DISPOSITION OF PEER REVIEW COMMENTS FOR TOXICOLOGICAL PROFILE FOR DINITROPHENOLS

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

August 2018

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Annotated Comments on the Profile

COMMENT: Use of the word 'pesticides' here is somewhat redundant and general: suggest deleting. The specified uses as wood preservatives (antifungal) and insecticides are the specific pesticidal uses of DNPs.

RESPONSE: *The word pesticides was deleted from Section 1.1.*

"1.1 OVERVIEW AND U.S. EXPOSURES

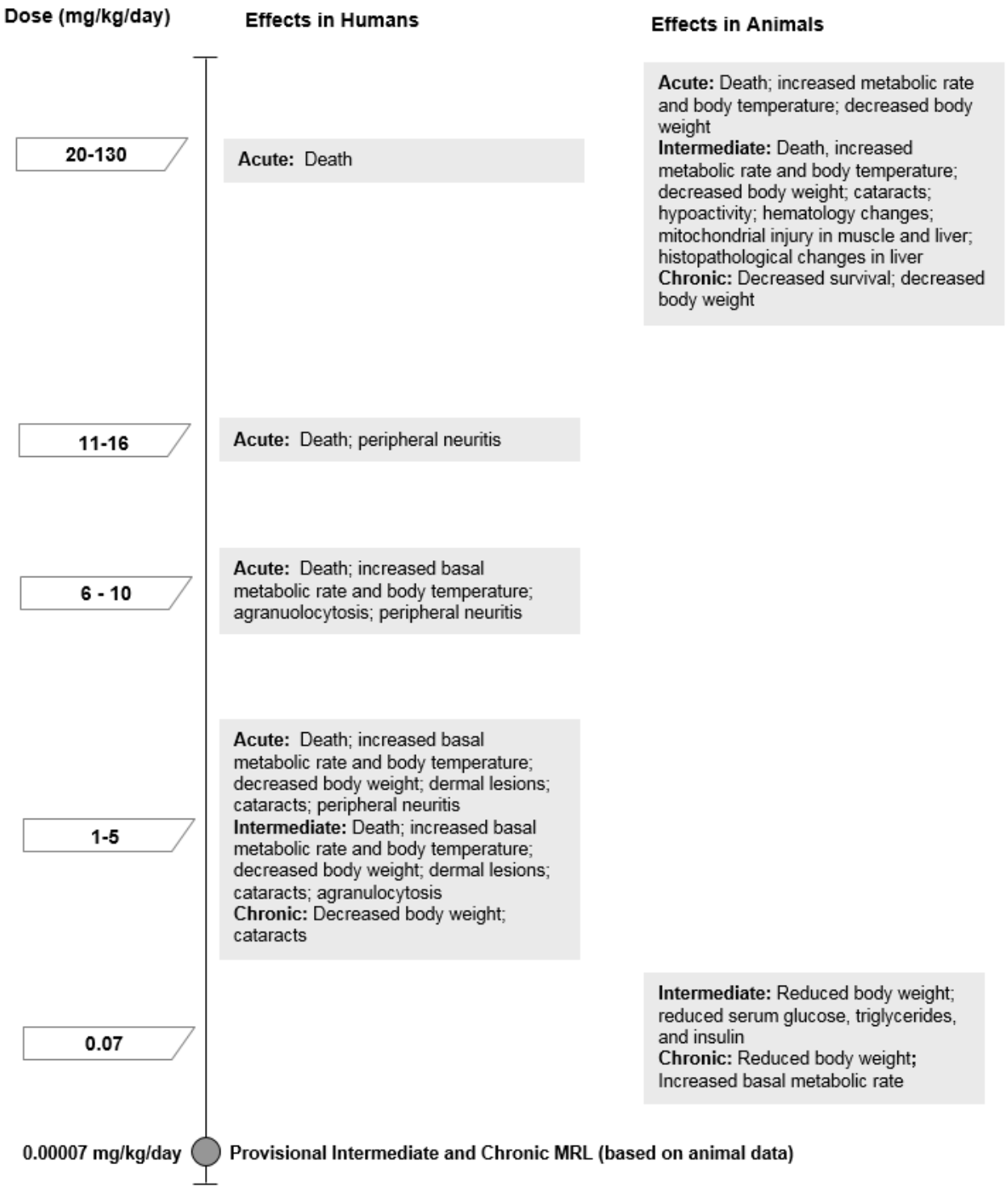
ATSDR's Toxicological Profile for Dinitrophenols was released in 1995. In order to update the literature in this profile, ATSDR conducted a literature search focused on health effects information as described in Appendix B. Chapters 2, 3, and 7 were revised to reflect the most current health effects and regulations/guidelines data. In some cases, other sections of the profile were updated as needed or for consistency with the updated health effects data. However, the focus of the update to this profile is on health effects information.

In the 1930s, 2,4 dintrophenol (DNP) was prescribed by physicians as a weight-reducing agent, but its use was discontinued due to health risks. In recent years, however, 2,4-DNP in tablet and powder form has been marketed for weight loss and body building by unregulated internet sources, leading to a number of human fatalities. These unregulated sources often provide information to potential users regarding dosing and how to combine with other stimulants, steroids, and growth hormone for body building purposes, without informing users of the risk of death. As a result of the growth in availability of 2,4-DNP to the general public, there is increased potential for exposure and health effects among police officers involved in seizure of material or arrest of users, mail and shipping company employees who handle shipments, health care providers who treat or decontaminate users, and family members who live with persons who purchase and/or use 2,4-DNP. In addition to the toxicity hazards associated with 2,4-DNP, this compound is explosive when dry and when heated or subjected to flame, shock, or friction (WHO 2015).

DNPs are also used in the manufacture of dyes, wood preservatives, photographic developers, explosives, and insecticides, and as a pH indicator. 2,4-DNP and other DNPs are released to the environment primarily during their manufacture and use, and from waste disposal sites. The most likely routes of exposure near hazardous waste sites would be breathing contaminated air, drinking contaminated water, eating contaminated food, or skin contact with contaminated soil. The toxicity of 2,4-DNP is greater at high ambient temperatures; therefore, susceptibility to the toxic effects may increase for workers at high workroom temperatures or in the general population at high environmental temperatures."

COMMENT: Are these health effects based solely on studies using 2,4-DNP? If so, specify in Figure caption.

RESPONSE: *The title of Figure 1-1 was corrected as follows: "**Health Effects Found in Humans and Animals Following Oral Exposure to 2,4-Dinitrophenol."***



COMMENT: This is a vague term. Suggest changing to increased serum enzymes that are markers of organ toxicity, or even specifying enzymes (e.g., GGT, ALT).

RESPONSE: *The text in Section 1.2 was revised as follows:*

"Hepatic Effects. Limited available data from humans do not suggest hepatic effects of 2,4-DNP apart from those related to its pyrexic effects; these data consist of case reports of poisonings, which lack information on pre-existing conditions, as well as clinical studies from the 1930s. Early human studies attributed yellow discoloration of the conjunctiva, sclera, and skin in exposed persons to jaundice, but these effects appear to result from direct discoloration by the compound itself. There are insufficient data to assess the hepatic effects of acute- or chronic-duration exposure to 2,4-DNP in animals, but well-conducted intermediate-duration studies in rats have shown increased serum enzymes indicative of liver toxicity and increased liver weights, along with microscopic changes (centrilobular hypertrophy, necrotic foci, and mitochondrial changes)."

COMMENT: This same comment was made previously about this Dow study. I'm wondering whether this is necessary. After reading through the summaries of other older studies that do not report doses, durations, sex of animals, etc, it would appear to me that the Dow study is not the only poorly reported study!

RESPONSE: *The statement about reporting was deleted from Section 2.10.*

"Renal effects have been reported in many case reports of human 2,4-DNP poisoning, as discussed below. In most cases, the effects appear to be part of the multiorgan dysfunction that accompanies severe and/or fatal hyperthermia (see Sections 2.18 and 2.18.1). Acute renal failure occurs commonly with heatstroke (Bunai et al. 2012). Tubular necrosis results from hypovolemia (as blood is moved to peripheral blood vessels) and diaphoresis, and there can also be direct thermal injury to the kidneys (Bunai et al. 2012). Hemorrhagic conditions precipitated by severe hyperthermia, including disseminated intravascular coagulation (in which the clotting cascade is activated throughout the small blood vessels of the body), also contribute to renal failure, as does myoglobinuria that results from rhabdomyolysis (Bunai et al. 2012).

Renal failure was noted in a case report of a fatality after DNP intake (~40 mg/kg) with suicidal intent (Bartlett et al. 2010), and in another fatality presumed to be associated with DNP exposure but without dose or duration information (Suozzi et al. 2005). Mild nephrotic changes were seen during histopathological examination of tissues from a man who died after ingesting two doses of 46 mg/kg 2,4-DNP as the sodium salt of 2,4-DNP 1 week apart (Tainter and Wood 1934). In other fatal cases, cloudy swelling, pyknosis, and necrosis in the renal tubules, edema in interstitial tissue, distention of capillary and arterial loops in the glomerulus, and hemorrhage were seen in the kidneys of a woman who took 7 mg/kg/day 2,4-DNP as the sodium salt for 5 days (Poole and Haining 1934); marked destruction of the epithelium lining the renal tubules with hemorrhage into the glomeruli was found in the kidneys of a woman who took an indeterminate dose of 2,4-DNP for 1 week (Lattimore 1934); and hemorrhagic nephritis was found in the kidneys of a young girl who took 1.0 mg/kg/day 2,4-DNP for 46 days (Goldman and Haber 1936). The blood nonprotein nitrogen level was normal in a psychiatric patient who subsequently died after being given 3 mg/kg/day 2,4-DNP as the sodium salt for 14 days (Masserman and Goldsmith 1934). Upon autopsy, no gross evidence of kidney damage was found, but microscopic examination was inconclusive due to autolysis, because autopsy was delayed by 4 days. Autopsies of workers who died from exposure to 2,4-DNP (via inhalation and dermal contact) in the French munitions industry did not reveal any consistent changes of the kidney; no information on exposure levels or durations was provided (Perkins 1919).

Nonfatal poisonings with 2,4-DNP also resulted in renal effects. A woman who ingested 2,4-DNP at a single dose of ≥10 mg/kg for weight loss developed transient renal failure, but recovered within 4 days (van Veenendaal et al. 2011). No changes in blood tests for renal function were seen in healthy male bodybuilders who took ~1 mg/kg/day 2,4-DNP daily for 10 days (Lee et al. 2014) or ~4 mg/kg/day for 6 days (Le et al. 2015). Similarly, the blood nonprotein nitrogen level was normal in a woman who took 2 mg/kg/day 2,4-DNP as the sodium salt for 14 days (Anderson et al. 1933). Moderate and marked albuminuria was found in 2 women who took 2 mg/kg/day (Beinhauer 1934) or 4 mg/kg/day 2,4-DNP (Imerman and Imerman 1936) for 37 or 35 days, respectively. In the woman who took 2 mg/kg/day, kidney function as determined by phenolsulfonphthalein retention was normal (Beinhauer 1934). Tests of renal function (examination of urine for albumin, red and white cells and casts; concentration-diuresis tests with measurement of specific gravity; phenolsulfonphthalein excretion; blood nonprotein nitrogen determinations) performed repeatedly on three patients over a period of 8 weeks while they underwent treatment with 4 mg/kg/day 2,4-DNP showed no changes; the data were not provided (MacBryde and Taussig 1935).

A few clinical studies found transient or no renal effects following 2,4-DNP exposure. In an extensive clinical study of 159 patients taking an average of 3 mg/kg/day 2,4-DNP as the sodium salt for 22–89 days, kidney function, as assessed by phenolsulfonphthalein retention, was normal in the 15 patients to whom the test was given (Simkins 1937a, 1937b). However, 4 of 15 had transient albuminuria and 2 of 15 had persistent albuminuria. In a group of psychiatric patients given 2,4-DNP at various doses for 34 months to determine whether the drug would have a beneficial effect on depression, no changes in urinary constituents were found (Masserman and Goldsmith 1934).

Two acute-duration animal studies reported mild or no renal effects following 2,4-DNP exposure, but lacked statistical analysis or dose data. Eight rats treated once by gavage with 20 mg/kg 2,4-DNP displayed very mild tubular necrosis in 5 of 16 kidneys examined 12 hours after dosing (Arnold et al. 1976). No statistical analysis of the data was reported. Two dogs repeatedly fed capsules of 2,4-DNP at dose levels of ≤20 mg/kg, with "recovery periods" of about 5 days between doses, followed by a "fatal dose" (dose level not reported) had no abnormalities with respect to gross and microscopic histology of the kidney (Tainter and Cutting 1933b).

Intermediate-duration animal studies generally reported mild or no renal effects following nonfatal exposures, but some had reporting limitations. A 28-day study in rats exposed to 2,4-DNP by gavage revealed increased kidney weight and histopathology changes (mineralization in the corticomedullary junction) at 80 mg/kg/day, a dose that also resulted in the death of 2/12 males and 6/12 females (Koizumi et al. 2002, 2001). A NOAEL of 1 mg/kg/day for kidney histology was reported for rats exposed for 5 days/week for 4 weeks; higher doses (not reported) produced chronic tubular necrosis characterized by degeneration of the tubular epithelium (Dow Chemical Co. 1940). The degeneration varied from slight cloudy swelling of the epithelium to complete necrosis with extensive desquamation and sloughing into the tubular lumina. Marked pyknosis and degeneration were observed in the nuclei of the epithelial cells, but the glomeruli were essentially normal. In rats exposed to 2,4-DNP by daily gavage for 40–47 days, relative kidney weights were increased by 11–14% in both males and females at a dose of 30 mg/kg/day but not at 10 mg/kg/day; histopathology was not evaluated (Takahashi et al. 2009). Rats exposed to 5–50 mg/kg/day 2,4-DNP in the diet for 6 months had no gross or histological evidence of damage to the kidney (Spencer et al. 1948). Blood urea nitrogen (BUN) was greatly elevated in 2 /14 and 2/9 rats exposed to 25 and 50 mg/kg/day, respectively, but the mean values in each group were similar to those of the controls (Spencer et al. 1948). Dogs

(three per dose group) exposed to 5 or 10 mg/kg/day 2,4-DNP via capsules for 6 months had normal levels of blood urea and urinary sugar; urinary albumin was increased at 12 weeks at both exposure levels but was otherwise normal throughout the experiment (Tainter et al. 1934b). In addition, no gross or histological evidence of kidney damage was observed. The authors concluded that the treatment did not produce progressive damage to the kidney (Tainter et al. 1934b). Rats exposed to 60 mg/kg/day 2,4-DNP in the diet for life had gross and histological findings in the kidney comparable to the control group (Tainter 1938).

In rats treated with a metabolite of 2,4-DNP (2-amino-4-nitrophenol) by gavage on 5 days/week, mineralization of the renal cortex and degeneration of the renal tubular epithelium were observed after 13 weeks at ≥ 500 mg/kg, and increased incidences of nephropathy and renal tubular cell hyperplasia were seen in males after 2 years of exposure to 250 mg/kg (NTP 1988a)."

COMMENT: Pruritus?

RESPONSE: *The spelling was corrected in Section 2.11.*

"Yellow discoloration of the skin and pruritic skin rashes were common findings in people taking 2,4-DNP for weight loss. Some early studies (e.g., Bayer and Gray 1935) attributed the yellow discoloration to jaundice, but this finding more likely results from 2,4-DNP excretion in sweat (e.g., Holborow et al. 2016). A woman who took 4 mg/kg/day 2,4-DNP for 4 days developed a rash on her chest (Dintenfass 1934). Two women who took 0.91 or 1.45 mg/kg/day 2,4-DNP for 8 days developed marked pruritic rashes that disappeared within 2–5 days after dosing was discontinued, but reappeared upon resumption of treatment (Nadler 1935). Generalized maculopapular rashes covering much of the body were observed in two young, healthy bodybuilders who ingested 2,4-DNP; one patient took 72 mg 2,4-DNP per tablet once per day for 10 days (~1 mg/kg/day Lee et al. 2014), while the other took 200–400 mg/day for 6 days (~4 mg/kg/day; Le et al. 2015). For the latter individual, the rash resolved within 5 days (Le et al. 2015). Severe skin lesions developed in two women who took 2 mg/kg/day 2,4-DNP for 14 days (Anderson et al. 1933; Hitch and Schwartz 1936). In one case, the lesions were characterized by severe exfoliating dermatitis with redness, edema, oozing of serum, scaling, and crusting over 100% of the body surface (Hitch and Schwartz 1936). In the other case, severe pruritus, edema, maculopapular eruptions covered the entire body, with the exception of the face and scalp (Anderson et al. 1933). No dermal effects were seen in 37 obese patients taking 1 mg/kg/day 2,4-DNP as the sodium salt of 2,4-DNP for an average of 14 days (Tainter et al. 1935b). Serious skin reactions (not otherwise specified) were observed in 3 of 15 obese patients taking 4 mg/kg/day 2,4-DNP for 1–8 weeks; the duration of 2,4-DNP treatment for the affected patients was not specified (MacBryde and Taussig 1935).

Case reports of people taking 2,4-DNP for longer durations reported pruritis and urticaria. A woman who took 4 mg/kg/day for 21 days developed pruritis (Nadler 1935). Urticaria developed in one or all of two women and one man who took 3 mg/kg/day for 41–49 days (Hunt 1934), in addition to one patient (sex not specified) taking 2 mg/kg/day for 110 days (Simkins 1937a, 1937b). Transient pruritic spots occurred in a woman who had been taking 100–200 mg of 2,4-DNP intermittently for 1 year (Imerman and Imerman 1936). Beinhauer (1934) reported a severe case of pruritus involving the entire body of a woman who took 2 mg/kg/day 2,4-DNP for 37 days The pruritus was characterized by swelling of both eyelids, lips, and neck; giant wheals covering the entire body, which were tense to the touch and marked by numerous deep excoriating and intense urticaria; distended and swollen hands and feet; and numerous herpetic lesions in the mouth.

Intermediate-duration clinical studies have also reported dermal effects following 2,4-DNP exposure. In an extensive clinical study of 159 patients taking an average of 3 mg/kg/day 2,4-DNP as the sodium salt for 22–89 days, 32 developed skin lesions, including 4 cases of pruritus, 3 of macular rashes, 12 of maculopapular rashes, 4 of swelling and redness of hands, and 10 of urticaria (Simkins 1937a, 1937b). Skin reactions were observed in 23 of 170 obese patients who ingested an average of 4 mg/kg/day 2,4-DNP from sodium 2,4-DNP for an average of 88 days (Tainter et al. 1935b). The treatment regimen involved an initial dose of 1 mg/kg/day 2,4-DNP, usually for 1 week, increasing to 2 mg/kg/day for several weeks, and then to 4 mg/kg/day with continued small incremental increases until symptoms or loss of body weight contraindicated further increases. The dermal effects occurred only among the 100 patients who took ≥4 mg/kg/day for ≥6 weeks. One-third of the 23 affected patients experienced transient itching without a rash; the remaining two-thirds experienced itching and visible urticarial or maculopapular skin lesions. In one case, the reaction was severe, with massive urticarial wheals covering the body and extensive localized edema. Patients sometimes recovered while remaining on treatment, but usually treatment was discontinued, and recovery ensued. In an experimental study involving 13 men of average weight given an average dose of 5 mg/kg/day 2,4-DNP for 20 days, no skin lesions were observed (Grant and Schube 1934).

