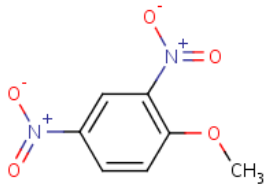
WORKPLACE ENVIRONMENTAL EXPOSURE LEVEL®

OARS WEEL

2,4-Dinitranisole (DNAN)(2014)

I. IDENTIFICATION

Chemical Name: 2,4-Dinitroanisole (DNAN)
Synonyms: 1-Methoxy-2,4-Dinitrobenzene; 2,4-Dinitrophenylmethyl Ether, DNAN
CAS Number: 119-27-7
Molecular Formula: C7H6N2O5
Structural Formula:



II. CHEMICAL AND PHYSICAL PROPERTIES[1, 2, 3, 4]

Physical State: tan to yellow crystalline solid.
Molecular Weight: 198.1
Conversion Factors: 1 ppm = 8.10 mg/m^3; 1 mg/m^3 = 0.12 ppm
Boiling Point: 351 °C (664 °F)
Melting Point: 94–96°C (201.2–204.8 °F)
Vapor pressure: 1.4E-4 mm Hg at 25 °C
Saturated Vapor Concentration: No data available
Odor Description and Threshold: No data available
Vapor Density: 6.8
Flash Point: 181°C (357.8°F)
Flammability Limits: LEL: No data; UEL: No data
Autoignition Temperature: 347 °C (656.6 °F)
Specific Gravity: 1.34 g/cm^3 at 25°C (77 °F)
Solubility: Slightly soluble in water; soluble in ethanol, ether, acetone, and benzene; very soluble in pyridine.
Log K_{ow}: 1.56 at 25°C (77 °F)
Stability: Stable
Reactivities and Incompatibilities: Oxidizing agents

III. USES

DNAN was historically used as an explosive in warheads containing Amatol 40 and is currently being investigated as a replacement for 2,4,6-Trinitrotoluene (TNT) in melt-cast insensitive munitions (IM) formulations. DNAN is also used industrially in the synthesis of dyes and insect repellants.[1, 5]

IV. ANIMAL TOXICITY DATA

A. Acute Toxicity

1. Lethality Data

Species	Route	LD_{50} or LC_{50}
Rat	Oral	199 mg/kg[6]
Rat	Inhalation	> 2.9 g/m^3[7]
Rat	Dermal	Not available

2. Eye Irritation

Assays to assess potential eye irritation in rabbits (following OPPTS 870.2400) indicated that DNAN was mildly irritating to the eye with a maximum average score of 12.0 at 1 hr and clearing by 48 hr.[6]

3. Skin Absorption

Steady state flux of pure DNAN was determined through dermatomed rat skin in static diffusion cells over six hours at 32 ºC. The rate of penetration was determined to be 1.55 µg/cm^2/hr. DNAN was applied to the skin as a powder, the same form encountered by workers. When DNAN was applied to skin as part of an explosive mixture (CBR-12, aka PAX-21 with a DNAN composition approximately 34%) the rate of DNAN dermal penetration was estimated at 0.74 µg/cm^2/hr. For comparison, steady state flux of TNT from Composition B in the same system was 1.14 µg/cm^2/hr.[6, 8]

4. Skin Irritation

Assays for skin irritation in rabbits (following OPPTS 870.2500) indicated that DNAN produced slight dermal irritation (primary irritation index range from 0.08 to 0.25), which was transient, clearing after 24-48 hours.[6]

5. Skin Sensitization

Dermal sensitization was not observed when DNAN was administered to guinea pigs in a sensitization assay (OPPTS 870.2600). [6]

6. Acute Inhalation Toxicity

An acute inhalation study was carried out in Sprague-Dawley rats by heating DNAN to 175°C to generate DNAN vapors. The target concentration was 1 to 5 mg/m^3 (actual average concentration, 2.8 mg/m^3). A second phase of the study involved dissolving DNAN in acetone and generating an

aerosol to achieve a target concentration of 2,000 mg/m^3 (actual average concentration, 2933 mg/m^3). No mortalities were observed in either group. No clinical signs of toxicity were observed at the lower vapor-based exposure. Clinical signs of toxicity during the aerosol exposure consisted of decreased activity and labored breathing. Clinical signs observed post-exposure included increased salivation, lacrimation, and red or clear nasal discharge. These symptoms resolved in all animals within several days. There were no macroscopic postmortem findings at the end of the 14-day post-exposure period that were considered treatment related. The inhalation LC_{50} was therefore judged to be greater than 2.9 mg/m^3.[7]

