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Provisional Peer-Reviewed Toxicity Values for

N,N-Diphenyl-1,4-Benzenediamine
(CASRN 74-31-7)

Superfund Health Risk Technical Support Center
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COMMONLY USED ABBREVIATIONS

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

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR N,N-DIPHENYL-1,4-BENZENEDIAMINE (CASRN 74-31-7)

Background

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

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

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

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

Disclaimers

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

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

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

Questions Regarding PPRTVs

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

INTRODUCTION

The U.S. EPA has not derived RfDs, RfCs, or estimates of carcinogenic potency for N,N-diphenyl-1,4-benzenediamine (diphenyl-p-phenylene diamine or DPPD) (see Figure 1 for chemical structure). No values are posted on IRIS (U.S. EPA, 2008a), the Drinking Water Standards and Health Advisories list (U.S. EPA, 2006), or the Health Effects Assessment Summary Tables (HEAST) (U.S. EPA, 1997). There are no entries for this chemical in the Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1991, 1994). U.S. EPA (2008b) has not derived Acute Exposure Guidelines (AEGLs) for DPPD for use in the event of a sudden, unexpected release, and there are no occupational exposure guidelines for DPPD (American Conference of Governmental Industrial Hygienists [ACGIH], 2007; National Institute for Occupational Safety and Health [NIOSH], 2008; Occupational Safety and Health Administration [OSHA], 2008).

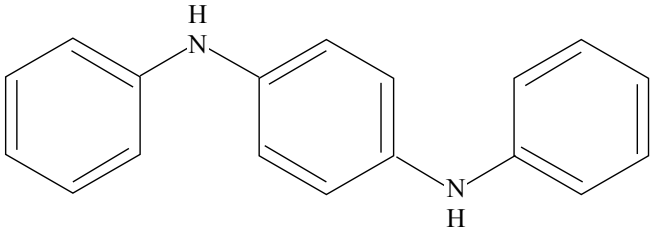

Figure 1. Structure of N,N-Diphenyl-1,4-Benzenediamine

The National Toxicology Program (NTP, 2008) has not assessed the toxicity or carcinogenicity of DPPD, and this compound is not included in the 11th Report on Carcinogens (NTP, 2005). DPPD has not been the subject of a monograph by the International Agency for Research on Cancer (IARC, 2008) or a toxicological profile by the Agency for Toxic Substances and Disease Registry (ATSDR, 2008). CalEPA (2002, 2008a,b) has not derived exposure levels for chronic toxicity or carcinogenic potency for DPPD.

To identify toxicological information pertinent to the derivation of provisional toxicity values for DPPD, literature searches were conducted on November 5, 2008, using the following databases: MEDLINE, TOXLINE, BIOSIS (1999–November 5, 2008), Chemical Abstracts (1999–November 2008), TSCATS1/2, CCRIS, DART, GENETOX, HSDB, RTECS, and Current Contents (May 2007–November 2008). Except where noted, the literature searches are not limited by date.

REVIEW OF PERTINENT DATA

Human Studies

Conde-Salazar et al. (2004) reported a case of contact dermatitis in association with occupational exposure to a black rubber mixture that contained DPPD, N-cyclohexyl-N'-phenyl-4-phenylenediamine, and N-isopropyl-N'-phenyl-4-phenylenediamine. The patient was an 18-year-old dental technician who developed dry hyperkeratotic lesions on the palm of one hand that was in contact with a container used to prepare molds for dental prostheses. On patch testing, the subject had a positive reaction to the black rubber mixture and its components. The lesions went away when the subject stopped touching the container.

No other studies concerning the toxicity of DPPD in humans are identified in the existing literature.

Animal Studies

Oral Exposure

There are no complete subchronic toxicity studies of DPPD, and there is only one chronic oral study. The reproductive toxicity of DPPD has been studied extensively in older studies that were conducted by the oral route of exposure.

Subchronic Studies—In a discussion of a reproductive toxicity study, Draper et al. (1956) noted briefly that a group of weanling rats (sex and number, not specified) fed 0.5% DPPD (about 250 mg/kg-day based on measured body weight and food consumption) in a vitamin E-deficient diet appeared healthy for the first 12 weeks on the diet, but then developed "porphyrin" staining of the whiskers and coat in the area of the head and neck, rough "haircoat" and moderate depression of weight gain. No significant changes in hemoglobin values or prothrombin times were detected. According to the study authors, palatability of the food did not appear to be a problem, based on food consumption and growth rate during the earlier portion of the study. Effect levels could not be defined due to the limited information available.

Chronic Studies—In the only chronic oral study of DPPD, groups of 50 male and 50 female F344 rats were fed diets containing 0, 0.5, or 2% DPPD (0, 5000, or 20,000 ppm; purity not reported, Tokyo Chemical Company, Inc.) dissolved in corn oil for 104 weeks (Hasegawa et al., 1989). The study authors described the diet as "a powdered basal diet" (Oriental M brand). Neither the constituents nor a dietary analysis was reported, but it appears that the diet may have been a semipurified or purified diet based on a statement in the discussion section of the report where the study authors attribute an observed endpoint, nephrocalcinosis, to dietary insufficiency (discussed later). Surviving animals were killed 8 weeks after the end of the treatment period. Daily intake of DPPD, based on food consumption and body-weight

measurements taken throughout the study, was reported to be 0, 194, or 857 mg/kg-day in males and 0, 259, or 1024 mg/kg-day in females. Urinalysis (pH, protein, glucose, ketone bodies, bilirubin, occult blood, urobilinogen, specific gravity, and sediments) was performed 1 week prior to the end of the treatment period. Hematology (erythrocyte, leukocyte and platelet counts; hematocrit and hemoglobin), blood chemistry (aspartate aminotransferase [AST], alanine aminotransferase [ALT]), alkaline phosphatase, total cholesterol, β-lipoprotein, total protein, albumin/globulin ratio, urea nitrogen, glucose, sodium, potassium, chloride and calcium), organ weights (brain, heart, liver, spleen, kidneys, adrenals, testes and ovaries) and comprehensive histology were evaluated following sacrifice at the end of the posttreatment period (Study Week 112). Necropsies and histological evaluations were also performed on the rats that died or became moribund during the study.