Studies of dermal irritation in animals following acute dermal exposure to 2,4-DNP had deficiencies in experimental protocol (statistical analysis was not performed) and reporting (strain, sex, and numbers of animals, duration of each application, and number of applications per day were not reported). Twenty applications of a 3% 2,4-DNP solution in 95% ethanol to the ears of rabbits produced no significant signs of dermal irritation (Spencer et al. 1948). When similar treatment was applied to a bandage on the shaved abdomen, the result was very slight irritation, including mild hyperemia, edema, and exfoliation. No evidence of toxic absorption was apparent, but the criteria used to assess toxicity were not reported (Spencer et al. 1948). Twenty applications of a 4% 2,4-DNP solution in propylene glycol to the ears of rabbits produced no significant signs of dermal irritation (Dow Chemical Co. 1940). In the same study, six applications of a similar solution onto the shaved abdomen resulted in a "moderate simple irritation," as indicated by hyperemia, edema, and denaturation."

COMMENT: Should be ^{131}I.

RESPONSE: *The text was corrected in Section 2.13.*

"A case report and two clinical studies reported thyroid and/or glucose tolerance effects following acute- and intermediate-duration exposures to 2,4-DNP. Autopsy of a woman who died after taking 1.03 mg 2,4-DNP for 46 days revealed extensive vascularization of the spleen and pituitary accompanied by goiter in the thyroid (Goldman and Haber 1936). Decreased glucose tolerance was seen in one clinical study in five of eight patients after 1–2 weeks of treatment and in four of four patients after 3–4 weeks of treatment with 4.3 mg/kg/day 2,4-DNP (MacBryde and Taussig 1935). An additional finding in humans given 2,4-DNP for short durations was a 21% decrease in serum protein-bound iodine in 11 non-obese subjects who ingested 3 mg/kg/day 2,4-DNP for 2 days (Castor and Beierwaltes 1956). Thyroidal uptake and fecal and urinary excretion of ^{131}I, tested in two of these subjects, did not appear to be affected. Hence, the toxicological significance of this finding is unclear.

Two intermediate-duration exposure rodent studies observed 2,4-DNP effects on glucose regulation. Rats exposed to 2,4-DNP (20 mg/kg/day) by daily gavage for 15 days exhibited increased blood glucose concentrations compared with controls (Haasio et al. 2002a, 2002b). In

contrast, mice given 2,4-DNP in drinking water at a concentration (1 mg/L) yielding doses between 0.03 and 0.105 mg/kg/day exhibited decreased levels of serum glucose and insulin after 14 weeks of exposure (Caldeira da Silva et al. 2008).

Four studies were located that addressed potentially toxic effects of 2,4-DNP on the hypothalamic-pituitary-thyroid axis in rats (Bakke and Lawrence 1965; England et al. 1973; Maayan 1968; Wilkens et al. 1974). In these studies, rats were exposed for 7–30 days to dietary 2,4-DNP at a concentration of 0.2%. These studies all reported extremely rapid body weight loss (as much as 1% of body weight per day), implying that the animals were starving and/or wasting away, and diet-matched control groups were not used. Investigation of subtle endpoints of toxicity (e.g., pituitary levels of thyroid-stimulating hormone, daily fractional turnover rates of thyroxin, serum protein-bound iodine, and pituitary cyclic adenosine monophosphate [cAMP] concentrations) are inappropriate in circumstances in which animals are starving and dying. Thus, these four studies (Bakke and Lawrence 1965; England et al. 1973; Maayan 1968; Wilkens et al. 1974) were considered inadequate to estimate the endpoints addressed.

In a yeast two-hybrid assay for estrogenic activity, 2,4-DNP was not active (Jung et al. 2004). No other information on potential estrogenic effects of 2,4-DNP, and no information on potential androgenic effects, was located."

COMMENT: This comment appears to be redundant with line 12 above.

RESPONSE: *The redundant line was deleted from Section 2.20.*
"No studies were located regarding genotoxic effects in humans after exposure to DNP.

Some chemical mutagens and carcinogens bind covalently to deoxyribonucleic acid (DNA) and inhibit DNA synthesis. DNA synthesis (as determined by rate of uptake of tritiated thymidine given as a 30-minute pulse, 3.5 hours after drug administration) was measured in testicular cells of male Swiss mice treated once by gavage with 0 or 20 mg/kg 2,4-DNP (Friedman and Staub 1976). The rate of DNA synthesis in testicular cells was essentially the same in treated and untreated mice. The authors concluded that 2,4-DNP was not genotoxic under these experimental conditions. In another study, DNA synthesis (as determined by the ratio of the rate of uptake of tritiated thymidine injected 3 hours after drug administration to the rate of uptake of 14C-thymidine injected 16 hours before drug administration) was measured in testicular cells of mice treated by gavage with a single dose of 0 or 30 mg/kg 2,4-DNP (Seiler 1981). The rate of DNA synthesis in testicular cells of mice treated with 2,4-DNP was 55% less than that of untreated mice. Based on further in vitro experiments, the author claimed that the inhibition of DNA synthesis by 2,4-DNP was due to some other mechanism than genotoxicity. It is likely that the 2,4-DNP-induced decrease in DNA synthesis in vivo resulted from the effects of 2,4-DNP on energy-dependent cellular processes in testicular cells, rather than from a genotoxic effect.

2,4-DNP has been tested for genotoxicity in several in vivo and numerous in vitro test systems; 2,3-, 2,5-, 2,6-, 3,4-, and 3,5-DNP were tested in vitro for mutagenicity (see Tables 2-6 and 2-7). Two studies assessed the effects of 2,4-DNP administered once by gavage on DNA synthesis in testicular cells (Friedman and Staub 1976; Seiler 1981). In one study, the rate of DNA synthesis in mice treated with 20 mg/kg 2,4-DNP was essentially the same as that of untreated mice. The authors concluded that 2,4-DNP was not genotoxic under these experimental conditions (Friedman and Staub 1976). In another study, DNA synthesis (as determined by the ratio of the rate of uptake of tritiated thymidine injected 3 hours after drug administration to the rate of uptake of 14C-thymidine injected 16 hours before drug administration) in testicular cells of mice

treated with 30 mg/kg 2,4-DNP was 55% less than that of untreated mice (Seiler 1981). Based on further in vitro experiments, the study author suggested that the inhibition of DNA synthesis by 2,4-DNP was due to some other mechanism than genotoxicity, probably produced by 2,4-DNP-induced suppression of cellular metabolism and, therefore, DNA synthesis. Mice were injected intraperitoneally with 0.25, 0.50, and 1 mL of a saturated solution of 2,4-DNP and then sacrificed 24 hours posttreatment for analysis of bone marrow cells for chromosomal aberrations (Mitra and Manna 1971). A dose-related increase in percentage of these aberrations was observed. The authors concluded that 2,4-DNP was clastogenic under the assay conditions and attributed the effect to the compound's electrophilic properties. No studies were located regarding in vivo testing for genotoxicity after exposure to 2,3-, 2,5-, 2,6-, 3,4-, or 3,5-DNP.

Table 2-6. Genotoxicity of Dinitrophenols In Vivo

Species (test system)	Endpoint	Results	Reference	Isomer
Mammalian cells:				
Mouse (intraperitoneal)	Chromosomal aberrations (bone marrow cells)	+	Mitra and Manna 1971	2,4-DNP
Mouse (gavage)	Reduced DNA synthesis (testicular cells)	+	Seiler 1981	2,4-DNP
Mouse (gavage)	Reduced DNA synthesis (testicular cells)	–	Freidman and Staub 1976	2,4-DNP

– = negative result; + = positive result; DNA = deoxyribonucleic acid; DNP = dinitrophenol

Table 2-7. Genotoxicity of Dinitrophenols In Vitro

Species (test system)	Endpoint	Results		Reference	Isomer
		Activation			
		With	Without		
Prokaryotic organisms:					
Salmonella typhimurium					
TA98	Reverse mutation	–	–	Kubo et al. 2002	2,4-DNP
TA100		–	–		
TA98	Reverse mutation	No data	–	Chiu et al. 1978	2,4-DNP
TA100		No data	–		
TA1538	Reverse mutation	–	–	Garner and Nutman 1977	2,4-DNP
TA98	Reverse mutation	–	No data	Anderson and Styles 1978	2,4-DNP
TA100		–	No data		
TA1535		–	No data		
TA1538		–	No data		
TA1530	Reverse mutation	No data	–	Kleinhofs and Smith 1976	2,4-DNP

Table 2-7. Genotoxicity of Dinitrophenols In Vivo

Species (test system)	Endpoint	Results		Reference	Isomer
TA98	*Reverse mutation*	–	–	*Probst et al. 1981*	*2,4-DNP*
TA100		–	–		
TA1535		–	–		
TA1538		–	–		
G46		–	–		
C7036		–	–		
D3052		–	–		
TA98	*Reverse mutation*	–	–	*De Flora 1981*	*2,4-DNP*
TA100		–	–		
TA1535		–	–		
TA1537		–	–		
TA1538		–	–		
TA98	*Reverse mutation*	–	*(+)*	*Kawai et al. 1987*	*2,4-DNP*
TA100		–	*(+)*		
TA98	*Reverse mutation*	+	+	*Kawai et al. 1987*	*2,3-DNP*
TA100		+	+		
TA98	*Reverse mutation*	+	+	*Kawai et al. 1987*	*2,5-DNP*
TA100		+	+		
TA98	*Reverse mutation*	–	–	*Kawai et al. 1987*	*2,6-DNP*
TA100		–	–		
TA98	*Reverse mutation*	–	–	*Kawai et al. 1987*	*3,4-DNP*
TA100		+	+		
TA98	*Reverse mutation*	+	+	*Neuwoehner et al. 2007*	*3,5-DNP*
TA100		+	+		
TA1535/Psk1002	*DNA damage (induction of sister chromatid exchange response)*	–	–	*Nakamura et al. 1987*	*2,4-DNP*
Salmonella choleraesius subsp. chol.					
TA1535/pSK1002	*DNA damage*	–	+	*Neuwoehner et al. 2007*	*3,5-DNP*
TA1535/pSK1002/ pNM12		+	+		
Escherichia coli					
WP2	*Reverse mutation*	–	–	*Probst et al. 1981*	*2,4-DNP*
WP2(uvrA-)		–	–		
B/Sd-4/1,3,4,5	*Reverse mutation*	*No data*	+	*Demerec et al. 1951*	*2,4-DNP*
B/Sd-4/3,4		*No data*	+		
K-12(lambda)	*Phage induction*	*No data*	–	*Heinemann and Howard*	*2,4-DNP*

Table 2-7. Genotoxicity of Dinitrophenols In Vivo

Species (test system)	Endpoint	Results		Reference	Isomer
PQ37	DNA damage	+	–	Neuwoehner et al. 2007	3,5-DNP
Eukaryotic organisms: Mammalian cells					
Human lymphoblasts (TK6)	Chromosomal aberrations	No data	(+)	Hilliard et al. 1998	2,4-DNP
Human blood peripheral lymphocytes	Chromosomal aberrations	No data	+	Huang et al. 1995 1996	2,4-DNP
CHO cells	Chromosomal aberrations	No data	+	Hilliard et al. 1998	2,4-DNP
CHO cells V79	DNA damage (alkali elution)	–	–	Swenberg et al. 1976	2,4-DNP
Rat hepatocytes	Unscheduled DNA synthesis	No data	–	Probst et al. 1981	2,4-DNP
Mouse leukemia L1210	DNA damage (alkali elution)	No data	+[a]	Hilton and Walker 1977	2,4-DNP
Human HeLa cells	DNA damage (alkali elution)	No data	+[a]	Hilton and Walker 1977	2,4-DNP
Chinese hamster V79 cells	Inhibition of replicative DNA synthesis	No data	+	Richard et al. 1991	2,4-DNP

[a]Removal of 2,4-DNP allowed for repletion of ATP pools and repair of DNA damage; therefore, positive finding is related to depletion of DNA pools.

– = negative result; + = positive result; (+) = weakly positive result; ATP = adenosine triphosphate; CHO = Chinese hamster ovary; DNA = deoxyribonucleic acid; DNP = dinitrophenol

In in vitro studies of prokaryotic organisms, 2,4-DNP was negative for reverse mutations using one or more standard stains of Salmonella typhimurium (TA98, TA100, TA1530, TA1535, TA1537, TA1538, G46, C7036, D3052) with and/or without metabolic activation by rat liver S9 microsomes (Anderson and Styles 1978; Chiu et al. 1978; De Flora 1981; Garner and Nutman 1977; Kleinhofs and Smith 1976; Probst et al. 1981). For reverse mutation, a weakly positive response was observed in Salmonella strains TA98 and TA100 without metabolic activation; with metabolic activation, 2,4-DNP was negative (Kawai et al. 1987). The negative results for mutagenicity of 2,4-DNP with S9 are surprising in light of the fact that the two major metabolites of 2,4-DNP (2-amino-4-nitrophenol and 4-amino-2-nitrophenol) are genotoxic in several test systems. The S9 fraction contains both microsomal and soluble enzymes that metabolize 2,4-DNP to amino nitrophenols (Eiseman et al. 1972). However, 2,4-DNP metabolism requires ATP; unless the S9 fraction contains an ATP regenerating system, 2,4-DNP may not be metabolized.

Among the other DNP isomers, 2,3-, 2,5-, and 3,5-DNP were positive for reverse mutations in the TA98 and TAl00 strains of S. typhimurium with or without metabolic activation; 2,6-DNP was negative in both strains with or without metabolic activation; and 3,4-DNP was negative in TA98

and positive in TAl00 both with and without metabolic activation (Kawai et al. 1987; Neuwoehner et al. 2007).

Using Escherichia coli as the test organism, 2,4-DNP was negative for reverse mutation in the Wp2 and Wp2(uvrA-) strains with and without metabolic activation (Probst et al. 1981). Positive results for mutagenicity were reported for reverse mutation in the B/Sd-4/1,3,4,5 and B/Sd-4/3,4 strains of E. coli without metabolic activation (Demerec et al. 1951). The authors concluded that 2,4-DNP was clearly positive for mutagenicity; however, the data appeared unreliable, based on extreme variation in survival and mutation rates within exposure groups.

In in vitro studies, 2,4-DNP generally did not produce DNA damage in prokaryotic or eukaryotic organisms, but 3,5-DNP did. 2,4-DNP was negative for DNA damage in the TA1535/pSK1002 strain of S. typhimurium (as determined by induction of the SOS response) with and without metabolic activation (Nakamura et al. 1987); in the K12(λ) strain of E. coli (as determined by phage induction) without metabolic activation (Heinemann and Howard 1964); in rat hepatocytes (as determined by unscheduled DNA synthesis) (Probst et al. 1981); and in Chinese hamster ovary cells (as determined by alkali elution) with or without metabolic activation (Swenberg et al. 1976). One study reported increases in DNA damage (as determined by alkali elution) in mouse leukemia L1210 cells and human HeLa cells (Hilton and Walker 1977); however, the observed effects were related to depletion of ATP pools, and the removal of the 2,4-DNP allowed for repletion of the pools and repair of DNA damage. Based on the weight of evidence presented, 2,4-DNP was negative for DNA damage, either with or without metabolic activation. In contrast, 3,5-DNP induced DNA damage in Salmonella choleraesius subsp. chol. (Neuwoehner et al. 2007).

Numerous in vitro studies reported decreased DNA synthesis and/or changes in the mitotic index in mammalian cells exposed to 2,4-DNP (Garrett and Lewtas 1983; Gautschi et al. 1973; Ghosh et al. 1989; Miyagawa 1977; Richard et al. 1991; Tsuda 1974). Typically, large decreases in ATP and/or protein synthesis were also observed. Because a primary effect of 2,4-DNP in cells is to uncouple oxidative phosphorylation, cellular processes dependent on production of ATP by oxidative phosphorylation likely will be adversely affected by the actions of 2,4-DNP. DNA synthesis depends, to some extent, on ATP. Thus, assessing this endpoint as an indicator of genotoxicity may lead to "false positives" for genotoxicity. In these studies, the effects of 2,4-DNP on mitosis and/or DNA synthesis were related to lower ATP levels in cells exposed to 2,4-DNP, resulting in decreases in energy-dependent processes, including mitosis and DNA synthesis (Garrett and Lewtas 1983; Gautschi et al. 1973; Ghosh et al. 1989; Miyagawa 1977; Richard et al. 1991; Tsuda 1974). Thus, these changes probably do not indicate a positive response for genotoxicity.

No studies were located regarding genotoxicity in humans after inhalation, oral, or dermal exposure or in animals after inhalation or dermal exposure to 2,4-DNP. 2,4-DNP was negative for genotoxicity in one in vivo gavage assay in mice assessing DNA synthesis in testicular cells (Friedman and Staub 1976) and positive in another (Seiler 1981); 2,4-DNP was negative for mutagenicity in assays on prokaryotic organisms; and DNP was negative for DNA damage in vitro using prokaryotic and mammalian cells (Anderson and Styles 1978; Chiu et al. 1978; De Flora 1981; Garner and Nutman 1977; Heinemann and Howard 1964; Kleinhofs and Smith 1976; Nakamura et al. 1987; Probst et al. 1981; Swenberg et al. 1976). In mice injected intraperitoneally with 2,4-DNP, the incidence of chromosomal aberrations was increased (Mitra and Manna 1971). Other studies producing positive results for genotoxicity were either equivocal for mutagenicity in prokaryotic organisms or were "false positives" for genotoxicity in assays measuring DNA synthesis or mitotic indices that could be explained by a 2,4-DNP induced

decrease in cellular metabolic rate (Demerec et al. 1951; Garrett and Lewtas 1983; Gautschi et al. 1973; Ghosh et al. 1989; Kawai et al. 1987; Miyagawa 1977; Seiler 1981; Tsuda 1974). Thus, the weight of evidence indicates that 2,4-DNP is not genotoxic. However, one study demonstrated an increase in chromosomal aberrations in vivo, indicating that it might be useful to further test 2,4-DNP for clastogenicity (Mitra and Manna 1971). Furthermore, considerable data indicates that the metabolites of 2,4-DNP (2-amino-nitrophenol, 4 amino-2-nitrophenol, and 2,4-diaminophenol) are mutagenic in S. typhimurium (Garner and Nutman 1977). Since 2,4-DNP was negative with metabolic activation with rat liver S9, which contains the enzymes that reduce 2,4-DNP to these metabolites, the positive results with the metabolites are difficult to reconcile. A study that specifically addresses the metabolism of 2,4-DNP in the presence of the S9 activating system and an appropriate ATP-regenerating system would resolve this apparent contradiction.

In a study screening 102 chemicals for reverse mutations of S. typhimurium, 2,3- and 2,5-DNP were positive for mutagenicity in the TA98 and TA100 strains, 2,6-DNP was negative for mutagenicity in the TA98 and TA100 strains, and 3,4-DNP was positive and negative for mutagenicity in the TA100 and TA98 strains, respectively (Kawai et al. 1987). Thus, data indicate a potential for mutagenicity in 2,3-, 2,5-, and 3,4-DNP. Further studies in bacterial and mammalian culture assays of these isomers would be useful to better determine their potential genotoxicity.

