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004 EPA STAR Graduate Fellowship Conference *Next Generation Scientists—Next Opportunities*

Reproductive Toxicity of TNX in Laboratory Deer Mice

verview

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

nvironmental Issue

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

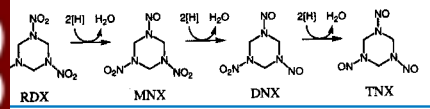

Figure 1. Anaerobic degradation of RDX

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 (χ^2~11.4, P~0.001-0.01), F1A birth to post natal day 4 mortality (χ^2~8.5, P~.02-.05), F1B overall mortality (χ^2~11.0, P~0.01-0.02), and F1B birth mortality (χ^2~17.3, P~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.

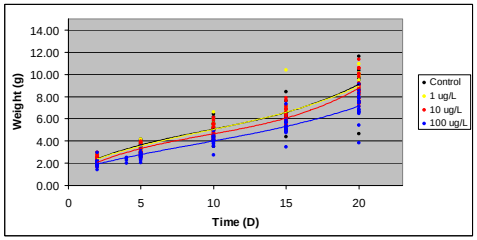

Figure 3. F1C Litter Growth Curves

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

nvironmental Issue

le Royale National Park (ISRO) is ntaminated with mercury (Hg)

nospheric deposition of Hg from human sources is taminating the remote wilderness landscape of ISRO
e State of Michigan has issued a fish consumption advisory six inland lakes

eproduction of fishes in the inland lakes ISRO may be impaired due to Hg

oratory studies have documented decreased spawning cess, delayed spawning, reduced fecundity, suppressed mone levels, and altered reproductive behavior in fish with rcury concentrations as low as 1.0 µg•g^{-1} wet weight
µg•g^{-1} wet weight is not an uncommon concentration of Hg iscivorous (fish-eating) fishes in inland lakes of ISRO

Every effort should be made to protect Isle yale's fish fauna" (Kallemeyn 2000)

inland lakes of ISRO contain ecologically and culturally ortant fish communities
vated concentrations of Hg in fish from the inland lakes is of at concern because ISRO has been designated as an rnational Biosphere Reserve, serving as a reference system 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

Citation: Kallemeyn, L.W. 2000. A comparison of fish communities from 32 inland lakes in Isle Royale National Park, 1929 and 1995-1997. U.S. Geological Survey, Biological Resources Division Biological Science Report USGS/BRD/BSR2000-0004.

Illustrations: Kraft, C.E., D.M. Carlson, and S.C. Brown. 2003. The On-line Fishes of New York State, Version 2.1. Department of Natural Resources, Cornell University, Ithaca, NY.

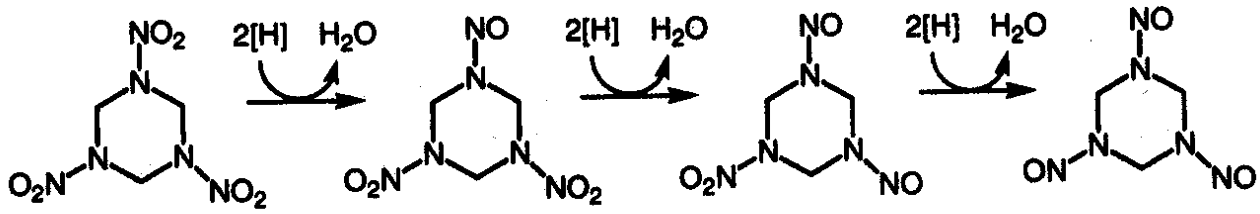

Figure 2. RDX

Breeding Pair of Deer Mice

Figure 3. Dosing Cages of Deer Mice.