Survival was not affected by DPPD; survival in the high-dose males was better than in controls (statistics not reported) (Hasegawa et al., 1989). Body-weight gain was reduced in treated males and females throughout the study. The decreases in weight gain were dose-related and more pronounced in the females; final body weights in the low- and high-dose groups were 5.9 and 8.9% lower than controls in males, respectively, and 22.5 and 25.2% lower than controls in females, respectively (statistical analysis not reported). Food consumption, however, was the same in treated as in control groups. All treated groups of rats reportedly had statistically significantly ($p < 0.05$) reduced absolute organ weights (heart, liver, and adrenal in both sexes; kidney in females; testis in males) and relative organ weights (liver in both sexes) in comparison with controls; data are not reported, so the magnitude of change is unknown. Hematological effects included small—but statistically significant ($p < 0.01$)—increases (compared with controls) in erythrocyte count (13–18%), hemoglobin (8–11%), and hematocrit (11–12%) in females at ≥259 mg/kg-day and increased platelet count (15%) in males at 857 mg/kg-day, but these changes were not considered toxicologically important by the study authors. Serum calcium levels were statistically significantly reduced (7.5%, $p < 0.01$) in males at 857 mg/kg-day. Histological examinations showed increased occurrence of renal calcification at the corticomedullary junction (nephrocalcinosis) in treated males; incidences were reported as 2.0, 57.1, and 65.3% in the 0-, 194-, and 857-mg/kg-day groups, respectively. Incidences of nephrocalcinosis were high (75–86%) in both control and treated female groups. The study authors observed no other treatment-related nonneoplastic changes upon histological examination of tissue samples. The results of this study indicate that the low dose, 5000 ppm (194 mg/kg-day in males and 259 mg/kg-day in females), is a LOAEL associated with an increased incidence of nephrocalcinosis in the males and biologically relevant decreased body weight in the females (>20% below control values at termination).

A limitation of this study is that the long observation period (8 weeks after termination of exposure) may have allowed for recovery of reversible lesions (Hasegawa et al., 1989). In addition, nephrocalcinosis has been shown to occur in untreated rats fed "semipurified" or "purified" diets (Phillips et al., 1986; NRC, 1995). The nature of the basal diet given to the rats in the study by Hasegawa et al. (1989) is not reported. The study authors stated that it was not clear whether the observed nephrocalcinosis was due to the feeding of DPPD or related to nutritional imbalances in calcium or phosphorus, which might suggest that a semipurified or purified diet was used. Feed intake rates in this study were lower than reference values and consistent with values observed in purified diet studies (see discussion under "*Studies using vitamin E-deficient diets, with or without supplementation*" below). In any event, the incidence

of nephrocalcinosis in control male rats fed the same basal diet was only 2%, compared with ≥57.1% in DPPD-treated male rats (Hasegawa et al., 1989), providing support that the nephrocalcinosis in males was treatment related.

All rats that died (or were killed) after the appearance of the first tumor at 57 weeks were included in the number of effective animals for statistical analysis of incidences of neoplastic lesions. The incidences of neoplastic lesions were not significantly ($p < 0.05$) different in the DPPD-treated groups as compared with controls. The incidence of squamous cell carcinoma of the skin in females was lower ($p < 0.05$) in the treated groups than in the controls. The incidences of testicular interstitial cell tumors were high in all groups of males (90, 89.8, and 87.8% in the control, low-, and high-dose groups, respectively). Thus, this study provides no evidence of carcinogenicity in rats of either sex fed the chemical at dietary levels to produce some toxicity (depression of body-weight gain, increased incidences of nephrocalcinosis in males) without affecting survival.

Reproductive/Developmental Studies—A number of early studies examining the effects of DPPD on reproduction were conducted decades ago. Some of these studies exposed animals to DPPD in conjunction with vitamin E-deficient diets (referred to by the study authors as "purified" or "semipurified" diets), in an effort to determine whether DPPD, an antioxidant, could prevent the adverse effects of vitamin E deficiency on reproduction. The available studies of reproductive effects of DPPD are discussed below; the studies are organized by whether stock diet (presumed to contain sufficient vitamin E) or "purified/semipurified" diet (deficient in vitamin E) was used in the study.

Studies using stock diets containing sufficient vitamin E

Bionetics Research Laboratories Inc. (1968) assessed the potential developmental toxicity of DPPD in two strains of mice exposed via gavage. A group of 12 pregnant C57BL6 mice was given 464 mg/kg of Agerite DPPD (no further characterization, purity not specified, dissolved in a 50% honey and water solution) on Days 6–14 of gestation. An additional study was attempted with AKR mice, but, due to the use of only one dam per dose group, no conclusions are possible. Both untreated (two groups of 31 or 37 dams) and vehicle (32 dams) controls were included in the study. All mice were fed a standard "baked diet" from a commercial supplier throughout the study. Dams were sacrificed and weighed on Day 18 of gestation, and uterine contents were examined. Maternal liver weights, but no other organ weights, were recorded. The study authors reported no mortality or treatment-related clinical signs among the dams. There were no statistically significant differences between DPPD-exposed dams and their vehicle controls with respect to maternal weight gain. Relative liver weight of dams was reported to be significantly ($p < 0.05$) increased compared with controls, but the difference was very small (6.37 ± 0.09% for DPPD-exposed dams vs. 6.14 ± 0.08% for vehicle controls; $p < 0.05$).

There were no treatment-related effects on mean implantations per litter, mean number of live fetuses per litter, or mean crown-rump length in C57BL6 mice (Bionetics Research Laboratories Inc., 1968). The study authors reported significant ($p < 0.05$) decreases in fetal mortality (7% mortality for DPPD-exposed; 14% for control litters); placental weight (100 ± 4 mg for DPPD-exposed; 107 ± 2 mg for controls) and amniotic fluid per fetus (174 ± 14 mg for DPPD-exposed; 202 ± 9 mg for controls). Mean fetal weight was significantly ($p < 0.05$) increased in DPPD-exposed mice (1051 ± 28 mg) relative to vehicle controls of the same strain (937 ± 24); the increase in fetal size may have contributed to the lower placental

weight and amniotic fluid. According to the study authors, there were no statistically significant differences between DPPD-exposed mice and their respective vehicle controls with regard to the numbers or types of developmental anomalies observed. Based on results for C57BL6 mice, the NOAEL for reproductive effects and changes in relative liver weights in this study is 464 mg/kg-day (the only dose tested). However, as shown in the reproductive toxicity studies discussed below, the effects of DPPD on reproduction are usually manifested during delivery; these effects include uterine hemorrhage, and maternal and fetal mortality. In this study, the dams were sacrificed prior to delivery such that the effects of DPPD during delivery would not have been observed.