Hilliard et al. (1998) reported that 2,4-DNP was an oxidative phosphorylation uncoupler that induced marked increases in chromosome aberrations with 26 and 38% cell aberrations. These were associated with considerable reductions in cell counts in Chinese hamster ovary cells.

Genotoxicity of DNP Metabolites. 2-Amino-4-nitrophenol was mutagenic in S. typhimurium strain TA98 both with and without metabolic activation (Shahin et al. 1982; Zeiger et al. 1987 and in strain TA1538 with and without activation (Ames et al. 1975; Garner and Nutman 1977; Shahin et al. 1982). 2-Amino-4-nitrophenol was not mutagenic in strains TAl00, TA1535, and TA1537, both with and without activation (Shahin et al. 1982; Zeiger et al. 1987). Results for 2-amino-4-nitrophenol were equivocal in a test of phage induction in E. coli without activation (Kvelland 1985). 2-Amino-4 nitrophenol was mutagenic in the neurospora Sorduriu brevicollis (Yu-Sun et al. 1981) without activation. In eukaryotic cells, 2-amino-4 nitrophenol was mutagenic without activation in mouse lymphoma L5178Y cells (NTP 1988a) and caused chromosomal aberrations and sister chromatid exchange in Chinese hamster ovary cells both with and without activation (Anderson et al. 1990; NTP 1988a). 2 Amino-4-nitrophenol was negative in a dominant lethal mutation test after intraperitoneal administration to rats (Burnett et al. 1977). Genotoxicity information is summarized in Tables 2-8 and 2-9.

Table 2-8. Genotoxicity of Dinitrophenol Metabolites In Vivo

Species (test system)	Endpoint	Results	Reference	Isomer
Mammalian cells:				
Rat (intraperitoneal)	Dominant lethal mutation	–	Burnett et al. 1977	2-a-4np
Rat (intraperitoneal)	Dominant lethal mutation	–	Burnett et al. 1977	2-a-5np
Rat (intraperitoneal)	Dominant lethal mutation	–	Burnett et al. 1977	4-a-2np

– = negative result; + = positive result; 2-a-4np = 2-amino-4-nitrophenol; 4-a-2np = 4-amino-2-nitrophenol; 2-a-5np = 2-amino-5-dinitrophenol

Table 2-9. Genotoxicity of Dinitrophenol Metabolites In Vitro

Species (test system)	Endpoint	Results: Activation: With	Results: Activation: Without	Reference	Isomer
Prokaryotic organisms:					
Escherichia coli					
B, CR63, K12(λh)	Phage induction	No data	(+)	Kvelland 1985	2-a-4np
Salmonella typhimurium					
TA98	Reverse mutation	(+)	+	Shahin et al. 1982	2-a-4np
TA100		–	–		
TA1535		–	–		
TA1537		–	–		
TA1538		(+)	+		
TA1538	Reverse mutation	+	No data	Ames et al. 1975	2-a-4np
TA1538	Reverse mutation	+	+	Garner and Nutman 1977	2-a-4np
TA98	Reverse mutation	+	(+)	Zeiger et al. 1987	2-a-4np
TA100		–	–		
TA1535		–	–		
TA1537		–	–		
Sordaria brevicollis	Reverse mutation	No data	+	Yu-Sun et al. 1981	2-a-2np
E. coli					
B, CR63, K12(λh)	Reverse mutation	–	–	Kvelland 1985	2-a-5np
S. typhimurium					
TA98	Reverse mutation	+	+	Shahin et al. 1982	2-a-5np
TA100		–	(+)		
TA1535		–	+		

Table 2-9. Genotoxicity of Dinitrophenol Metabolites In Vitro

Species (test system)	Endpoint	Results		Reference	Isomer
		Activation			
		With	Without		
TA1537		+	+		
TA1538		+	+		
TA1538	Reverse mutation	No data	+	Ames et al. 1975	2-a-5np
TA98	Reverse mutation	No data	+	Chiu et al 1982	2-a-5np
TA100		No data	–		
TA98	Reverse mutation	+	+	Zeiger et al. 1987	2-a-5np
TA1000		(+)	(+)		
TA1535		–	–		
TA1537		(+)	(+)		
TA98	Reverse mutation	+	+	Garner and Nutman 1977	4-a-2np
TA1538		+	+		
TA97	Reverse mutation	+	+	Zeiger et al. 1987	4-a-2np
TA98		+	+		
TA98	Reverse mutation	–	–	Shahin et al. 1982	4-a-2np
TA100		–	–		
TA1535		–	–		
TA1537		–	–		
TA1538		–	–		
TA1538	Reverse mutation	+	–	Dybing and Thorgeirsson 1977	2,4-dap
Eukaryotic organisms:					
Mammalian cells					
Mouse lymphoma L518Y cells	Gene mutation	No data	+	NTP 1988a	2-a-4np
CHO cells	Sister chromatid exchange	+	+	NTP 1988a	2-a-4np
CHO cells	Sister chromatid aberrations	+	+	NTP 1988a	2-a-4np
CHO cells	Sister chromatid aberrations	+	+	Anderson et al. 1990	2-a-4np
Mouse lymphoma L518Y cells	Gene mutation	No data	+	NTP 1988a	2-a-5np
CHO cells	Sister chromatid exchange	+	+	NTP 1988a	2-a-5np
CHO cells	Chromosomal aberrations	+	+	NTP 1988a	2-a-5np

Table 2-9. Genotoxicity of Dinitrophenol Metabolites In Vitro

Species (test system)	Endpoint	Results: Activation: With	Results: Activation: Without	Reference	Isomer
Rat 344 hepatocyte primary culture	Unscheduled DNA synthesis	No data	–	Williams et al. 1982	4-a-2np
Mouse lymphoma L518Y cells	Gene mutation	+	+	Mitchell et al. 1988	4-a-2np

– = negative result; + = positive result; (+) = weakly positive or equivocal result; 2-a-4np = 2-amino-4-nitrophenol; 2-a-5np = 2-amino-5-nitrophenol; 4-a-2np = 4-amino-2-nitrophenol; 2,4-dap = 2,4-diaminophenol; CHO = Chinese hamster ovary; DNA =deoxyribonucleic acid

Commercial-grade 4-amino-2-nitrophenol was reported to be mutagenic in S. typhimurium strains TA98 and TA1538 with and without activation (Garner and Nutman 1977). Highly purified 4-amino-2 nitrophenol was not mutagenic in strains TA98, TAl00, TA1535, TA1537, or TA1538 (Shahin et al. 1982), leading the authors to conclude that the mutagenic activity of the commercial grade was due to a contaminant. However, in other studies, highly purified 4-amino-2-nitrophenol was mutagenic with and without activation in strains TA97 and TA98 (Zeiger et al. 1987). 4-Amino-2-nitrophenol also caused forward mutations at the TK locus in mouse lymphoma L5178Y cells with and without activation (Mitchell et al. 1988). 4-Amino-2-nitrophenol was negative when administered intraperitoneally in a dominant lethal mutation study (Burnett et al. 1977) and did not induce unscheduled DNA synthesis in Fischer 344 rat primary hepatocyte cultures (Williams et al. 1982).

In a phage induction test for mutagenicity in E. coli, 2-amino-5-nitrophenol was mutagenic without activation (Kvelland 1985). In S. typhimurium, 2-amino-5-nitrophenol was mutagenic in strain TA98 with and without activation (Chiu et al. 1978; Shahin et al. 1982; Zeiger et al. 1987); negative or equivocal in strain TA100 without activation, and negative or equivocal with activation (Chiu et al. 1982; Shahin et al. 1982; Zeiger et al. 1987); positive or negative in strain TA1535 without activation (Shahin et al. 1982; Zeiger et al. 1987) and negative with activation (Shahin et al. 1982; Zeiger et al. 1987); positive or weakly positive in strain TA1537 with and without activation (Shahin et al. 1982; Zeiger et al. 1987); and positive in strain TA1538 with and without activation (Ames et al. 1975; Shahin et al. 1982). 2 Amino-5-nitrophenol was also mutagenic in the mouse lymphoma L5178Y cell mutation test without activation (NTP 1988b) and caused sister chromatid exchange and chromosomal aberrations in Chinese hamster ovary cells with and without activation. 2-Amino-5-nitrophenol was negative in a dominant lethal mutation test in CD rats given the test chemical intraperitoneally (Burnett et al. 1977).

2,4-Diaminophenol was reported to be mutagenic only with activation in S. typhimurium strain TA1538 (Dybing and Thorgeirsson 1977). Another report (Kawai et a1. 1987) stated that 2,4-diaminophenol was mutagenic, but did not provide further information."

COMMENT: Or even 1000-fold, as in the current MRL!

RESPONSE: *No change was made; this is the boilerplate introduction to the MRL worksheets. The 100-fold uncertainty factor was given as an example.*

COMMENT: Might consider specifying here and in subsequent worksheets the exposure duration.

RESPONSE: *Text on the acute-, intermediate-, and chronic-duration inhalation MRL worksheets (respectively) was revised as follows:*

> *"Therefore, data are insufficient to derive MRLs for acute-duration inhalation exposure to DNP";*
> *"Therefore, data are insufficient to derive MRLs for intermediate-duration inhalation exposure to DNP"; and*
> *"Therefore, data are insufficient to derive MRLs for chronic-duration inhalation exposure to DNP" (Chronic-duration Inhalation MRL Worksheet)."*

COMMENT: Should include Point of Departure (POD) here.

RESPONSE: *The Glossary was revised to include POD as follows:*

> *"Point of Departure (POD) – The point on the dose-response curve that defines where low-dose extrapolation commences. The POD may be a NOAEL, LOAEL, or benchmark dose estimated from mathematical modeling of the dose-response relationship."*

ATSDR Charge Questions and Responses (Peer Reviewer 1)

General Comments

COMMENT: I have read the pertinent sections of the document, and provide my specific comments below. In addition, I have offered several comments/edits within the document for consideration. Overall, the document is thorough and well-written. In my role as senior editor of a toxicological journal, and many other reviewer activities, it is unusual (and a good thing) that I found very few typographical errors or major grammatical issues in the document. My main comment is related to the use of an uncertainty factor of 1000 for the intermediate exposure MRL, as discussed in detail below.

RESPONSE: *See response~~s~~ where the Reviewer discusses related text below.*

Chapter 1. Relevance to Public Health

QUESTION: Do you agree with those effects known to occur in humans as reported in the text?

COMMENT: I agree with the effects listed and am not aware of studies observing any additional responses in humans.

RESPONSE: *No response necessary.*

QUESTION: Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

COMMENT: Although there may be differences in certain specific responses between animals and humans, given the central role of oxidative phosphorylation to produce ATP in aerobic organisms it is

highly likely that the effects observed in animals will also be observed in humans at an appropriate dose and/or duration of exposure. Thus, I agree that effects observed in animals are of concern to humans.

RESPONSE: *No response necessary.*

QUESTION: Have exposure conditions been adequately described? If you disagree, please explain.

COMMENT: Yes. The document provides detailed accounts of exposure conditions used for the various studies reviewed.

RESPONSE: *No response necessary.*

QUESTION: Do you believe the derived intermediate oral MRL value is justifiable? If you disagree, please explain (see also Appendix A).

COMMENT: In my opinion the intermediate oral MRL value might be overly conservative. It is based solely on a well-conducted study published in a very good journal (Caldeira da Silva et al 2008). Essentially, the median/mean exposure in this study (0.07 mg/kg/d) resulted in lesser body mass/body mass gain in mice compared to untreated controls; reduced serum glucose, triglycerides, and insulin concentrations; and increased longevity in treated mice. The authors concluded that mild mitochondrial uncoupling produces beneficial responses, and that identification of appropriate agents in this regard may be useful therapeutically. In other words, the effects observed in the Caldeira da Silva et al (2008) study can be viewed as beneficial, not toxic, and this appears to cloud the issue of deriving a MRL from these data. Nevertheless, the study clearly observed physiological changes in mice at this exposure level that could be considered as indications that other subclinical effects would occur that may be toxic, as has been seen with other studies in humans and animals exposed to much higher doses of DNP. Following are some specific comments for consideration:

a) In my opinion the uncertainty factor should be 100, not 1000. This is because the factor of 10 for LOAEL, based on Caldeira da Silva et al (2008), is not clearly an adverse effect level.
b) Chronic exposure is usually defined as the majority (>90%) of lifespan in laboratory animals. In my opinion, the 50-week (350 day) study, when compared to the strict definition in the ATSDR document (365 days) is moot. In other words the Caldeira da Silva et al (2008) study is for all intents and purposes a chronic study, and thus the intermediate MRL derived should also be considered a chronic MRL.
c) Since the Caldeira da Silva et al (2008) study exposed mice to 2,4-DNP via drinking water, it is hard to believe that a drinking water standard cannot also be derived from these data.

RESPONSE: *No changes were made. An uncertainty factor of 10 for use of a LOAEL was used because ATSDR considers a body weight decrease of 10% to be clearly adverse, and because the mice in the study were housed at a reduced ambient temperature to prevent hyperthermia, potentially mitigating adverse effects that might otherwise have occurred in the animals.*

ATSDR defines intermediate exposure as >14 and <365 days, and defines chronic exposure as ≥365 days. ATSDR agrees that the exposure duration in the study by Caldeira da Silva et al. (2008) is close to the boundary between intermediate and chronic, and this is one reason why ATSDR concluded that the intermediate-duration MRL is likely to be protective for chronic exposures.

ATSDR does not derive drinking water standards.

QUESTION: Do you agree that the data do not support derivation of acute, intermediate, and chronic inhalation MRLs?

COMMENT: I agree that data are insufficient to derive inhalation MRLs for different exposure durations.

RESPONSE: *No response necessary.*

Chapter 2. Health Effects

QUESTION: Do the health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature for DNPs?

COMMENT: Yes, the literature has been extensively reviewed and I am not aware of additional studies not listed/cited within the document.

RESPONSE: *No response necessary.*

QUESTION: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? Were the major study limitations sufficiently described in the text without going into lengthy discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT: Human studies are almost always subject to limitations, and these were identified in the text. Many of the human studies are cases involving n=1 individual, with uncertain dosage and biological data.

RESPONSE: *No response necessary.*

QUESTION: Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.

COMMENT: In essence, the only study being considered here for application to a MRL is the Caldeira da Silva et al (2008) study. The problem with many of the other animal studies cited is that they were conducted a long time ago (before 1950), and do not always have strict experimental design as occurs in more recent toxicological research. In addition, most of the earlier studies used extremely high exposure levels that are only relevant to human poisonings/suicide/intentional use as weight loss agents.

RESPONSE: *No response necessary.*

QUESTION: Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?

COMMENT: Yes, mice are appropriate. They are the basis of experimental medicine.

RESPONSE: *No response necessary.*

QUESTION: Are you aware of any studies that are not included in the profile that may be important in evaluating the toxicity of DNPs? Please provide a copy of each study and indicate where in the text each study should be included.

COMMENT: No.

RESPONSE: *No response necessary.*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the DNP isomers?

COMMENT: No.

RESPONSE: *No response necessary.*

QUESTION: Were all appropriate NOAELs and/or LOAELs identified for each study (both in the text and the Levels of Significant Exposure (LSE) tables and figures)? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

COMMENT: Yes.

RESPONSE: *No response necessary.*

QUESTION: Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables?

COMMENT: Yes. Mortality, stillbirths, and cataracts are serious (irreversible), whereas changes in body mass, organ mass and biochemical markers are less serious (generally reversible).

RESPONSE: *No response necessary.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain.

COMMENT: Yes. I am not aware of other MOAs other than mitochondrial uncoupling.

RESPONSE: *No response necessary.*

QUESTION: The updated DNPs profile includes an unpublished study by Eli Lilly and Co. unavailable to ATSDR when preparing the original profile. Please comment on the quality of the study, namely:

Did the study use an adequate number of animals and practice good animal care?
Did the study account for competing causes of death?
Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?
If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.
Do you agree with the conclusions of the author? If not, please explain.

COMMENT: The study was essentially a 14-day LD50 study using 4 doses of "Dinitrophenol I" (I assume this is 2,4-DNP) and 4 rats per dose. There was no control group! In my opinion the study is of little use and does not add any significant information to the ATSDR MRL derivation. Cite it, report the estimated LD50, and don't dwell on it.

RESPONSE: *No response necessary.*

Chapter 7. Regulations and Guidelines

QUESTION: Are you aware of any additional regulations or guidelines that we should add? Please provide citations.

COMMENT: No.

RESPONSE: *No response necessary.*

QUESTION: Are there any that should be removed? Please explain.

COMMENT: No.

RESPONSE: *No response necessary.*

Appendix A. ATSDR Minimal Risk Level Worksheets

QUESTION: Acute-duration oral MRL: The updated data evaluation includes a number of fatal human case studies involving lower exposure levels than were documented in the original profile (within an order of magnitude of the point of departure used for the original MRL).

Do you agree that these human fatality data adequately support ATSDR's decision to remove the original acute oral MRL? In not, please explain.

COMMENT: Yes. These more recent human cases clearly indicate that "abuse" of DNPs can occur at lower acute exposure levels than previously thought. Intraspecific differences among humans likely play an important role in the uncertainty associated with an acute MRL.

RESPONSE: *No response necessary.*

QUESTION: Acute-duration oral MRL: The updated data evaluation includes a number of fatal human case studies involving lower exposure levels than were documented in the original profile (within an order of magnitude of the point of departure used for the original MRL).

Please comment on any aspect of our MRL database assessment that you would like us to address.

COMMENT: None.

RESPONSE: *No response necessary.*

QUESTION: Intermediate-duration oral MRL: ATSDR derived a new intermediate-duration oral MRL of 0.00007 mg/kg/day was derived for 2,4-DNP based on decreased body weight in mice exposed to 0.07 mg/kg/day 2,4-DNP in drinking water for 50 weeks (Caldeira da Silva et al. 2008). The MRL is based on a LOAEL of 0.07 mg/kg/day and a total uncertainty factor of 1,000 (10 for interspecies extrapolation, 10 for human variability, and 10 for use of a LOAEL). An intermediate-duration oral MRL was not derived in the 1995 toxicological profile due to the lack of intermediate duration studies involving doses lower than those known to cause death in humans.

Do you agree or disagree with the proposed intermediate-duration oral MRL value? Explain. If you disagree, please specify the MRL value that you propose.

COMMENT: See my response to Chapter 1, question 4 above. In my opinion, an uncertainty factor of 100 (10 for mouse to human, and 10 for human variability) and an intermediate oral MRL of 0.0007 mg/kg/d should be used.

RESPONSE: *No changes were made. An uncertainty factor of 10 for use of a LOAEL was used because ATSDR considers a body weight decrease of 10% to be clearly adverse, and because the mice in the study were housed at a reduced ambient temperature to prevent hyperthermia, potentially mitigating adverse effects that might otherwise have occurred in the animals.*

QUESTION: Intermediate-duration oral MRL: ATSDR derived a new intermediate-duration oral MRL of 0.00007 mg/kg/day was derived for 2,4-DNP based on decreased body weight in mice exposed to 0.07 mg/kg/day 2,4-DNP in drinking water for 50 weeks (Caldeira da Silva et al. 2008). The MRL is based on a LOAEL of 0.07 mg/kg/day and a total uncertainty factor of 1,000 (10 for interspecies extrapolation, 10 for human variability, and 10 for use of a LOAEL). An intermediate-duration oral MRL was not derived in the 1995 toxicological profile due to the lack of intermediate duration studies involving doses lower than those known to cause death in humans.

