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Provisional Peer-Reviewed Toxicity Values for

4,6-Dinitro-*o*-cresol
(CASRN 534-52-1)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
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Cincinnati, OH 45268

COMMONLY USED ABBREVIATIONS

BMD	Benchmark Dose
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
LOAEL	lowest-observed-adverse-effect level
$LOAEL_{ADJ}$	LOAEL adjusted to continuous exposure duration
$LOAEL_{HEC}$	LOAEL adjusted for dosimetric differences across species to a human
NOAEL	no-observed-adverse-effect level
$NOAEL_{ADJ}$	NOAEL adjusted to continuous exposure duration
$NOAEL_{HEC}$	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional reference concentration (inhalation)
p-RfD	provisional reference dose (oral)
POD	point of departure (oral)
RfC	reference concentration (inhalation)
RfD	reference dose
UF	uncertainty factor
UF_A	animal to human uncertainty factor
UF_C	composite uncertainty factor
UF_D	incomplete to complete database uncertainty factor
UF_H	interhuman uncertainty factor
UF_L	LOAEL to NOAEL uncertainty factor
UF_S	subchronic to chronic uncertainty factor
WOE	weight of evidence

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PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR 4,6-DINITRO-*O*-CRESOL (CASRN 534-52-1)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1) EPA's Integrated Risk Information System (IRIS).
2) Provisional Peer-Reviewed Toxicity Values (PPRTVs) used in EPA's Superfund Program.
3) Other (peer-reviewed) toxicity values, including
   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's IRIS. PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multiprogram consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a 5-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV documents conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and Resource Conservation and Recovery Act (RCRA) program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV document and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of

Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

IRIS (U.S. EPA, 2008), the HEAST (U.S. EPA, 1997), and the Drinking Water Standards and Health Advisories List (U.S. EPA, 2006) do not report an RfD, an RfC, or a cancer assessment for 4,6-dinitro-*o*-cresol (DNOC; chemical structure shown in Figure 1). The Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1991, 1994) includes a Health and Environmental Effects Profile (HEEP) for Dinitrocresols (U.S. EPA, 1986) that derived an acceptable daily intake (ADI) of 0.35×10^{-3} mg/kg-day based on a LOAEL of 0.35 mg/kg-day for elevated basal metabolic rate, sweating, fatigue, and thirst, and discoloration of the conjunctiva in exposed humans (Plotz, 1936). The ATSDR (1995) Toxicological Profile for Dinitrocresols derived acute and intermediate oral MRLs of 0.004 mg/kg-day that are also based on the LOAEL identified in the Plotz (1936) study; a chronic oral MRL was not derived due to the lack of chronic data. Due to a lack of suitable data, neither the HEEP nor the Toxicological Profile derives inhalation toxicity values.

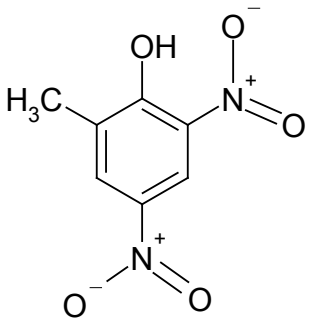

Figure 1. Chemical Structure for 4,6-Dinitro-*o*-cresol

The American Conference of Governmental Industrial Hygienists (ACGIH, 2008), National Institute for Occupational Safety and Health (NIOSH, 2008), and Occupational Safety and Health Administration (OSHA, 2008) have published a threshold limit value (TLV), a recommended exposure limit (REL), and a permissible exposure limit (PEL), respectively, for DNOC of 0.2 mg/m^3 (time-weighted average [TWA]) to protect against metabolic disorders. The older human literature was reviewed by NIOSH (1978). A World Health Organization (WHO, 2000) monograph endorsed a biological threshold limit of 20 µg/mL of DNOC in the blood, but it does not derive an oral or inhalation toxicity value for DNOC. Assessments of DNOC carcinogenicity have not been performed by the National Toxicology Program (NTP, 2005, 2008) or the International Agency for Research on Cancer (IARC, 2008).

Literature searches were conducted from the 1960s through September 2009 in the following databases for studies relevant to the derivation of provisional toxicity values for DNOC: MEDLINE, TOXLINE (with NTIS), BIOSIS, TSCATS/TSCATS2, CCRIS, DART, GENETOX, HSDB, RTECS, Chemical Abstracts, and Current Contents (last 6 months).

REVIEW OF PERTINENT DATA

Human Studies

Oral Exposure

During the 1930s, DNOC, along with dinitrophenol, was used therapeutically as a weight-loss agent after animal experiments had demonstrated that dinitrophenols increased the basal metabolic rate (BMR). The earliest mention of the use of these compounds for weight loss is a publication by Cutting and Tainter (1933) in which the authors reported clinical studies of dinitrophenol for this purpose. Dodds and Robertson (1933) reported that the related compound, DNOC, exhibited a greater effect on metabolism than dinitrophenol, leading to the marketing of DNOC for weight loss. Following the publication of these reports, dinitrophenol, and to a lesser extent, DNOC, began selling in drug stores and was prescribed by physicians for weight loss. In a recent review, Colman (2007) detailed the marketing of dinitrophenol and DNOC, along with their ultimate ban by the U.S. Food and Drug Administration (FDA). According to the review, the FDA banned sale of these compounds in 1938 and found no evidence of their availability for sale by 1940.

While data on the prevalence of dinitrophenol and DNOC usage for weight loss are not available, numerous reports at the time suggested "widespread" use of the compounds (i.e., Tainter et al., 1934; Tainter and Wood, 1934; Plotz, 1936; Quick, 1937). Tainter et al. (1934) estimated that at least 100,000 people in the United States had been treated with dinitrophenol during the first year of its therapeutic use. Horner (1942) described "extensive" use of DNOC and mixtures containing DNOC in Europe. Colman (2007) reported that more than 550,000 capsules of a product called Improved Formula 281, containing 0.5 g of DNOC, were sold in 1935 alone.

Several case reports and clinical trials were published during the early years of DNOC usage for weight loss, providing a database of human experience with oral exposure to DNOC. Dodds and Robertson (1933) undertook a study to establish a safe and effective dosage that would elevate the BMR above normal for long periods of time for weight reduction. The study

was conducted in young adults (individual ages and genders unknown) of normal weight or with some degree of obesity. The number of patients tested was not specified; "typical charts" of BMR and other data were shown for two patients. A dosage of 3 mg/kg-day elevated the BMR by >50% (≈70−100% for the two patients whose charts were shown) by the third day of dosing. Sweating, lethargy, severe headache, loss of appetite, and greenish-yellow coloration of the conjunctivae occurred when the BMR exceeded 50% of normal. Dosing was discontinued because of the severity of the effects. Blood and urine were examined for bile pigments, and the results were negative. A single dose of 3 mg/kg raised the BMR by 20−25%. Extended dosing with 50 or 100 mg/day (0.5−1.0 mg/kg-day according to the investigators) for ≈48 days did not result in signs or symptoms of toxicity and elevated the BMR by ≈5−35%. In the two typical charts shown, dosing with 50 mg/day on alternate days for 10 days and then daily up to 28 days resulted in a 15% or lower elevation of BMR. This treatment was followed immediately by 100 mg/day up through Day 48. At the higher dosage, the BMR rose by about 30−50% during the first 8 days at that dose and then appeared to level off. A LOAEL of 0.5 mg/kg-day is identified in this study for increased BMR.

