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Provisional Peer Reviewed Toxicity Values for

2,4-Dinitrophenol
(CASRN 51-28-5)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
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Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
kg	kilogram
L	liter
LEL	lowest-effect level
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
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level
MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
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 inhalation reference concentration
p-RfD	provisional oral reference dose

PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR 2,4-DINITROPHENOL (CASRN 51-28-5)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) 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 (PPRTV) 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 Integrated Risk Information System (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 multi-program 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 five-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 manuscripts 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 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 manuscript 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

The HEAST (U.S. EPA, 1997) listed a chronic RfD of 2E-3 mg/kg-day for 2,4-dinitrophenol (DNP) based on the IRIS database. The assessments were based on a LOAEL of 2 mg/kg-day for cataract in humans treated with 2,4-DNP as a weight reducing agent (Horner, 1942). The derivation for the chronic RfD included an uncertainty factor (UF) of 1000 (10 for extrapolation from subchronic exposure to chronic exposure, 10 for protecting sensitive individuals, and 10 for the use of a LOAEL). The HEAST also lists a subchronic RfD by adopting the chronic oral RfD from IRIS. The chronic RfD for 2,4-DNP was developed by U.S. EPA in 1991, and is listed on the IRIS database. However, it was not listed on the Drinking Water Standards and Health Advisories list (U.S. EPA, 2006). The toxicity of 2,4-DNP has been summarized in the Ambient Water Quality Criteria for Nitrophenols by U.S. EPA (1980) and the Toxicological Profile for Dinitrophenol by ATSDR (1995). ATSDR proposed an acute-duration Minimal Risk Level (MRL) of 0.01 mg/kg-day, but not for intermediate- or chronic-duration oral exposure to 2,4-DNP.

An RfC for 2,4-DNP was not listed on the HEAST (U.S. EPA, 1997). The health effect data for 2,4-DNP were reviewed by the U.S. EPA RfD/RfC Work Group in 1991 and were determined to be inadequate for the derivation of an inhalation RfC. The American Conference of Governmental Industrial Hygienists has not established a Threshold Limit Value (TLV)-Time-Weighted Average (TWA) and the National Institute for Occupational Safety and Health (NIOSH, 2005) has not established a REL-TWA for this chemical. The Occupational Safety and Health Administration (OSHA) has not developed a Permissible Exposure Limit (PEL)-TWA for 2,4-DNP either.

The 1997 HEAST did not include a cancer assessment for 2,4-DNP. No cancer assessment for this chemical has been developed by IRIS or IARC.

The WHO (2007) has not reviewed the toxicology of 2,4-DNP. Literature searches were conducted for the period from 1980 to August 2007 to identify data relevant for the derivation of provisional RfD, RfC, and cancer assessments for 2,4-DNP. The following databases were searched: TOXLINE, MEDLINE, CANCERLIT, TOXLIT/BIOSIS, Registry of Toxic Effects of Chemical Substances (RTECS), HSDB, GENETOX, CCRIS, TSCATS, EMIC/EMICBACK, and DART/ETICBACK.

This document has passed the Superfund Health Risk Technical Support Center (STSC) quality review and peer review evaluation indicating that the quality is consistent with the SOPs and standards of the STSC and is suitable for use by registered users of the PPRTV system.

REVIEW OF PERTINENT LITERATURE

Human Studies

Numerous occasions of human poisoning by 2,4-DNP have been reported in the literature. The earliest cases of fatal 2,4-DNP intoxication related to its usage as a component of explosives during World War I. Thirty-six cases of fatal occupational dinitrophenol poisoning occurred among employees of the munitions industry in France between 1916 and 1918 (Perkins, 1919). A literature review by von Oettingen (1949) revealed 27 reported cases of fatal occupational dinitrophenol poisoning in the United States for the years 1914 to 1916. In addition, Gisclard and Woodward (1946) reported two fatal cases of dinitrophenol poisoning during manufacture of picric acid where 2,4-DNP was produced as an intermediate. Swamy (1953) also described a case of suicidal poisoning by 2,4-DNP.

Early in the 1930s, 2,4-DNP was widely recommended as a treatment for obesity, and it resulted in both toxic side effects and fatalities. Horner (1942) reported a total of nine deaths resulting from the use of dinitrophenol as a slimming agent. The toxic manifestations of dinitrophenol exposure as reviewed by Horner (1942) included subacute symptoms such as gastrointestinal disturbances (nausea, vomiting, colic, diarrhea, anorexia), profuse sweating, weakness, dizziness, headache, and loss of weight. Acute poisoning has resulted in the sudden onset of pallor, burning thirst, agitation, dyspnea, profuse sweating, and hyperpyrexia. Intense and rapid onset of rigor mortis after death has also been described.

