I. IDENTIFICATION\(^{(1,2)}\)

Chemical Name: 3-NITRO-1,2,4-TRIAZOL-5-ONE  
Synonyms: (NTO)  
CAS Number: 932-64-9  
Molecular Formula: C\(_2\)H\(_2\)N\(_4\)O\(_3\)  
Structural Formula:

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\begin{array}{c}
\text{N} \\
\text{N} \\
\text{O} \\
\text{O} \\
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II. CHEMICAL AND PHYSICAL PROPERTIES\(^{(1–3)}\)

Physical State: White to pale yellow crystalline powder  
Odor Description: None  
Molecular Weight: 130 g/mol  
Conversion Factors: 1 ppm = 5.31 mg/m\(^3\);  
1 mg/m\(^3\) = 0.188 ppm  
Melting Point: 268–271 °C (514.4–519.8 °F)  
Boiling Point: Undergoes thermal degradation at temperatures above its melting point  
Vapor Pressure: No data available  
Saturated Vapor Concentration: No data available  
Flammability Limits: No data available  
Flash Point: 181 °C (357.8 °F)  
Autoignition Temperature: 347 °C (656.6 °F)  
Specific Gravity: 1.93 at 25 °C (77 °F)  
Log K\(_{ow}\): 0.858 at 25 °C (77 °F)  
Vapor Density: No data available  
Solubility: 2000 mg/L in water.  
Stability: No data available  
Reactivities and Incompatibilities: No data available

III. USES

NTO was developed as a potential replacement for RDX and other energetics in military munitions. It is a component of IMX-101, an insensitive munition designed to prevent unplanned explosions. IMX-101 and other insensitive formulations exhibit a reduced potential for detonation resulting from impact and fires in military combat vehicles and aircraft. NTO is synthesized by nitration of a keto-triazine derivative in an enclosed system. The NTO is mixed with the other IMX compounds and melted in a large heated kettle; the molten IMX-101 is dispensed onto a moving belt where it is cooled and solidified. The solid IMX-101 is then broken into flakes and shipped to a load and pack (LAP) facility where it is re-melted and poured into various warheads and projectiles. Potential worker exposures include particulates from the initial mixing process with dry components and NTO vapors associated with the molten NTO and the IMX-101 mixture.

IV. ANIMAL TOXICITY DATA

A. Acute Toxicity and Irritancy

1. Lethality Data

Mouse and rat LD\(_{50}\) > 5000 mg/kg\(^{(4)}\)

2. Eye Irritation

Eye irritation was tested in white rabbits using the Draize protocol. NTO (100 mg) was placed into the conjunctival envelop of 6 rabbits; the compound was not rinsed out in 2 animals, but was rinsed out of the eyes at 30 seconds for 2 animals and 5 minutes for the other 2 animals. Ocular irritation was graded at 24, 48 and 72 hours. All rabbits showed erythema at 1 and 4 hours and by 72 hours the response had resolved in all but 1 rabbit.\(^{(4)}\)

3. Skin Absorption

The dermal absorption of NTO was evaluated in an in vitro test. The test system used frozen human cadaver epidermal membranes in a static Franz cell system based on OECD 428 testing guidelines.\(^{(5)}\) NTO was applied to the skin as a powder and liquid samples were withdrawn from the receptor fluid at 1, 2, 4, 6 or 8 hours and analyzed for NTO. The authors estimated a steady state flux of 332 µg/cm\(^2\)/hr for NTO.\(^{(6)}\) In contrast, McDougal\(^{(7)}\) used a similar diffusion cell apparatus but with rat skin as the test membrane and estimated a dermal flux for RDX, another common munition, to be approximately 0.086 µg/cm\(^2\)/hr; for TNT, which is known to readily penetrate human skin the flux was approximately 1.14 µg/cm\(^2\)/hr. The reasons for the difference in steady state flux estimates from the similar compound may be due to differences in the skin sample preparation methods for human cadaver skin and rat skin. Based on the contrasting data, the dermal penetration of NTO should be confirmed using in vivo uptake studies before definitive conclusions can be made regarding potential systemic toxicity following dermal exposures.
4. Skin Irritation

Rabbit skin irritation was evaluated using the Draize procedure. A paste of NTO in corn oil was applied to the backs of six New Zealand white rabbits. The sites were occluded for 24 hours then scored for erythema and edema. The primary irritation score averaged for the 24 hour and 72 hour readings was 0.34, indicative of mild irritation.(4)