Another study reports results of patients taking DNOC to lose weight (Ibrahim et al., 1934). Fifteen patients (eight males and seven females, 11 to 38 years old) took initial DNOC doses of 50 mg/day, increasing to 100 mg/day, for an average of 5.5 weeks. The initial male body weights ranged from 58−114 kg, while the females ranged from 66−118 kg. Information on body weight, total dose of DNOC received during treatment, and treatment duration were available for six males and six females. Using these data, the average daily dose was calculated to be 1.05 mg/kg-day for males (range 0.85−1.41 mg/kg-day) and females (range 0.80−1.27 mg/kg-day). Males and females exhibited an average body weight loss of 4% and 3%, respectively. All of the patients developed signs and symptoms of DNOC toxicity within a few days, including excessive sweating, fatigue, decreased appetite, elevated BMR, and greenish-yellow coloration of the conjunctivae. This study identifies a LOAEL of 0.8 mg/kg-day for DNOC based on these effects.

Plotz (1936) treated four patients (three men weighing 82 kg, 91 kg, and unspecified, and a woman weighing 90 kg) for obesity with DNOC. All four were started at a dosage of 0.75 mg/kg-day. Two patients tolerated this dosage for 6 weeks (a male patient) or 8 weeks (a female patient) with no outward signs of toxicity, but reported mild symptoms (i.e., slight headache or fatigue). The female patient then ingested 1 mg/kg-day, which resulted in an elevation of body temperature, weight loss, and green coloration of the sclerae. Decrease of the dosage to 0.5 mg/kg-day resulted in disappearance of the green coloration. Subsequent increase in dosage to 1.5 mg/kg-day resulted in a severe rash within 3 days, at which time, treatment was discontinued. The remaining two male patients experienced elevated body temperature, fatigue, and a greenish tint in the sclerae within days of starting treatment at the initial dose of 0.75 mg/kg-day. BMR was elevated in one of these patients but not reported in the other. After 2 weeks at the initial dosage, one of the affected patients was put on a decreased dosage of 0.5 mg/kg-day. At the end of 4 weeks on the reduced dosage of 0.5 mg/kg-day, the patient's temperature and BMR were still elevated and symptoms persisted; treatment was discontinued because of his discomfort. The other affected patient was taken off treatment for 2 weeks and then given 0.35 mg/kg-day. On the fifth day at this reduced dosage, the BMR was normal, but, by the seventh day, the greenish tinge to the sclerae had reappeared and the patient complained of sweating and fatigue. His icteric index was normal, and the urine contained no bile pigments.

He discontinued treatment because of the side effects. The study author ingested 1.0 mg/kg-day of DNOC for 4 weeks and experienced elevated body temperature, sweating, and a sensation of fullness of the head. The data from this study suggest variable sensitivity among individuals to DNOC. In this study, 0.35 mg/kg-day is identified as the LOAEL, which is the lowest dosage of DNOC tested.

In a case report, Quick (1937) reported bilateral cataract formation and resultant blindness in a 28-year old English woman who ingested DNOC for 3 years; the dosage was specified only as one capsule/day for 6 months and then two capsules/day of "dekrysil," with occasional 1-month intermissions if yellow pigmentation of the conjunctivae or tachycardia developed.

Mahlen (1938) reported on 73 patients in Sweden who had received DNOC at weight-reduction dosages (≈1 mg/kg-day) during a clinical study. Durations of treatment for these patients are not specified. After an unknown time on treatment, a nonjaundice yellowing of the skin and sclera occurred; however, reduction of the dosage eliminated this symptom. Six of the patients were examined for cataracts by other physicians and were found to be negative. The remaining 67 patients were examined by the study author. Of these 67, 11 were eliminated from consideration because they had senile cataracts, had other conditions such as diabetes, or in the case of one patient, were not yet on DNOC treatment at the time of the examinations. Of the remaining 56 patients, one had bilateral cataracts attributable to DNOC. The affected patient (a 31-year old woman) had taken 83 mg/day (1.2 mg/kg-day) of DNOC for 56 days and developed cataracts about 8 months later. Based on this case, a LOAEL of 1.2 mg/kg-day is identified for DNOC-induced cataract development.

Six other cases of cataracts associated with ingestion of DNOC in Sweden were also described by Mahlen (1938). These cases were identified through a questionnaire sent by the Swedish Board of Health to Swedish ophthalmologists. In all six cases, the patients were women (ages ranged from 34−57 years), and their daily doses of DNOC ranged from 50−125 mg/day. Although body weights were not specified, assuming body weights of 60 kg would result in doses of 0.8−2.1 mg/kg-day. Durations of treatment ranged from 5−12 months, with occasional additional treatment periods after 1−2 months without treatment. Symptoms and diagnosis of cataracts occurred 2−10 months after the end of treatment.

Horner (1942) reported that he had "collected" 13 cases of DNOC-induced cataracts from the literature up through January 1941. The study author provided no details.

In a study by Harvey et al. (1951), five healthy male volunteers (ages 19−36 years) weighing 59−81.4 kg were given 75 mg/day (0.92−1.27 mg/kg-day) of DNOC on 5 consecutive days. Two subjects receiving doses at the low end of the range continued to receive treatment for 2 more days. Blood levels of DNOC, pulse and respiratory rate, blood pressure, body weight, red blood cell (RBC) counts, and signs and symptoms of toxicity were monitored. Blood levels of DNOC rose steadily over the 5-day treatment period. Following 3−5 days of treatment, blood levels of DNOC reached 15−20 μg/g; additional doses then produced temporary high concentrations of 40−48 μg/g (within 4 hours of dosing) that were associated with symptoms of lassitude, headache, and malaise. Blood levels at 24 hours after dosing never exceeded 24 μg/g. No marked changes were seen in RBC counts. Yellow staining of the conjunctivae was observed

in all subjects on the third or fourth day of treatment. A LOAEL of 0.92 mg/kg-day is identified for clinical signs in this study (i.e., lassitude, headache, malaise, and conjunctivae yellowing).

Taken together, the available studies of human experience with use of DNOC suggest LOAEL values in the range of 0.35 to 1.2 mg/kg-day for effects ranging from increased BMR to clinical signs and cataracts. These LOAEL values are similar to the therapeutic dose range initially recommended by Dodds and Robertson (1933) as a "safe" dose range for weight loss, prior to the identification of side effects in humans.