B.F. Goodrich Company sponsored reproductive toxicity studies of DPPD (Ashe, 1956). Several studies of rats and mice were conducted with both chemically pure DPPD and several commercially available DPPD mixtures that contained small amounts of contaminants. Similar studies were conducted for rats and mice, and the results were similar for both species. Reporting of the mouse study was incomplete, while reporting for the rat study was relatively complete; so only the rat study is reported in detail in this review. Groups of approximately 20 female Wistar rats were fed diets of Ralston-Purina Laboratory chow alone (controls) or with chemically pure DPPD (99.5% pure) at concentrations of 300 or 1000 ppm (Ashe, 1956). Based on default body weight and food consumption values[1], these dietary concentrations are equivalent to approximate doses of 31 and 103 mg/kg-day, respectively. Additional groups of 10 female Wistar rats were fed the control diet plus a commercial DPPD mixture (purity not specified; contained small amounts of diphenylamine [DPA], hydroxydiphenylamine [HDA] and blue tar; 300 or 1000 ppm), or the control diet plus various contaminants such as blue tar (characterized as "mostly oxidized DPPD", 100 ppm), pure DPA (100 ppm), pure HDA (100 ppm), or various combinations of DPPD (1000 ppm) with the aforementioned contaminants (0.5% DPA, 3% HDA and/or 2% blue tar).

Ashe (1956) did not clearly indicate the length of time that these females were fed the various diets before they were paired with males to initiate the reproductive phase of the study. However, the report stated that all animals were observed for a week to 10 days before starting the study. Test diets were fed to the females throughout pregnancy and lactation. Males were fed the control diet except when they were paired with females, at which time they received whatever diet the females were exposed to. Females were given up to five chances to become pregnant (five 5-day pairings with males with 10–12-day intervals between the 5-day pairings). The study authors acknowledged that they could not determine with accuracy the timing of conception and gestation[2]. Females that did not become pregnant after the fifth pairing were sacrificed and necropsied.

Ashe (1956) did not report maternal food consumption or body weights. Because the timing of conception was not accurately determined for all test animals, the gestation times for individual animals were reported as approximate ranges. In addition, the numbers of live and dead births were not determined due to instances of undetected deliveries and subsequent cannibalism (frequency of occurrence not reported). Individual animal data reported by the

[1]Body weight of 0.156 kg and food consumption rate of 0.016 kg/day for female Wistar rats exposed for subchronic duration (U.S. EPA, 1988).
[2]Ashe (1956) stated that "Careful examination of the vagina and the presence of a so-called 'copulation plug' was not a reliable index of pregnancy in our hands."

study authors are minimum, maximum, and midpoint of estimated gestation times; number of live and dead offspring; number of pups raised to 21 days; and weight of pups on Day 21. A summary table compared the results of each test group, reporting % fertility rate, % maternal mortality, mean live births, mean dead births, mean gestation time, number of litters weaned per number pregnant, and mean weanling weight on Postnatal Day 21. Table 1 summarizes these data. No statistical analyses are reported, and neither are the variance data that would be needed to conduct an independent statistical analysis.

Table 1. Reproductive Data from Rats Fed Diets Containing DPPD and its Contaminants[a]

Group	Fertility Rate (%)	Maternal Mortality Rate (%)	Mean Live Births[b]	Mean Dead Births[b]	Mean Gestation Time (days)	Percent of Litters Weaned[c]	Mean Weanling Weight[d] (grams)
Control	85	0	9.0	0.4	20.9	80	46.1
Pure DPPD							
300 ppm	75	5	4.6	4.1	22.6	38	47.0
1000 ppm	81	10[e]	1.9	5.4	22.6	7.7	36.0
Commercial DPPD							
300 ppm	60	5	6.1	4.9	22.5	25	ND
1000 ppm	63	0	3.4	5.0	22.7	25	40.5
1000 ppm DPPD + Contaminants							
HDA (3%)	70	0	1.1	7.5	24.1	14	38.8
DPA (0.5%)	80	20	6.0	2.6	25.1	12	37.0
Blue tar (2%)	70	0	3.0	4.1	22.8	14	35.6
DPA (0.5%) + HDA (3%)	70	20	0.9	6.0	24.1	0	NA
HDA (3%) + Blue tar (2%)	66	33	5.7	4.0	23.9	16	36.9
DPA (0.5%) + Blue Tar (2%)	80	20	1.1	6.5	23.3	0	NA
Contaminants Alone							
100-ppm HDA	100	0	4.9	2.3	22.2	20	40.6
100-ppm DPA	80	0	7.8	0.9	23.9	62	35.7
100-ppm Blue Tar	90	0	8.7	0.3	21.7	78	39.1

[a]Ashe (1956), Table XVI, page 22 of report. Please note that slightly different numbers are reported in a second version of this table shown on page 53 that appears to be a draft for review. No information other than mean values is reported.

[b]Ashe (1956) reports that these values may not be absolutely accurate due to unquantifiable cannibalism, but they are still useful for comparing across groups

[c]Number litters weaned divided by the number dams pregnant × 100; determined on Postnatal Day 21.

[d]Recorded on Postnatal Day 21.

[e]Table XVI, page 22 of the report shows a maternal mortality of 15% for this group; an examination of the individual animal data, however, indicates that 2/20 dams in this group died, for a mortality rate of 10%.

ND = Not Determined; NA = Not Applicable

The data from Ashe (1956) suggest the following effects due to pure DPPD: (1) possible treatment-related maternal mortality (0, 1/20, and 2/20 in control, 300-ppm, and 1000-ppm pure DPPD groups, respectively; all maternal deaths occurred during labor); (2) possible slight decrease in fertility (85%, 75%, and 81%, in control, 300 ppm and 1000 ppm pure DPPD groups, respectively); (3) dose-related offspring mortality (increase in dead births and decreases in live births and ratio of litters weaned to number of pregnant dams at 300 and 1000 ppm; offspring mortality in DPPD-treated groups occurred primarily at birth), although there is some uncertainty in these data due to problems determining the timing of conception; (4) possible increased gestation time, although these data are highly uncertain due to problems determining the timing of conception; and (5) an apparent decrease in pup weight on PND 21 at 1000 ppm DPPD. Increased maternal and offspring mortality and reduced fertility were also observed in dams fed commercial DPPD or DPPD preparations containing the various contaminants. Among the contaminants alone, HDA appears to have effects on offspring viability as well. There is no strong indication of adverse effects associated with the contaminants DPA or blue tar alone.