Perkins (1919) reported that postmortem examination of dinitrophenol victims demonstrated no characteristic lesions. Acute edema of the lungs was mentioned but was believed to be secondary to the toxic effects on the vasomotor system. Microscopic lesions of the liver and kidney cells were inconstant and typical changes were lacking elsewhere.

The widespread use of 2,4-DNP as a weight reducing agent in humans during the 1930s also provided some information regarding the chronic effects of this compound in humans. Recommended therapeutic doses of 2,4-DNP for weight control on humans ranged from 2 to 5 mg/kg-day (Dunlop, 1934; Horner, 1942; Tainter et al., 1933). Tainter et al. (1933) administered 2,4-DNP (average daily dose of 0.3 g) to 113 obese patients for as long as four months without demonstrating evidence of cumulative or toxic effects. Based on an assumption of body weight

of 70 kg, the corresponding average daily dose was 4.3 mg/kg-day. The most important side effect noted by the investigators was a skin rash observed in about 7% of the patients treated. The rash was manifested usually after a one-day period of mild itching and consisted of a maculopapular or urticarial type of rash. The itching was intense and in some cases there was considerable swelling. Symptoms subsided in 2 to 5 days following withdrawal from the drug. The next most important side effect noted by the authors was a loss of taste for salt and sweets observed in 5.3% of the patients. This effect also subsided following withdrawal from 2,4-DNP. The investigators failed to detect any effect of 2,4-DNP on liver or kidney function, pulse, blood pressure, or oxygen capacity of the blood. No cases of anemia, agranulocytosis, or malignant neutropenia appeared. Three cases of mild gastrointestinal upset were reported, however.

In a later publication, Horner (1942) reviewed the acute and chronic toxicity of use of 2,4-DNP (including cataract formation) resulting from therapeutic use of the compound. Gastrointestinal symptoms consisting of nausea, vomiting, and loss of appetite were common as a result of 2,4-DNP administration. Cutaneous lesions were the most frequent side effect with an incidence of 8 to 23%. Although the majority of lesions were mild, others were severe. Bone marrow effects of dinitrophenol have also been reported. Eight cases of agranulocytosis were reported, with three fatalities. Thirty cases of neuritis including aberrations of taste and multiple regional involvement, particularly affecting the feet and legs, were recorded. Symptoms appeared after an average of ten weeks, followed ordinary therapeutic doses and persisted for weeks or months. Electrocardiographic evidence of functional heart damage was offered by several investigators and fragmentation of the heart muscle was reported at autopsy in one fatal case. It was generally agreed that 2,4-DNP was rarely injurious to the liver and kidneys when administered in therapeutic doses.

The development of cataracts following dinitrophenol therapy was first described by Horner et al. (1936). Later, over 100 cases of cataract formation following dinitrophenol therapy were reviewed by Horner (1942). Horner described the following characteristic features of 2,4-DNP induced cataracts: (1) they occurred in young women who were physically normal except suffering varying degrees of obesity and were in an age group in which senile cataracts do not occur; (2) they were bilateral and appeared either during or after period of dinitrophenol treatment; (3) an interval of months or years might elapse between the time the last dose was taken and the onset of blurred vision; (4) lenticular changes were strikingly similar and could be demonstrated with the biomicroscope at a time when vision for distance and reading was still normal; (5) after gradual onset, the lenticular changes progressed with startling rapidity until the vision was obscured; (6) treatment was without effect in staying their progress; and (7) surgical removal of the lens was uniformly successful in restoring vision. Cataract formation appears to be the primary reason 2,4-DNP was withdrawn from medical use.

The length of time that 2,4-DNP was taken and the amount of the drug consumed varied widely among cataract victims. In 29 cases, the duration of treatment varied from 3 months to 24 months with an average of 11 months. Neither the length of treatment nor the total dose seemed to have any bearing on the occurrence of cataracts. Individual susceptibility appeared to be a more important factor. Horner (1942) estimated that the incidence of cataracts in patients who had taken dinitrophenol exceed one percent.

The available data do not allow the calculation of a minimum effect level for 2,4-DNP-induced cataract formation in humans. Cataractogenic activity in humans has been observed in a small proportion of patients receiving as little as 2 mg/kg-day. An assessment of the no-effect-level for cataract formation awaits further investigation. Such an assessment is further complicated by the fact that cataract formation in humans, following DNP administration, differs significantly from the situation seen in experimental animal studies.