Do you agree/disagree with each component of the total uncertainty factor? Explain. If you disagree, please specify the uncertainty factor(s) that you propose.

COMMENT: See above.

RESPONSE: *No changes were made. An uncertainty factor of 10 for use of a LOAEL was used because ATSDR considers a body weight decrease of 10% to be clearly adverse, and because the mice in the study were housed at a reduced ambient temperature to prevent hyperthermia, potentially mitigating adverse effects that might otherwise have occurred in the animals.*

QUESTION: Intermediate-duration oral MRL: ATSDR derived a new intermediate-duration oral MRL of 0.00007 mg/kg/day was derived for 2,4-DNP based on decreased body weight in mice exposed to 0.07 mg/kg/day 2,4-DNP in drinking water for 50 weeks (Caldeira da Silva et al. 2008). The MRL is based on a LOAEL of 0.07 mg/kg/day and a total uncertainty factor of 1,000 (10 for interspecies extrapolation, 10 for human variability, and 10 for use of a LOAEL). An intermediate-duration oral

MRL was not derived in the 1995 toxicological profile due to the lack of intermediate duration studies involving doses lower than those known to cause death in humans.

Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT: None.

RESPONSE: *No response necessary.*

QUESTION: Chronic-duration oral MRL: ATSDR believes the chronic-duration oral exposure database does not provide sufficient data for derivation of a chronic oral MRL. Specifically, the lowest LOAELs for effects of chronic exposure are higher than doses known to cause fatalities in humans. However, we believe the intermediate-duration oral MRL is protective for chronic exposures.

Do you agree with ATSDR that the intermediate oral MRL of 0.00007 mg/kg/day would be sufficiently protective for chronic exposures? If not, please explain.

COMMENT: Yes. See my previous comments. In my opinion the Caldeira da Silva et al (2008) study should be used for chronic as well as intermediate exposure MRLs.

RESPONSE: *ATSDR defines intermediate exposure as >14 and <365 days, and defines chronic exposure as ≥365 days. ATSDR agrees that the exposure duration in the study by Caldeira da Silva et al. (2008) is close to the boundary between intermediate and chronic, and this is one reason why ATSDR concluded that the intermediate-duration MRL is likely to be protective for chronic exposures.*

Appendix B. Literature Search Framework

QUESTION: Does Appendix B provide a sufficiently clear documentation of ATSDR's health effects literature search strategy and inclusion/exclusion criteria?

COMMENT: Yes, very detailed search criteria.

RESPONSE: *No response necessary.*

QUESTION: Does it provide enough transparency regarding ATSDR's implementation of its inclusion and exclusion criteria (e.g. how ATSDR chose the studies it included in the health effects chapter)?

COMMENT: Yes.

RESPONSE: *No response necessary.*

Overall Usability of the Profile

QUESTION: Does the new chapter organization make it easy for you to find the information you need? For example, are you satisfied with the organization of the health effects chapter by organ system rather than exposure route?

COMMENT: Yes, organizing by organ system is a much better way to summarize the health effects.

RESPONSE: *No response necessary.*

QUESTION: Does the profile contain all of the information you need? Is there information you would like to see that is not currently included?

COMMENT: Yes, the profile is very detailed.

RESPONSE: *No response necessary.*

QUESTION: Having read this Toxicological Profile (and others, if applicable), which chapter(s) or content do you find most valuable and why? If you have previously used any Toxicological Profile(s) for your work, which chapter(s) or content have you used the most and for what purpose(s)?

COMMENT: This would depend on the intended use of the Profile (e.g., human health effects, environmental fate/behavior, regulatory criteria/guidelines, etc). Overall the Profiles provide a nice balance of "conciseness" and detail, and this serves the stated purpose of Profiles well.

RESPONSE: *No response necessary.*

QUESTION: Are the new tables and figures clear and useful? Do they make the Toxicological Profile easier to read?

COMMENT: Yes, I like the way Figures and Tables are presented in the Profile.

RESPONSE: *No response necessary.*

Comments Provided by Peer Reviewer #2

Annotated Comments on the Profile

COMMENT: Although covered earlier there is a bit of interchange here between DNP, 2,4-DNP and DNPs which is at first glance confusing.

RESPONSE: *Section 1.1 was revised for clarity.*

> *"In the 1930s, 2,4-dinitrophenol (DNP) was prescribed by physicians as a weight-reducing agent, but its use was discontinued due to health risks. In recent years, however, 2,4-DNP in tablet and powder form has been marketed for weight loss and body building by unregulated internet sources, leading to a number of human fatalities. These unregulated sources often provide information to potential users regarding dosing and how to combine with other stimulants, steroids, and growth hormone for body building purposes, without informing users of the risk of death. As a result of the growth in availability of 2,4-DNP to the general public, there is increased potential for exposure and health effects among police officers involved in seizure of material or arrest of users, mail and shipping company employees who handle shipments, health care providers who treat or decontaminate users, and family members who live with persons who purchase and/or use 2,4-DNP. In addition to the toxicity hazards associated with 2,4-DNP, this compound is explosive when dry and when heated or subjected to flame, shock, or friction (WHO 2015).*
>
> *DNPs are also used in the manufacture of dyes, wood preservatives, photographic developers, explosives, and insecticides, and as a pH indicator. 2,4-DNP and other DNPs are released to the environment primarily during their manufacture and use, and from waste disposal sites. The most likely routes of exposure near hazardous waste sites would be breathing contaminated air, drinking contaminated water, eating contaminated food, or skin contact with contaminated soil. The toxicity of 2,4-DNP is greater at high ambient temperatures; therefore, susceptibility to the toxic effects may increase for workers at high workroom temperatures or in the general population at high environmental temperatures."*

COMMENT: Also in powder form.

RESPONSE: *Section 1.1 was revised as follows:*

> *"In recent years, however, 2,4-DNP in tablet and powder form has been marketed for weight loss and body building by unregulated internet sources…"*

COMMENT: I would probably stress this more here, as this is of very great clinical significance with an increasing number of deaths in the UK (not reported but from my clinical experience).

RESPONSE: *Section 1.1 was revised to add the following introductory paragraph in response to this comment pertaining to unregulated internet sources and the occurrence of hyperpyrexia as well as later comments pertaining to individuals who may experience high exposures:*

> *"In the 1930s, 2,4-DNP was prescribed by physicians as a weight-reducing agent, but its use was discontinued due to health risks. In recent years, however, 2,4-DNP in tablet and powder form has been marketed for weight loss and body building by unregulated internet sources, leading to a number of human fatalities. These unregulated sources often provide information to potential*

users regarding dosing and how to combine with other stimulants, steroids, and growth hormone for body building purposes, without informing users of the risk of death. As a result of the growth in availability of 2,4-DNP to the general public, there is increased potential for exposure and health effects among police officers involved in seizure of material or arrest of users, mail and shipping company employees who handle shipments, health care providers who treat or decontaminate users, and family members who live with persons who purchase and/or use 2,4-DNP. In addition to the toxicity hazards associated with 2,4-DNP, this compound is explosive when dry and when heated or subjected to flame, shock, or friction (WHO 2015)."

COMMENT: Put in about skin discolouration here as well?

RESPONSE: *Section 1.2 was revised as follows:*
"Human case reports of poisoning with 2,4-DNP after acute and intermediate oral exposures document yellow discoloration of skin, erythema, and pruritis, as well as maculopapular eruptions of the skin, sometimes covering the entire body."

COMMENT: Probably specifically state here that no human effects.

RESPONSE: *Section 1.2 was revised as follows:*
"No information was located on developmental effects of 2,4-DNP in humans."

COMMENT: Need reference here.

RESPONSE: *Section 2.1 was revised as follows:*
"As discussed in detail in Section 2.18.1 (Mechanisms of Action—Oxidative Phosphorylation Uncoupling), 2,4-DNP exerts its toxic effects via uncoupling of oxidative phosphorylation (e.g., Ilivicky and Casida 1969; Loomis and Lipmann 1948; Lou et al. 2007; Muscatello et al. 1975; Pinchot 1967; Stryer 1988; Weinbach and Garbus 1969)."

COMMENT: There is no link in the text to Table 2-1 and Figure 2-2.

RESPONSE: *No change was made; call-outs for Table 2-1 and Figure 2-2 are in the introduction.*
"As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figure 2-1 provides an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to DNPs but may not be inclusive of the entire body of literature.

Oral studies (human case reports and animal studies) are presented in Table 2-1 and Figure 2-2. Animal dermal studies are presented in Table 2-2."

COMMENT: 1. Need to put in something about the time course and that death can be very rapid after onset of symptoms and/or ingestion; 2. Need to put in something about the agitation, restlessness, fidgeting that often preceeds onset of hyperpyrexia and death.

RESPONSE: *Section 2.2 was revised as follows:*

"The time course between exposure by ingestion and the onset of serious symptoms and/or death can be very rapid. Symptoms preceding death consisted of fever progressing to hyperthermia, agitation or restlessness, excessive sweating (diaphoresis), increased respiratory rate and gasping (dyspnea and tachypnea), increased heart rate (tachycardia), extreme thirst, nausea, and vomiting."

COMMENT: What is the basis for the order of these cases – it is not alphabetical, not year of publication or age of patient – seems random.

RESPONSE: *Table 2-3 was re-ordered to show studies first by exposure duration and then alphabetically.*

Table 2-3. Case Reports of Human Fatalities after Oral Exposure to 2,4-DNP

Gender and age	Approximate lethal dose (mg/kg/day)	Exposure duration	Notes	Reference
Acute-duration exposure				
Male, 46 years old	~40	Once	DNP intake with suicidal intent. The patient consumed 2,800 mg. An average male body weight of 70 kg was assumed.	Bartlett et al. 2010
Male, 21 years old	~43	Once	DNP intake with suicidal intent. The patient consumed 4,250 mg. His BMI was 38 kg/m^2; a body weight of 100 kg was assumed for an obese male.	Holborow et al. 2016
Female, 17 years old	~31–38	Once	DNP intake with suicidal intent. The patient consumed 12–15 tablets each containing 192 mg 2,4-DNP. The authors reported the patient's body weight as 75 kg. The concentration of 2,4-DNP in the serum was 315 µg/mL.	Hsiao et al. 2005
Male, adult	~36–71	Once	DNP intake for weight loss. The intake estimated by an expert after death was 2,500–5,000 mg; upon hospital admission, the patient reported consuming 300 mg. An average male body weight of 70 kg was assumed.	Geiger 1933
Female, 21 years old	~64	Once	DNP intake with suicidal intent. The patient characterized as obese, consumed 45 capsules each containing 100 mg. A body weight of 70 kg was assumed for an obese female. The concentration of 2,4-DNP in the blood was 12 mg/mL.	Purvine et al. 1936
Male, adult	~40	Once	DNP intake with suicidal intent. The patient consumed 2,800 mg. An average male body weight of 70 kg was assumed.	Siegmueller and Narasimhaiah 2010

Table 2-3. Case Reports of Human Fatalities after Oral Exposure to 2,4-DNP

Gender and age	Approximate lethal dose (mg/kg/day)	Exposure duration	Notes	Reference
Male, 37 years old	46	Twice	DNP intake for weight loss. The patient ingested 3,700 mg 2,4-DNP as the sodium salt on two occasions 1 week apart; the study authors reported his weight as 80 kg.	Tainter and Wood 1934
Male, 22 years old	~6	3 days	DNP intake for weight loss. The patient, characterized as obese, consumed 600 mg 2,4-DNP per day. Body weight was assumed to be 100 kg for an obese male.	McFee et al. 2004
Female, 25 years old	7	5 days	DNP intake for weight loss. The patient ingested 2,880 mg over 5 days; the study authors reported her weight as 66.7 kg.	Poole and Haining 1934
Female, 31 years old	0.8–3.8 (TWA 2.7)	14 days	DNP intake for potential antidepressive effects. The patient ingested a total of 5,820 mg over 14 days; the study authors reported that her body weight ranged between 130 and 127 kg (average of 128.5 kg).	Masserman and Goldsmith 1934
Intermediate-duration exposure				
Female, 25 years old	0.6–4	41 days	DNP intake for weight loss. The patient ingested increasing doses from 90 to 540 mg/day 2,4-DNP sodium salt (74–440 mg/day as 2,4-DNP) for 41 days. Her body weight was reported as 120 kg at the beginning of dosing and 117 kg at the end. Agranulocytosis diagnosed.	Silver 1934
Female, 46 years old	3–4	42 days	DNP intake for weight loss. The patient ingested 200 mg/day increasing to 300 mg/day. The patient was characterized as obese; body weight of 70 kg for obese female was assumed. Agranulocytosis diagnosed.	Dameshek and Gargill 1934
Male, 50 years old	1.7–5.4 (TWA 2.7)	43 days	DNP intake for weight loss. Doses were calculated from intakes and body weights reported by study authors.	Zack et al. 2016
Female, "young"	1.0	46 days	DNP intake for weight loss. The patient ingested 5,400 mg 2,4-DNP over 46 days; the study authors reported that her weight ranged between 120 and 109 kg (average of 114.5 kg). Agranulocytosis was diagnosed.	Goldman and Haber 1936

Table 2-3. Case Reports of Human Fatalities after Oral Exposure to 2,4-DNP

Gender and age	Approximate lethal dose (mg/kg/day)	Exposure duration	Notes	Reference
Fatalities lacking dose or duration Information				
Female, 27 years old	*ND*	*ND*	*DNP intake for weight loss. The patient reportedly took twice the dose recommended by the website from which she purchased the chemical. No other information on exposure was provided, nor were blood levels.*	*Tewari et al. 2009*
Male, 30 years old	*ND*	*ND*	*DNP intake for body building. DNP was considered to be a contributing factor in the death, along with citalopram. Postmortem blood level was 48.4 mg/L.*	*Politi et al. 2007*
Female, 17 years; male, 28 years old	*ND*	*ND*	*DNP intake for weight loss (female) and body building (male). Blood levels were 36.1 and 28 mg/L on admission to the hospital.*	*Miranda et al. 2006*
Male, 24 years old	*ND*	*ND*	*DNP intake for weight loss/body building. No information on exposure or blood levels was provided.*	*Suozzi et al. 2005*
Female, 29 years old	*ND*	*See notes*	*DNP intake for weight loss. Patient took 3–5 tablets a day for several months, discontinued its use for 3 months, and then resumed taking 5 tablets/day for 1 week. The dose per tablet was not provided.*	*Lattimore 1934*

BMI = body mass index; DNP =dinitrophenol; ND = no data; TWA = time-weighted average

COMMENT: What does this mean? Put in the from and to numbers to give more context.

RESPONSE: *Section 2.2 was revised as follows:*

"Following the institution of better ventilation, use of masks, and other industrial hygiene measures to minimize exposure, the numbers of deaths per 10,000 tons 2,4-DNP manufactured per year decreased from 16.3 to 1.2."

COMMENT: Might be worth explaining what this is for a generalist who may not understand**.**

RESPONSE: *Section 2.2 was revised as follows:*

"Animal studies of mortalities after acute gavage (stomach tube) exposure to 2,4-DNP..."

COMMENT: Consider putting in °C as well for non-US readers?

RESPONSE: *Table 2-5 was revised to include °C as suggested.*

Table 2-5. Temperature Dependence of Intraperitoneal LD_{50} Values in Mice

Ambient temperature		LD_{50} (mg/kg)					
(°F)	(°C)	2,4-DNP	2,6-DNP	3,5-DNP	3,4-DNP	2,3-DNP	2,5-DNP
64–70[a]	18–21	36	45	50	112	200	273
95–99	35–37	35	37	47	115	160–175	250
102–106	39–41	<5 (all died)	<10 (all died)	50	100–110	160–175	200

[a]*Experiments in rats at this temperature yielded LD_{50} values very similar to the LD_{50} values in mice. Rats were not tested at other temperatures.*

Source: Harvey (1959)

COMMENT: Is this over whole time or per week?

RESPONSE: *Section 2.3 was revised as follows*

"When four volunteers were placed on various diets (balanced, high carbohydrate, high fat, or high protein) and given an average dose of 4 mg/kg/day 2,4-DNP for 7–16 days, the average weight loss during 2,4-DNP treatment was ~2 pounds (0.92 kg) (Cutting and Tainter 1933)."

COMMENT: What changes were seen?

RESPONSE: *Section 2.5 was revised as follows:*

"An intermediate-duration clinical study of six patients treated for 1–8 weeks with 2,4-DNP at 4 mg/kg/day showed definite changes in the electrocardiograms (increased size or inversion of T wave, depression of ST interval, notching of QRS complex) of three patients (MacBryde and Taussig 1935)."

COMMENT: Rhabdomyolysis from hyperpyrexia should be included here.

RESPONSE: *Section 2.8 was revised as follows:*

"A number of case studies have documented muscle weakness or pain following oral exposure to 2,4-DNP, as well as rhabdomyolysis resulting from hyperpyrexia."

COMMENT: Need to also include hyperpyrexia induced acute liver injury.

RESPONSE: *Section 2.9 was revised as follows:*

"These limited data in humans do not suggest that 2,4-DNP exerts strong effects, if any, on the liver. In human cases of hyperpyrexia associated with ingestion of 2,4-DNP, however, acute liver injury can occur (see Section 2.2)."

COMMENT: I know covered elsewhere but as first time used in this section consider spelling out what this is?

RESPONSE: *No change was made. LOAEL is defined in the text of the introduction to Chapter 2. "The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of DNPs. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.*

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figure 2-1 provides an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to DNPs but may not be inclusive of the entire body of literature.

Oral studies (human case reports and animal studies) are presented in Table 2-1 and Figure 2-2. Animal dermal studies are presented in Table 2-2.

Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an endpoint should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these endpoints. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health."

COMMENT: Worth mentioning yellow skin discoloration?

RESPONSE: *The text of Section 2.11 was revised as follows:*

"Yellow discoloration of the skin and pruritic skin rashes were common findings in people taking 2,4-DNP for weight loss. Some early studies (e.g., Bayer and Gray 1935) attributed the yellow discoloration to jaundice, but this finding more likely results from 2,4-DNP excretion in sweat (e.g., Holborow et al. 2016)."

COMMENT: Make the time course for development more clearer.

RESPONSE: *Section 2.12 was reorganized such that the introductory paragraph begins as follows:*

"Cataracts developed in some patients who took 2,4-DNP or sodium 2,4-DNP as a weight reduction aid for acute, intermediate, and chronic durations. The cataracts developed rapidly, sometimes while the patient was still ingesting the drug and sometimes after cessation of treatment, and were bilateral and irreversible, progressing to total blindness."

COMMENT: What percentage?

RESPONSE: *Information on the percentage of patients who developed cataracts was not located. Section 2.12 was revised as follows:*

"Cataracts developed in some patients who took 2,4-DNP or sodium 2,4-DNP as a weight reduction aid for acute, intermediate, and chronic durations."

COMMENT: What does this mean?