5. Skin Sensitization

Skin sensitization was evaluated in 10 female guinea pigs that received a series of 10 sensitizing injections with NTO dissolved in corn oil. Two weeks following the last injection the animals received a challenge injection of NTO and the injection site was graded after 24 hours. NTO did not induce sensitization in this test. (4)

6. Inhalation Toxicity

O'Neil & Crouse performed an acute inhalation study with NTO in order to estimate a 4-hour LC₅₀ value in rats. (5) Since NTO is an explosive material the authors could not generate atmospheres of vapor or dry dust. They were able to solubilize NTO in water and aerosolize this solution in order to expose rats in a nose only system. At the highest air concentration tested (0.184 mg/L) there were no compound related animal deaths.

B. Subacute Toxicity

1. Inhalation

No Information available.

2. Oral Toxicity

The Army performed a 14-day repeated dose oral gavage study in male and female rats. (3) Rats were dosed daily with 0 (PEG 200 control), 250, 500, 1000, 1500 or 2000 mg/kg/day of NTO for 14-days. No compound-related lethality was observed throughout the 14-day dosing period and the majority of the changes were found in the male reproductive organ weights and weight ratios. Results from the 14-day subacute oral toxicity study in rats showed significantly smaller testes weights in the high dose groups (≥500 mg/kg).

C. Subchronic Toxicity

1. Inhalation

No Information available.

2. Oral Toxicity

A 90-day oral (gavage) subchronic study (7 days a week/13 weeks) was conducted in male and female rats. Doses were selected based on the results from the 14-day repeated dose study and were set at 0 (PEG 200 control), 30, 100, 315 or 1000 mg/kg/day for both sexes. No compound-related mortality was observed in any of the dose groups throughout the 90-day dosing period. All surviving animals were euthanized on day 91 at which time a complete necropsy was performed, organs were removed and weighed, and blood was collected for clinical and hematological analysis. Statistical significance versus controls was observed at doses of 315 mg/kg/day and above mainly limited to the male reproductive organs and organ weight ratios. Sporadic significant changes both between dose groups and compared to controls were observed in the following parameters: body weight, body weight gain, various organ weights and weight ratios, hematology, and clinical chemistry. (3)

Preliminary histopathology results indicated testicular hypoplasia in all dose groups with the incidence and severity increasing with dose. The histopathology was initially performed by a contract laboratory; however based on inconsistencies in the report, the testes slides were re-evaluated by a board certified veterinary pathologist and this evaluation was subsequently peer reviewed by a second board certified veterinary pathologist. (6) Based on this re-evaluation the groups treated with 315 mg/kg/day and 1000 mg/kg/day had moderate to severe degeneration and atrophy of the seminiferous tubules and these changes were statistically significant when compared to the control group. The incidence of changes in the testes of the rats dosed with 30 and 100 mg/kg/day was not different when compared to controls. The testicular effects were considered to be the critical effect for deriving various exposure criteria and a benchmark dose analysis of the re-evaluated incidence data (0/10, 1/10, 1/9, 9/9 and 10/10 at 0, 30, 100, 315 and 1000 mg/kg/day, respectively) from this study was performed. Three models (Logistic, Probit and Multistage) were selected based on goodness-of-fit and statistical parameters. Based on this analysis the BMDL₁₀ for testicular hypoplasia was estimated to range from 22 to 47 mg/kg/day depending on the subset of acceptable models used. (9)

D. Chronic Toxicity

No data available.

E. Reproductive/Developmental Toxicity

Only limited developmental and reproductive toxicity information is available for NTO. In a 90-day subchronic study, rats were exposed to doses of 0, 30, 100, 315 or 1000 mg/kg/day by oral gavage. (10) These data are discussed below in detail but there were marked effects on the male reproductive system.