The existing review documents (U.S. EPA, 1980, 1984; ATSDR, 1995) and an updated literature search did not identify relevant studies regarding the carcinogenicity of 2,4-dinitrophenol in humans following oral or inhalation exposure.

Animal Studies

Short Term Animal Studies

Attempts to find a suitable animal to study cataract development in humans exposed to 2,4-DNP have generally been unsuccessful. Normal mammalian animals have not developed cataracts after oral exposure to 2,4-DNP, although cataracts could be induced in a special strain of mouse (yellow adipose), in vitamin C-deficient guinea pigs, in ducks, and in chickens (ATSDR 1995). Formation of cataracts by acute exposure to DNP was first demonstrated in animals almost 10 years after the problem was known to exist in humans (Gehring and Buerge, 1969a; Ogino and Yasukura, 1957; Feldman et al., 1959, 1960; Bettman, 1946). Experimental cataracts, first produced in ducks and chickens, differ from DNP-induced human cataracts in that they can be formed in acute exposures and may appear in less than one hour. Furthermore, these lesions will disappear spontaneously in animals within 25 hours (Howard et al., 1976). Hence, the usefulness of data on the formation of cataracts in experimental animals following DNP administration in assessing human hazard to dinitrophenol is questionable.

Langerspectz and Tarkkonen (1961) failed to detect histological changes in the adrenals or the liver during 30 day treatment of Swiss albino male mice with twice daily doses of 10 mg of 2,4-DNP/kg (20 mg/kg-day) via the subcutaneous injection.

Subchronic Animal Studies

Tainter and Cutting (1933) administered 2,4-DNP to dogs at intervals of three or more days over a period of 2 to 3 months. Abnormal liver and kidney pathology were not detected but an effect on spleen tissue was noted. Over large areas of the material containing "numerous large faintly staining cells with vesicular polyhedral nuclei." This study is limited due to the lack of dose information in the summary document (U.S. EPA, 1980).

Groups of three male dogs received daily oral dose of 0, 5 or 10 mg/kg 2,4-DNP in capsules for 6 day/week for 27 weeks (Tainter et al., 1934). There were no important changes in body weight as a result of the continuous administration of 2,4-DNP. Estimations at intervals of three weeks of the amount of sugar, and albumin in the urine and of the hemoglobin and red, white and differential blood cell counts, urea content, icteric index and oxygen capacity of the

blood and fragility of the red cells showed no significant or consistent deviations from the normal or control values. At the end of treatment, the dogs were killed for complete necropsy and histological study of the tissues. No significant pathologic changes were noticed grossly or microscopically. Thus, the highest dose of 10 mg/kg-day (equivalent to continuous dose of 8.6 mg/kg-day) is considered a free-standing NOAEL.

Spencer et al. (1948) studied the subchronic toxicity of 2.4-DNP in rats. Male rats (10-20/dose) were fed diets containing 0, 0.01, 0.02, 0.05, 0.1, or 0.2 g of 2,4-DNP per 100g of food. Rats were maintained on diets containing 2,4-DNP for six months and both hematological and pathological investigations on surviving animals were performed. Based on rat food intake in a subchronic study (U.S. EPA, 1988), the average daily food intake factor for male rat of unknown species is assumed to be 0.091 kg food/kg body weight/day. Thus, the estimated doses were 0, 9.1, 18, 46, 91, and 182 mg/kg-day, respectively. Hematological examination included erythrocyte counts, hemoglobin concentrations, leukocyte counts, differential counts, and bone marrow counts at autopsy. Both gross and microscopic examination of liver, kidney, spleen, lung, heart, adrenal, pancreas, and stomach tissues were also performed. Rats maintained on diets containing 0.02% 2,4-DNP (18 mg/kg-day) grew at a normal rate and the investigators failed to detect discernible ill effects of pathological changes at autopsy. Similarly, pathological changes were not found upon microscopic examination of tissues from rats receiving diets containing 0.05% 2,4-DNP (46 mg/kg-day) although growth of these rats fell 5 to 10% below that of the controls throughout the six-month experimental period. At autopsy the only changes observed in these animals were a very slight depletion of body fat and a very slight increase in the average weight of the kidneys. More reduced growth was also seen in the rats treated with 0.1% of 2,4-DNP (91 mg/kg-day). At the highest dose of 2,4-DNP in their diets (182 mg/kg-day) rats occasionally died and survivors lost weight rapidly. Examination of surviving animals revealed marked emaciation, an empty gastrointestinal tract, a slightly enlarged and dark spleen, and undersized testes. Microscopic examination showed slight congestion and cloudy swelling of the liver, very slight parenchymatous degeneration of the epithelium of the renal tubules, slight congestion and hemosiderosis of the spleen and testicular atrophy. No significant pathological changes were observed in the lung, heart, adrenals, pancreas, or stomach of these animals. Based on these observations, a NOAEL for 2,4-DNP in rats was 18 mg/kg-day.

