05/26/1988

Verification Date — 08/27/1997

Screening-Level Literature Review Findings — A screening-level review conducted by an EPA contractor of the more recent toxicology literature pertinent to the RfD for 1,3,5-Trinitrobenzene conducted in August 2003 did not identify any critical new studies. IRIS users who know of important new studies may provide that information to the IRIS Hotline at hotline.iris@epa.gov or 202-566-1676.

I.A.7. EPA Contacts (Oral RfD)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (FAX), or hotline.iris@epa.gov (internet address).

I.B. Reference Concentration for Chronic Inhalation Exposure (RfC)

Substance Name — 1,3,5-Trinitrobenzene
CASRN — 99-35-4

Not available at this time.

II. Carcinogenicity Assessment for Lifetime Exposure

Substance Name — 1,3,5-Trinitrobenzene
CASRN — 99-35-4

Not available at this time.

III. [reserved]
IV. [reserved]
V. [reserved]

VI. Bibliography

Substance Name — 1,3,5-Trinitrobenzene
CASRN — 99-35-4

VI.A. Oral RfD References

Cooper, K.R. and D.J. Caldwell. 1995. Developmental toxicity evaluation of 1,3,5-trinitrobenzene in Sprague-Dawley rats. Final Report, U.S. Army, Wright-Patterson AFB, OH.

Kinkead, E.R., R.E. Wolfe, C.D. Flemming, D.J. Caldwell, C.R. Miller and G.B. Marit. 1994. Reproductive toxicity screen of 1,3,5-trinitrobenzene administered in the diet of Sprague-Dawley rats. Al/OE-TR-1994-0144, WRAIR-TR-1994-0016. U.S. Army, Wright-Patterson AFB, OH.

Kinkead, E.R., R.E. Wolfe, C.D. Flemming, D.J. Caldwell, C.R. Miller and G.B. Marit. 1995. Reproductive toxicity screen of 1,3,5-trinitrobenzene administered in the diet of Sprague-Dawley rats. Toxicol. Ind. Health. 11: 309-323.

Reddy, T.V., F.B. Daniel, M. Robinson, G.R. Olson, B. Wiechman and G. Reddy. 1994a. Subchronic toxicity studies on 1,3,5-trinitrobenzene, 1,3-dinitrobenzene and tetryl in rats: Subchronic toxicity evaluation of 1,3,5-trinitrobenzene in Fischer 344 rats. ADA 283663. U.S. Army Project Order MIPR No. 92MM2525. U.S. Environmental Protection Agency, Cincinnati, OH.

Reddy, T.V., J.A. Torsella, F.B. Daniel, G.R. Olson, B. Wiechman and G. Reddy. 1994b. Subchronic toxicity evaluation of 1,3,5-trinitrobenzene (TNB) in Fischer 344 rats. Toxicologist. 14(1): 117. (Abstract)

Reddy, T.V., J. Torsell, F.B. Daniel, G.R. Olson, B. Wiechman and G. Reddy. 1995. Ninety-day Toxicity Evaluation of 1,3,5-Trinitrobenzene (TNB) in Peromyscus leucopus. Second Society of Environmental Toxicology and Chemistry World Congress. November 5-9, 1995, Vancouver, British Columbia, Canada, (Abstract), p. 189.

Reddy, T.V., F.B. Daniel, G.R. Olson, B. Wiechman and G. Reddy. 1996. Chronic toxicity studies of 1,3,5-trinitrobenzene in Fischer 344 rats. Final Report, U.S. Army, Fort Detrick, MD.

Reddy G., T.V. Reddy, H. Choudhury, F.B. Daniel and G. Leach. 1997. Assessment of environmental Hazards of 1,3,5-trinitrobenzene (TNB). J. Toxicol. Environ. Health 52:101-114.

U.S. EPA. 1989. Health and Environmental Effects Document for Trinitrobenzenes (o-, m-, p-). Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC.

U.S. EPA, 1997. Support Document for Trinitrobenzene. National Center for Environmental Assessment, Cincinnati, OH. Contact the IRIS Hotline at (202)566-1676 (phone), (202)566-1749 (FAX), or hotline.iris@epa.gov (internet address).

VI.B. Inhalation RfC References

None

VI.C. Carcinogenicity Assessment References

None

VII. Revision History

Substance Name — 1,3,5-Trinitrobenzene
CASRN — 99-35-4

Date	Section	Description
09/07/1988	I.A.	Oral RfD summary on-line
10/01/1997	I.A.	Oral RfD summary replaced; new assessment
10/28/2003	I.A.6	Screening-Level Literature Review Findings message has been added.

VIII. Synonyms

Substance Name — 1,3,5-Trinitrobenzene
CASRN — 99-35-4
Last Revised — 09/07/1988

- 99-35-4
- BENZENE, 1,3,5-TRINITRO-
- RCRA WASTE NUMBER U234
- TNB
- TRINITROBENZEEN
- TRINITROBENZENE
- 1,3,5-Trinitrobenzene
- Trinitrobenzene, 1,3,5-
- TRINITROBENZOL