The observation of male reproductive organ effects in the 90-day study was confirmed in a screening study for reproductive and developmental effects using the OECD 422 test guidelines. (11) Groups of 10 male and 10 female rats were administered NTO at 0, 31, 125 or 500 mg/kg/day. An additional 20 males were included to serve as a satellite group in order to evaluate recovery and/or delayed effects from NTO. Male rats were dosed for 28 days including 2 weeks prior to and following mating. A complete necropsy was performed at the end of the dosing period. Female rats were dosed for 2 weeks prior to mating, during pregnancy and through day 4 post-partum. Treatment with NTO resulted in significant reductions in testes and epididymides mass and mass ratios in male rats given 500 mg/kg/day. Microscopic evaluation of these tissues revealed severe degeneration and atrophy of the testicular
control animals, compared to 6.8% for the 500 mg/kg/day main
counts in this group recovered slightly to 28.6% of recovery
spermatids were only variably present. Cauda epididymal sperm
spermatocytes were present through all stages but maturing
developmental effects. The NOAEL from this study was 125
mg/kg/day.(10)

external examinations of the offspring NTO did not cause any
related changes in male rats exposed to 125 mg/kg/day and
complete recovery. There were no statistically significant dose
the duration of the recovery period was insufficient for a more
study males, but no motile sperm were detected. It is likely that
satellite group exhibited a partial recovery following the 4-week
manifest on the testes and developing sperm. Rats from the
in the mating period the NTO effects were likely not completely
in all cases.(13)

F. Genotoxicity/Mutagenicity
NTO was evaluated for mutagenicity in Salmonella
typhimurium and Escherichia coli plate incorporation assays
both with and without S-9 activation. The results were negative
in Salmonella at concentrations up to 500 µg/plate without
activation, and up to 5000 µg/plate with activation. In E. coli,
results were also negative at maximum concentrations up to
2500 µg/plate without activation and 5000 µg/plate with
activation. Under the test conditions, NTO was negative with
and without activation.(12)

NTO was tested for its potential to induce mutations in the
L5178Y TK⁺/- mouse lymphoma mutation assay. Cells were
 treated with NTO at concentrations up to 5000 µg/mL, both
with and without activation. Results of the assay were negative
in all cases.(13)

NTO was tested in Chinese Hamster Ovary (CHO) cells for
elastogenicity. The test was conducted both with and without
exogenous metabolic activation at concentrations up to 5000
µg/mL with negative results.(14)

A rat micronucleus assay was conducted in conjunction a 14-
day oral subacute study described below. The frequency of
micronucleated reticulocytes ranged from 0.20 to 0.23% in
female rats and 0.21 to 0.27% in male rats treated with 1000,
1500, and 2000 mg/kg/day of NTO in PEG. Treatment with
NTO did not produce a statistically significant increase in the
frequency of micronucleated reticulocytes in the peripheral
blood of female or male rats. These results indicate that NTO is
not genotoxic in rat peripheral blood at these doses.(15)

G. Metabolism/Pharmacokinetics
1. Absorption
Crouse & O’Neill(7) included an investigation into the time
course of NTO absorption and clearance from both oral and
inhalation exposure. NTO blood concentrations for male and
female rats exposed via inhalation peaked at the 4-hr sampling
time (immediately upon removal from the chamber) and
averaged 41.5 µg/mL. Blood concentrations gradually increased
during the exposure from 13.08 µg/mL at 1-hr to 23.0 µg/mL at
2-hr. Upon removal from the chamber (4-hr), blood
concentrations rapidly decreased to 4.75, 1.42, and 0.68 µg/mL
at 8, 12, and 24 hours post-exposure. In the oral gavage dosing
component of the study, a calculated dose equivalent to the 4-
hour inhaled dose was given in a single bolus. NTO blood
concentrations peaked at 1-hr post-dosing (first sample time)
and averaged 6.38 µg/mL. Average blood concentrations
dropped to 3.52 µg/mL at 2-hr post-dosing and remained below
the limit of detection at 5, 8, and 24 hours post-dosing. In a
study with orally dosed primates, similar doses of NTO were
also eliminated by 8 hours.(15) Together these data suggest rapid
takeup and elimination following inhalation and oral dosing.
In terms of absorption and elimination rates, the profile of NTO in
rodents and primates was reported to be similar; male Rhesus
monkeys given doses of 21 or 24 mg/kg showed peak profiles at
around 2 hours in blood with elimination by 8 hours, while male
rats given oral doses 25 mg/kg showed peak NTO in blood at
approximately one hour with elimination from blood by 4
hours.