Chronic Animal Studies

Groups of 5-6 white rats (sex unknown) received 2,4-DNP in the food beginning shortly after weaning when they weighted about 30 g, and continuing until death (Tainter, 1938). The treatment doses included 0, 0.001, 0.005, 0.01, 0.02, 0.04, 0.06, 0.08, 0.12 and 0.24% of 2,4-DNP in the diet. Based on rat food intake in a chronic study (U.S. EPA, 1988), the average daily food intake factor for rat of unknown sex and speices is assumed to be 0.078 kg food/kg body weight. Thus, the estimated doses were 0, 0.78, 3.9, 7.8, 16, 31, 47, 62, 94 and 187 mg/kg-day, respectively. The food intakes, growth curves, final weights, and life spans were compared with those of untreated controls. At the time of death, necropsies and histological studies of the tissues were made. The rats were observed closely throughout the entire duration of the experiment. The food intakes were similar for all the groups. Doses of 2,4-DNP in the diet ranging from 0.78 to 31 mg/kg-day did not appreciably modify the growth curves or final weights. Doses of 47 to 94 mg/kg-day decreased the rate of growth, and diminished the final

average weight about 75 g. The average duration of life of about two years was not decreased by doses of 2,4-DNP up to 62 mg/kg-day; a dose of 94 mg/kg-day decreased it by about one-half and 187 mg/kg-day killed the rats in about one month. There was no evidence of any toxic effects of 2,4-DNP on the eyes of these rats, as indicated by direct observations, and ophthalmoscopic study or slit lamp microscopy. At necropsy, and histologically, the tissues of the treated rats were indistinguishable from those of the untreated controls, there being no lesions which could be ascribed to the action of the 2,4-DNP. The NOAEL in rats was identified to be 31 mg/kg-day based on significant decreases in body weight.

Reproductive and Developmental Animal Studies

Based on the available data it appears unlikely that the 2,4-DNP pose a teratogenic hazard to humans. Gibson (1973) examined developmental toxicity of 2,4-DNP in mice. Groups of pregnant mice (7-8 animals/dose) received intraperitoneal (7.7 or 13.6 mg/kg) or oral (25.5 or 38.3 mg/kg) administration of 2,4-DNP during early organogenesis (gestation day 10-12). Nine pregnant females received water served as control. Caesarean section was performed on day 19 of gestation, and the number and position of live, dead and resorbed fetuses was examined. Individual fetuses were weighted, and examined for external anomalies. Fetal crown-rump distance was measured for each fetus. Each litter was divided into two sub-groups for further examination for soft-tissue or skeletal anomalies. Very limited results were provided in the original report. Among the all the endpoints (including number of implantations, resorptions, fetal body weight and fetal crown-rump length) presented in a summary table (Table 8 in the original paper), increased resorptions (mean response/litter), decreased fetal body weight, and fetal crown-rump length occurred in almost all the treated groups (including both i.p. and oral treatments); however, only the decreases in the fetal body weight and crown-rump length in the high i.p. dose group (13.6mg/kg-day) was statistically significant. No other details were provided in the original report. Gibson (1973) ambiguously concluded that dinitrophenol does not produce morphological defects in the offspring, but embryo toxicity occurs at the higher dose levels. The higher doses also produced overt toxic signs (hyperexcitability and hyperthermia) in the dams, but were not lethal. However, it is not clear whether the author referred the higher doses to i.p. high dose of 13.6 mg/kg or to doses ≥13.6 mg/kg-day including i.p. high dose and two oral doses (25.5 and 38.3 mg/kg-day). Based on limited information available from the original report, the low i.p. dose of 7.7 mg/kg-day was considered NOAEL.

