



# Reproductive Toxicity of TNX in Laboratory Deer Mice

## Overview

The Environmental Protection Agency (EPA) has classified Royal Demolition Explosive (RDX) as a possible human carcinogen (C). The carcinogenic mechanism of RDX is unknown and could be due to nitroso compounds (MNX, DNX, & TNX) formed via anaerobic transformation of RDX. There is little known of the toxicity of these nitroso metabolites. In order to further our understanding of the nature of these compounds, this project utilizes molecular genetic techniques as a means of detecting potential genetic and other toxicological effects.

## Environmental Issue

RDX has been used as an explosive for both military purposes and civilian applications worldwide. Environmental explosive contamination is estimated to be located at more than 1,200 sites in the United States (Schmelling *et al.* 1997) and over 2,000 sites in Europe (Held *et al.* 1997). Using anaerobic sludge, RDX has been shown to biodegrade progressively to into the following nitroso intermediates: MNX, DNX, and TNX (hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine; hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine; and hexahydro-1,3,5-trinitroso-1,3,5-triazine respectively) (Figure 1) (McCormick *et al.* 1981). Nitroso compounds are known carcinogens via first bioactivation of the compound through hydrololation, then carcinogenicity through alkylation of general macromolecules (Kotsonis *et al.* 2001). In addition, these nitroso compounds have been found in groundwater beneath the Iowa Army Ammunition Plant (Beller and Tiemeier 2002). MNX was the most abundant compound at concentrations up to 430 µg/l.

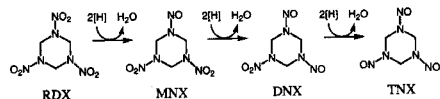


Figure 1. Anaerobic degradation of RDX

## Scientific Approach

In order to further our understanding of the nature of these compounds, this project utilizes molecular genetic techniques as a means of detecting potential genetic and other toxicological effects. In a preliminary study, breeding pairs of laboratory deer mice were exposed to TNX *ad libitum* via drinking water in four doses-100 µg/L, 10 µg/L, 1 µg/L, and control (0 µg/L) (Figure 2). Paired mice bred and produced three litters of offspring. Effects of exposure to TNX are being measured using the following endpoints: reproductive success, offspring survival, offspring weight gain, organ weights, and microsatellite mutation rate. A follow up multigenerational study is currently being planned, pending results from the first study.



Figure 2. Breeding Pair of Deer Mice and Dosing Cages of Deer Mice

## Preliminary Results

Preliminary results from the first study show a dose response in the growth period from birth to post natal day 21 (Figure 3). These results have yet to be statistically analyzed. Overall mortality rate was increased in all dose groups for all litters when compared to the control. When using a chi square analysis ( $\alpha = 0.05$ ) to evaluate statistical significance, F1A overall mortality ( $\chi^2 \sim 11.4$ ,  $P \sim 0.001-0.01$ ), F1A birth to post natal day 4 mortality ( $\chi^2 \sim 8.5$ ,  $P \sim .02-.05$ ), F1B overall mortality ( $\chi^2 \sim 11.0$ ,  $P \sim 0.01-0.02$ ), and F1B birth mortality ( $\chi^2 \sim 17.3$ ,  $P \sim 0.0001-0.001$ ) showed a 'not independent' relationship among dose groups. This could be due to a limited sample size and thus limited statistical power. The microsatellite analysis is currently underway, and will hopefully shed some light on the genotoxic potential of these compounds.

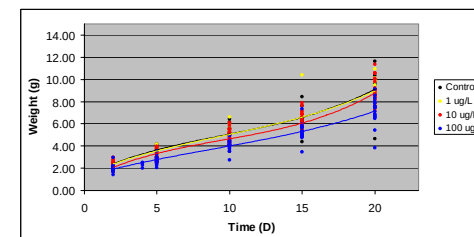


Figure 3. F1C Litter Growth Curves

## Impact

Results could provide insight on potential teratogenic, reproductive, and mutagenic effects of RDX nitroso metabolites. These results could also play an important role in future policy decisions for remediation of contaminated sites.