Ashe (1956) reported that all of the DPPD-treated dams—but none of the controls—hemorrhaged abnormally during delivery; dams that survived were reported to be severely anemic for many weeks (data not shown). The observed uterine hemorrhage is likely to have caused the recorded maternal deaths, although this is not explicitly stated by the study author. The study author reports that affected offspring were deeply cyanotic and concludes that fetal deaths were due to anoxia resulting from partial or complete placental separation at term with inadequate uterine contraction.

Ashe (1956) conducted gross and microscopic examinations of rats that failed to become pregnant and those that died in labor. Data for these findings are not clearly summarized, and the narrative mixes results for mice and rats. Based on the available information, a total of 26 rats were examined, of which three were controls that did not become pregnant, and 23 were fed diets containing DPPD or DPPD contaminants (no further specification of what specific diets these rats received). Of the 23 experimental (i.e., exposed to DPPD- or DPPD contaminants) rats that were examined, 12 died during labor and the remainder were killed in moribund condition before or after delivery of their litters. Histological examinations revealed no compound-related effects on the heart, stomach or spleen (data not shown). Tubular degeneration of the kidneys with casts in the upper and lower nephrons was observed in 11 of the 23 dams that died or were killed; hyaline necrosis of the liver was also observed in five of these. The histology data are of limited use given that the study authors do not clearly indicate the test material (pure DPPD, commercial DPPD, or one of several contaminants) to which the 23 rats were exposed. The low dose in this study (300 ppm, or 31 mg/kg-day) is a FEL for maternal and fetal mortality during parturition.

In another reproductive study, groups of 10 female rats (strain not reported) were fed diets containing 0, 0.025, 0.10, 0.40, or 1.60% (0, 250, 1000, 4000, or 16,000 ppm) commercial grade DPPD (purity ≥95%) for 2 weeks prior to mating to untreated males, and subsequently throughout gestation, parturition and lactation (Oser and Oser, 1956). The female rats were selected from an established breeding colony; each female had previously produced and weaned a normal litter. The basal diet was a stock-type diet consisting of a mixture of grains (whole wheat, corn, alfalfa meal), nonfat dry milk, meat meal, liver, hydrogenated cottonseed oil, brewer's yeast, vitamin supplements (B complex, A, D, E, and K in cottonseed oil providing linoleic acid), and sodium chloride and manganese sulfate. Based on default values for body

weight and food consumption[3], estimated DPPD intakes were 0, 22, 88, 350, or 1400 mg/kg-day, respectively. The endpoints evaluated in the study are duration of pregnancy; numbers of resorptions, live and dead pups, and complete and partial litters; pup body weight; and maternal (during parturition and postpartum) and pup (Days 4 and 21) survival.

The duration of gestation was prolonged in the DPPD-treated groups compared with controls (Oser and Oser, 1956). The incidence of maternal mortality during parturition increased with dose, as did pup mortality at birth, which was significantly increased at all dose levels. There were postpartum maternal deaths in controls, 350-, and 1400-mg/kg-day groups, but the incidence in treated groups was not statistically distinguishable from controls. Table 2 shows the details of these findings. The pups born dead or found in the uterus of females that died at parturition were reportedly 10–20% heavier than controls, although there was no clear indication of abnormal weight in pups born alive (insufficient data were shown to conduct statistical analyses). The study authors reported that the pups appeared large but, otherwise, normal. This finding is consistent with the longer duration of gestation. To determine whether damage to the posterior pituitary might be involved in the delay of parturition, histopathological examinations of the posterior pituitary were performed in five of the females that died in parturition after gestations of 24 or 25 days' duration, and in control rats killed on the 22nd day of gestation. The study authors found no histological differences. The lowest dose of DPPD tested, 22 mg/kg-day, is a FEL based on maternal and fetal mortality during parturition.

Table 2. Selected Reproductive Data from Rats Fed DPPD in Stock Diets[a]

Effect	DPPD Dose (mg/kg-day)				
	0	**22**	**88**	**350**	**1400**
Mean duration of gestation (± SEM) (days)	22.1 ± 0.23	22.9 ± 0.23[b]	24.1 ± 0.30[b]	25.2 ± 0.68[b]	24.7 ± 0.54[b]
Maternal mortality during parturition	0/10	1/10	3/10	3/10	5/10[c]
Pup mortality at birth	18/107 (17%)	42/79[c] (53%)	21/35[c] (60%)	18/20[c] (90%)	20/24[c] (83%)
Maternal postpartum mortality	2/10	0/9	0/7	1/7	2/5

[a]Source: Oser and Oser (1956).
[b]Statistically significant difference from controls, Student's *t*-test, *p*-value not specified.
[c]Statistically significant difference from controls, ($p < 0.05$) by Fisher exact test conducted for this review.

Groups of 10–17 female rats of unspecified strain and body weight were fed 0, 0.0125, 0.0625, 0.313, or 1.55% (0, 125, 625, 3130, or 15,500 ppm) of DPPD ("feed grade", purity and supplier not reported) in the diet starting 10 days prior to mating and continuing through parturition and lactation (Ames et al., 1956). The diet was a "stock" diet consisting of corn, wheat, dry skim milk, casein, "meat-bone scraps," alfalfa meal, liver, yeast, linseed meal,

[3]Food consumption of 0.022 kg/day and body weight of 0.25 kg for mature female rats (U.S. EPA, 1988). Values for mature animals were used because the rats were from an established breeding colony, and, as such, were older and weighed more than the assumed body weight for a young rat in a subchronic duration study.

partially hydrogenated vegetable oil, calcium carbonate, and iodized salt (Ames et al., 1952, 1956). Based on default values for body weight and food consumption[4], estimated average DPPD intakes were 0, 11, 55, 275, and 1360 mg/kg-day. The study endpoints include a fertility index (number of females pregnant/number mated), the litter efficiency (% pregnant animals with at least 1 viable fetus), a mortality index (number of dams dying at parturition/number pregnant), the duration of pregnancy, the litter size, a viability index (number of pups alive at Neonatal Day 3/number born), and a lactation index (number of young weaned/number alive at 3 days).

Like Oser and Oser (1956), Ames et al. (1956) also observed prolonged gestation associated with DPPD exposure as well as increased maternal mortality (at doses ≥55 mg/kg-day) and markedly increased pup mortality on or before PND 3 (95–100% at all doses, compared with 32% in controls). Table 3 shows the details. Deaths of the dams and pups occurred mainly during parturition, and signs of difficult parturition (vaginal bleeding and prolapse of the uterus) were occasionally observed. The study authors suggested that the prolongation of gestation may have resulted in unusually large fetuses, such that the birth process was difficult and prolonged. Pup weights are not reported. The lowest dose tested (11 mg/kg-day) is a FEL based on markedly increased pup mortality.