The toxicity of 2,4-DNP was examined in newborn rats by Koizumi et al. (2001). Groups of Sprague-Dawley rats (6/sex/dose) were administered 2,4-DNP at 0, 3, 10 or 20 mg/kg-day by gastric intubation daily from days 4 to 21 after birth, and killed after overnight starvation following the last treatment. Recovery-maintenance groups at the same dosages were maintained for 9 weeks without chemical treatment and fully examined at 12 weeks old. General behavior was observed daily, and body weight and food consumption were measured more than once a week. At treatment day 17 or 18, papillary reflex, corneal reflex, surface righting, mid-air righting and auricular reflexes were examined as parameters of reflex ontogeny. Furthermore, fur appearance, incisor eruption and eye opening were noted in the lactating period as evidence of physical development, and testes descent and vaginal opening during the early recovery-maintenance period for assessment of sexual maturation. Color, pH, occult blood, protein, glucose, ketone bodies, bilirubin, urobilinogen, urine sediment and volume of the urine were

examined only at the end of the recovery-maintenance period. The blood samples were analyzed for complete hematological parameters as well as blood biochemistry. All the organs were weighted and examined for gross and histopathological changes at the end of treatment. No clinical signs or deaths were encountered. The body weights at 20 mg/kg-day were significantly below control values from dosing day 7 in the males and dosing day 10 in females in the scheduled-sacrifice group. There was also statistically significant lowering of body weight in the 20 mg/kg-day males for the first quarter of the recovery-maintenance period, but not in females. No definitive changes in abdominal fur appearance, incisor eruption, eye opening and testis descent or vaginal opening as well as reflex ontogeny parameters were detected in any dose groups. There were significant changes in absolute weights of testes at ≥10 mg/kg-day, and changes in absolute and relative organ weights in several other organs at 20 mg/kg-day. No chemical-related histopathological changes were noted in either scheduled-sacrifice or recovery-maintenance groups. Significant increase in RBC was observed in females receiving 20 mg/kg-day after the treatment but not after the recovery-maintenance period. Although increases in serum glutamate oxaloacetate transaminase (GOT) in males and total bilirubin in females were detected at 10 and 20 mg/kg-day after treatment, those were not considered to be chemical-induced because they were very slight and there was no dose-relationship. The authors considered the dose of 10 mg/kg-day as the NOAEL at which only the lowering of absolute testis weight was observed.

The toxicity of 2,4-DNP was also examined in young rats by Koizumi et al. (2001). Groups of 5 to 6-week old Sprague-Dawley rats (6/sex/dose) were administered 2,4-DNP at 0, 3, 10, 30 or 80 mg/kg-day by gastric intubation daily for 28 days, and killed after overnight starvation following the last treatment. Recovery-maintenance groups at the 0, 30 or 80 mg/kg-day were maintained for 2 weeks without chemical treatment and fully examined at 11-12 weeks of age. Rats were examined for general behavior, body weight, food consumption, urinalysis, hematology and blood biochemistry, necropsy finding, organ weights and histopathological finding. Clear toxic signs, such as decrease in locomotor activity, prone position, ptosis, panting, crawling position and salivation, were observed repeatedly during the dosing period at 80 mg/kg-day in both sexes, and two males and six females died in the same dose group. However, decrease in locomotor activity and salivation in the 30 mg/kg-day group were mostly observed only after the first dosing. The relative liver weights were increased in both sexes of the 80 mg/kg-day scheduled-sacrifice group, and this persisted through the recovery period. Relative organ weights for brain, kidneys and testes were increased only in 80 mg/kg-day males. On histopathological examination, mineralization of the corticomedullary junction in kidneys was observed in both sexes at 80 mg/kg-day in the scheduled-sacrifice and recovery groups, but the change was only statistically significant in males of the scheduled-sacrifice group. On hematological examination, increase in hemoglobin and hematocrit in the recovery period were observed, limited to 80 mg/kg-day males. Although blood chlorine levels were slightly decreased in 30 and 80 mg/kg-day males and total bilirubin was slightly increased in females receiving 10 mg/kg and more, no changes in histopathology or organ weights were observed at 30 mg/kg-day or lower. Prior to the this experiment, a dose-finding study in the same age group (4/sex/group) at doses of 0, 0.6, 2, 6, 20 or 60 mg/kg-day had been conducted. The study results from these animals after treatment for 14days were consistent with the main study. Thus, the authors considered 20 mg/kg-day from the dose-finding study as the NOAEL based on decrease in locomotor activity and salivation at 30 mg/kg-day.