Hoyt et al(15) studied NTO absorption in primates, including a
brief screen for ATO and urazole in urine samples with high
NTO exposure levels, and found no indication of urazole and
only slight traces of ATO. It is therefore unlikely that in
primates hepatic metabolism of NTO takes place and more
probably that it is excreted unchanged. Metabolism of NTO in
primates was also examined in adult male rhesus monkeys that
received oral doses of NTO of 5, 25 or 50 mg/kg followed by
serial blood and urine sampling up to 48 hours post exposure.
Doses were orally administered under sedation; at 5 hours,
animals were recovered and returned to their cages with follow
up samples at 8, 24, and 48 hours. At all three doses, NTO was
absorbed quickly, peaked at 4 hours and was eliminated by 8
hours, with urinary concentrations at least 100-fold higher than
that of blood or serum. Overall, these results show that there is
little metabolism of NTO in primates and that for monitoring
exposure, urine samples are optimal.(15) Together these data
describe significant potential species differences in the
metabolism of NTO. However, there is no clear evidence
whether the toxicity of NTO observed in rodents is due to NTO
or a metabolite, and thus, the implications of species differences
in the metabolic profiles is uncertain.

2. Metabolism
A study using ¹⁴C-NTO found that metabolic degradation of this
compound using rat liver microsomes in vitro appeared to
involve two separate enzymatic pathways. In the presence of
oxygen, NTO was metabolized to two separate products: 5-
amino-1,2,4-triazol-3-one (ATO) and 5-hydroxy-1,2,4-triazol-3-
one (urazole). The presence of oxygen did not affect the overall conversion of NTO, but did alter the proportion of the metabolites. Under anaerobic conditions, the ATO was the primary product while urazole comprised only 5 percent of the product. Under aerobic conditions, urazole represented 40 percent of the product with a decrease in nitroreduction. Two separate pathways were represented here, since incubation of ATO with activated microsomes did not result in production of urazole, indicating that ATO did not represent an intermediate in this pathway and that urazole was formed directly from NTO in mammalian in vitro systems in the body.(16) Other explosives, such as RDX, have been shown to display aerobic and anaerobic pathways in P450 metabolism.(17) It is therefore important, for mode of action, to fully describe the metabolic pathways of NTO given that LeCampion et al demonstrated oxygen sensitive metabolism in vitro using rat tissues.

H. Other

1. Endocrine Disruption Studies

NTO was tested in a battery of in vitro and in vivo tests for endocrine disruption.

NTO was tested in a battery of 5 in vitro tests, including assays for estrogen receptor binding, androgen receptor binding, estrogen transactivation, aromatase and steroidogenisis. All of these in vitro tests were negative with NTO.(18)

In vivo endocrine disruption assays included a pubertal assay, Hershberger assay and uterotrophic assay. The pubertal test evaluated the potential for NTO to interact with the endocrine system to affect pubertal development and thyroid function in male and female rats. NTO did not affect pubertal development in either sex and the measured hormone levels were also not affected. Male rats exhibited reduced testis mass, tubular degeneration and these changes were associated with less pronounced reductions in the mass of androgen dependent accessory reproductive tissues.(19) The Hershberger and uterotrophic assays involved short term in vivo exposure screens that assessed NTO’s potential to act as an estrogen agonist or an androgen antagonist through changes in sex steroid sensitive organ weights in ovariectomized or castrated Sprague-Dawley rats. The results of these two screens do not provide evidence for endocrine disrupting activity at the dose levels tested.(20)

2. Biomarker Studies

Rhesus monkeys were used to identify potential biomarkers of exposure. NTO was dissolved in deionized water and administered through a gastric tube to anesthetized animals. Serial blood and urine samples were taken for 5 hours. Based on this limited data set it appears that NTO is readily detectable in blood and urine even at the lowest (5 mg/kg) dose tested in this study.(15)

3. IMX-101 Mixture Studies

IMX-101 is one of the first of the insensitive munitions to contain NTO. It is a mixture of NTO, 2,4-dinitroanisole and nitroguanidine. Acute and 14-day repeated dose studies were conducted to determine if the toxicity of the mixture was based on the additive toxicity of the individual components. IMX-101 had an LD₅₀ in male rats of 1237 mg/kg and in female rats of 924 mg/kg, with a combined (male-female average) value of 1100 mg/kg.(21) In the 14-day IMX-101 repeated oral dose study the notable adverse events were: 1) lethality in the 500 mg/kg/day and 1000 mg/kg/day dose groups; 2) splenomegaly (increased spleen weight) primarily in females; and 3) testicular atrophy and decreased sperm density and motility. The LOAEL for IMX-101 from this study was 100 mg/kg/day based on changes in testicular mass with a calculated BMDL₁₀₀ of 30.6 mg/kg/day. The IMX-101 mixture data provided some indication of dose-relationships that were more than additive. For example, the testicular effects from IMX-101 exposure occurred at much lower doses (based on the NTO equivalent dose after reducing the LOAEL for concentrations in the IMX-101 mixture) than seen in tests with NTO alone. Toxic interactions are dependent on the doses present, and the IMX-101 dosing may not reflect actual ratios of workplace exposures to IMX-101 components. In addition, doses in both tests were much higher than those likely to occur in an occupational setting. Thus, the results from the IMX-101 study are not considered adequate for derivation of the WEEL.