Inhalation Exposure

No inhalation studies in humans were identified in the available literature for DNOC.

Animal Studies

Oral Exposure

Subchronic Studies—Groups of 5−10 male Wistar rats were fed 0, 7.8, 15.6, 31.2, 62.5, 125, 250, 500, or 1,000 ppm 3,5-dinitro-*o*-cresol (purity unspecified) in the diet for 105 days (Ambrose, 1942). The study authors stated that 3,5-dinitro-*o*-cresol is synonymous with DNOC. Using the reported values for food consumption, body weight, and total daily drug ingestion, the daily DNOC intake was calculated to be 0, 0.4, 0.9, 1.8, 4, 8.4, or 20 mg/kg-day for the 0- to 250-ppm groups, respectively[1]. Food consumption and body-weight data for the two highest treatment groups are not reported because these rats refused to eat. Body weight, food consumption, and clinical signs were evaluated weekly. At the end of the study, gross and histopathological examinations were given to all surviving rats, but no details of tissues examined or results were reported. No incidence data (apart from mortality) or statistical analyses were reported. Mortality incidences at 0, 0.4, 0.9, 1.8, 4, 8.4, and 20 mg/kg-day were 0/10, 0/10, 0/10, 2/10, 2/10, 6/10, and 6/10, respectively. All rats in the two highest dose groups (500 and 1,000 ppm) died within 2−3 days. Food consumption was the same as controls at ≤1.8 mg/kg-day, and 9%, 15%, and 27% higher in the 4-, 8.4-, and 20-mg/kg-day dose groups, respectively. Body weights at ≤1.8 mg/kg-day were the same as controls, but they were 5% and 7% higher at 4 and 8.4 mg/kg-day. At 20 mg/kg-day, body weights were 13% lower than controls. No gross or histopathological changes were noted in treated animals—except for possible yellow coloring of skeletal muscle and blood serum at ≥20 mg/kg-day. The food consumption and body-weight data suggest a LOAEL of 4 mg/kg-day and a NOAEL of 1.8 mg/kg-day. However, the occurrence of unexplained mortality at both of these dose levels makes it difficult to interpret the results of this study; as a result, a reliable NOAEL or LOAEL could not be identified.

Additional endpoints were investigated in a study by Spencer et al. (1948). Groups of 10−20 male white rats were fed 0, 20, 50, 100, 200, 500, or 1,000 ppm DNOC (98.7% pure) in the diet for up to 182 days. Using terminal body weights reported by the study authors and the EPA (1988) allometric equation for calculation of food consumption from rat body weights, the approximate daily doses calculated for this PPRTV document were 0, 1.7, 4.2, 8.4, 17.3, or 44.9 mg/kg-day for the 0- to 500-ppm groups, respectively. The daily dose of DNOC in mg/kg-day for the 1,000-ppm group is not estimated because no terminal body weight is

[1]As an example, the 250-ppm group had an average rate of food consumption/rat of 14.8 g, an average rate of food consumption/kg body weight of 80 g, and an average daily DNOC consumption rate of 3.70 mg/rat. Average body weight = 14.8 g/rat ÷ 80 g/kg = 0.185 kg. Thus, 3.70-mg DNOC/rat-day ÷ 0.185 kg = 20-mg DNOC/kg-day.

reported, and clinical and gross postmortem examinations suggest these animals were not eating prior to death. Clinical signs were evaluated, and body weight and food consumption measured twice weekly. Blood was collected monthly and observed for RBC and white blood cell (WBC) (total and differential) counts, hemoglobin (Hgb), and blood urea nitrogen (BUN) levels. At the time of death, liver, kidney, heart, and testes weights were recorded. Gross and histological observations were made of liver, kidneys, lung, heart, spleen, adrenals, pancreas, testes, stomach and bone marrow.

No adverse effects were purportedly seen at 1.7−8.4 mg/kg-day (Spencer et al., 1948). At 17.3 mg/kg-day, terminal body weights were significantly reduced (by 8%) in comparison to controls (see Table 1). At 44.9 mg/kg-day, all rats appeared emaciated and unkempt, terminal body weights were significantly less than controls (21%), and mortality was high (8/20 rats died prior to Day 77, and 4 more were sacrificed moribund at that time). BUN levels were increased 54−122% in comparison to controls. Splenic congestion was seen in rats dying prematurely, and fat depletion was observed in animals surviving to the end of the study. In the 1,000-ppm dose group, all rats appeared weak, hungry, thin, and unkempt; 10/20 rats died within 10 days, and the remainder were sacrificed at that time. Gross examination showed marked emaciation and empty gastrointestinal tract. BUN levels in this group were roughly 3-fold higher than controls; cloudy hepatic swelling, renal tubule degeneration, and splenic congestion were observed. However, these effects were likely due to severe malnutrition rather than a specific effect of the chemical. A LOAEL of 17.3 mg/kg-day and a NOAEL of 8.4 mg/kg-day were identified in this study for reduced body weight.

Table 1. Body Weight and Serum Chemistry Changes in Male Rats Fed 4,6-Dinitro-*o*-cresol for up to 6 Months[a]

	Dose in mg/kg-day (ppm in diet)						
	Control	**1.7 (20)**	**4.2 (50)**	**8.4 (100)**	**17.3 (200)**	**44.9 (500)**	**NC (1000)**
No. Animals Examined	16	18	20	18	16	9	5
Terminal Body Weight (g) [b]	300 ± 8	292 ± 8	293 ± 8	304 ± 8	277 ± 7[c]	247 ± 7[d]	NM
Serum Chemistry							
BUN (mg/100 mL)	15.8	17.1	17.8	14.0	16.1	24.4−35.0	44.4

[a]Spencer et al., 1948
[b]Mean ± standard error
[c]Significantly different from control at $p < 0.05$
[d]Significantly different from control at $p < 0.01$

NC = Not calculated due to absence of terminal body-weight data
NM = Not measured

In a more recent study, groups of Wistar rats (10/group/sex) were administered 0, 50, 100, 200, or 400 ppm DNOC (98.7% pure) in the diet for 90 days (Den Tonkelaar et al., 1983). Using reference values for body weight (0.217 kg for males, 0.156 kg for females) and food consumption (0.02 kg for males, 0.016 kg for females) (U.S. EPA, 1988), DNOC doses were estimated to be 0, 4.6, 9.2, 18.4, or 36.9 mg/kg-day for males and 0, 5.1, 10.3, 20.5, or 41.0 mg/kg-day for females. These estimates are more uncertain in the higher dose groups because dose-related decreases in food consumption and growth were observed. Body weights were measured weekly, while food consumption was measured during Weeks 1, 2, 5, 9, and 12. Body temperature was measured on Days 1, 2, 14, 28, 42, 56, 70, and 84. At 90 days, blood was drawn and measured for levels of Hgb, hematocrit (Hct), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), RBC, and WBC (total and differential) counts, as well as levels of protein, pyruvate, lactate, triiodothyronine (T_3), and thyroxine (T_4). Urine was collected and measured for glucose, protein, and creatinine levels. Organ weights were measured for the brain, heart, liver, spleen, pituitary gland, thyroid, thymus, adrenals, ovaries, testes, uteri, and prostate. Histopathological observations were made of these and 16 other unspecified tissues. Measurements were made of liver glycogen levels and glucose-6-phosphate dehydrogenase activity.