Wulff et al. (1935) examined the effects of 2,4-DNP on the fertility, gestation, and fetal life of rats in an one-generation study. A group of 20 female rats (unknown strain) received 20 mg/kg 2,4-DNP 8 days prior the introduction of males. Nine females received no treatment and five females received 1% sodium bicarbonate solvent served as control. Dinitrophenol was administered intragastrically twice daily throughout cohabitation, and gestation until the respective litters were weaned. The daily average dose was estimated to be 40 mg/kg-day. The average number born in each litter was not affected by dinitrophenol treatment, and the treatment did not appreciably affect the body weight gains of mothers during pregnancy. Neonatal malformations were not detected. Among 2,4-DNP treated rats, however, 25% of the total number of pups were stillborn while only 6.8% of the pups were stillborn in the control group (two groups combined). In addition, the mortality during the nursing period of viable pups born to mothers administering 2,4-DNP was 30.9% as compared with 13.4% for young of control mothers. Two possible explanations for this latter phenomenon were offered by the authors: treated mothers neglected their pups while in a febrile state, and only the more vigorous of the offspring manage to reach the mother for nursing; or, a toxic agent was passed to the young through the milk. Data to distinguish between the two possibilities are not available. Based on developmental toxicity (stillbirth and mortality during lactation), this study provided a free standing LOAEL of 40 mg/kg-day.

Other Studies

Bowman (1967) has studied the effect of 2,4-DNP on the developing chick embryo in vitro. At 2,4-DNP concentrations of 18 mg/L or 370 mg/L a syndrome of abnormalities resulted consisting of degeneration and sometimes complete absence of neural tissue accompanied by a reduction in the number of somites. The 2,4-DNP concentrations used in this study are extremely high and the relevance of the experimental findings to the *in vivo* situation in mammals is not clear.

Genotoxicity data for 2,4-DNP were reviewed by ATSDR (1995). Test results were negative for 2,4-DNP in multiple assays for reverse mutation in *Salmonella typhimurium*, with or without metabolic activation. However, the two major metabolites of 2,4-DNP are mutagenic in *S. typhimurium* (and other systems), suggesting that the negative results for 2,4-DNP may indicate failure of the S9 activating system used in these assays to metabolize this chemical. Mixed results were reported for 2,4-DNP in studies of reverse mutation in *Escherischia coli*. There is little evidence that 2,4-DNP produces DNA damage. Assays for phase induction in *E. coli*, SOS response in *S. typhimurium*, unscheduled DNA synthesis in rat hepatocytes, and DNA damage (alkali elution) in Chinese hamster V79 cells were negative. DNA damage (alkali elution) was reported in mouse leukemia L1210 cells and human HeLa cells, but was associated with depletion of ATP. Depletion of ATP was also observed in studies showing decreases in DNA synthesis and mitotic index after exposure to 2,4-DNP. Therefore, positive findings in these studies probably reflects cytotoxicity (decreased cellular metabolic rate), rather than genotoxicity. *In vivo*, 2,4-DNP produced chromosomal aberrations in bone marrow cells of mice treated by intraperitoneal injection.

In a study designed to measure tumor promoting activity, Boutwell and Bosch (1959) examined the ability of 2,4-DNP to promote tumor formation following a single initiating dose

of dimethylbenzanthracene. The 2,4-DNP failed to promote skin tumors in mice in this experiment. In a similar experiment, Stenback and Garcia (1975) also examined the ability of 2,4-DNP to promote skin tumor formation in mice, and found no tumor promoting activity.

The existing review documents (U.S. EPA, 1984; ATSDR, 1995) and an updated literature search did not identify relevant studies regarding the carcinogenicity of 2,4-dinitrophenol in animals following oral exposure.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR 2,4-DINITROPHENOL

2,4-DNP is considered a classic uncoupler of oxidative phosphorylation and is widely used by biochemists to determine whether a given biochemical process is energy dependent. The toxic action of the dinitrophenol is generally attributed to their ability to uncouple oxidative phosphorylation. It prevents the utilization of the energy provided by cellular respiration and glycolysis by inhibiting the formation of high energy phosphate bonds. All energy dependent biochemical processes are therefore affected by the action of the compounds. The large number of clinical effects attributed to dinitrophenol toxicity result essentially from the shortcircuiting of metabolism in cells which absorb sufficient dinitrophenol. At concentrations higher than those necessary to uncouple oxidative phosphorylation, a number of inhibitory effects of the dinitrophenol isomers on certain enzymatic reactions may occur. The dinitrophenol may also act directly on the cell membrane, thus causing toxic effects on cells which do not depend on oxidative phosphorylation for their energy requirements. More detailed information on the mechanism of toxicity has been summarized by U.S. EPA (1980).