RESPONSE: *Section 2.13 was revised to add the following sentence to place the unclear information in context:*

"No other information on potential estrogenic effects of 2,4-DNP, and no information on potential androgenic effects, was located."

COMMENT: Maybe stress that often this is the first clinical feature and can then continue rapidly to hyper-pyrexia and/or cardiovascular collapse

RESPONSE: *Section 2.15 was revised to add the following introductory sentence:*

"Often, the earliest clinical sign of poisoning with 2,4-DNP is agitation or restlessness, progressing rapidly to hyperpyrexia and/or cardiovascular collapse."

COMMENT: What is the animal data to support this – may be put in some more detail if available. Also risk that this suggests even though says unlikely to be of therapeutic benefit that if seen by someone with untreatable neurological disease may try to see if helps. Would suggest maybe making stronger the concluding sentence

RESPONSE: *Upon reconsideration, this paragraph in Section 2.16 was deleted. These studies do not contribute information on mechanisms of adverse effects, and as such are not important to the Toxicological Profile.*

COMMENT: What was the trimester? Could this have been unrelated?

RESPONSE: *The pregnancy loss occurred at approximately 14 weeks of pregnancy, based on the timeline provided by the study authors. The text was revised as follows:*

"After taking the drug for an additional 45 days (at approximately 14 weeks of pregnancy), she was hospitalized for profuse vaginal bleeding, and no evidence of a fetus was found (Epstein and Rosenblum 1935). The authors suggested that 2,4-DNP caused a premature separation of the placenta, resulting in miscarriage; however, the precise cause of the miscarriage is uncertain."

COMMENT: Would be good to define what this means.

RESPONSE: *No change was made. PND was defined at first use in Section 2.2.*

"Studies regarding death in animals after gavage exposure to 2,4-DNP for intermediate durations were limited, but studies conducted under modern protocols showed that daily gavage doses of 30 mg/kg/day for 18 days were fatal to most (6/10) newborn rats (postnatal day [PND] 4 at exposure initiation), but older rats (5–6 weeks old at exposure initiation) survived for 6 weeks at this dose, succumbing (8/24 died) only at doses of 80 mg/kg/day for up to 28 days (Koizumi et al. 2001, 2002; Takahashi et al. 2009). Earlier studies by Dow Chemical Co. provided contradictory information; in one study, rats of unspecified age survived gavage doses of 30 mg/kg/day 2,4-DNP, 5 days/week for 4 weeks (Dow Chemical Co. 1940), but in another study (Dow Chemical Co. 1950), the LD50 in rats was reported as 30 mg/kg 2,4-DNP; no explanation was given for this apparent contradiction."

COMMENT: This section covers a whole range of different effects and is difficult to read as stands. I would put in sub-divisions and group the topics that you are talking about.

RESPONSE: *Section 2.18 was reorganized and subheadings were added as follows:*

2.18.1 Metabolic Effects from Uncoupling of Oxidative Phosphorylation
2.18.2 Mechanism of Action—Oxidative Phosphorylation Uncoupling
2.18.3 Effects on Hearing

COMMENT: See comment above – for example this would be better before discussing hearing.

RESPONSE: *Section 2.18 was revised to move this discussion ahead of the hearing section (see Response above).*

COMMENT: Put in restlessness. This is not true confusion or agitation and clinically is often the first warning sign that significant toxicity may occur

RESPONSE: *Section 2.18.2 was revised as follows:*

"Symptoms reported in humans who poisoned with 2,4-DNP exposure are similar to those seen in heat stroke, including headache; nausea and vomiting; muscle pain or weakness; and behavioral changes such as restlessness, confusion, agitation, and delirium."

COMMENT: Duplicate of sentence above the paragraph above.

RESPONSE: *Redundant line was deleted from Section 2.20.*

COMMENT: 1. Dermal? 2. What about any info from case reports and/or deaths in relation to the onset of symptoms which is likely to indicate something about the absorption?

RESPONSE: *Section 3.1.1 was revised as follows:*

"Qualitative evidence for absorption of DNPs after inhalation and/or dermal exposure is provided by reports of health effects in workers exposed via inhalation of vapor and dust; however, the exposures may have included uptake by the dermal route and possibly the oral route. A metabolite of 2,4-DNP, 2-amino-4-nitrophenol, was commonly detected in the urine of workmen (predominantly male) exposed to 2,4-DNP in the munitions industry in France (Perkins 1919). Results of autopsies performed on workers who died indicated the presence of 2,4-DNP and its metabolites in blood, unspecified organs, and urine, but quantitative data were not provided (Perkins 1919). In a case of fatal occupational poisoning from exposure to mists and airborne dust of 2,4-DNP in the U.S. chemical industry, the urine contained 2.08 g/L of 2,4-DNP and 50 mg/L of 2-amino-4 nitrophenol (Gisclard and Woodward 1946). Workroom air levels of 2,4-DNP, determined subsequent to the death, were "normally" ≥40 mg/m^3. More recent reports (Jiang et al. 2011; Lu et al. 2011) of occupational poisoning with 2,4-DNP provide additional support for absorption via dermal and inhalation routes. Two of these victims were exposed by recycling nylon bags that had contained 2,4-DNP, while wearing only facial masks and no protective covering on the skin (Jiang et al. 2011).

The data regarding absorption in humans after oral exposure are limited. Case reports of poisoning have documented symptoms of toxicity as early as 4–9 hours, and death as soon as 10–15 hours, after a single oral exposure (Holborow et al. 2016; Hsiao et al. 2005; Siegmueller and Narasimhaiah 2010), suggesting rapid oral absorption."

COMMENT: Some of the next section is a combination of distribution, elimination and metabolism and so is slightly confusing as not laid out in a true pharmacokinetic way

RESPONSE: *Section 3.1.2 was revised and reorganized for clarity and to focus on distribution.*

"No reliable information on the distribution of 2,4-DNP in humans after inhalation or dermal exposure was identified in the literature. 2,4-DNP and its metabolites were reportedly detected in the blood and organs of workmen who died from exposure to 2,4-DNP in the munitions industry in France (Perkins 1919); however, the organs, concentrations, and details of extraction and analytical methods were not reported. Analysis of unspecified organs from two workmen who died following exposure to 2,4-DNP in the United States did not demonstrate the presence of the chemical or its metabolites, despite the fact that 2,4-DNP and its metabolite were detected in the urine of one worker (Gisclard and Woodward 1946).

Limited information is available regarding distribution in animals after oral exposure to 2,4-DNP. In mice given a gavage dose of 22.5 mg/kg of 2,4-DNP, concentrations of 2,4-DNP were much lower in liver and kidney than in serum (Robert and Hagardom 1983), despite similar half-times for absorption (t1/2=0.50–0.62 hours) in all three tissues. Elimination of 2,4-DNP from kidney was very slow compared with liver and serum (see Section 3.1.4). The authors suggested that the apparent persistence of 2,4-DNP in the kidney could be related to tissue binding of the compound.

The time course of plasma concentrations of 2,4-DNP following oral administration to dogs (one per dose) at 5, 12.5, or 25 mg/kg gave no evidence of a trend towards higher plasma levels with continued daily dosing (Kaiser 1964). Hence, 2,4-DNP did not appear to accumulate.

In coordination with toxicity studies (Gehring and Buerge 1969a) (see Section 2.12, Ocular Effects), a study was performed to determine whether susceptibility to 2,4-DNP cataractogenesis could be related to the concentrations of 2,4-DNP in the compartments of the eye (aqueous humor, vitreous humor, lens) after intraperitoneal injection (Gehring and Buerge 1969b). The concentration of 2,4-DNP in the ocular compartments appeared to be more important than the elimination rates (see Section 3.1.4) in determining susceptibility to developing cataracts. Although initial concentrations of 2,4-DNP in the serum of all three animal models were similar, initial concentrations of 2,4-DNP in the compartments of the eye were higher in the more susceptible immature rabbits (~10 µg/g in all compartments) and ducklings (~3, 10, and 10 µg/g in lens, aqueous humor, and vitreous humor, respectively) than in the less susceptible mature rabbits (~1, 4, and 3 µg/g, respectively).

Additional experiments, including in vitro investigations and pharmacokinetic analysis, indicated that some of the 2,4-DNP in serum was bound to protein and some was free; the fraction of free DNP was similar among the animals tested (mature and immature rabbits, ducklings) (Gehring and Buerge 1969b). The concentration of DNP in the aqueous humor was related to, but lower than, the concentration of free 2,4-DNP in the serum; hence, there appeared to be a blood-aqueous humor barrier preventing free diffusion. This barrier appeared to be most effective in the mature rabbit and least effective in the duckling."

COMMENT: Put in where this occurs in the body.

RESPONSE: *Section 3.1.3 was revised as follows:*

"In both humans and animals, available data indicate that 2,4-DNP is metabolized by gut microflora by sequential nitro group reduction via the enzyme nitroreductase to form 2-amino-4-nitrophenol and 4-amino-2-nitrophenol and 2,4-diaminophenol."

COMMENT: 1. Percentage eliminated unchanged? 2. What about extracorporeal elimination – is this possible / beneficial.

RESPONSE: *No change was made. Quantitative information on the percentage of 2,4-DNP eliminated unchanged was not located. Extracorporeal elimination was not discussed, as this pertains to methods to reduce exposure, and ATSDR no longer includes this information in Toxicological Profiles.*

COMMENT: I found this section confusing – could it be better with sub-sections?

RESPONSE: *Some text in Section 3.2 was moved to improve organization. In addition, the following subsections were added:*

3.2.1 Increased Susceptibility due to Age

3.2.2 Pre-existing Conditions that Increase Susceptibility

3.2.3 Factors Increasing Susceptibility to Cataracts

COMMENT): Put at start of section. Also not just a marker of poisoning, as may be seen in just exposure without poisoning

RESPONSE: *Section 3.3.1 was revised to introduce the Biomarkers of Exposure section as follows:*

"Yellow staining of the skin or sclera has occurred in humans exposed to 2,4-DNP, and may be an initial indicator of exposure and/or or poisoning."

COMMENT: Make clearer that no data from humans. Anything from the case reports about potential interactions?

RESPONSE: *Text was revised as follows to clarify that there are no other data (including from case reports) on potential interactions from human studies.*

"It was suggested in an early study that alcoholics are more susceptible to the toxicity of 2,4-DNP during occupational exposure (Perkins 1919); no other human data on potential interactions between 2,4-DNP and other chemicals were located."

COMMENT: What does white mean – no NPL sites or no data available?

RESPONSE: *No change was made. States without pattern in Figure 5-1 are those for which there were no NPL sites with reported 2,4-DNP contamination as indicated by the title.*

COMMENT: Not done or not detected?

RESPONSE: *Section 5.1 was revised as follows:*

"Other than in workplace air, information on DNPs in ambient air in the United States was not located; however, DNPs have been detected in air in other countries (see Section 5.5.1)."

COMMENT: Any data from outside the US to support this paragraph?

RESPONSE: *Section 5.1 was revised to refer the reader to Section 5.5.1, which reports air detections of DNPs in Japan and several European locations.*

COMMENT: This is very hypothetical and could be seen as controversial to those living near these sites if nothing to support.

RESPONSE: *Section 5.1 was revised as follows:*

"Within the overall population, occupational workers at facilities producing or using DNPs, law enforcement officials who seize illegally-obtained 2,4-DNP, mail or shipping employees who handle unregulated shipments of 2,4-DNP, healthcare workers who treat or decontaminate exposed individuals, and people residing with or otherwise encountering persons who purchase 2,4-DNP via unregulated internet sources are likely to be exposed to higher concentrations of DNPs than the rest of the general population. It is possible that people who live near hazardous waste sites that contain these pollutants may also have higher exposures; however, the extent of such exposures for residents around waste sites has not been documented."

COMMENT: When is this data from?

RESPONSE: *Section 5.2.1 was revised as follows:*
"Table 5-1 reports data from 2016 on the number of facilities in each state that manufacture and process 2,4-DNP and the range of maximum amounts of 2,4-DNP stored on-site."

COMMENT: No export then?

RESPONSE: *Section 5.2.2 was revised as follows:*
"During 1985, 102,000 pounds of 2,4-DNP were imported into the United States (HSDB 1994); no information on export of 2,4-DNP from the United States was located, nor were more recent import data."

COMMENT: What does UI mean?

RESPONSE: *No change was made. UI is defined in the footnotes to Table 5-2, as shown below.*
"UI = underground injection"

COMMENT: Need to put in here about:
- Law enforcement and others who may handle seizures
- Healthcare workers who treat and/or decontaminate people who are exposure
- Shipping risk from unregulated internet suppliers and those who work in mail / shipping companies

RESPONSE: *Section 5.7 was revised as follows:*
"Within the general population, workers in facilities using DNPs have potentially high exposures to DNPs. Other persons who may experience high exposures include law enforcement officials who seize illegal supplies of 2,4-DNP, and workers in mail or shipping facilities who handle shipments from unregulated internet suppliers. In addition, healthcare workers who treat or decontaminate exposed persons may be inadvertently exposed. Finally, persons who reside with, or otherwise come into regular contact with, persons who work with DNPs or obtain 2,4-DNP illegally may have potentially high exposures."

COMMENT: Is there any evidence of this as this could be used by those living near these areas?

RESPONSE: *Section 5.7 was revised as follows:*
"It is possible that people who live near hazardous waste sites that contain these pollutants may also have higher exposures from inhaling contaminated air or ingesting contaminated groundwater; however, the extent of such exposures for residents around waste sites has not been documented."

COMMENT: Redefine what this means and other abbreviations in this section when first used.

RESPONSE: *No change was made. The acronym MRL is defined in the beginning of the document in accordance with ATSDR guidance.*

COMMENT: I would define what this means when first used to save reader having to find elsewhere.

RESPONSE: *No change was made. The acronym MRL is defined in the beginning of the document.*

ATSDR Charge Questions and Responses (Peer Reviewer 2)

Chapter 1. Relevance to Public Health

QUESTION: Do you agree with those effects known to occur in humans as reported in the text?

COMMENT: Yes I agree with the effects that are discussed. In my comments on the manuscript, I think that inclusion and discussion of restlessness and fidgeting should be more apparent as this is often an early clinical sign that things may deteriorate

RESPONSE: *Revisions to the text were made throughout the document as suggested by the Reviewer to emphasize restlessness as an early clinical sign.*

QUESTION: Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

COMMENT: The majority of the effects described in animals are comparable to those in humans; of note weight gain has not been described in humans

RESPONSE: *No response necessary.*

QUESTION: Have exposure conditions been adequately described? If you disagree, please explain.

COMMENT: I think it would be worth including more about the internet availability of this product and that often information is provided to potential users about how to dose this product and also combination with other stimulants, steroids, growth hormone in those who are body builders and using this for increase muscle definition alongside weight loss. In addition, there is need to discuss exposure to those handling seized / transported DNP (not only health risks but also explosive risks of this compound) and those who may have to treat / decontaminate people who are unwell.

RESPONSE: *The text was revised to add the suggested information in the first paragraph of Section 1.1, as follows:*

> *"In the 1930s, 2,4-DNP was prescribed by physicians as a weight-reducing agent, but its use was discontinued due to health risks. In recent years, however, 2,4-DNP in tablet and powder form has been marketed for weight loss and body building by unregulated internet sources, leading to a number of human fatalities. These unregulated sources often provide information to potential users regarding dosing and how to combine with other stimulants, steroids, and growth hormone for body building purposes, without informing users of the risk of death. As a result of the growth in availability of 2,4-DNP to the general public, there is increased potential for exposure and health effects among police officers involved in seizure of material or arrest of users, mail and shipping company employees who handle shipments, health care providers who treat or decontaminate users, and family members who live with persons who purchase and/or use 2,4-DNP. In addition to the toxicity hazards associated with 2,4-DNP, this compound is explosive when dry and when heated or subjected to flame, shock, or friction (WHO 2015)."*

QUESTION: Do you believe the derived intermediate oral MRL value is justifiable? If you disagree, please explain (see also Appendix A).

COMMENT: Based on the data presented here this appears to be justified.

RESPONSE: *No response necessary.*

QUESTION: Do you agree that the data do not support derivation of acute, intermediate, and chronic inhalation MRLs?

COMMENT: I agree that there is probably insufficient data on inhalation related MRLs. There was no comment about dermal exposure and MRLs.

RESPONSE: *No change was made. ATSDR does not derive dermal MRLs.*

Chapter 2. Health Effects

QUESTION: Do the health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature for DNPs?

COMMENT: Yes I think that these do discuss the health related effects of DNP exposure.

RESPONSE: *No response necessary.*

QUESTION: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? Were the major study limitations sufficiently described in the text without going into lengthy discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT: The information provided is appropriate – I have made specific comments in the attached commented on version about specific areas where additional information may be useful.

RESPONSE: *See responses to specific comments in the Annotated Comments section.*

QUESTION: Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.

COMMENT: The information provided is appropriate – I have made specific comments in the attached commented on version about specific areas where additional information may be useful.

RESPONSE: *See responses to specific comments in the Annotated Comments section.*

QUESTION: Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?

COMMENT: I am not sure whether any other animal species would be appropriate, but the information summarized is that which has been previously published. Given the clear understanding of the acute and chronic toxicity of these compounds, I am not sure that it would be ethical to suggest additional animal studies would be required.

RESPONSE: *No response necessary.*

QUESTION: Are you aware of any studies that are not included in the profile that may be important in evaluating the toxicity of DNPs? Please provide a copy of each study and indicate where in the text each study should be included.

COMMENT: No.

RESPONSE: *No response necessary.*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the DNP isomers?

COMMENT: No.

RESPONSE: *No response necessary.*

QUESTION: Were all appropriate NOAELs and/or LOAELs identified for each study (both in the text and the Levels of Significant Exposure (LSE) tables and figures)? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

COMMENT: Yes.

RESPONSE: *No response necessary.*

QUESTION: Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables?

COMMENT: Yes.

RESPONSE: *No response necessary.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain.

COMMENT: I think that everything has been explained well, particularly around the effects that are not related to uncoupling of oxidative phosphorylation (e.g. why cataracts develop).

RESPONSE: *No response necessary.*

QUESTION: The updated DNPs profile includes an unpublished study by Eli Lilly and Co. unavailable to ATSDR when preparing the original profile. Please comment on the quality of the study, namely:
Did the study use an adequate number of animals and practice good animal care?

COMMENT: From the information in the additional paper it is not possible for me to comment on the quality of the animal care. I think that there were appropriate numbers of animals as they appear to be similar to other animal studies in the ATSDR document

RESPONSE: *No response necessary.*

QUESTION: The updated DNPs profile includes an unpublished study by Eli Lilly and Co. unavailable to ATSDR when preparing the original profile. Please comment on the quality of the study, namely:
Did the study account for competing causes of death?

COMMENT: There is no information on the exclusion of other causes, although the animals were treated with only DNP and therefore this would be the most likely cause of death. However it is possible that the way that the animals were housed and/or cared for may have contributed to the deaths

RESPONSE: *No response necessary.*

QUESTION: The updated DNPs profile includes an unpublished study by Eli Lilly and Co. unavailable to ATSDR when preparing the original profile. Please comment on the quality of the study, namely:
Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: There appeared to sufficient animals in each group. I am unclear why the doses of 10, 36.5, 140 and 500mg/Kg were chosen. There was a large gap between 140 and 500mg/Kg and given that the animals with 140mg/Kg did not die whereas all of those given 500mg/Kg this means that it is not clear where the threshold for fatalities occurs as it is likely to be somewhere between 140 and 500mg/Kg.