With the exception of mortality, data are only reported semiquantitatively by Den Tonkelaar et al. (1983) and are also not reported by sex. Numbers and statistical significance are not reported; direction, relative magnitude, and dose-relatedness of changes were indicated graphically. Mortality incidences in the 0-, 50-, 100-, 200-, and 400-ppm dose groups were 0/20, 0/20, 1/20, 2/20, and 5/20, respectively. Food consumption was similar to controls in the 50- to 100-ppm dose groups, higher than controls at 200 ppm, and markedly lower at 400 ppm. Body weight gain was lower than controls at ≥100 ppm; the change from controls increased with dose. Food efficiency (g weight gained per g food consumed) likewise decreased with dose from 100−400 ppm. Body temperature was decreased only in the 400-ppm dose group. In blood, there were slight increases in Hgb and Hct at 100 and 200 ppm and a larger increase at 400 ppm. BUN also increased with dose from 100−400 ppm. Alanine transaminase (ALT) was increased in the 400-ppm dose group. Serum glucose was increased at 200−400 ppm, while serum pyruvate was decreased in all dose groups. The study authors suggested that the changes in blood pyruvate and glucose levels indicated reduced glycolysis, probably secondary to reduced availability of ATP due to uncoupling of oxidative phosphorylation (the known mechanism of action for DNOC). The study authors interpreted this as a sensitive indicator of metabolic derangement by DNOC in this study. Serum levels of the thyroid hormones T_3 and T_4 were also decreased in all dose groups; the differences from controls increased with dose for T_3, and large increases were observed at all doses for T_4. Organ weight changes reflect the change in body weight, with dose-related decreases in absolute weights and increases in relative weights in most organs, primarily at 200 and 400 ppm. In the 400-ppm dose group, histopathological lesions were noted in a number of tissues but seemed to reflect poor general condition rather than any specific target organ effects of DNOC; incidences and severity scores are not provided. Only incidental lesions were observed at lower doses. This study identifies a LOAEL of 4.6 mg/kg-day for DNOC based on indicators of metabolic effects (decreased blood pyruvate, T_3, and T_4 levels) that were more sensitive than body weight and food intake.

Groups of 18 male and female mice (strain not reported) were fed a diet containing 0, 1, 5, or 10 mg/kg-day DNOC in an unpublished 13-week range-finding study (Kelly, 1995, as described in a WHO [2000] monograph). Observations were made for mortality, clinical signs, food consumption, body weight gain, blood T_3 and T_4 levels, and unspecified histological endpoints. No mortality, clinical signs, or treatment-related histological changes are reported, and food consumption and body weight gain appear similar for all groups. Male mice exhibited decreased blood T_4 levels at 5 and 10 mg/kg-day, relative to controls, while a treatment-related decrease in blood T_3 levels was observed in females. However, the magnitude of T_3 and T_4 changes in mice is not reported. Insufficient study details are reported by WHO (2000) for identifying a NOAEL or LOAEL.

Chronic Studies—No published chronic oral studies in animals are available for review. A WHO (2000) monograph describes an unpublished 2-year study in rats from the United Kingdom (Broadmeadow, 1991). Groups of Fischer-344 rats (50/sex/group) were given 0, 2.5, 15, or 100 ppm DNOC (99.5% pure) in the diet for 104 weeks. According to WHO (2000), the approximate daily doses of DNOC were 0, 0.1, 0.6, or 4 mg/kg-day in males and 0, 0.12, 0.8, or 5 mg/kg-day in females. No details of study methods or statistical evaluation are reported. No significant changes in mortality were noted at any exposure level. No clinical signs or changes in body weight or weight gain were noted, although food consumption in males at 4 mg/kg-day was increased by 6% over controls. This may suggest that the male rat metabolism compensates for possible increases in BMR and energy consumption (as seen in humans) by increasing energy (food) intake. No marked treatment-related changes in histopathology were noted, and no increase in the incidence of any tumor was seen in treated groups, compared to controls. Using the data available from WHO (2000), a LOAEL of 4 mg/kg-day and a NOAEL of 0.6 mg/kg-day were tentatively identified for treatment-related increased food consumption in male rats. However, the study report is not available, precluding a complete review of the study data.

Reproductive/Developmental Studies—The effect of DNOC on sperm morphology was examined in two rodent studies. Groups of 24, 24, and 36 Sprague-Dawley male rats were given gavage doses of 0, 10, or 15 mg/kg-day DNOC (90% purity) in corn oil for 5 days (Takahashi et al., 2006). On Days 1, 7, and 14 postdosing, groups of 7−8 control, 8 low-dose, and 11−12 high-dose rats were sacrificed. Organ weights were recorded for the testes, epididymides, seminal vesicles, and ventral prostates. Sperm suspensions were obtained from the corpus, caput, and cauda epididymides, and were examined using light microscopy. Sperm from control and high-dose rats were also examined for abnormalities using scanning microscopy (SEM) and transmission electron microscopy (TEM). DNOC treatment did not markedly affect reproductive organ weights. Effects on sperm morphology were found at both dose levels, but they were more prominent in the high-dose group (see Table 2). The effect was manifest first as an increase in incidence of "peeled" sperm in the caput epididymides (Day 1 postdosing), then by increases in "peeled" sperm in the corpus and cauda epididymides (Day 7 postdosing), and finally by increases in "peeled" and tailless sperm in the cauda epididymides (Day 14 postdosing). By light microscopy, the "peeled" sperm had a thin, dark appearance near the distal end of the middle piece of the cell. SEM and TEM analyses on Day 1 postdosing revealed that the mitochondrial sheath was missing from the proximal end of the middle piece of the "peeled" sperm. The study authors suggested that the affected sperm became tailless by Day 14 after reaching the cauda epididymides. For this study, a LOAEL of 10 mg/kg-day was identified for increased percentages of abnormal sperm in rats.