Table 3. Selected Reproductive Data from Rats Fed DPPD in Stock Diets[a]

Effect	DPPD Dose (mg/kg-day)				
	0	**11**	**55**	**275**	**1360**
Mean duration of gestation (days)	23	24	25	25	25
Mean litter size	10.6	7.9	4.9	5.3	4.7
Maternal mortality	1/17	0/12	5/17	5/10[b]	7/13[b]
Pup mortality (Postnatal Day 3)	33/104 (32%)	75/79[b] (95%)	49/49[b] (100%)	16/16[b] (100%)	14/14[b] (100%)

[a]Source: Ames et al. (1956); no statistical analyses are reported.
[b]Statistically significantly different from control ($p < 0.05$) by Fisher exact test conducted for this review.

In a study of the effects of antioxidants on fetal resorptions, a total dose of 0.5 g (500 mg) of DPPD (purity not specified) was administered in the diet to mated female 200 g Walter Reed-Carworth Farms rats (group size was not reported), starting after mating and continuing through necropsy on Day 22 of gestation (Telford et al., 1962). The type of diet was not specified, and so was assumed for this review to be a stock diet. The daily dose was estimated to be 114 mg/kg-day (500 mg/ [0.2 kg × 22 days]). The young were delivered by caesarian section after the dams were sacrificed and the uterus inspected for resorption sites. In comparison with the control group (126 litters), the DPPD-treated group (23 litters) appeared to have an increased rate of resorption (litters with resorptions = 40.8% in controls vs. 60.8% in DPPD-treated; resorptions as % of total number of implantations = 10.6% control vs. 15.3% DPPD). The study

[4]Food consumption of 0.022 kg/day and body weight of 0.25 kg for mature female rats (U.S. EPA, 1988).

authors considered this result to indicate a substantial increase in resorptions, but no additional details were provided and statistical analyses were not reported. As such, effect levels were not determined for this study.

Studies using vitamin E-deficient diets, with or without supplementation

A number of studies conducted in the 1950s and early 1960s were aimed at assessing whether administration of DPPD, an antioxidant, could prevent the adverse effects of vitamin E deficiency on reproduction (sterility). In these studies, "purified" or "semipurified" diets (deficient in vitamin E) were used both with or without vitamin E supplementation. The doses of vitamin E that were used in supplementation varied from 0.7 to 30 mg/week. The available studies do not clearly discuss what level of dietary vitamin E is considered to be "sufficient" for laboratory rodents. However, Ames (1974) reported that 0.7 mg DL-α-tocopherol acetate, given 6 times per week, was required for normal reproduction in older female rats. This translates to a weekly requirement for 4.5 mg/week.

A study (Draper et al., 1956) that measured intake of a purified diet reported lower consumption rates than for stock diets. Food consumption was measured for female Sprague-Dawley rats fed DPPD in a purified diet from weaning through mating, gestation, and parturition. Rats weighed 0.188 kg and had an estimated food consumption of 0.009 kg/day[5]. This intake is about half of the reference value for food consumption predicted by the allometric equations in U.S. EPA (1988) for a rat of that weight (0.018 kg/day). One possible explanation for the reduced intake is that the purified diet was more concentrated (no fiber source was included). Due to the possibility that intake of purified diets was lower than for stock diets, default values for food consumption were not used to estimate doses of DPPD administered in purified or semipurified diets. Instead, a food factor of 0.05 kg diet/kg bw-day (0.009 kg diet per day divided by body weight of 0.188 kg) based on empirical data from Draper et al. (1956) was used to estimate doses for studies where DPPD was administered in vitamin-E deficient diets.

Draper et al. (1956) fed weanling female Sprague-Dawley rats 0.005, 0.025, or 0.1% (50, 250, or 1000 ppm) of DPPD in a purified, vitamin E-deficient diet. An additional group of 10 rats was given 0.385 mg/week of DPPD orally, divided into three equal doses, to simulate the level of intake that would result from 0.0006% (6 ppm) DPPD in the diet. Controls received just the purified diet (25 females), or the purified diet supplemented with vitamin E at 30 mg/week (19 females). The vitamin E supplementation at 30 mg/week appears to be adequate to support successful reproduction in this study based on the requirement of 4.5 mg/week reported by Ames (1974). The basal purified diet contained 64.6% cerelose (glucose), 20% casein, 10% tocopherol-low (distilled) lard, salts (unspecified), and vitamins (including B, A, D, and K). Using the food factor of 0.05 kg diet/kg bw-day derived above, concentrations of 6, 50, 250, and 1000 ppm DPPD in the purified diets are estimated to correspond to DPPD doses of 0.3, 2.5, 12.5, and 50 mg/kg-day. The rats were fed the DPPD-containing and control diets starting at weaning and continuing through mating and parturition. The females, weighing 175–200 g (188-g median) were then mated to healthy male rats that had been maintained on a stock diet.

[5]A dose of 0.385 mg/week was estimated by the authors to correspond to a dietary level of 0.0006% (6 ppm) DPPD (Draper et al., 1956). Dividing 0.385 mg/week by 7 days and by the median weight of the female rats at mating (0.188 kg) gives an estimated DPPD dose of 0.3 mg/kg-day. A daily food ingestion value (kg-diet/day) can be estimated using these values, as follows: (0.3 mg/kg bw/day × 0.188 kg bw) ÷ 6 mg DPPD/kg diet = 0.009 kg diet/day.

Endpoints include clinical observations, serum hemoglobin, prothrombin time, white blood cell counts, number of conceptions (number pregnant), gestation length, number of pups born, and live births.