B. Subacute Toxicity

1. Inhalation

Male and female Sprague-Dawley rats (5/sex/group) were exposed to DNAN dissolved in acetone at target nominal concentrations of 150, 500, or 1500 mg/m^3 of aerosol/vapor for 6 hours/day; 5 days/week, for a total of 11 days. Actual average exposure concentrations were 165, 535 or 1313 mg/m^3. Controls were exposed to acetone aerosol/vapor alone (approximately 23,727 mg/m^3). Exposures and analysis were carried out consistent with the methods outlined in OECD 412. All animals in the 1500 mg/m^3 and 8/10 animals in the 500 mg/m^3 group were found dead or euthanized in moribund condition during the exposure period. Clinical signs of toxicity observed prior to euthanasia included decreased food consumption, prostration, irregular gait, lethargy, head bobbing, poor condition, pallor, backwards walking, labored breathing, and red nasal discharge. Animals exposed to 500 mg/m^3 gained less weight and consumed less feed during the first week of exposure than the acetone controls. Male rats exposed to 150 mg/m^3 had significantly decreased blood urea nitrogen (BUN) and increased kidney weight. Females in the 150 mg/m^3 had statistically significant decreases, relative to the acetone control group, in mean hemoglobin concentrations, mean corpuscular volume, and mean corpuscular hemoglobin, and increases in mean absolute monocytes and liver weight. The urine of both male and female rats exposed to 150 mg/m^3 was darker than in acetone-treated controls. Irregular gait was periodically noted in the 150 mg/m^3 dose group but this was also observed in controls and was attributed to the CNS depressant effects of the acetone. The only reported DNAN-related microscopic finding was non-specific minimal metaplasia of laryngeal epithelium in rats exposed to nominal concentrations of 500 and 150 mg/m^3. The concentration of 150 mg/m^3 (actual average concentration: 165 mg/m^3) was therefore the LOAEL for this study.[9]

2. Oral Toxicity

Male and female Sprague-Dawley rats (6/sex/group) were administered DNAN in corn oil at 0, 1.56, 3.13, 6.25, 12.5, 25, 50, or 100 mg/kg-day via oral gavage for 14 days. Male rats exposed to 100 mg/kg-day of DNAN exhibited reduced body weight and reduced testes weight. Liver-to-body and spleen-to-body weight ratios were significantly increased in males at 100 mg/kg-day and in females at 50 and 100 mg/kg-day DNAN. In males, serum albumin concentrations were significantly increased in the 50 and 100 mg/kg-day dose groups. Female rats in the 100 mg/kg-day dose group exhibited significantly elevated total bilirubin concentrations while serum cholesterol concentrations were significantly increased in female rats exposed to 50 (but not 100) mg/kg-day DNAN. In females, changes in hematology indicative of anemia, including decreased red blood cell count, hematocrit, and hemoglobin, and increased red cell distribution width were observed in the 100 mg/kg-day group. Increased alanine aminotransferase levels suggesting hepatocellular injury were also evident in female rats given 50 and 100 mg/kg-day DNAN. The NOAEL from this study was therefore 25 mg/kg-day.[10]

C. Subchronic Toxicity

1. Inhalation

No data available.

2. Oral Toxicity

Male and female Sprague-Dawley rats were dosed with DNAN via oral gavage at 0,1.25, 5, 20, or 80 mg/kg-day for 90 days. Mortality occurred in three male rats (days 50, 63, and 77) and one female rat (day 26), all from the 80 mg/kg-day dose group. Rats in the highest dose group (80 mg/kg-day) experienced lethargy, labored/rapid respiration, prostrate and/or recumbent posture, hunched posture, ear twitching, squinting, curled tail, and gait irregularities. A functional observation battery (FOB) and analysis of motor activity at week 13 indicated that rats given 80 mg/kg-day had altered neuromuscular function and decreased activity levels. In the 80 mg/kg-day group, female rats also had reduced sensorimotor responses while male rats had increased excitability responses. The neurobehavioral evaluations indicated no treatment-related effects at 20 mg/kg-day or below.

Although food intake was similar among groups for male rats, animals from the 80 mg/kg-day dose group exhibited reduced body weight and a reduced food efficiency ratio. Body weight did not differ among dose groups for female rats. Female rats in the 80 mg/kg-day dose group also had a reduced food efficiency ratio, but had elevated food consumption at several time points during the study.