Table 2. Changes in Sperm Morphology on Days 1, 7, and 14 Postdosing for Male Sprague-Dawley Rats Treated with 4,6-Dinitro-*o*-cresol via Oral Gavage for 5 Days[a]

Lesion / Tissue	Dose in mg/kg-day		
	Control	10	15
Day 1			
Caput Epididymides			
Total normal sperm (%)	97.2 ± 1.5	95.8 ± 2.1	86.8 ± 9.3[b]
Peeled sperm (%)	0.5 ± 0.5	2.3 ± 1.7[b]	11.7 ± 8.3[c]
Tailless sperm (%)	0.5 ± 0.3	1.6 ± 1.2 [b]	0.6 ± 0.5
Day 7			
Corpus Epididymides			
Total normal sperm (%)	96.7 ± 3.6	90.6 ± 4.8[b]	63.1 ± 12.5[c]
Peeled sperm (%)	1.4 ± 1.9	8.4 ± 4.3[c]	34.9 ± 12.1[c]
Cauda Epididymides			
Total normal sperm (%)	94.4 ± 4.5	94.6 ± 5.5	92.0 ± 4.5
Amorphous sperm (%)	0.8 ± 0.5	0.2 ± 0.4[b]	0.2 ± 0.3[b]
Peeled sperm (%)	0.6 ± 1.2	2.9 ± 3.0[b]	5.5 ± 3.2[c]
Day 14			
Cauda Epididymides			
Total normal sperm (%)	97.1 ± 1.6	94.4 ± 3.5[b]	78.5 ± 18.7[c]
Peeled sperm (%)	0.9 ± 0.4	2.5 ± 2.0[b]	9.2 ± 4.6[c]
Tailless sperm (%)	1.4 ± 1.0	2.0 ± 1.9	11.5 ± 17.8[c]

[a]Takahashi et al., 2006
[b]Significantly different from control at $p < 0.05$
[c]Significantly different from control at $p < 0.01$

Values presented as mean ± standard deviation

Groups of male 11−15 week-old C3B6F1 mice (6/group) were administered 0, 3, 6, or 12 mg/kg-day DNOC in distilled water for 5 days by both the oral and intraperitoneal routes (Quinto et al., 1989). Testes weight, sperm counts, and frequency of sperm abnormalities were measured. DNOC was found to have no marked effect on the percentage of sperm abnormalities, sperm counts or testicular weight by either oral or i.p. administration. A NOAEL of 12 mg/kg-day was identified in this study. The contradictory results observed in this study versus Takahashi et al. (2006) could be due to a species-dependent effect (mouse versus rat) or differences in the methods utilized (i.e., assessment of sperm morphology and the time of assessment relative to dose administration).

In a developmental toxicity study, 13 pregnant DBA mice were given gavage doses of Krezonit E herbicide (containing only 50% DNOC; equivalent to 8 mg/kg-day) on Gestation Days (GD) 11−14 (Nehez et al., 1981). A group of 20 pregnant controls was included. On GD 18, the dams were sacrificed and uteri observed for number of corpus lutea, implantations, living and dead fetuses, resorptions, and fetus weight. Fetuses were observed for gross developmental abnormalities and were examined for skeletal malformations. Inspection of the data showed that none of the observed endpoints in treated animals was significantly different from controls. A NOAEL of 8 mg/kg-day is identified in this study.

Observations from other unpublished reproductive and developmental studies listed below are reported in the WHO (2000) monograph. However, because these study reports are not available, a complete review of the studies could not be performed. Additionally, because these studies are unpublished and the information is incompletely reported in the available WHO (2000) monograph (e.g., numbers of animals/treatment group is unknown, DNOC purity is unknown, unknown compliance with Good Laboratory Practice standards, etc.), these studies are deemed unacceptable for the toxicity assessment of DNOC. Thus, these studies are only used as supporting studies and do not wield any quantitative impact on the toxicity assessment of DNOC.

Coles and Brooks (1997) exposed groups of male and female Sprague Dawley CD rats (number/group not reported) to technical grade (97.45% pure) DNOC at concentrations of 15, 30, and 100 mg of DNOC/kg diet throughout maturation, mating, gestation (total exposure duration not reported) in a two-generation reproductive toxicity study. The study authors concluded that no toxicologically significant effects occurred at any dose level for adults and in both generations. According to the WHO (2000) monograph, however, the reported data actually indicate effects at the high dose including a significant reduction in group mean body weight in F0 females, a significant reduction in group mean litter size in the F0 generation on Days 14 and 21 of lactation, and a statistically significant reduction in group mean litter weight in the F0 and F1 generations on Days 14 and 21 of lactation.

In a developmental toxicity study, groups of pregnant SPF Wistar rats (number/group not reported) were administered 0, 1, 5, or 25 mg/kg-day DNOC (purity not specified) via drinking water on GD 6−15 (Dickhaus and Heisler, 1984). The authors concluded that no toxicologically significant effects occurred at any dose level for the dams and pups. In a study using chinchilla rabbits (Kfm: CHIN.SPF), 16 females/group received doses of 0, 4, 10, and 25 mg/kg-day DNOC (purity not specified) by gavage on GD 6−18. External and visceral fetal malformations were observed at the high dose. However, as stated above, the study report is not available, precluding a complete review of the study data.

Inhalation Exposure

Data on inhalation studies of DNOC in animals are limited to a single study in Russian (Burkatskaya, 1965) that was reported both by ATSDR (1995) and the WHO (2000). Death occurred in 2/3 cats exposed to 2 mg/m^3 of DNOC for 4 hours/day for 30 days. However, no mortality or "severe adverse effects" were reported for the 3 cats exposed to 0.2 mg/m^3 for 4 hours/day for 60 or 90 days. No further details of this study are available.

Other Studies

Acute or Short-term Studies

Groups of 10−30 male rats (strain unspecified) were given single gavage doses of 20, 30, 40, 50, or 100 mg/kg DNOC (purity unspecified) and observed for mortality for up to 12 hours (Ambrose, 1942). The use of controls is not reported. The mortality incidences and time-to-death at 20, 30, 40, 50, or 100 mg/kg were 0/10, 3/30 (within 4−12 hours), 15/15 (within 1−8 hours), 10/10 (within 15 minutes−4 hours), and 10/10 (within 2−2.5 hours), respectively. No clinical signs were observed at 20 mg/kg. CNS depression, dyspnea, and convulsions were observed just before death at ≥30 mg/kg.

Other Routes

Intraperitoneal injection of Krezonit E herbicide (containing only 50% DNOC; equivalent to 15 mg/kg-day) of this formulation into DBA or ABH-J mice on Day 11 or Days 11−14 or Days 4, 9, and 11 of gestation did not result in changes from controls in viable pregnancies, gross abnormalities, or skeletal malformations (Nehez et al., 1981).