Draper et al. (1956) reported that during the first 6 weeks on the diets (the growth period), all the rats gained weight normally and appeared healthy, with no signs of DPPD toxicity or vitamin deficiency. None of the rats in the control group that did not receive vitamin E became pregnant. The conception rate for the other groups was not different from vitamin E-supplemented controls. During gestation, signs of toxicity (anorexia, rough haircoat, and anemia) were observed at the highest exposure level (1000 ppm DPPD). Table 4 shows the other pertinent findings of the study. The average duration of gestation was increased among dams fed the two highest concentrations of DPPD, and the incidence of stillbirths was increased in all but the lowest dose group. The incidence of stillbirths at the low dose (6 ppm) was low (12%) and less than the vitamin E-supplemented control group (43%); however, at the higher doses, the incidence of stillbirth was ≥81%. At parturition, vaginal hemorrhage was observed in the 1000-ppm dams; 3/10 animals in this group died and the rest were semimoribund. The three deaths were attributed to hemorrhaging (only seven of these animals were pregnant, however, and it is not clear whether hemorrhages were seen in the nonpregnant rats). The hemorrhagic rats were acutely anemic, but their prothrombin times were normal. Hematological parameters in the other groups were not affected. The total number of pups born per pregnant rat was comparable to vitamin E-supplemented controls at the lower levels of DPPD, but it was very low in the 1000-ppm group. Whether this was due to failure to deliver some of the pups (because the dams died or were semi-moribund) is not discussed. During gestation and at parturition, no adverse effects on any endpoint were seen in the 0.3 mg/kg-day (6 ppm) group; this dose is a NOAEL. The next higher dose (2.5 mg/kg-day or 50 ppm) is considered to be a FEL based on the marked increase in stillborn pups.

Table 4. Selected Reproductive Data from Rats Fed DPPD in Vitamin E-Deficient Diets[a]

	DPPD Dose (mg/kg-day)					
Effect	**0 (no vitamin E)**	**0 (+ 30 mg vitamin E/wk)**	**0.3**	**2.5**	**12.5**	**50**
Mean duration of gestation (days)	No pregnancies	22.8	Not reported	23.8	25.4	Not reported
Vaginal hemorrhage, death	Not applicable	None reported	None reported	None reported	None reported	3/10
Stillborn pups	Not applicable	67/155 (43%)	8/69 (12%)	93/108[b] (86%)	66/71[b] (93%)	17/21[b] (81%)

[a]Source: Draper et al. (1956); no statistical analyses are reported.
[b]Statistically significantly different from vitamin E-supplemented control ($p < 0.05$) by Fisher exact test conducted for this review.

Draper et al. (1958) conducted a series of four studies to determine whether vitamin E deficiency could be reversed by supplementation with vitamin E or other antioxidants such as DPPD. In the first study, groups of 10 weanling Sprague-Dawley rats were fed a purified, vitamin E-deficient diet supplemented with (1) no supplement; (2) 0.7 mg D-α-tocopheryl acetate; (3) 0.7 mg DL-α-tocopheryl acetate; (4) 0.4 mg DPPD/week given once weekly in triacetin and estimated to be equivalent to 0.0006% DPPD (6 ppm) in the diet; or (5) 0.1% (1000 ppm) DPPD in the diet, reduced in the second and third reproductive cycles to 0.025% (250 ppm) and 0.0025% (25 ppm), respectively. Using the food factor of 0.05 kg diet/kg bw-day for purified diets developed from data in the Draper et al. (1956) study, DPPD dietary levels of 6 ppm (0.4 mg/week) and 1000 ppm (followed by 250 and 25 ppm) correspond to estimated doses of 0.3 and 50 mg/kg-day (followed by 12.5 and 1.3 mg/kg-day). The amount of vitamin E that was added to the diets of groups 2 and 3 (0.7 mg, once per week) is well below the 4.5 mg/week reported to be required to support female reproduction in rats (Ames, 1974). The diets were fed starting at weaning and continuing for 8 weeks, at which time the rats were mated to normal males fed a stock diet; the female rats were continued on their respective diets as above through two to four reproductive cycles (not specifically defined by the study authors; however, a reproductive cycle appears to include mating and the production of a litter) over three generations. There were five weanling females (F1) from the first litters of the lowest-dose DPPD group that were fed the same diet for two reproductive cycles, and five of the weanling female pups (F2) from their first litters were then maintained on the diet through mating and parturition. Criteria for reproductive performance included number of pregnancies and of resorbed litters, number of litters, number of pups born (total and per pregnancy), and percent born alive. Table 5 summarizes the results for this study. The reproductive performance of the females receiving DPPD at an oral dose equivalent to 6 ppm in the diet is comparable to that of the groups receiving vitamin E supplements (D-α- or DL-α-tocopheryl acetate) through two reproductive cycles for each of two generations (Draper et al., 1958). Reproductive failure (resorption) occurred in the third generation (F2 females); it was similar to that seen in the first generation control group that received no vitamin E supplementation (controls were studied only for one generation). The reproductive failure seen in the F2 females was of the type characteristic of vitamin E deficiency (resorptions), and, thus, appears to reflect an inability of DPPD to substitute totally for vitamin E during prolonged vitamin E deficiency rather than any toxicity of DPPD itself (as the latter would typically be manifest as maternal or pup mortality at delivery). Accordingly, this study provides support for a NOAEL 0.3 mg/kg-day (equivalent to 6 ppm) for reproductive effects of DPPD in a vitamin E-deficient purified diet.

The group that started on 1000 ppm (50 mg/kg-day) DPPD at weaning through the first reproductive cycle, with decreased dietary levels (250 and 25 ppm, corresponding to 12.5 and 1.3 mg/kg-day) for the second and third cycles, experienced a high rate of stillbirth (calculated from data regarding total pups born and the percent born alive) in the first two cycles, and a low pregnancy rate in the third (Draper et al., 1958). Additional effects in the dams were the prolongation of gestation time by 2–3 days, vaginal hemorrhages, and anemia. It was not specified whether the prolongation of gestation, hemorrhages, and anemia occurred only in the first cycle or in subsequent cycles as well. The number of dams dropped from 10 for the first cycle to 8 for the second and third; whether this attrition was due to mortality or morbidity also was not specified, but it appears likely from the description of effects in the dams.

Table 5. Reproductive Performance of Female Rats Fed DPPD in Vitamin E-Deficient Diets[a]

Cycle No.[b]	Group No.	Treatment	No. Females	No. Females with Implantations	No. Females with Resorptions	No. of Pups Born		Percent Born Alive
						Total	Per Implantation	
First Generation								
1	1	None	10	7	7	0	0	0
1	2	0.7 mg D-α-tocopheryl acetate/wk	10	4	0	30	7.5	100
1	3	0.7 mg DL-α-tocopheryl acetate/wk	10	7	0	64	9.1	100
1	4	0.3 mg DPPD/kg-day	10	5	0	35	7.0	100
1	5	50 mg DPPD/kg-day	10	10	0	21	2.1	15
2	1	None	10	10	7	15	1.5	100
2	2	0.7 mg D-α-tocopheryl acetate/wk	10	10	1	69	6.9	99
2	3	0.7 mg DL-α-tocopheryl acetate/wk	10	10	0	92	9.2	97
2	4	0.3 mg DPPD/kg-day	9	9	1	61	6.8	87
2	5	12.5 mg DPPD/kg-day	8	7	0	50	7.1	50
3	5	1.3 mg DPPD/kg-day	8	2	1	5	2.5	100
Second Generation								
1	4	0.3 mg DPPD/kg-day	5	4	0	36	9.0	100
2	4	0.3 mg DPPD/kg-day	5	3	0	18	6.0	100
Third Generation								
1	4	0.3 mg DPPD/kg-day	5	2	2	0	0	0

[a]Source: Draper et al. (1958), Table 1; no statistical analyses are reported.
[b]Includes mating and gestation period/litter.