RESPONSE: *No response necessary.*

QUESTION: The updated DNPs profile includes an unpublished study by Eli Lilly and Co. unavailable to ATSDR when preparing the original profile. Please comment on the quality of the study, namely:
If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: There is very limited information as this is not a full published report, as commented on above and below, the spacing of doses is not clear and this may have impacted on the results of the study and in particular the median lethal dose.

RESPONSE: *No response necessary.*

QUESTION: The updated DNPs profile includes an unpublished study by Eli Lilly and Co. unavailable to ATSDR when preparing the original profile. Please comment on the quality of the study, namely:

Do you agree with the conclusions of the author? If not, please explain.

COMMENT: The authors report a median lethal dose of 340mg/Kg which I am unclear about since the doses used could have been better spaced and there was a large gap between 140 and 500mg/Kg. This median dose is based on all those with lower doses surviving compared to none of those with higher doses, but actually if more doses were used and/or better spacing of doses was used then the median dose may be very different.

RESPONSE: *No response necessary.*

Chapter 7. Regulations and Guidelines

QUESTION: Are you aware of any additional regulations or guidelines that we should add? Please provide citations.

COMMENT: No.

RESPONSE: *No response necessary.*

QUESTION: Are there any that should be removed? Please explain.

COMMENT: No.

RESPONSE: *No response necessary.*

Appendix A. ATSDR Minimal Risk Level Worksheets

QUESTION: Acute-duration oral MRL: The updated data evaluation includes a number of fatal human case studies involving lower exposure levels than were documented in the original profile (within an order of magnitude of the point of departure used for the original MRL).

Do you agree that these human fatality data adequately support ATSDR's decision to remove the original acute oral MRL? In not, please explain.

COMMENT: The issue is that not having anything from an MRL perspective is not necessarily helpful for a clinician (or a member of the public) who reads this document. Whilst there is limited data and there is the issue of how accurate the weights are, at least including something puts into context the information from the case reports about how potentially toxic this compound is. Additionally the values quoted for intermediate and chronic toxicity are well below those reported by users – could we include those lower values as this would confer significant safety?

RESPONSE: *No change was made. ATSDR endeavored to address the hazard associated with acute exposure in its Rationale for Not Deriving an Acute MRL, by documenting the low doses at which fatalities occurred.*

QUESTION: Acute-duration oral MRL: The updated data evaluation includes a number of fatal human case studies involving lower exposure levels than were documented in the original profile (within an order of magnitude of the point of departure used for the original MRL).

Please comment on any aspect of our MRL database assessment that you would like us to address.

COMMENT: No.

RESPONSE: *No response necessary.*

QUESTION: Intermediate-duration oral MRL: ATSDR derived a new intermediate-duration oral MRL of 0.00007 mg/kg/day was derived for 2,4-DNP based on decreased body weight in mice exposed to 0.07 mg/kg/day 2,4-DNP in drinking water for 50 weeks (Caldeira da Silva et al. 2008). The MRL is based on a LOAEL of 0.07 mg/kg/day and a total uncertainty factor of 1,000 (10 for interspecies extrapolation, 10 for human variability, and 10 for use of a LOAEL). An intermediate-duration oral MRL was not derived in the 1995 toxicological profile due to the lack of intermediate duration studies involving doses lower than those known to cause death in humans.

Do you agree or disagree with the proposed intermediate-duration oral MRL value? Explain. If you disagree, please specify the MRL value that you propose.

COMMENT: The issue is that uncertainty factors makes the intermediate duration exposure amounts VERY low. For example for a 100mg male that would be 0.007mg/day. How does this equate to potential environmental exposure? Also the determination of 0.07mg/Kg/Day prior to application of the uncertainty factor appears to be derived from one study reporting impact on weight in a mouse model.

RESPONSE: *No change was made. MRLs are health-based numbers and environmental exposure levels were not considered in the derivation of MRLs. While the MRL is derived based on a single study, the selected study is of high quality and identifies an effect seen also in humans and in other laboratory animals. No other suitable animal studies were identified.*

QUESTION: Intermediate-duration oral MRL: ATSDR derived a new intermediate-duration oral MRL of 0.00007 mg/kg/day was derived for 2,4-DNP based on decreased body weight in mice exposed to 0.07 mg/kg/day 2,4-DNP in drinking water for 50 weeks (Caldeira da Silva et al. 2008). The MRL is based on a LOAEL of 0.07 mg/kg/day and a total uncertainty factor of 1,000 (10 for interspecies extrapolation, 10 for human variability, and 10 for use of a LOAEL). An intermediate-duration oral MRL was not derived in the 1995 toxicological profile due to the lack of intermediate duration studies involving doses lower than those known to cause death in humans.

Do you agree/disagree with each component of the total uncertainty factor? Explain. If you disagree, please specify the uncertainty factor(s) that you propose.

COMMENT: I am not an expert in this area and I do not know whether these are reasonable factors to include.

RESPONSE: *No response necessary.*

QUESTION: Intermediate-duration oral MRL: ATSDR derived a new intermediate-duration oral MRL of 0.00007 mg/kg/day was derived for 2,4-DNP based on decreased body weight in mice exposed to 0.07 mg/kg/day 2,4-DNP in drinking water for 50 weeks (Caldeira da Silva et al. 2008). The MRL is based on a LOAEL of 0.07 mg/kg/day and a total uncertainty factor of 1,000 (10 for interspecies extrapolation, 10 for human variability, and 10 for use of a LOAEL). An intermediate-duration oral MRL was not derived in the 1995 toxicological profile due to the lack of intermediate duration studies involving doses lower than those known to cause death in humans.

Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT: No.

RESPONSE: *No response necessary.*

QUESTION: Chronic-duration oral MRL: ATSDR believes the chronic-duration oral exposure database does not provide sufficient data for derivation of a chronic oral MRL. Specifically, the lowest LOAELs for effects of chronic exposure are higher than doses known to cause fatalities in humans. However, we believe the intermediate-duration oral MRL is protective for chronic exposures.

Do you agree with ATSDR that the intermediate oral MRL of 0.00007 mg/kg/day would be sufficiently protective for chronic exposures? If not, please explain.

COMMENT: In Appendix A it states there is insufficient data for derivation. Is this therefore based on the extrapolated of intermediate to chronic exposures. If so given how low this is value is, it is therefore likely to be protective but needs to be appropriately caveated in that this is extrapolation of data interpretation from other scenarios.

RESPONSE: *No change was made. ATSDR did not to derive a chronic-duration MRL because it is the policy of the Agency not to extrapolate from intermediate- to chronic-duration effects. Instead, Appendix A describes the reasons why ATSDR expects that the intermediate-duration MRL is protective for chronic-duration exposures.*

Appendix B. Literature Search Framework

QUESTION: Does Appendix B provide a sufficiently clear documentation of ATSDR's health effects literature search strategy and inclusion/exclusion criteria?

COMMENT: Yes.

RESPONSE: *No response necessary.*

QUESTION: Does it provide enough transparency regarding ATSDR's implementation of its inclusion and exclusion criteria (e.g. how ATSDR chose the studies it included in the health effects chapter)?

COMMENT: Yes.

RESPONSE: *No response necessary.*

Overall Usability of the Profile

QUESTION: Does the new chapter organization make it easy for you to find the information you need? For example, are you satisfied with the organization of the health effects chapter by organ system rather than exposure route?

COMMENT: Overall it is useful in the way that it is laid out – I agree that discussing systems affected is better for the reader than doing by route of exposure, given that the overall effects of each route of exposure is largely similar across the routes so would lead to lots of repetitive text. I have annotated in the commented on manuscript where I think that the use of sub-headings may be useful and may help to clarify the reading of those sections.

RESPONSE: *No response necessary.*

QUESTION: Does the profile contain all of the information you need? Is there information you would like to see that is not currently included?

COMMENT: As a clinician there is no reference to treatment which would be useful. Also in the pharmacokinetics section, I have mentioned about extracorporeal removal, but it would be useful to also include something about reducing absorption (e.g. is activated charcoal beneficial).

RESPONSE: *No change was made. ATSDR no longer includes information on methods to reduce exposure (e.g., activated charcoal) or effects in Toxicological Profiles. Appendix D provides resources for clinicians.*

QUESTION: Having read this Toxicological Profile (and others, if applicable), which chapter(s) or content do you find most valuable and why? If you have previously used any Toxicological Profile(s) for your work, which chapter(s) or content have you used the most and for what purpose(s)?

COMMENT: The area that is of most interest to me is the discussion of the acute and chronic health effects and the information that is summarized from the available published literature without me having to undertake my own literature searching. Whilst this is useful to know what the risks are, there is nothing to help me know what I need to do to treat someone if they develop those clinical features. In addition, the rapidity of deterioration once someone becomes unwell is not clear in the reading of this, whereas this is my own clinical experience in managing these patients

RESPONSE: *The text has been revised in several places to emphasize the speed with which clinical signs develop, as suggested by the Reviewer. ATSDR no longer includes information on methods to reduce exposure or effects in Toxicological Profiles. Appendix D provides resources for clinicians.*

QUESTION: Are the new tables and figures clear and useful? Do they make the Toxicological Profile easier to read?

COMMENT: I liked the figures and tables and they helped to summarise the data. However I have commented that the basis for ordering of cases etc in the tables is not clear and may suggest that the first ones discussed are of more significance rather than if they were in a clear ordering. Also some of the figures and tables are not referred to in the text and so appear as standalone and unrelated to the main text.

RESPONSE: *The table of cases was reordered by duration and alphabetical by author. The figure and table call-outs were provided in the boilerplate text for the section.*

Comments provided by Peer Reviewer #3

Annotated Comments on the Profile

COMMENT: Delete "High energy phosphate bonds", replace with "chemical potential."

RESPONSE: *Section 1.2 was revised as suggested.*

"Mechanistic data indicate that DNP effects are related to the uncoupling of mitochondrial electron transport from oxidative phosphorylation, which results in the release of energy as heat, rather than storage in the chemical potential of adenosine triphosphate (ATP) (see Section 2.18.1). The uncoupling of oxidative phosphorylation has the potential to affect all tissues and organs. Exposure of humans to 2,4-DNP results in increased basal metabolic rate, increased perspiration, weight loss, and, at higher doses, increased heart and respiratory rates and hyperthermia. These effects occur rapidly (over several hours), and may present a significant risk of death. Stopping exposure to 2,4-DNP often leads to a complete recovery. Very limited data on the other DNP isomers indicate that 2,6-, 3,4-, and 3,5-DNP may have equivalent potential for increasing basal metabolic rate than 2,4-DNP, while 2,3- and 2,5-DNP appear to have lower potential.

Health endpoints that may not be related to increases in body temperature and basal metabolic rate are discussed below."

COMMENT: Change "which is normally stored in high energy phosphate bonds in ATP" to "which is normally stored as the chemical energy of ATP"

RESPONSE: *Section 2.2 was revised as suggested.*

"Mechanistic data indicate that DNP effects are related to the uncoupling of mitochondrial electron transport from oxidative phosphorylation, which results in the release of energy as heat, rather than storage in the chemical potential of adenosine triphosphate (ATP) (see Section 2.18.1). The uncoupling of oxidative phosphorylation has the potential to affect all tissues and organs. Exposure of humans to 2,4-DNP results in increased basal metabolic rate, increased perspiration, weight loss, and, at higher doses, increased heart and respiratory rates and hyperthermia. These effects occur rapidly (over several hours), and may present a significant risk of death. Stopping exposure to 2,4-DNP often leads to a complete recovery. Very limited data on the other DNP isomers indicate that 2,6-, 3,4-, and 3,5-DNP may have equivalent potential for increasing basal metabolic rate than 2,4-DNP, while 2,3- and 2,5-DNP appear to have lower potential.

Health endpoints that may not be related to increases in body temperature and basal metabolic rate are discussed below."

COMMENT: Change "diffusing across the inner mitochondrial membrane and deprotonating, thereby dissipating…" to "diffusing across the inner mitochondrial membrane, deprotonating, and returning to pick up more protons, thereby dissipating…".

RESPONSE: *Section 2.18.2 was revised as suggested.*

"The ability of 2,4-DNP to uncouple oxidative phosphorylation underpins many of the clinical observations and physiological effects of its toxicity in both humans and animals. Such effects include elevated basal metabolic rate or oxygen consumption, elevated respiration and pulse rates, increased perspiration, and increased body temperature, and are related to the uncoupling of oxidative phosphorylation. In vitro demonstrations of 2,4-DNP's ability to uncouple oxidative phosphorylation began as early as 1948 (Loomis and Lipmann 1948; see also Ilivicky and Casida 1969; Muscatello et al. 1975; Pinchot 1967; Weinbach and Garbus 1969). During the Krebs cycle, 2,4-DNP and other lipophilic weak acids uncouple oxidative phosphorylation from electron transport by picking up protons, diffusing across the inner mitochondrial membrane, deprotonating, and returning to pick up more protons, thereby dissipating the pH gradient and membrane electrochemical potential needed for the formation of ATP (Lou et al. 2007; Stryer 1988). During this uncoupling, electron transport from NADH to oxygen can increase several-fold, but the energy produced, which is normally stored as the chemical potential of ATP, is released as heat. The prevention of ATP formation by 2,4-DNP means that all energy-dependent biochemical processes are likely to be affected. In addition, the depletion of ATP associated with mitochondrial uncoupling may produce hyperkalemia. 2,4-DNP has been reported to induce potassium accumulation in rabbit kidney slices (Mudge 1951), and hyperkalemia has been observed in humans poisoned with 2,4-DNP (Jiang et al. 2011).

The body attempts to dissipate the increased heat by inducing dilation of blood vessels. When heat production exceeds the organism's capacity to dissipate heat, fatal hyperthermia may result (Murphy 1986). Symptoms reported in humans who poisoned with 2,4-DNP exposure are similar to those seen in heat stroke, including headache; nausea and vomiting; muscle pain or weakness; and behavioral changes such as restlessness, confusion, agitation, and delirium. Hyperkalemia in turn may produce muscle pain and weakness often reported by humans after exposure to 2,4-DNP. With both 2,4-DNP exposure and hyperthermia, these symptoms may progress to seizures and coma, and may be accompanied by rhabdomyolysis and acute renal failure and other signs of multiorgan failure; the cause of death is typically cardiac arrest (see Section 2.2 for effects of 2,4-DNP, and Power et al. [2014] and Trujillo and Fragachán [2011] for hyperthermia). Autopsy findings after fatal 2,4-DNP poisoning often show widespread tissue hyperemia, congestion, and hemorrhage resulting from vasodilation (see Section 2.2).

Both endogenous and exogenous chemicals that uncouple oxidative phosphorylation, including 2,4-DNP, have recently been explored as potential therapies for prevention or mitigation of obesity, Type II diabetes, and aging (reviewed by Divakaruni and Brand 2011). Uncoupling of oxidative phosphorylation has been shown to induce weight loss and improve glucose homeostasis, effects also seen with exposure to 2,4-DNP (Caldeira da Silva et al. 2008; Goldgof et al. 2014; see also Sections 2.3 and 2.13). Furthermore, because superoxide anion is a byproduct of oxidative phosphorylation as well as a potent cellular oxidant, uncoupling has been shown to reduce oxidative stress, thought to be an important mechanism of aging (reviewed by Divakaruni and Brand 2011). Indeed, several measures of oxidative stress were decreased, and survival was prolonged, in mice exposed to low levels of 2,4-DNP for their natural lifespan (Caldeira da Silva et al. 2008). However, the narrow margin of safety between a 2,4-DNP dose that is beneficial and that which is toxic, even lethal, limits the potential therapeutic uses of 2,4-DNP (Divakaruni and Brand 2011; Lou et al. 2007).

Little information is available on the uncoupling potency of other DNP isomers. In experiments designed to facilitate development of a quantitative structure-activity relationship (QSAR) for uncoupling activity of substituted phenols, Escher et al. (1999) showed that 3,4-DNP uncoupled oxidative phosphorylation in Rhodobacter membrane vesicles, with higher rates of uncoupling observed at lower pH (tested from pH 5.3 to 8.25). In isolated rat liver mitochondria, the

effective concentrations for uncoupling by the DNPs were 20, 30, 40, 40, 100, and 100 µM for 3,5-, 2,4-, 2,6-, 3,4-, 2,3-, and 2,5-DNP (Burke and Whitehouse 1967). This order is not entirely congruent with the acute lethality of the isomers in animals exposed via intraperitoneal injection (LD50 values were 45, 35, 38, 98, 190, and 150 mg/kg in rats for the same order of DNPs; Harvey 1959), but does provide support for the lower relative potency of 2,3- and 2,5-DNP compared with the others. The lower potency of 2,3- and 2,5-DNP is also supported by the observation that mouse intraperitoneal LD50 values for these isomers did not change with increasing temperature (Harvey 1959), suggesting lower potential for inducing chemical hyperthermia.

Metabolic effects of 2,4-DNP also appear to influence intracellular calcium levels. Hudman et al. (2002) investigated the basis for DNP-induced increase in cytoplasmic calcium in rat cardiac myocytes. Their results indicated that the increase in cytoplasmic calcium occurs in two phases. The first phase appears to result from the release of mitochondrial calcium due to mitochondrial depolarization. The second phase appears to be the result of a progressive release of calcium from the sarcoplasmic reticulum following depletion of intracellular ATP.

In addition, 2,4-DNP-induced toxicity may involve activation of ATP-sensitive K+ channels (Ravesloot and Rombouts 2000). Wu et al. (2000) reported increased ATP-sensitive K+ channel activity in pituitary GH3 cells treated with 2,4-DNP.

The DNP metabolites for which toxicity information is available appear to have much lower systemic toxicity than 2,4-DNP. This is most likely due to the fact that they are much less potent in uncoupling oxidative phosphorylation than 2,4-DNP. However, while 2,4-DNP is metabolized to compounds with lower systemic toxicity, the aminonitrophenols produced are mutagenic in test systems and show some evidence of carcinogenicity in chronic-duration tests in rats and mice. These data are difficult to reconcile with the generally negative results obtained with 2,4-DNP with S9 activation since the S9 fraction contains both microsomes and the soluble enzymes that metabolize 2,4-DNP to the aminonitrophenols (Eiseman et al. 1972). The generally negative results for genotoxicity of 2,4-DNP in test systems where metabolic activation was present may be related to the dependence of 2,4-DNP reduction on ATP. 2,4-DNP metabolism requires ATP; unless the S9 fraction contains an ATP-regenerating system, 2,4-DNP may not be metabolized."