Genotoxicity

WHO (2000), ATSDR (1995), and EPA (1986) have reviewed the mutagenic effects of DNOC. Evaluation of DNOC for reverse mutation in *Salmonella typhimurium* has produced mixed results, both with and without activation, that do not appear to be strain-specific. Reverse mutation assays in *Escherichia coli* have generally been negative. In vitro evaluations of mutations and chromosomal damage in mammalian cells have also reported both positive and negative results. DNOC resulted in increases in sex-linked recessive lethal mutations in *Drosophila* in vivo. Injection studies of chromosomal aberrations in rats and mice and an oral gavage study in rats have generally yielded positive results, as have injection studies of micronucleus formation in mice.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR 4,6-DINITRO-*O*-CRESOL

Subchronic p-RfD

As noted earlier, the available human studies collectively suggest LOAELs in the range of 0.35 to1.2 mg/kg-day for humans ingesting DNOC for up to 1 year (see Table 3). The limitations across all of the human studies include deficiencies in reporting, lack of control groups, small numbers of exposed individuals, inconsistent dosing regimens, and brief exposure durations. Subchronic and chronic animal studies support metabolic effects as the key endpoint for DNOC, most commonly manifested in these studies by changes in food consumption and body-weight gain (Ambrose, 1942; Spencer et al., 1948; Den Tonkelaar et al., 1983; Broadmeadow, 1991, as summarized in WHO, 2000), but also by more subtle indicators of metabolic disturbance (Den Tonkelaar et al., 1983). Effective doses in the animal studies are approximately an order of magnitude higher than in the human studies (see Table 3).

Given that the human studies identified effect levels well below those observed in the animal studies, and that human data are preferred over animal data for derivation of toxicity values, the human data (i.e., Dodds and Robertson, 1933; Ibrahim et al., 1934; Plotz, 1936; Mahlen, 1938; Harvey et al., 1951) are considered as the basis for the subchronic p-RfD. Of

these studies, Ibrahim et al. (1934) has been chosen as the principal study because on the whole, it was the best conducted and utilized an adequate number of human subjects (eight males and seven females). This study identifies DNOC-induced metabolic critical effects including reduced body weight, excessive perspiration and fatigue, and elevated BMR and body temperature, as well as ocular effects (i.e., greenish-yellow coloration of the conjunctivae). Ibrahim et al. (1934) identifies a LOAEL of 0.8 mg/kg-day for DNOC based on these effects, and this study is also supported by several other human studies (Dodds and Robertson, 1933; Plotz, 1936; Harvey et al., 1951) that identify analogous critical effects occurring in a similar dose range. Although the study by Mahlen (1938) utilized more subjects (56 cases), this study did not evaluate DNOC-induced metabolic effects, and a higher LOAEL of 1.2 mg/kg-day was identified based on cataract development. Thus, the lower LOAEL of 0.8 mg/kg-day for DNOC-induced metabolic and ocular effects distinguished in Ibrahim et al. (1934) is identified as the point of departure (POD). The **subchronic p-RfD** for DNOC is derived by dividing this POD by a composite UF of 1,000, as follows:

Subchronic p-RfD = LOAEL ÷ UF
= 0.8 mg/kg-day ÷ 1,000
= **0.0008 mg/kg-day or 8×10^{-4} mg/kg-day**

The composite UF of 1,000 consists of the following:

- UF_H: A factor of 10 is applied for extrapolation to a potentially susceptible human subpopulation because the data for evaluating susceptible human responses are unavailable.
- UF_D: A factor of 10 is applied for database deficiencies because the data for evaluating developmental/reproductive toxicity are incomplete (specifically lacking an acceptable two-generation reproductive toxicity study and an acceptable developmental study).
- UF_L: A factor of 10 is applied for using a LOAEL as the POD because the data for establishing a NOAEL are unavailable.

Confidence in the principal study (Ibrahim et al., 1934) as well as the supporting studies (Dodds and Robertson, 1933; Plotz, 1936; Harvey et al., 1951) is low because the studies consist of case reports and limited clinical trials, and they do not define a NOAEL. Confidence in the database is low. While multiple human studies indicate similar sensitive effects (i.e., metabolic and ocular effects) and are supported by multiple animal studies showing similar effects, no acceptable two-generation reproductive function studies are available in animals, and acceptable developmental toxicity studies are also limited. Therefore, confidence in the subchronic p-RfD is low.

Chronic p-RfD

The LOAEL of 0.8 mg/kg-day identified from the Ibrahim et al. (1934) study and used to derive the subchronic p-RfD above could also be used to derive a chronic p-RfD for DNOC. However, in this case, the composite UF would increase to 10,000. Based on current guidelines and standard operating procedures, composite UFs > 3000 cannot be considered for provisional reference value derivation. As such, while a chronic p-RfD cannot be derived here, the appendix of this document contains a chronic oral "screening value" that may be useful in certain instances. Please see the attached Appendix for details.

FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR 4,6-DINITRO-*O*-CRESOL

Due to a lack of suitable human or animal data, a subchronic or chronic p-RfC for DNOC cannot be derived

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR 4,6-DINITRO-*O*-CRESOL

Weight-of-Evidence Descriptor

Under the 2005 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), there is "***Inadequate Information to Assess* [the] *Carcinogenic Potential of DNOC.***" The WHO (2000) monograph describes an unpublished 2-year feeding study in rats from the United Kingdom (Broadmeadow, 1991). In this study, no increase in the incidence of any tumor was seen in treated groups. However, because the study report is not available, a complete review of the study could not be performed. No other studies examining the carcinogenicity of DNOC in animals have been located, and no relevant human studies have been located. The mutagenicity and genotoxicity data for DNOC, as reviewed by WHO (2000), ATSDR (1995), and EPA (1986), are inconclusive.

Quantitative Estimates of Carcinogenic Risk

Derivation of quantitative estimates of cancer risk for DNOC is precluded by the lack of suitable data.

REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). 2008. Threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati, OH.

Ambrose, A.M. 1942. Some toxicological and pharmaceutical studies on 4,6-dinitro-*o*-cresol. J. Pharmacol. Exp. Ther. 76:245−251.

ATSDR (Agency for Toxic Substances and Disease Registry). 1995. Toxicological Profile for Dinitrocresols. U.S. Department of Health and Human Services, Public Health Service. Online. http://www.atsdr.cdc.gov/toxprofiles/tp63.html.

Broadmeadow, A. 1991. Technical DNOC: Combined oncogenicity and toxicity study by dietary administration to F-344 rats for 104 weeks (Life Science Research study no. PTN/003/DNOC). Eye, UK. (unpublished report prepared for Pennwalt Corporation) (Cited in WHO, 2000).

Burkatskaya, E.N. 1965. [Maximum permissible concentration of dinitro-*o*-cresols in air.] Gig. Sanit. 30:34−37. (Article in Russian) (Described in WHO, 2000).

Coles, R.J. and P.N. Brooks. 1997. Technical DNOC: dietary two generations reproduction study in the rat (Safepharm Laboratory project no.764/010). Derby, UK (unpublished report prepared for Elf Atochem Agri SA) (Cited in WHO, 2000).

Colman, E. 2007. Dinitrophenol and obesity: an early twentieth-century regulatory dilemma. Regulatory Toxicology and Pharmacology 48(2):115−117.

Cutting, W.C. and M.L. Tainter. 1933. Metabolic actions of dinitrophenol with the use of balanced and unbalanced diets. J. Am. Med. Assoc. 101(27):2,099−2,102.

Den Tonkelaar, E.M., F.X.R. Van Leeuwen and C. Kuiper. 1983. Semichronic toxicity testing of DNOC in the rat. Meded. Fac. Landbouwwet. Rijksuniv. Genet. 48(4):1,015−1,022.