The second experiment tested the ability of DPPD at dietary levels of 0.005% or 0.025% (50 or 250 ppm, corresponding to 2.5 or 12.5 mg/kg-day estimated as above) to maintain reproductive performance in female rats fed vitamin E-deficient purified diets through four complete reproductive cycles (Draper et al., 1958). Diets were similar to those reported above for other experiments in the same study; controls included a vitamin E-deficient group and a group that received an oral supplement of vitamin E (30 mg D-α-tocopheryl acetate/week; sufficient to support female reproduction). From weaning through the end of the second reproductive cycle, the only fat source in the diet was cod-liver oil, which was known to accelerate the appearance of vitamin E deficiency. These diets produced signs of essential fatty acid deficiency (tails became scaly and developed cracks) in all groups by the time the animals were mated, so supplementation with methyl linoleate was instituted. Nevertheless, the percentage of dams that produced litters during the first cycle was low in all groups (≤44%). For cycles three and four, lard, rather than cod liver oil, was used as the source of dietary fat.

Table 6 shows the results from the second experiment reported by Draper et al. (1958). No litters were born to the vitamin E-deficient controls. In all four reproductive cycles, the percentage of stillbirths, calculated from data regarding total pups born and the percent born alive, was higher in the 50-ppm (2.5 mg/kg-day) DPPD group than in the vitamin-E-supplemented controls. The percentage of stillbirths was even higher in the 250-pm (12.5 mg/kg-day) DPPD group—reaching 100% in the fourth cycle—and the number of pups born in this cycle to this group was low relative to the vitamin E controls. The number of dams decreased somewhat in all groups over the four cycles, but the decrease was more marked in the 250-pm group; whether the decrease was due to treatment-related mortality was not discussed but in any event, was not statistically significant (Fisher exact test conducted for this review). The study authors noted that chronic respiratory infection affected an unspecified number of rats in each group as age advanced.

Additional studies in which one generation of female rats fed Vitamin E deficient diets were allowed to mate, but were unable to reproduce. Their diet was supplemented with nothing, with vitamin E (30 mg D-α-tocopherol acetate/week; adequate to support female reproduction) or with 0.005% DPPD (50 ppm or 2.5 mg/kg-day) during the second reproductive cycle of mating and litter production. The results showed that DPPD restored fertility in terms of supporting the ability to carry litters to term (Draper et al., 1958). The percentage of stillbirths, however, was elevated in the DPPD group (37%) relative to vitamin E-supplemented controls (14%).

The Draper et al. (1956, 1958) series of studies defines a FEL of 2.5 mg/kg-day (50 ppm) based on markedly increased incidence of stillbirths (see Table 6). The NOAEL for this series of studies is 0.3 mg/kg-day (6 ppm). An important observation from the studies by Draper et al. (1958) is that the effects of vitamin E deficiency on reproduction appear earlier in gestation than the effects of DPPD. Specifically, vitamin E deficiency results in marked increases in the rate of resorptions. In contrast, DPPD toxicity is manifested as stillbirths or pup mortality at birth (see data in Tables 5 and 6).

Table 6. Reproductive Performance of Female Rats fed DPPD in Vitamin E-Deficient Diets[a]

Cycle No.[b]	Group No.	Treatment Group	No. Females	No. of Litters	No. of Pups Born		Percent Born Alive
					Total	Per Litter	
1	1	Control	25	0	0	0	0
1	2	30 mg D-α-tocopheryl acetate/wk	25	9	78	8.7	75
1	3	2.5 mg DPPD/kg-day	25	11	78	7.1	25
1	4	12.5 mg DPPD/kg-day	25	4	13	3.2	15
2	1	None	25	0	0	0	0
2	2	30 mg D-α-tocopheryl acetate/wk	23	17	155	9.1	57
2	3	2.5 mg DPPD/kg-day	22	16	108	6.7	14
2	4	12.5 mg DPPD/kg-day	21	10	71	7.1	7
3	1	None	21	0	0	0	0
3	2	30 mg D-α-tocopheryl acetate/wk	21	4	33	8.2	55
3	3	2.5 mg DPPD/kg-day	15	7	44	6.3	27
3	4	12.5 mg DPPD/kg-day	14	4	14	3.5	4
4	1	None	17	0	0	0	0
4	2	30 mg D-α-tocopheryl acetate/wk	17	6	39	6.5	67
4	3	2.5 mg DPPD/kg-day	14	7	43	6.1	35
4	4	12.5 mg DPPD/kg-day	11	6	11	1.8	0

[a]Source: Draper et al. (1958), Table 1; no statistical analyses are reported.
[b]Includes mating and gestation period/litter.

In a preliminary study of DPPD's ability to substitute for vitamin E, Ames et al. (1956) fed nine vitamin E-depleted female rats DPPD at 0.2% (2000 ppm, or 100 mg/kg-day, estimated as above for purified diets) in a purified vitamin E-deficient diet starting 7 days before mating and continuing through gestation, parturition and lactation. A vitamin E-supplemented control group of nine vitamin E-depleted female rats received 0.002% D-α-tocopheryl acetate in the diet starting 7 days before mating, and a negative control group (three females) received only the vitamin E-deficient diet. Although the negative controls became pregnant, the fetuses were resorbed such that there were no viable fetuses at term. In the DPPD group, 3/9 dams had a least one viable fetus versus 9/9 for the vitamin E controls. Gestation was prolonged to 25 days in the DPPD group (as compared with a normal duration of 22 days), and 2/9 dams died (versus 0/9 in the vitamin E controls and 0/3 in the negative controls). The viability index (number pups alive at 3 days/number born) was 0/7 for the DPPD group and 63/70 for the vitamin E controls. The only dose tested in this study (100 mg/kg-day) is a FEL for maternal and pup mortality.