V. HUMAN USE AND EXPERIENCE

NTO is a component of the IMX-101 mixture. There is very little information related to humans exposed to IMX-101 or its components. The Army published an Industrial Health survey, which investigated a number of jobs in a melt-pour operation at the Iowa Army Ammunition Plant (IAAP). Processes included emptying large boxes of IMX-101 onto a table, sifting the IMX 101 and dumping the powder into a kettle hopper where it was melted. Once the IMX-101 reached the correct temperature it was poured into projectiles. Air samples were taken at a number of workstations throughout the plant and analyzed for the IMX-101 components. NTO concentrations in all of the air samples were below the current recommended Army OEL of 1.6 mg/m³.(21)

VI. RATIONALE

NTO is a crystalline powder with no odor and decomposes before melting. It was developed as a potential replacement for RDX and other military munitions as a component of IMX-101, which is an insensitive munition designed to prevent unplanned explosions. NTO is not acutely toxic: the oral the LD₅₀ (mouse and rat) is > 5000 mg/kg and inhalation studies using an NTO:water mixture resulted in no deaths at a concentration of 0.184 mg/L. No dermal acute lethality studies are available and data on dermal penetration is inconclusive. NTO is mildly irritating to the eyes and skin. It is not a sensitizer and is negative is a variety of assays for genotoxicity and mutagenicity. Based on a subchronic oral toxicity study and a reproductive and developmental toxicity screening study in rats, the primary effect of concern is male reproductive toxicity. These studies revealed severe degeneration and atrophy of the testicular seminiferous tubules and moderate to severe hypospermia. No effects on male fertility were noted, but the absence of such effects likely reflects the duration of dosing
prior to mating in the reproductive study protocol. No developmental effects were seen on gross external examinations.

The testicular effects serve as the point of departure (POD) for deriving the WEEL for NTO. A benchmark dose analysis modeled the lower 95% confidence interval on the 10% response rate for the testicular histopathology in the oral subchronic study in rats. The resulting BMDL10 for WEEL derivation is 40 mg/kg/day (reflecting an average of several acceptable models).

The derivation of the WEEL considers several key uncertainties. Although there is evidence for interspecies differences in metabolism overall clearance rates of NTO from the blood are similar between rodents and primates. The absence of information on mode of action for reproductive effects precludes direct use of the toxicokinetic data to modify the POD to account for species differences. Adjustments based on toxicokinetic data are also limited by the absence of data on human variability in susceptibility. The POD is based on testicular effects observed in a subchronic study in male rats. Although the toxicokinetic data suggest limited potential for dose accumulation with chronic exposure, the possibility of effects occurring at a lower dose is not known in the absence of data on tissue repair rates or the availability of a longer-duration study.

VII. RECOMMENDED WEEL GUIDE

8-hour Time-Weighted Average: 2 mg/m³. No additional hazard notations are assigned.

VIII. REFERENCES


(2) BAE Systems. MSDS 6000.


(8) Wallace, S. M. *The Subchronic Oral Toxicity of 3-Nitro-1,2,4-Triazol-5-One (NTO) In Rats (Rattus Norvegicus)*. Protocol No. OA6W-38-08-02-01.; American College of Veterinary Pathologists: Madison, WI, 2011.

(9) U.S. Army Armament Research. Revised Preliminary Occupational Exposure Level for 3-Nitro-1,2,4-Triazol-5-One (NTO),. 2011.


(20) USAPHC. *Toxicology Portfolio Assessment of 3-Nitro-1,2,4-Triazol-5-One (NTO) as a Potential Endocrine Disrupting Chemical In Rats Using the Hershberger and Uterotrophic Bioassays.* Toxicology Study No. 87-XE-0FV5-12.; U.S. Army Public Health Command: Aberdeen Proving Ground, MD, 2012.