Female rats in the 80 mg/kg-day dose group and male rats in the 20 mg/kg-day group produced higher volumes of urine with lower specific gravity. Despite the increase in volume, urine color was darker in the 20 and 80 mg/kg-day dose groups for both sexes. Increased mean kidney, liver, and spleen weight were observed in male and female rats given 80 mg/kg-day DNAN. In male rats, increased mean kidney and liver weight were also noted in the 20 mg/kg-day dose group. These changes were not associated with treatment-related microscopic abnormalities or alterations in clinical chemistry parameters but it was observed that a number of clinical chemistry parameters in the controls were above normal reported levels for this strain. Decreased weight of the testes and epididymides as well as degeneration and atrophy of the testicular seminiferous tubules and aspermia were also observed in rats from the 80 mg/kg-day group. In females, changes in hematology indicative of anemia, including decreased red blood cell count, hematocrit, and

hemoglobin, and increased red cell distribution width were observed in the 80 mg/kg-day group. A dose related increase in extramedullary hematopoiesis (EMH) was noted in spleens of female rats at 20 and 80 mg/kg-day. Glial lesions within the cerebellum were noted in four rats (1 female/3 males) in the 80 mg/kg-day group.[10]

While the NOAEL from this study was 5 mg/kg-day, extramedullary hematopoiesis (EMH) was observed at the lowest dose with no significant dose-response relationship observed in the lower dose range. Benchmark Dose Software (BMDS v.2.1.2) was used to fit mathematical models to the EMH incidence dose response data and calculate a lower-bound 95% confidence limit on a dose corresponding to a 10% response rate ($BMDL_{10}$).[17,18] The EMH incidence and dose data used in the modeling were: 0/10, 1/10, 3/10, 4/10 and 9/10 at 0, 1.25, 5, 20 and 80 mg/kg-day, respectively. The Log Logistic model was selected based on goodness-of-fit and statistical parameters resulting in a $BMDL_{10}$ value of 0.93 mg/kg-day.[10]

D. Chronic Toxicity/Carcinogenicity

No data available.

E. Reproductive/Developmental Toxicity

No data from specific reproductive or developmental toxicity studies are available for DNAN. In the 90-day systemic toxicity study (described above), effects on the male reproductive system were observed but these occurred at a higher dose than the EMH that is the basis for the NOAEL. A prenatal developmental toxicity study (OPPTS 870.3700) has been conducted with CRB-12 (i.e., PAX-21) which contains approximately 34% DNAN. In this study, pregnant rats were dosed *via* gastric intubation with 0, 15, 30, or 60 mg/kg-day CRB-12 (i.e., approximately 0, 5.1, 10.2, 20.4 DNAN) on gestation days 6 to 19. Maternal toxicity was observed at 60 mg/kg-day CRB-12, including maternal mortality. Dams dosed with 30 mg/-day exhibited yellow ano-genital staining, decreased fecal volume, alopecia, decreased body weight and decreased food consumption. In the 15 mg/kg-day group, the only adverse clinical sign was decreased food consumption on gestational days 6 to 9. No treatment-related macroscopic post-mortem findings were observed in female animals treated at 15 and 30 mg/kg-day CRB-12. A slight decrease in fetal body weight was observed in the 30 mg/kg-day group which was attributed to maternal toxicity. No external, visceral and/or skeletal malformations or variations were observed in fetuses of the 15 and 30 mg/kg-day groups. The maternal and fetal NOAEL values were therefore both 15 mg/kg-day CRB-12 (effective DNAN concentration: approximately 5.1 mg/kg-day).[6]

F. Genotoxicity/Mutagenicity

1. In vitro

DNAN was evaluated in the Ames *Salmonella* assay (using strains TA98, TA100, TA102, TA1535, and TA1537), with and without S9 metabolic activation (OPPTS 870.5100). Concentrations ranged from 0.003 to 3 mg/plate. All strains tested gave a positive response when exposed to DNAN and inclusion of the S9 fraction had little effect on the test result.[6] Evaluation using Chinese Hamster Ovary (CHO) cells (AS52/XPRT) at concentrations of 0.0625 to 1.0 mg/ml with and without S9 activation indicated no mutagenic induction in the tested cells.[6]

2. In vivo

The potential genotoxicity of DNAN was also assessed *in vivo* using the mouse bone marrow micronucleus test (OPPTS 870.5395). Male and female Swiss CD-1 mice were orally dosed with DNAN at 10 to 90 mg/kg. There was no significant increase in micronucleated cell frequency with DNAN treatment. The highest dose was considered to be toxic to the hematopoietic system, inducing a change in the ratio of polychromatic erythrocytes to normochromatic erythrocytes (this is consistent with the oral toxicity studies described above). DNAN was judged to have caused no chromosomal damage and to be non-genotoxic in the *in vivo* mouse bone marrow assay.[6]

G. Metabolism/Pharmacokinetics

Detailed information on the metabolism of pharmacokinetics of DNAN is not available. Studies have demonstrated that DNAN is metabolized *in vivo* to 2,4-dinitrophenol (DNP) via oxidative cleavage of the methoxy group.[11] The extent and rate of metabolism are not known.