The database for 2,4-DNP toxicity is relatively comprehensive, and it covers human case studies and results from experimental exposure. In addition, the database also includes animals studies ranging from short-term studies, subchronic studies in dogs and rats, to a chronic study in rats, accompanied by developmental studies in mice and young rats, and an one-generation study in rats.

The toxicity of 2,4-DNP in humans ranges from mortality due to high dose exposure to minor effects such as gastrointestinal symptoms and cutaneous lesions due to therapeutic use of the compound as a weight reducing agent. The development of cataracts following the dinitrophenol therapy was first described by Horner et al. (1936), and similar response was also reported from treated animals. The length of time that 2,4-DNP was taken and the amount of the drug consumed varied widely among cataract victims. In 29 cases, the duration of treatment with the compound varied from 3 months to 24 months. Neither the length of treatment nor the total dose seemed to have any bearing on the occurrence of cataracts. The available data do not allow the calculation of a minimum effect level for 2,4-DNP-induced cataract formation in humans. Since cataractogenic activity in humans has been observed in a small proportion of patients receiving as little as 2 mg/kg-day dose of 2,4-DNP, this dose is considered as a LOAEL for cataract in humans after subchronic exposure to the chemical.

The formation of cataracts by acute exposure to 2,4-DNP has been reported in ducks and chickens treated with the compound. However, the experimental cataracts in the animals differ from DNP-induced human cataracts in that they can be formed in acute exposures and may appear in less than one hour. Furthermore, these lesions will disappear spontaneously in animals within 25 hours.

Subchronic studies in dogs and rats did not identify a specific target organ for 2,4-DNP, and a free-standing NOAEL of 8.6 mg/kg-day in dogs and a NOAEL of 18 mg/kg-day in rats based on decreased growth rate were identified (Spencer et al., 1948; Tainter et al., 1938). Similar to the subchronic studies, the chronic study in rats (Tainter et al., 1938) identified a NOAEL of 31 mg/kg-day based on significant decreases in body weight. There was no evidence of any toxic effect on the eyes in the rats treated chronically with 2,4-DNP at dose levels up to 187 mg/kg-day. Since the subchronic rat study (Spencer et al., 1948) did not include a dose at 30 mg/kg-day range, the subchronic NOAEL of 18 mg/kg-day from that study is considered consistent with the chronic NOAEL of 31 mg/kg-day (Tainter et al., 1938) because the latter study included smaller dose spacing in the experiment which allowed identification of a NOAEL higher than that from the subchronic study.

The toxicity of 2,4-DNP in developmental studies did not demonstrate more sensitive responses than the systemic effects observed in the subchronic or chronic studies. 2,4-DNP does not produce morphological defects in the offspring, but it could produce embryo toxicity at dose level of ≥ 13.6 mg/kg-day, and the same doses also produced overt toxic signs (hyperexcitability and hyperthermia) in the dams (Gibson, 1973). The developmental study provided a NOAEL of 7.7 mg/kg-day based on i.p. treatment dose. Short-term treatment (7 days) with 2,4-DNP in newborn rats resulted in decreased testis weight and body weight at the dose of 20 mg/kg-day, and the next lower dose of 10 mg/kg-day was identified as the NOAEL (Koizumi et al., 2001). 2,4-DNP treatment (28 days) in young rats (5-6 weeks old) resulted in decreases in locomotor activity and salivation at the dose level of ≥ 30 mg/kg-day (Koizumi et al., 2001), and this study identified a NOAEL of 20 mg/kg-day in young rats. Comparison of the effective doses in newborn and young rats suggested a less sensitivity to 2,4-DNP in young rats than newborn rats.

The one-generation reproductive study (Wulff et al., 1935) showed that 2,4-DNP treatment at dose level of 40 mg/kg-day resulted in increased stillbirth and mortality during lactation. It is not clear whether the mortality during lactation was due to toxicity in dams or fetuses exposed to the compound through milk. Therefore, this dose (40 mg/kg-day) is considered a free-standing LOAEL.