COMMENT: Change "proceeds normally" to "can increase several-fold"

RESPONSE: *Section 2.18.2 was revised as suggested.*

"The ability of 2,4-DNP to uncouple oxidative phosphorylation underpins many of the clinical observations and physiological effects of its toxicity in both humans and animals. Such effects include elevated basal metabolic rate or oxygen consumption, elevated respiration and pulse rates, increased perspiration, and increased body temperature, and are related to the uncoupling of oxidative phosphorylation. In vitro demonstrations of 2,4-DNP's ability to uncouple oxidative phosphorylation began as early as 1948 (Loomis and Lipmann 1948; see also Ilivicky and Casida 1969; Muscatello et al. 1975; Pinchot 1967; Weinbach and Garbus 1969). During the Krebs cycle, 2,4-DNP and other lipophilic weak acids uncouple oxidative phosphorylation from electron transport by picking up protons, diffusing across the inner mitochondrial membrane, deprotonating, and returning to pick up more protons, thereby dissipating the pH gradient and membrane electrochemical potential needed for the formation of ATP (Lou et al. 2007; Stryer 1988). During this uncoupling, electron transport from NADH to oxygen can increase several-fold, but the energy produced, which is normally stored as the chemical potential of ATP, is released as heat. The prevention of ATP formation by 2,4-DNP means that all energy-dependent

biochemical processes are likely to be affected. In addition, the depletion of ATP associated with mitochondrial uncoupling may produce hyperkalemia. 2,4-DNP has been reported to induce potassium accumulation in rabbit kidney slices (Mudge 1951), and hyperkalemia has been observed in humans poisoned with 2,4-DNP (Jiang et al. 2011).

The body attempts to dissipate the increased heat by inducing dilation of blood vessels. When heat production exceeds the organism's capacity to dissipate heat, fatal hyperthermia may result (Murphy 1986). Symptoms reported in humans who poisoned with 2,4-DNP exposure are similar to those seen in heat stroke, including headache; nausea and vomiting; muscle pain or weakness; and behavioral changes such as restlessness, confusion, agitation, and delirium. Hyperkalemia in turn may produce muscle pain and weakness often reported by humans after exposure to 2,4-DNP. With both 2,4-DNP exposure and hyperthermia, these symptoms may progress to seizures and coma, and may be accompanied by rhabdomyolysis and acute renal failure and other signs of multiorgan failure; the cause of death is typically cardiac arrest (see Section 2.2 for effects of 2,4-DNP, and Power et al. [2014] and Trujillo and Fragachán [2011] for hyperthermia). Autopsy findings after fatal 2,4-DNP poisoning often show widespread tissue hyperemia, congestion, and hemorrhage resulting from vasodilation (see Section 2.2).

Both endogenous and exogenous chemicals that uncouple oxidative phosphorylation, including 2,4-DNP, have recently been explored as potential therapies for prevention or mitigation of obesity, Type II diabetes, and aging (reviewed by Divakaruni and Brand 2011). Uncoupling of oxidative phosphorylation has been shown to induce weight loss and improve glucose homeostasis, effects also seen with exposure to 2,4-DNP (Caldeira da Silva et al. 2008; Goldgof et al. 2014; see also Sections 2.3 and 2.13). Furthermore, because superoxide anion is a byproduct of oxidative phosphorylation as well as a potent cellular oxidant, uncoupling has been shown to reduce oxidative stress, thought to be an important mechanism of aging (reviewed by Divakaruni and Brand 2011). Indeed, several measures of oxidative stress were decreased, and survival was prolonged, in mice exposed to low levels of 2,4-DNP for their natural lifespan (Caldeira da Silva et al. 2008). However, the narrow margin of safety between a 2,4-DNP dose that is beneficial and that which is toxic, even lethal, limits the potential therapeutic uses of 2,4-DNP (Divakaruni and Brand 2011; Lou et al. 2007).

Little information is available on the uncoupling potency of other DNP isomers. In experiments designed to facilitate development of a quantitative structure-activity relationship (QSAR) for uncoupling activity of substituted phenols, Escher et al. (1999) showed that 3,4-DNP uncoupled oxidative phosphorylation in Rhodobacter membrane vesicles, with higher rates of uncoupling observed at lower pH (tested from pH 5.3 to 8.25). In isolated rat liver mitochondria, the effective concentrations for uncoupling by the DNPs were 20, 30, 40, 40, 100, and 100 µM for 3,5-, 2,4-, 2,6-, 3,4-, 2,3-, and 2,5-DNP (Burke and Whitehouse 1967). This order is not entirely congruent with the acute lethality of the isomers in animals exposed via intraperitoneal injection (LD50 values were 45, 35, 38, 98, 190, and 150 mg/kg in rats for the same order of DNPs; Harvey 1959), but does provide support for the lower relative potency of 2,3- and 2,5-DNP compared with the others. The lower potency of 2,3- and 2,5-DNP is also supported by the observation that mouse intraperitoneal LD50 values for these isomers did not change with increasing temperature (Harvey 1959), suggesting lower potential for inducing chemical hyperthermia.

Metabolic effects of 2,4-DNP also appear to influence intracellular calcium levels. Hudman et al. (2002) investigated the basis for DNP-induced increase in cytoplasmic calcium in rat cardiac myocytes. Their results indicated that the increase in cytoplasmic calcium occurs in two phases. The first phase appears to result from the release of mitochondrial calcium due to mitochondrial

depolarization. The second phase appears to be the result of a progressive release of calcium from the sarcoplasmic reticulum following depletion of intracellular ATP.

In addition, 2,4-DNP-induced toxicity may involve activation of ATP-sensitive K+ channels (Ravesloot and Rombouts 2000). Wu et al. (2000) reported increased ATP-sensitive K+ channel activity in pituitary GH3 cells treated with 2,4-DNP.

The DNP metabolites for which toxicity information is available appear to have much lower systemic toxicity than 2,4-DNP. This is most likely due to the fact that they are much less potent in uncoupling oxidative phosphorylation than 2,4-DNP. However, while 2,4-DNP is metabolized to compounds with lower systemic toxicity, the aminonitrophenols produced are mutagenic in test systems and show some evidence of carcinogenicity in chronic-duration tests in rats and mice. These data are difficult to reconcile with the generally negative results obtained with 2,4-DNP with S9 activation since the S9 fraction contains both microsomes and the soluble enzymes that metabolize 2,4-DNP to the aminonitrophenols (Eiseman et al. 1972). The generally negative results for genotoxicity of 2,4-DNP in test systems where metabolic activation was present may be related to the dependence of 2,4-DNP reduction on ATP. 2,4-DNP metabolism requires ATP; unless the S9 fraction contains an ATP-regenerating system, 2,4-DNP may not be metabolized."

COMMENT: Change **"**Which is normally stored in high-energy phosphate bonds in ATP" to "which is normally stored as the chemical potential of ATP"

RESPONSE: *Section 2.18.2 was revised as suggested.*

"The ability of 2,4-DNP to uncouple oxidative phosphorylation underpins many of the clinical observations and physiological effects of its toxicity in both humans and animals. Such effects include elevated basal metabolic rate or oxygen consumption, elevated respiration and pulse rates, increased perspiration, and increased body temperature, and are related to the uncoupling of oxidative phosphorylation. In vitro demonstrations of 2,4-DNP's ability to uncouple oxidative phosphorylation began as early as 1948 (Loomis and Lipmann 1948; see also Ilivicky and Casida 1969; Muscatello et al. 1975; Pinchot 1967; Weinbach and Garbus 1969). During the Krebs cycle, 2,4-DNP and other lipophilic weak acids uncouple oxidative phosphorylation from electron transport by picking up protons, diffusing across the inner mitochondrial membrane, deprotonating, and returning to pick up more protons, thereby dissipating the pH gradient and membrane electrochemical potential needed for the formation of ATP (Lou et al. 2007; Stryer 1988). During this uncoupling, electron transport from NADH to oxygen can increase several-fold, but the energy produced, which is normally stored as the chemical potential of ATP, is released as heat. The prevention of ATP formation by 2,4-DNP means that all energy-dependent biochemical processes are likely to be affected. In addition, the depletion of ATP associated with mitochondrial uncoupling may produce hyperkalemia. 2,4-DNP has been reported to induce potassium accumulation in rabbit kidney slices (Mudge 1951), and hyperkalemia has been observed in humans poisoned with 2,4-DNP (Jiang et al. 2011).

The body attempts to dissipate the increased heat by inducing dilation of blood vessels. When heat production exceeds the organism's capacity to dissipate heat, fatal hyperthermia may result (Murphy 1986). Symptoms reported in humans who poisoned with 2,4-DNP exposure are similar to those seen in heat stroke, including headache; nausea and vomiting; muscle pain or weakness; and behavioral changes such as restlessness, confusion, agitation, and delirium. Hyperkalemia in turn may produce muscle pain and weakness often reported by humans after exposure to 2,4-DNP. With both 2,4-DNP exposure and hyperthermia, these symptoms may progress to seizures and coma, and may be accompanied by rhabdomyolysis and acute renal failure and

other signs of multiorgan failure; the cause of death is typically cardiac arrest (see Section 2.2 for effects of 2,4-DNP, and Power et al. [2014] and Trujillo and Fragachán [2011] for hyperthermia). Autopsy findings after fatal 2,4-DNP poisoning often show widespread tissue hyperemia, congestion, and hemorrhage resulting from vasodilation (see Section 2.2).

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In addition, 2,4-DNP-induced toxicity may involve activation of ATP-sensitive K+ channels (Ravesloot and Rombouts 2000). Wu et al. (2000) reported increased ATP-sensitive K+ channel activity in pituitary GH3 cells treated with 2,4-DNP.

The DNP metabolites for which toxicity information is available appear to have much lower systemic toxicity than 2,4-DNP. This is most likely due to the fact that they are much less potent in uncoupling oxidative phosphorylation than 2,4-DNP. However, while 2,4-DNP is metabolized to compounds with lower systemic toxicity, the aminonitrophenols produced are mutagenic in test systems and show some evidence of carcinogenicity in chronic-duration tests in rats and mice. These data are difficult to reconcile with the generally negative results obtained with 2,4-DNP

with S9 activation since the S9 fraction contains both microsomes and the soluble enzymes that metabolize 2,4-DNP to the aminonitrophenols (Eiseman et al. 1972). The generally negative results for genotoxicity of 2,4-DNP in test systems where metabolic activation was present may be related to the dependence of 2,4-DNP reduction on ATP. 2,4-DNP metabolism requires ATP; unless the S9 fraction contains an ATP-regenerating system, 2,4-DNP may not be metabolized."

COMMENT: Change "decoupling" to "uncoupling".

RESPONSE: *The Worksheet for the chronic oral MRL was revised as suggested.*

*"**MINIMAL RISK LEVEL (MRL) WORKSHEET***

Chemical Name: *2,4-Dinitrophenol*
CAS Numbers: *51-28-5*
Date: *June 2018*
Profile Status: *Fourth draft*
Route: *Oral*
Duration: *Intermediate*
MRL *0.00007 mg/kg/day*
Critical Effect: *Decreased body weight*
Reference: *Caldeira da Silva et al. 2008*
Point of Departure: *LOAEL of 0.07 mg/kg/day*
Uncertainty Factor: *1,000*
LSE Graph Key: *73*
Species: *Mouse*

MRL Summary: A provisional intermediate-duration oral MRL of 0.00007 mg/kg/day was derived for 2,4-DNP based on decreased body weight in mice exposed to 0.07 mg/kg/day 2,4-DNP in drinking water for 50 weeks (Caldeira da Silva et al. 2008). The provisional MRL is based on a LOAEL of 0.07 mg/kg/day and a total uncertainty factor of 1,000 (10 for interspecies extrapolation, 10 for human variability, and 10 for use of a LOAEL).

Selection of the Critical Effect: Human fatalities have been reported after acute- and intermediate-duration oral exposures to 2,4-DNP doses as low as 1–5 mg/kg/day (Dameshek and Gargill 1934; Goldman and Haber 1936; Masserman and Goldsmith 1934; Silver 1934; Zack et al. 2016). The only effects reported at lower doses in humans or animals were decreased body weight (at least 10% lower than controls after 20 weeks of exposure) and decreased serum glucose, triglycerides, and insulin (after 12 weeks of exposure) in a chronic mouse study in which 2,4-DNP was administered at an average dose of 0.07 mg/kg/day in drinking water (Caldeira da Silva et al. 2008). The mice were maintained at a lower ambient temperature (22°C) than normal to enable heat to dissipate; thus, it is not possible to assess whether hyperthermia might also occur at this exposure level. A clinical study in humans provides limited support for the effects of 2,4-DNP on serum glucose and insulin, as decreased glucose tolerance was seen after acute and intermediate exposures to 4 mg/kg/day 2,4-DNP (MacBryde and Taussig 1935).

In contrast, there are abundant data indicating that oral 2,4-DNP exposure results in decreased body weight or body weight gain in humans and animals. Decreases in body weight have been reported in humans exposed to acute- (Anderson 1933; Bortz 1934; Cutter and Tainter 1933; Tainter et al. 1935b), intermediate- (Beinhauer 1934; Boardman 1935; Cutting et al. 1933, 1934; Horner et al. 1935; Looney and Hoskins 1934; Masserman and Goldsmith 1934; Nadler 1935;

Simkin 1937a, 1937b; Tainter et al. 1935; Whalman 1936), and chronic-duration (Horner et al. 1935) exposures of 1–4 mg/kg/day. Likewise, body weight decrements have been reported in intermediate- (Bakke and Lawrence 1965; Goldgof et al. 2014; Koizumi et al. 2002, 2001; Pugsley 1935; Spencer et al. 1948; Tainter and Borley 1938; Takahashi et al. 2009) and chronic-duration (Tainter 1938) studies in rats and mice, albeit at much higher doses (≥20 mg/kg/day). Furthermore, the mechanism for 2,4-DNP-induced decreases in body weight is well established: 2,4-DNP uncouples oxidative phosphorylation, leading to an increase in basal metabolic rate and an increase in carbohydrate consumption as the body endeavors to produce ATP needed for cell functions. In summary, decreased body weight occurs in both humans and animals exposed to 2,4-DNP by a well-studied mode of action, and was selected as the critical effect for provisional MRL derivation.

Selection of the Principal Study: The Caldeira da Silva et al. (2008) study provided the lowest adverse effect level.

Summary of the Principal Study:

Caldeira da Silva CC, Cerqueira FM, Barbosa LF, et al. 2008. Mild mitochondrial uncoupling in mice affects energy metabolism, redox balance and longevity. Aging Cell 7(4):552-560.

Six groups (three pairs of control and exposed groups) of 30 female Swiss mice were exposed to DNP in drinking water at a concentration of 0 or 1 mg/L beginning at 18 weeks of age (Caldeira da Silva et al. 2008). The animals were housed at 22°C to allow heat due to uncoupling to dissipate and to prevent hyperthermia. Food and water intake and body weight were recorded weekly. The authors reported that the animals received doses between 0.03 and 0.105 mg/kg/day (midpoint of the dose range is 0.07 mg/kg/day) based on body weight and water intake. At 22 and 32 weeks of age (after 4 and 14 weeks of exposure), one exposed and one control group each were sacrificed for evaluation of oxygen consumption and oxidative stress in the heart, brain, and liver (oxidative stress was measured as peroxide release, protein carbonyl signal, and oxidative DNA adducts). In the groups sacrificed at 32 weeks of age (14 weeks of exposure), blood was collected for analysis of triglycerides, glucose, and insulin. The third sets of exposed and control groups were observed until natural death; the only endpoints assessed in these animals were food and water intake, body weight, body temperature, and survival.

Serum levels of glucose, triglycerides, and insulin were significantly lower than controls after 14 weeks of DNP treatment. Oxygen consumption was significantly increased in the brain and liver of exposed mice, but not in the heart. In all three organs, oxidative stress was reduced by exposure to DNP. In the groups exposed for their natural lifespans, food and water intake, and body temperature at 75 weeks of age did not differ from controls. Exposed mice exhibited significantly reduced body weight (~8–13% less than controls based on digitization of data shown graphically; see Table A-1) after the first 20 weeks of exposure; the decrease persisted throughout the study. The last body weight measurement reported in the study was ~week 68, reflecting at least 50 weeks of exposure. Mean lifespan was significantly higher in exposed mice than in controls.

Table A-1. Body Weight Changes Over Time in Mice Exposed to 2,4-Dinitrophenol in Drinking Water from 18 Weeks of Age until Natural Death[a]

Mouse age (weeks)	Exposure duration (weeks)	Approximate body weight (g)		Approximate percent change from control
		Control	Exposed	
18	Pretreatment	40	39	Not applicable
28	10	48	45	6%
38	20	51	47[b]	8%
48	30	55	50[b]	9%
58	40	56	51[b]	9%
68	50	55	48[b]	13%[c]

[a]Mice were exposed for ~140 weeks, but the only body weight data presented were measurements through the first 50 weeks of exposure.
[b]Significantly different from control mean, $p<0.05$.
[c]Exceeds ATSDR benchmark for adverse change in body weight (10% change from control mean).

Source: Caldeira da Silva et al. (2008). Data digitized from Figure 1 using Grab It!™.

Selection of the Point of Departure for the MRL: The LOAEL of 0.07 mg/kg/day (midpoint of the dose range reported by the authors) for decreased body weight was selected as the POD for deriving a provisional MRL for intermediate-duration oral exposure to DNP.

Calculations: None.

Intermittent Exposure: Not applicable.

Uncertainty Factor: The LOAEL of 0.07 mg/kg/day was divided by a total uncertainty factor of 1,000:

- *10 for interspecies extrapolation*
- *10 for use of a LOAEL*
- *10 for human variability*

Other Additional Studies or Pertinent Information that Lend Support to this MRL: See Selection of the Critical Effect above.

Agency Contacts (Chemical Managers): Susan Ingber

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 2,4-Dinitrophenol
CAS Numbers: 51-28-5
Date: August, 1995
April, 2017—Updated literature search
Profile Status: Fourth draft

Route: *Oral*
Duration: *Chronic*

MRL Summary: There are insufficient data for derivation of a chronic-duration oral MRL for 2,4-DNP.

Rationale for Not Deriving an MRL: An MRL derived based on the lowest LOAELs for effects of chronic oral exposure to 2,4-DNP would not provide adequate protection, because the POD would be at or above dose levels known to cause death in humans. However, the provisional intermediate-duration oral MRL is believed to be protective for chronic exposures.

Only two chronic-duration studies of 2,4-DNP (Horner et al. 1935; Tainter 1938) provided enough information to identify effect levels, and the effect levels were at or above doses associated with human fatalities. The NOAELs and LOAELs in the Horner et al. (1935) human study and Tainter (1938) were 2 and 30 mg/kg/day, respectively, and human fatalities have been reported after acute- or intermediate-duration exposures of 1–5 mg/kg/day (Dameshek and Gargill 1934; Goldman and Haber 1936; Masserman and Goldsmith 1934; Silver 1934; Zack et al. 2016).

Caldeira da Silva et al. (2008) included an experiment with chronic-duration exposure (mice were exposed from 18 weeks of age until their natural deaths), but the only endpoints evaluated in the groups exposed until natural death were food and water intake, body weight, body temperature, and survival. Furthermore, the authors reported data on food and water intake and body temperature only at 75 weeks of age (57 weeks of exposure), and on body weights only through 68 weeks of age (50 weeks of exposure). The provisional intermediate-duration MRL is based on the LOAEL for decreased body weight in mice in the study by Caldeira da Silva et al. (2008). In this study, the mice exhibited body weight decrements of 8–13% less than controls between 20 and 50 weeks of exposure. The body weight data, reported graphically, showed a persistent or slightly expanding decrement from control weights over the exposure period.