Dickhaus, S. and E. Heisler. 1984. [Teratogenic/embryotoxic study with the product "Trifocide liquid 50%" following oral administration in the rat.] (Pharmatox study no. 2-4-240-83) Hamburg, Germany (unpublished report prepared for Pennwalt Holland bv) in German (Cited in WHO, 2000).

Dodds, E.C. and J.D. Robertson. 1933. The clinical applications of dinitro-*o*-cresol. Lancet. 2:1,137−1,139.

Harvey, D.G., P.L. Bidstrup and J.A. Bonnell. 1951. Poisoning by dinitro-ortho-cresol. Some observations on the effects of dinitro-ortho-cresol administration by mouth to human volunteers. Br. Med. J. 2:13−16.

Horner, M.D. 1942. Dinitrophenol and its relation to formation of cataracts. Arch. Ophthal. 27:1,097.

IARC (International Agency for Research on Cancer). 2008. Search IARC Monographs. Online. http://monographs.iarc.fr/ENG/Monographs/allmonos90.php.

Ibrahim, H., H. Avad and M.A. Mahdi. 1934. The new treatment of obesity with dinitro-*o*-cresol or dekrysil. J. Egypt. Med. Assoc. 17:969−990.

Kelly, J. 1995. DNOC: 13-week oral (dietary administration) range-finding study in the mouse. (Corning Hazelton project no. CHE 1151/8). Harrogate, UK (unpublished report prepared for Elf Atochem Agri SA) (Described in WHO, 2000).

Mahlen, S. 1938. Zur Kenntnis der Katarakto bei Dinitroorthocresolbehandlung. Acta Ophthal. 16:563−572.

Nehez, M., A. Paldy, A. Selypes et al. 1981. The teratogenic and mutagenic effects of dinitro-*o*-cresol-containing herbicide on the laboratory mouse. Ecotoxicol. Environ. Safety. 5:38−44.

NIOSH (National Institute for Occupational Safety and Health). 1978. Criteria for a Recommended Standard—Occupational Exposure to Dinitro-ortho-cresol. U.S. Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, NIOSH. DHEW (NIOSH) Publication No. 78-131.

NIOSH (National Institute for Occupational Safety and Health). 2008. NIOSH Pocket Guide to Chemical Hazards. Online. http://www.cdc.gov/niosh/npg/.

NTP (National Toxicology Program). 2005. 11th Report on Carcinogens. Online. http://ntp.niehs.nih.gov/index.cfm?objectid=32BA9724-F1F6-975E-7FCE50709CB4C932.

NTP (National Toxicology Program). 2008. Management Status Report. Online. http://ntp.niehs.nih.gov/index.cfm?objectid=78CC7E4C-F1F6-975E-72940974DE301C3F.

OSHA (Occupational Safety and Health Administration). 2008. OSHA Standard 1910.1000 Table Z-1. Part Z, Toxic and Hazardous Substances. Online. http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=9992.

Plotz, M. 1936. Dinitro-ortho-creosol: A metabolic stimulator and its toxic side-actions. N.Y. State J. Med. 36:266−268.

Quick, H.E. 1937. Slimming drugs and cataract—With notes of a case. Br. Med. J. 1:1,203−1,204.

Quinto, I., E. De Marinis, M. Mallardo, A. Arcucci, R. Della Morte and N. Staiano. 1989. Effect of DNOC, Ferbam and Imidan exposure on mouse sperm morphology. Mutat. Res. 224:405−408.

Spencer, H.C., V.K. Rowe, E.M. Adams et al. 1948. Toxicological studies on laboratory animals of certain alkyldinitrophenols used in agriculture. J. Ind. Hyg. Toxicol. 30:10−25.

Tainter, M.L. and D.A. Wood. 1934. A case of fatal dinitrophenol poisoning. J. Am. Med. Assoc. 102 (14):1,147−1,149.

Tainter, M.L., W.C. Cutting, A.B. Stockton. 1934. Use of dinitrophenol in nutritional disorders. A critical survey of clinical results. Am. J. Public Health 24:1,045−1,053.

Takahashi, K., M. Harigae, N. Takahashi et al. 2006. Pathogenetic transition in the morphology of abnormal sperm in the testes and the caput, corpus, and cauda epididymides of male rats after treatment with 4,6-dinitro-*o*-cresol. Reprod. Toxicol. 22:501−507.

U.S. EPA. 1986. Health and Environmental Effects Profile (HEEP) for Dinitrocresols. 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. 1988. Recommendations for and Documentation of Biological Values for Use in Risk Assessment. 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. EPA/600/6-87/008. NTIS PB 88-179874.

U.S. EPA. 1991. Chemical Assessments and Related Activities (CARA). Office of Health and Environmental Assessment, Washington, DC. April.

U.S. EPA. 1994. Chemical Assessments and Related Activities (CARA). Office of Health and Environmental Assessment, Washington, DC. December.

U.S. EPA. 1997. Health Effects Assessment Summary Tables. FY-1997 Update. Prepared by the Office of Research and Development, National Center for Environmental Assessment, Cincinnati OH for the Office of Emergency and Remedial Response, Washington, DC. July. EPA/540/R-97/036. NTIS PB97-921199.

U.S. EPA. 2005. Guidelines for carcinogen risk assessment, Final Report. Risk Assessment Forum, U.S. Environmental Protection Agency. Washington, DC. EPA/630/P-03/001F. Online. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid =1 16283.

U.S. EPA. 2006. 2006 Edition of the Drinking Water Standards and Health Advisories. Office of Water, Washington, DC. EPA 822-R-06-013. Washington, DC. Available at http://www.epa.gov/waterscience/drinking/standards/dwstandards.pdf.

U.S. EPA. 2008. Integrated Risk Information System (IRIS). Online. Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Online. http://www.epa.gov/iris/.

WHO (World Health Organization). 2000. Environmental Health Criteria Monograph on Dinitro-*ortho*-cresol. Monograph 220. International Programme on Chemical Safety. Geneva, Switzerland.

APPENDIX. DERIVATION OF A SCREENING VALUE FOR 4,6-DINITRO-*O*-CRESOL

For reasons noted in the main PPRTV document, it is inappropriate to derive provisional toxicity values for 4,6-dinitro-*o*-cresol. However, information is available for this chemical which, although insufficient to support derivation of a provisional toxicity value under current guidelines, may be of limited use to risk assessors. In such cases, the Superfund Health Risk Technical Support Center summarizes available information in an appendix and develops a "screening value." Appendices receive the same level of internal and external scientific peer review as the PPRTV documents to ensure their appropriateness within the limitations detailed in the document. Hazard identification and dose-response information contained in an Appendix receives the same level of internal and external scientific peer review as the main body of PPRTV documents, to ensure their appropriateness within the limitations detailed in the document.