Based on all the data available, the cataracts developed in humans after therapeutic use of the compound as a weight reducing agent is considered the critical effect, and the estimated minimal dose of 2 mg/kg-day causing this effect is considered as the point of departure in deriving a provisional subchronic RfD. Using this point of departure is further supported by the relative rich data from animal studies. A **provisional subchronic RfD of $2x10^{-2}$ mg/kg-day** for 2,4-DNP is derived by applying a composite uncertainty factor of 100 (10 for the use of a LOAEL, and 10 to protect sensitive individuals) to the point of departure of 2 mg/kg-day. Because the database for 2,4-DNP included subchronic studies, chronic studies, developmental studies and an one-generation reproductive study, it is unlikely to identify more sensitive

responses from additional animal studies. Therefore, an uncertainty factor of 1 is used as the database factor. Because the critical effect in humans was observed after subchronic treatment of the compound, no extra uncertainty factor for exposure duration is needed for deriving a provisional subchronic RfD.

subchronic p-RfD = POD / UF
= (subchronic LOAEL) / (100)
= 2 mg/kg-day / 100
= 0.02 or $2x10^{-2}$ mg/kg-day

The current chronic RfD of 2E-3 mg/kg-day on IRIS (U.S. EPA, 1991) was based on the same critical effect and point of departure with an extra uncertainty factor of 10 to cover the extrapolation from subchronic to chronic duration. A chronic RfD of $2x10^{-3}$ mg/kg-day for 2,4-DNP was derived by applying to the human LOAEL of 2 mg/kg-day a composite uncertainty factor of 1000 (10 for the use of a LOAEL, 10 for extrapolation from subchronic to chronic duration, and 10 to protect sensitive individuals). The chronic RfD on IRIS is still valid.

Chronic RfD = POD / UF
= (subchronic LOAEL) / (1000)
= 2 mg/kg-day / 1000
= 0.002 or $2x10^{-3}$ mg/kg-day

Confidence in the principal study is low. Higher study confidence is precluded because the principal study only describes anecdotal data which provides limited formation in the dosing and exposure duration, minimal data reporting, and the lack of reliable data on no effect levels for the critical effect in critical study. Confidence in the database is high because the database for 2,4-DNP toxicity not only includes experimental studies in humans, but also covers relative comprehensive studies in animals including short-tem studies, subchronic studies in multiple species, a chronic study, developmental studies in pregnant mice and young rats, as well as a one-generation study. Therefore, it is unlikely to identify other more sensitive responses from additional animal studies. Overall confidence in the subchronic p-RfD values is medium, as the strengths in the database, particularly the supportive data from comprehensive animal studies, somewhat outweigh the low confidence in the principal human study. A chronic p-RfD based on the same point of departure with an additional uncertainty factor to cover the extrapolation from subchronic to chronic duration. The overall confidence for the chronic p-RfD would be the same as the one for subchronic p-RfD. The using of the subchronic data as the point of departure for chronic p-RfD is appropriate because neither the length of treatment nor the total dose seems to have any bearing on the occurrence of critical effect.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR 2,4- DINITROPHENOL

No data were located for the subchronic or chronic inhalation toxicity of 2,4-DNP in humans or animals. Due to the lack of data, no provisional RfC was derived for 2-4-DNP.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR 2,4- DINITROPHENOL

Weight-of-Evidence Descriptor

No studies were located examining associations between cancer and exposure of humans to 2,4-DNP. Thus, there were inadequate human data to assess the carcinogenicity of 2,4-DNP.

Data examining the potential for 2,4-DNP to produce cancer in animals were restricted to several mouse skin tumor assays that found no DNP-induced increases in incidence of skin tumors. U.S. EPA guidelines (2005) indicated that, in order to classify the compound as not likely to be carcinogenic to humans, no increased incidence of neoplasms should be found in at least two-well designed and well-conducted animal studies of adequate power and dose in different species. Thus, the available animal data for 2,4-DNP were not sufficient to classify them as providing no evidence of carcinogenicity. Additional well-conducted testing in other animal species with long-term exposure, preferably via oral and inhalation exposure, is necessary to provide reasonable assurance as to whether 2,4-DNP may or may not be carcinogenic in animals or humans.

Mixed results in genotoxicity of 2,4-DNP were reported in several short-term mutagenesis assays in bacteria, and in *in vitro* and *in vivo* mammalian systems. The majority of the *in vitro* genotoxicity studies showed negative responses with some exceptions in DNA damage in mouse leukemia cells and human HeLa cells, although the positive findings in these studies probably reflects cytotoxicity rather than genotoxicity. An *in vivo* study produced chromosomal aberrations in bone marrow cells.

Following U.S. EPA (2005) guidelines for compounds with inadequate human data and inadequate animal data, 2,4-DNP was classified as having *inadequate information to assess carcinogenic potential*.

Quantitative Estimates of Carcinogenic Risk for 2,4-DNP

Due to inadequate information to assess carcinogenic potential, a quantitative cancer risk estimate for neither an oral slope factor nor an inhalation unit risk could be derived for 2,4-DNP.

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