The provisional intermediate-duration MRL is believed to be protective for chronic exposures, for several reasons. The exposure duration in the critical experiment was just short of 1 year (50 weeks, or 350 days), and thus approximated a chronic duration (≥365 days). In addition, as noted above, the body weight data showed a continued trend in reduced weight throughout the experiment that is likely to have persisted with longer exposure. Furthermore, available chronic-duration studies in humans (Horner et al. 1935) and rodents (Tainter 1938) indicated that weight loss is a common and sensitive effect of chronic exposure to 2,4-DNP. Finally, the mechanism for the weight loss (uncoupling of oxidative phosphorylation and increased basal metabolic rate) is very well characterized, and is expected to be operant at all exposure durations.

Agency Contacts (Chemical Managers): Susan Ingber"

ATSDR Charge Questions and Responses (Peer Reviewer 3)

General comments

COMMENT: Minor technical comments: ATP is a good store of chemical energy because the equilibrium constant for the hydrolysis reaction (K' ~ 105 M) is such that when displaced 10 orders of magnitude from equilibrium (as it is in cells, where the reaction can be harnessed to do work), the concentrations of ATP and ADP differ only 1000-fold (and can therefore be maintained in the range at

which diffusion does not limit reaction kinetics too much). The text incorrectly repeats a common textbook error of referring to "high energy bonds" in ATP. On page 2 line 24, page 49 line 34 and page 84 line 17 "high energy phosphate bonds" could be replaced by "chemical potential" as indicated to retain the general meaning without committing the error.

RESPONSE: *The Reviewer's annotated comments on this topic were addressed throughout the text.*

COMMENT: On page 84 lines 14-17 I suggest clarifying the description of uncoupling to indicate that it is a cycle of protonation, diffusion of the acid, deprotonation, and diffusion back of the anion. The result is that respiration goes faster, which is the cause of the increased metabolic rate and hyperpyrexia. (At constant rate, uncoupling does not produce extra heat, because even if ATP was made it would later be hydrolyzed to do work, resulting in the same steady-state heat production by either mechanism).

RESPONSE: *Section 2.18.2 was revised as suggested by the Reviewer's annotated comments.*

"The ability of 2,4-DNP to uncouple oxidative phosphorylation underpins many of the clinical observations and physiological effects of its toxicity in both humans and animals. Such effects include elevated basal metabolic rate or oxygen consumption, elevated respiration and pulse rates, increased perspiration, and increased body temperature, and are related to the uncoupling of oxidative phosphorylation. In vitro demonstrations of 2,4-DNP's ability to uncouple oxidative phosphorylation began as early as 1948 (Loomis and Lipmann 1948; see also Ilivicky and Casida 1969; Muscatello et al. 1975; Pinchot 1967; Weinbach and Garbus 1969). During the Krebs cycle, 2,4-DNP and other lipophilic weak acids uncouple oxidative phosphorylation from electron transport by picking up protons, diffusing across the inner mitochondrial membrane, deprotonating, and returning to pick up more protons, thereby dissipating the pH gradient and membrane electrochemical potential needed for the formation of ATP (Lou et al. 2007; Stryer 1988). During this uncoupling, electron transport from NADH to oxygen can increase several-fold, but the energy produced, which is normally stored as the chemical potential of ATP, is released as heat. The prevention of ATP formation by 2,4-DNP means that all energy-dependent biochemical processes are likely to be affected. In addition, the depletion of ATP associated with mitochondrial uncoupling may produce hyperkalemia. 2,4-DNP has been reported to induce potassium accumulation in rabbit kidney slices (Mudge 1951), and hyperkalemia has been observed in humans poisoned with 2,4-DNP (Jiang et al. 2011).

The body attempts to dissipate the increased heat by inducing dilation of blood vessels. When heat production exceeds the organism's capacity to dissipate heat, fatal hyperthermia may result (Murphy 1986). Symptoms reported in humans who poisoned with 2,4-DNP exposure are similar to those seen in heat stroke, including headache; nausea and vomiting; muscle pain or weakness; and behavioral changes such as restlessness, confusion, agitation, and delirium. Hyperkalemia in turn may produce muscle pain and weakness often reported by humans after exposure to 2,4-DNP. With both 2,4-DNP exposure and hyperthermia, these symptoms may progress to seizures and coma, and may be accompanied by rhabdomyolysis and acute renal failure and other signs of multiorgan failure; the cause of death is typically cardiac arrest (see Section 2.2 for effects of 2,4-DNP, and Power et al. [2014] and Trujillo and Fragachán [2011] for hyperthermia). Autopsy findings after fatal 2,4-DNP poisoning often show widespread tissue hyperemia, congestion, and hemorrhage resulting from vasodilation (see Section 2.2).

Both endogenous and exogenous chemicals that uncouple oxidative phosphorylation, including 2,4-DNP, have recently been explored as potential therapies for prevention or mitigation of obesity, Type II diabetes, and aging (reviewed by Divakaruni and Brand 2011). Uncoupling of oxidative phosphorylation has been shown to induce weight loss and improve glucose

homeostasis, effects also seen with exposure to 2,4-DNP (Caldeira da Silva et al. 2008; Goldgof et al. 2014; see also Sections 2.3 and 2.13). Furthermore, because superoxide anion is a byproduct of oxidative phosphorylation as well as a potent cellular oxidant, uncoupling has been shown to reduce oxidative stress, thought to be an important mechanism of aging (reviewed by Divakaruni and Brand 2011). Indeed, several measures of oxidative stress were decreased, and survival was prolonged, in mice exposed to low levels of 2,4-DNP for their natural lifespan (Caldeira da Silva et al. 2008). However, the narrow margin of safety between a 2,4-DNP dose that is beneficial and that which is toxic, even lethal, limits the potential therapeutic uses of 2,4-DNP (Divakaruni and Brand 2011; Lou et al. 2007).

Little information is available on the uncoupling potency of other DNP isomers. In experiments designed to facilitate development of a quantitative structure-activity relationship (QSAR) for uncoupling activity of substituted phenols, Escher et al. (1999) showed that 3,4-DNP uncoupled oxidative phosphorylation in Rhodobacter membrane vesicles, with higher rates of uncoupling observed at lower pH (tested from pH 5.3 to 8.25). In isolated rat liver mitochondria, the effective concentrations for uncoupling by the DNPs were 20, 30, 40, 40, 100, and 100 µM for 3,5-, 2,4-, 2,6-, 3,4-, 2,3-, and 2,5-DNP (Burke and Whitehouse 1967). This order is not entirely congruent with the acute lethality of the isomers in animals exposed via intraperitoneal injection (LD50 values were 45, 35, 38, 98, 190, and 150 mg/kg in rats for the same order of DNPs; Harvey 1959), but does provide support for the lower relative potency of 2,3- and 2,5-DNP compared with the others. The lower potency of 2,3- and 2,5-DNP is also supported by the observation that mouse intraperitoneal LD50 values for these isomers did not change with increasing temperature (Harvey 1959), suggesting lower potential for inducing chemical hyperthermia.

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COMMENT: On page A10 line 42 "decoupling" is not strictly correct (it refers to non-protonophoric mechanisms), and should be replaced by the correct term, "uncoupling" (protonophoric mechanism, as for DNP).

RESPONSE: *The Worksheet for the chronic oral MRL was revised as suggested.*

*"**MINIMAL RISK LEVEL (MRL) WORKSHEET***

Chemical Name: *2,4-Dinitrophenol*
CAS Numbers: *51-28-5*
Date: *August, 1995*
April, 2017—Updated literature search
Profile Status: *Fourth draft*
Route: *Oral*
Duration: *Chronic*

MRL Summary: There are insufficient data for derivation of a chronic-duration oral MRL for 2,4-DNP.

Rationale for Not Deriving an MRL: An MRL derived based on the lowest LOAELs for effects of chronic oral exposure to 2,4-DNP would not provide adequate protection, because the POD would be at or above dose levels known to cause death in humans. However, the provisional intermediate-duration oral MRL is believed to be protective for chronic exposures.

Only two chronic-duration studies of 2,4-DNP (Horner et al. 1935; Tainter 1938) provided enough information to identify effect levels, and the effect levels were at or above doses associated with human fatalities. The NOAELs and LOAELs in the Horner et al. (1935) human study and Tainter (1938) were 2 and 30 mg/kg/day, respectively, and human fatalities have been reported after acute- or intermediate-duration exposures of 1–5 mg/kg/day (Dameshek and Gargill 1934; Goldman and Haber 1936; Masserman and Goldsmith 1934; Silver 1934; Zack et al. 2016).

Caldeira da Silva et al. (2008) included an experiment with chronic-duration exposure (mice were exposed from 18 weeks of age until their natural deaths), but the only endpoints evaluated in the groups exposed until natural death were food and water intake, body weight, body temperature, and survival. Furthermore, the authors reported data on food and water intake and body temperature only at 75 weeks of age (57 weeks of exposure), and on body weights only through 68 weeks of age (50 weeks of exposure). The provisional intermediate-duration MRL is based on the LOAEL for decreased body weight in mice in the study by Caldeira da Silva et al. (2008). In this study, the mice exhibited body weight decrements of 8–13% less than controls between 20 and 50 weeks of exposure. The body weight data, reported graphically, showed a persistent or slightly expanding decrement from control weights over the exposure period.

The provisional intermediate-duration MRL is believed to be protective for chronic exposures, for several reasons. The exposure duration in the critical experiment was just short of 1 year (50 weeks, or 350 days), and thus approximated a chronic duration (≥365 days). In addition, as noted above, the body weight data showed a continued trend in reduced weight throughout the experiment that is likely to have persisted with longer exposure. Furthermore, available chronic-duration studies in humans (Horner et al. 1935) and rodents (Tainter 1938) indicated that weight loss is a common and sensitive effect of chronic exposure to 2,4-DNP. Finally, the mechanism for the weight loss (uncoupling of oxidative phosphorylation and increased basal metabolic rate) is very well characterized, and is expected to be operant at all exposure durations.

Agency Contacts (Chemical Managers): Susan Ingber"

Chapter 1. Relevance to Public Health

QUESTION: Do you agree with those effects known to occur in humans as reported in the text?

COMMENT: Yes.

RESPONSE: *No response necessary.*

QUESTION: Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

COMMENT: Yes. Mechanism is biophysical (DNP is a protonophore that catalyzes net transport of H+ ions across lipid bilayers) so depends little on genetically-encoded proteins and is therefore not very species-dependent.

RESPONSE: *No response necessary.*

QUESTION: Have exposure conditions been adequately described? If you disagree, please explain.

COMMENT: Yes.

RESPONSE: *No response necessary.*

QUESTION: Do you believe the derived intermediate oral MRL value is justifiable? If you disagree, please explain (see also Appendix A).

COMMENT: Yes.

RESPONSE: *No response necessary.*

QUESTION: Do you agree that the data do not support derivation of acute, intermediate, and chronic inhalation MRLs?

COMMENT: Yes.

RESPONSE: *No response necessary.*

Chapter 2. Health Effects

QUESTION: Do the health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature for DNPs?

COMMENT: Yes.

RESPONSE: *No response necessary.*

QUESTION: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? Were the major study limitations sufficiently described in the text without going into lengthy discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT: Limitations of the historical human data are appropriately discussed.

RESPONSE: *No response necessary.*

QUESTION: Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.

COMMENT: Yes.

RESPONSE: *No response necessary.*

QUESTION: Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?

COMMENT: Yes.

RESPONSE: *No response necessary.*

QUESTION: Are you aware of any studies that are not included in the profile that may be important in evaluating the toxicity of DNPs? Please provide a copy of each study and indicate where in the text each study should be included.

COMMENT: No.

RESPONSE: *No response necessary.*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the DNP isomers?

COMMENT: No.

RESPONSE: *No response necessary.*

QUESTION: Were all appropriate NOAELs and/or LOAELs identified for each study (both in the text and the Levels of Significant Exposure (LSE) tables and figures)? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

COMMENT: Yes.

RESPONSE: *No response necessary.*

QUESTION: Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables?

COMMENT: Yes.

RESPONSE: *No response necessary.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain.

COMMENT: Yes.

RESPONSE: *No response necessary.*

QUESTION: The updated DNPs profile includes an unpublished study by Eli Lilly and Co. unavailable to ATSDR when preparing the original profile. Please comment on the quality of the study, namely:
Did the study use an adequate number of animals and practice good animal care?

COMMENT: Yes.

RESPONSE: *No response necessary.*

QUESTION: The updated DNPs profile includes an unpublished study by Eli Lilly and Co. unavailable to ATSDR when preparing the original profile. Please comment on the quality of the study, namely:
Did the study account for competing causes of death?

COMMENT: Yes.

RESPONSE: *No response necessary.*

QUESTION: The updated DNPs profile includes an unpublished study by Eli Lilly and Co. unavailable to ATSDR when preparing the original profile. Please comment on the quality of the study, namely:
Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: Adequate.

RESPONSE: *No response necessary.*

QUESTION: The updated DNPs profile includes an unpublished study by Eli Lilly and Co. unavailable to ATSDR when preparing the original profile. Please comment on the quality of the study, namely:

If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: N/A.

RESPONSE: *No response necessary.*

QUESTION: The updated DNPs profile includes an unpublished study by Eli Lilly and Co. unavailable to ATSDR when preparing the original profile. Please comment on the quality of the study, namely:

Do you agree with the conclusions of the author? If not, please explain.

COMMENT: Yes

RESPONSE: *No response necessary.*

Chapter 7. Regulations and Guidelines

QUESTION: Are you aware of any additional regulations or guidelines that we should add? Please provide citations.

COMMENT: No.

RESPONSE: *No response necessary.*

QUESTION: Are there any that should be removed? Please explain.

COMMENT: No.

RESPONSE: *No response necessary.*

Appendix A. ATSDR Minimal Risk Level Worksheets

QUESTION: Acute-duration oral MRL: The updated data evaluation includes a number of fatal human case studies involving lower exposure levels than were documented in the original profile (within an order of magnitude of the point of departure used for the original MRL).

Do you agree that these human fatality data adequately support ATSDR's decision to remove the original acute oral MRL? In not, please explain.

COMMENT: Yes.

RESPONSE: *No response necessary.*

QUESTION: Acute-duration oral MRL: The updated data evaluation includes a number of fatal human case studies involving lower exposure levels than were documented in the original profile (within an order of magnitude of the point of departure used for the original MRL).

Please comment on any aspect of our MRL database assessment that you would like us to address.

COMMENT: No comment provided.

RESPONSE: *No response necessary.*

QUESTION: Intermediate-duration oral MRL: ATSDR derived a new intermediate-duration oral MRL of 0.00007 mg/kg/day was derived for 2,4-DNP based on decreased body weight in mice exposed to 0.07 mg/kg/day 2,4-DNP in drinking water for 50 weeks (Caldeira da Silva et al. 2008). The MRL is based on a LOAEL of 0.07 mg/kg/day and a total uncertainty factor of 1,000 (10 for interspecies extrapolation, 10 for human variability, and 10 for use of a LOAEL). An intermediate-duration oral MRL was not derived in the 1995 toxicological profile due to the lack of intermediate duration studies involving doses lower than those known to cause death in humans.

Do you agree or disagree with the proposed intermediate-duration oral MRL value? Explain. If you disagree, please specify the MRL value that you propose.

COMMENT: Yes

RESPONSE: *No response necessary.*

QUESTION: Intermediate-duration oral MRL: ATSDR derived a new intermediate-duration oral MRL of 0.00007 mg/kg/day was derived for 2,4-DNP based on decreased body weight in mice exposed to 0.07 mg/kg/day 2,4-DNP in drinking water for 50 weeks (Caldeira da Silva et al. 2008). The MRL is based on a LOAEL of 0.07 mg/kg/day and a total uncertainty factor of 1,000 (10 for interspecies extrapolation, 10 for human variability, and 10 for use of a LOAEL). An intermediate-duration oral MRL was not derived in the 1995 toxicological profile due to the lack of intermediate duration studies involving doses lower than those known to cause death in humans.

Do you agree/disagree with each component of the total uncertainty factor? Explain. If you disagree, please specify the uncertainty factor(s) that you propose.

COMMENT: Yes. (If required, species uncertainty could be reduced to 3-fold given the known biophysical mechanism of action)

RESPONSE: *ATSDR considered whether a lower species uncertainty factor could be justified. However, available data indicate that effect levels for virtually all endpoints are lower in humans than in animals (see Figure 2-2). Coupled with the scarcity of toxicokinetic information comparing humans and laboratory rodents, this observation suggests that retaining a species uncertainty factor of 10 is warranted to ensure that the MRL is adequately protective.*

QUESTION: Intermediate-duration oral MRL: ATSDR derived a new intermediate-duration oral MRL of 0.00007 mg/kg/day was derived for 2,4-DNP based on decreased body weight in mice exposed to 0.07 mg/kg/day 2,4-DNP in drinking water for 50 weeks (Caldeira da Silva et al. 2008). The MRL is based on a LOAEL of 0.07 mg/kg/day and a total uncertainty factor of 1,000 (10 for interspecies extrapolation, 10 for human variability, and 10 for use of a LOAEL). An intermediate-duration oral MRL was not derived in the 1995 toxicological profile due to the lack of intermediate duration studies involving doses lower than those known to cause death in humans.

Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT: No comment provided.

RESPONSE: *No response necessary.*

QUESTION: Chronic-duration oral MRL: ATSDR believes the chronic-duration oral exposure database does not provide sufficient data for derivation of a chronic oral MRL. Specifically, the lowest LOAELs for effects of chronic exposure are higher than doses known to cause fatalities in humans. However, we believe the intermediate-duration oral MRL is protective for chronic exposures.

Do you agree with ATSDR that the intermediate oral MRL of 0.00007 mg/kg/day would be sufficiently protective for chronic exposures? If not, please explain.

COMMENT: Yes.

RESPONSE: *No response necessary.*

Appendix B. Literature Search Framework

QUESTION: Does Appendix B provide a sufficiently clear documentation of ATSDR's health effects literature search strategy and inclusion/exclusion criteria?

COMMENT: Yes.

RESPONSE: *No response necessary.*

QUESTION: Does it provide enough transparency regarding ATSDR's implementation of its inclusion and exclusion criteria (e.g. how ATSDR chose the studies it included in the health effects chapter)?

COMMENT: Yes.

RESPONSE: *No response necessary.*

Overall Usability of the Profile

QUESTION: Does the new chapter organization make it easy for you to find the information you need? For example, are you satisfied with the organization of the health effects chapter by organ system rather than exposure route?

COMMENT: Yes.

RESPONSE: *No response necessary.*

QUESTION: Does the profile contain all of the information you need? Is there information you would like to see that is not currently included?

COMMENT: 1) Yes. 2) No.

RESPONSE: *No response necessary.*

QUESTION: Having read this Toxicological Profile (and others, if applicable), which chapter(s) or content do you find most valuable and why? If you have previously used any Toxicological Profile(s) for your work, which chapter(s) or content have you used the most and for what purpose(s)?

COMMENT: (Responding to "which chapter(s) or content do you find most valuable and why?") Chapter 1. Summary.

RESPONSE: *No response necessary.*

QUESTION: Are the new tables and figures clear and useful? Do they make the Toxicological Profile easier to read?

COMMENT: Yes.

RESPONSE: *No response necessary.*