H. Other

As noted above, DNAN is metabolized to DNP. Data on the toxicity of DNP may nonetheless be informative. DNP is an uncoupler of oxidative metabolism and was once used in human populations as a diet drug. There are therefore considerable data on the health effects of direct DNP exposure in humans.[11] The primary effects reported in humans so exposed are anemia, cataracts, and metabolic alterations (e.g., increased body temperature, weight loss). The threshold for these effects lies in the 1 to 2 mg/kg-day range among individuals taking DNP for up to 18 months (note that most studies of these effects are case reports published in the 1930s so dose levels are estimated). Studies and case reports of women taking DNP as a weight loss drug (e.g., at doses in the range of 3 mg/kg-day) have suggested some reproductive effects (e.g., pregnancy loss, altered menstrual cycles), but these were reported at doses intended to affect maternal metabolism and body weight.[11] While potentially informative, data on DNP should be applied with caution for evaluating risks of DNAN. While DNAN will be converted to DNP in the body, the process is not instantaneous and DNP that is produced will be eliminated. Thus DNP exposure *via* DNAN can be expected to be somewhat attenuated relative to an exposure to an identical weight of DNP itself.

V. HUMAN USE AND EXPERIENCE

DNAN was used as a component of MYL louse powder (0.2% pyrethrins, 2.0% IN-930, 0.3% Phenol-S, 2.0% DNAN, and

pyrophyllite inert diluent) until the longer-acting DDT replaced it. In its use for control of human lice, MYL powder was applied to clothing at 30 g/suit, resulting in a dermal application of DNAN of 600 mg/man or 9 mg/kg. MYL powder was demonstrated to be safe through testing and use.[12] DNAN is currently being investigated as a replacement for TNT in a variety of insensitive munitions formulations. Limited air sampling in buildings where melt-pour and drilling operations for munitions containing DNAN are conducted revealed mean air levels at all operations of 1.2 mg/m^3 with a range of <0.1 to 8.5 mg/m^3.[13,14] During the melt and pour process, work involves potential for dermal contact with powdered DNAN.

VI. RATIONALE

The available acute data for DNAN suggest that it is moderately toxic via the oral route and slightly toxic via inhalation. Occupational exposure to DNAN is likely to occur primarily through inhalation (aerosol, vapor) and potentially through dermal exposure. However, no long-term toxicity data are available for the inhalation route and no systemic data are available for the dermal route. Subacute inhalation data are available; however, a dose response assessment is not reliable since few animals from more than one exposure group survived at tested levels. Although this study indicated possible portal of entry effects, systemic effects similar to those observed following oral exposures were also observed, indicating that route-to- route extrapolation may be appropriate.[15] No chronic studies are available; one subchronic oral toxicity study was conducted in the rat. Extramedullary hematopoiesis (EMH) can be associated with anemia in female rats and as such was identified as the critical endpoint in this study with a BMDL10 value of 0.93 mg/kg-day.[16,17] This resulting BMDL is equivalent to an airborne concentration of 6 mg/m^3 based on a body weight of 55 kg and breathing rate of 8 m^3 per work shift. After application of appropriate uncertainty factors, a WEEL value of 0.1 mg/m^3 is obtained. Key areas of uncertainty included: intraspecies variability which is limited by the nature of the exposed population (i.e., healthy working adults); interspecies variability; uncertainty due to subchronic to chronic effect extrapolation, which is limited by the nature of the effect observed (i.e., anemia, which is a short-term effect on blood formation); and database uncertainty, reflecting the absence of specific reproductive studies on DNAN.

An alternate WEEL derivation was considered based on the potential for DNP, a DNAN metabolite, to produce cataracts and other adverse effects in humans. The LOAEL for this effect in humans is reported to be approximately 1 to 2 mg/kg-day. If this is divided by safety factors to account for LOAEL to NOAEL extrapolation as well as the less than lifetime exposure, an alternative WEEL value of 0.015 mg/kg-day or 0.11 mg/m^3 could be derived. This value is similar to the WEEL derived based on data for DNAN itself. In addition, because DNAN must first be metabolized to DNP and the extent of that metabolism is unknown, use of DNAN data for the WEEL is preferred.

VII. RECOMMENDED WEEL

8-hour Time-Weighted Average: 0.1 mg/m^3 (0.01 ppm)

VIII. REFERENCES

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