Screening values are intended for use in limited circumstances when no Tier 1, 2, or 3 values are available. Screening values may be used, for example, to rank relative risks of individual chemicals present at a site to determine if the risk developed from the associated exposure at the specific site is likely to be a significant concern in the overall cleanup decision. Screening values are not defensible as the primary drivers in making cleanup decisions because they are based on limited (e.g., scope, depth, validity, etc.) information. Questions or concerns about the appropriate use of screening values should be directed to the Superfund Health Risk Technical Support Center.

Screening Chronic p-RfD

As noted earlier, the available human studies collectively suggest LOAELs in the range of 0.35 to 1.2 mg/kg-day for humans ingesting DNOC for up to 1 year (see Table 3). The longest exposure duration of a human subject in the principal study (Ibrahim et al., 1934) was only up to 63 days. Thus, in addition to the aforementioned recommendations on the intended and appropriate use of screening values, it is important to note that due to the particularly short exposure duration employed in the principal study, the certitude of the screening chronic p-RfD derived below is particularly diminished. A **screening chronic p-RfD** for DNOC is derived by dividing the LOAEL of 0.8 mg/kg-day identified in Ibrahim et al. (1934) by a composite UF of 10,000, as follows:

$$\begin{aligned} \textbf{Screening Chronic p-RfD} &= \text{LOAEL} \div \text{UF} \\ &= 0.8 \text{ mg/kg-day} \div 10{,}000 \\ &= \textbf{0.00008 mg/kg-day or } \mathbf{8 \times 10^{-5}} \textbf{ mg/kg-day} \end{aligned}$$

The composite UF of 10,000 was composed of the following UFs:

- UF_D: A factor of 10 is applied for database deficiencies because the data for evaluating developmental/reproductive toxicity are incomplete (specifically lacking an acceptable two-generation reproductive toxicity study and an acceptable developmental study).
- UF_H: A factor of 10 is applied for extrapolation to a potentially susceptible human subpopulation because the data for evaluating susceptible human responses are unavailable.

- UF_L: A factor of 10 is applied for using a LOAEL as the POD because data for establishing a NOAEL are unavailable.
- UF_S: A factor of 10 is applied for using data from less-than-lifetime exposure to assess potential effects from chronic exposure.

Table 3. Summary of Oral Noncancer Dose-Response Information for 4,6-Dinitro-*o*-cresol

Species and Study Type (*n*/sex/group)	Exposure	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Responses at the LOAEL	Reference
Human Short-term					
Human Short-term 5 male volunteers	0.92−1.27 mg/kg-day administered for 5−7 days	Not identified	0.92	Lassitude, headache, malaise, yellow conjunctival staining	Harvey et al., 1951
Human Short-term Unspecified number of subjects	administered 0.5−1 mg/kg-day for 48 days or 3 mg/kg-day for 3 days	Not identified	0.5	Modest elevation of BMR with no associated clinical signs	Dodds and Robertson, 1933
Human Short-term 8 males and 7 females	0.6−1.3 mg/kg-day administered for up to 63 days	Not identified	0.8	Reduced body weight, excessive sweating, fatigue, decreased appetite, elevated BMR, and greenish-yellow coloration of the conjunctivae	Ibrahim et al., 1934
Human Short-term 4 patients	0.35−1.5 mg/kg-day administered orally for up to 8 weeks	Not identified	0.35	Headache, excessive perspiration, and fatigue, elevated BMR, body temperature, and greenish coloration of sclera	Plotz, 1936
Human Short- to long-term oral 56 cases	50−125 mg/day for 56 days to 12 months	Not identified	1.2	Bilateral cataracts developed 8 months after DNOC treatment of a 31-year-old woman with 83 mg/day (1.2 mg/kg-day) for 56 days. Six other 34−57-year-old women developed cataracts 2−12 months after cessation of 5−12 months of DNOC treatments ranging from 50−125 mg/day	Mahlen, 1938
Animal Subchronic					
Rat Subchronic oral (5−10 males/group)	0-, 7.8-, 15.6-, 31.2-, 62.5-, 125-, 250-, 500-, or 1,000-ppm DNOC (estimated as 0-, 0.4-, 0.9-, 1.8-, 4-, 8.4-, or 20-mg DNOC/kg-day for the 0- to 250-ppm groups)	Not identified	Not identified	N/A (the food consumption and body-weight data suggest a LOAEL of 62.5 ppm (4 mg/kg-day) and NOAEL of 31.2 ppm (1.8 mg/kg-day). However, the occurrence of unexplained mortality at both of these dose levels makes it difficult to interpret the results of this study)	Ambrose, 1942

Table 3. Summary of Oral Noncancer Dose-Response Information for 4,6-Dinitro-*o*-cresol

Species and Study Type (*n*/sex/group)	Exposure	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Responses at the LOAEL	Reference
Rat Subchronic oral (10−20 males/group)	0-, 20-, 50-, 100-, 200-, 500-, or 1,000-ppm DNOC in the diet for up to 182 days (estimated as 0, 1.7, 4.2, 8.4, 17.3, or 44.9 mg DNOC/kg-day for the 0−500 ppm groups)	8.4	17.3	Reduced body weight	Spencer et al., 1948
Wistar rat Subchronic oral (10/sex/group)	0-, 50-, 100-, 200-, or 400-ppm DNOC in diet for 90 days (estimated as 0-, 4.6-, 9.2-, 18.4-, or 36.9-mg DNOC/kg-day in males and 0-, 5.1-, 10.3-, 20.5-, or 41.0-mg DNOC/kg-day in females)	Not identified	4.6	Decreased blood pyruvate, T_3, and T_4 levels	Den Tonkelaar, et al., 1983
Animal Chronic					
F344 rats Chronic oral (50/sex/group) Note: details of study methodology and resulting data are not available	0, 2.5, 15, or 100 ppm in diet for 104 weeks WHO (2000) reported the following daily doses: Males: 0, 0.1, 0.6, or 4 mg/kg-day Females: 0, 0.12, 0.8, or 5 mg/kg-day	0.6	4	Increased food consumption, possibly as a response to increased BMR	Broadmeadow, 1991, as reported in WHO, 2000
Reproductive/Developmental					
SD rats Short-term oral Reproductive (24−36 males/group)	0-, 10- or 15-mg DNOC/kg-day for 5 days	Not identified	10	Abnormal sperm morphology	Takahashi et al., 2006
C3B6F1 mice Short-term oral Reproductive	0-, 3-, 6- or 12-mg DNOC/kg-day for 5 days	12	Not identified	N/A (no effect observed on testicular weight, sperm counts or percentage of sperm abnormalities)	Quinto et al., 1989
DBA mice Short-term oral Developmental	0- or 8-mg DNOC/kg-day (in Krezonit E herbicide) on GD 11−14	8	Not identified	N/A (no effect on number of corpus luteum, implantations, living and dead embryos, resorptions, and embryo weight, gross abnormalities or skeletal malformations)	Nehez et al., 1981