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# Toxicological Effects of Methylmercury on Fishes in Inland Lakes of Isle Royale National Park

## Environmental Issue

- Isle Royale National Park (ISRO) is contaminated with mercury (Hg)
  - atmospheric deposition of Hg from human sources is contaminating the remote wilderness landscape of ISRO
  - the State of Michigan has issued a fish consumption advisory for six inland lakes
- Reproduction of fishes in the inland lakes of ISRO may be impaired due to Hg
  - laboratory studies have documented decreased spawning success, delayed spawning, reduced fecundity, suppressed hormone levels, and altered reproductive behavior in fish with mercury concentrations as low as  $1.0 \mu\text{g}\cdot\text{g}^{-1}$  wet weight
  - $1.0 \mu\text{g}\cdot\text{g}^{-1}$  wet weight is not an uncommon concentration of Hg in piscivorous (fish-eating) fishes in inland lakes of ISRO
- “Every effort should be made to protect Isle Royale’s fish fauna” (Kallemeyn 2000)
  - the inland lakes of ISRO contain ecologically and culturally important fish communities
  - elevated concentrations of Hg in fish from the inland lakes is of great concern because ISRO has been designated as an International Biosphere Reserve, serving as a reference ecosystem that has been minimally affected by pollution

## Scientific Approach

- Hypothesis: Reproduction of piscivorous fishes is impaired in inland lakes with elevated concentrations of Hg at ISRO
- Research Plan:
  - Determine the mechanisms responsible for impaired reproduction in fish due to Hg
    - novel techniques will be used to determine the action of Hg on the reproductive system of laboratory-exposed fish
    - zebrafish (*Danio rerio*) will be used as the model fish species
    - methylmercury will be used as the chemical form of Hg
  - Develop biomarkers based on mechanisms
    - it is difficult to quantify the reproductive success of wild fish
    - biomarkers are useful because they provide quantifiable measures of physiological changes due to contaminants
  - Assess the reproduction of piscivorous fishes in inland lakes of ISRO with biomarkers
    - reproduction of adult northern pike (*Esox lucius*) and yellow perch (*Perca flavescens*) will be assessed in lakes that span a gradient of Hg contamination within the Park



Isle Royale National Park is a remote island archipelago located in Lake Superior, Michigan, USA

## Impact

- ISRO ecosystem
  - information gained from this study will assist the U.S. National Park Service in assessing the relative effects of Hg on year-class strengths of piscivorous fishes in inland lakes
- Other ecosystems
  - Hg contamination is a global environmental problem
  - this problem is magnified in ecosystems that are “mercury sensitive”, such as boreal ecosystems like ISRO
  - fish consumption advisories are common for lakes in boreal regions of Canada, Scandinavia, and the United States
  - information from this study will also help assess the effects of Hg on fish in these ecosystems

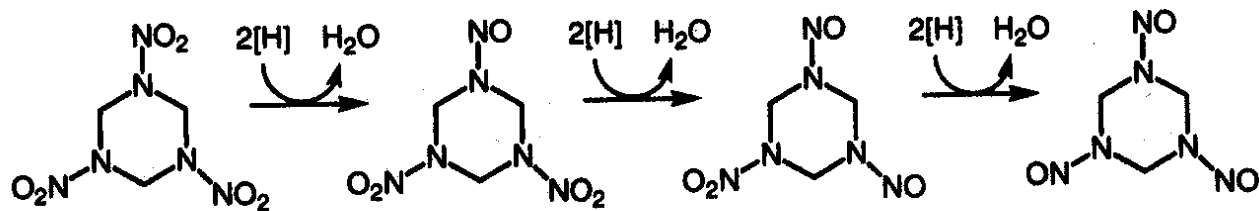


Figure 2. RDX Reducing Pair of Deer Mice

DNX Figure 3. Dosing Cages of Deer Mice