In another study by these study authors, groups of 10–17 female rats of unspecified strain and body weight were fed DPPD at 0, 0.0125, 0.0625, 0.313, or 1.55% (0, 125, 625, 3130, or 15,500 ppm) of DPPD ("feed grade", purity and supplier not reported) in a purified diet to which 0.001% vitamin E was added, starting on the day of mating and continuing through parturition and lactation (Ames et al., 1956). It is not clear whether the amount of vitamin E added to this diet could be considered "sufficient" because it is half the amount of vitamin E used in the previously reported preliminary study in the positive control group. In that study, 0.002% vitamin E added to the basal vitamin E-depleted diet was sufficient to prevent the adverse effects on reproduction that were observed among dams fed the vitamin E-depleted diet. The purified diet used in this and the preliminary study consisted of 60% cerelose (glucose), 24% casein, 12% distilled lard, supplemental vitamins (B, K, A, and D), and a salt mixture (content not specified) (Ames et al., 1956; Harris and Ludwig, 1949). Using the food factor of 0.05 kg diet/kg bw-day from the study of Draper et al., (1956), concentrations of 0-, 125-, 625-, 3130-, and 15,500-ppm DPPD in the purified diets of Ames et al. (1956) are estimated to correspond to doses of 0, 6.3, 31, 157, and 775 mg/kg-day. Study endpoints included fertility index (number of females pregnant/number mated), litter efficiency (% pregnant animals with at least 1 viable fetus), mortality index (number of dams dying at parturition/number pregnant), duration of pregnancy, litter size, viability index (number of pups alive at Neonatal Day 3/number born), and lactation index (number of young weaned/number alive at 3 days).

Results from the latter experiment using purified diet were similar to those from the experiment using stock diet and longer-term DPPD exposure (see description under "*Studies using stock diets containing sufficient vitamin E*"), but most of the effects were less severe at any given DPPD dietary level (Ames et al., 1956). Table 7 presents pertinent findings. As the table shows, DPPD exposure resulted in a dose-related increase in gestation duration and pup mortality. Maternal mortality (1–2 dams/dose) was observed in the 6.3-, 157-, and 775-mg/kg-day groups—but not in the 31 mg/kg-day group or in controls. Pup mortality was 42% at the lowest dose (6.3 mg/kg-day), compared with 14% in controls. Higher doses were associated with 85–100% pup mortality. The lowest dose in this study (6.3 mg/kg-day) is a FEL for markedly increased pup mortality.

Table 7. Selected Reproductive Data from Rats fed DPPD in Vitamin E-Deficient Diets Partially Supplemented with Vitamin E (0.001%)[a]

	DPPD Dose (mg/kg-day)				
Effect	**0**	**6.3**	**31**	**157**	**775**
Mean duration of gestation (days)	22	23	24	25	26
Mean litter size	7.7	6.2	5.1	6.0	7.3
Maternal mortality	0/17	1/11	0/11	2/11	1/11
Pup mortality (Postnatal Day 3)	12/85 (14%)	13/31[b] (42%)	43/51[b] (85%)	54/54[b] (100%)	29/29[b] (100%)

[a]Source: Ames et al. (1956); no statistical analyses are reported.
[b]Statistically significantly different from control ($p < 0.05$) by Fisher exact test conducted for this review.

Draper et al. (1964) maintained female Sprague-Dawley rats on a vitamin E-deficient purified diet from weaning through mating and gestation so that sterility could be demonstrated; they were then remated and given 0.75 mg DPPD/day until Day 21 of gestation, at which time they were killed and the uteri examined for live fetuses and resorptions. Reproductive performance of the DPPD-treated group was better than that of control groups supplemented with vitamin E (5 or 10 mg/day of DL-α-tocopheryl acetate) in the same manner. However, because dams were not allowed to go through the birthing process, the parturition-related maternal and pup mortality observed in other studies of DPPD-exposure are not able to be evaluated in this study.

King (1964) fed three dietary levels of DPPD (0.025, 0.050, and 0.075% [250, 500, and 750 ppm]) in purified, vitamin E-deficient diets to weanling female Holtzman rats through mating and 21 days of gestation. These concentrations correspond to 12.5, 25, and 37.5 mg/kg-day calculated using the food factor of 0.05 kg diet/kg bw-day for purified diets. Each concentration of DPPD was tested at three different levels of vitamin E supplementation: none, 2 mg/day given on the first five days of gestation, and 2 mg given on Day 10 of gestation. As with the previous study, the dams were not allowed to give birth. Results are variable, but they did not reveal a pattern of significant differences in terms of percentages of live normal or abnormal fetuses or dead or resorbed fetuses (per total number of implantation sites) among the three dietary levels of DPPD tested, or within each dietary level under different levels of vitamin E supplementation. The examination of fetuses for abnormalities was conducted under a dissecting binocular microscope, and, thus, was not a rigorous examination for skeletal and soft tissue abnormalities. Although the study included control groups receiving the three different levels of vitamin E and no DPPD, the DPPD groups were not compared statistically with the control groups, and the data are not presented in enough detail to support independent statistical analysis. In addition, the author's statement that the experiments lasted for more than 2-years raises the question of whether the various DPPD dietary levels and the controls were tested concurrently. As such, effect levels cannot be determined from this study. The use of this study is also limited because the critical event affected by DPPD exposure (i.e., parturition) was not allowed to take place.

Inhalation Exposure

No inhalation studies of DPPD were located in the available literature.

Other Studies

Although DPPD has been tested for antioxidant properties in a number of feeding studies with animals, these studies were focused narrowly to address endpoints such as the effects of DPPD on the prevention of atherosclerosis (e.g., Sparrow et al., 1992; Tangirala et al.,1995) or on the distribution of mercury in body tissues (Welsh, 1979). As such, the usual toxicological endpoints necessary for a reliable assessment of dose-response and the definition of "adverse" effect levels (such as clinical chemistry, urinalysis, and gross and microscopic examination of major organs) are not presented in these studies. A mouse study (Tangirala et al., 1995) suggested some mortality and reduced body weights at a high dose, but the study used mice that were genetically modified to develop atherosclerosis, and. as such, the results from the study are of questionable value with respect to the general population.

Mechanistic Studies

Orally administered DPPD was found to inhibit processes indicative of inflammatory response, including paw edema in rats, adjuvant arthritis in rats, and serum sickness in rabbits (Levy and Kerley, 1974). The doses used in the studies ranged from 50 to 200 mg/kg with durations of exposure ranging from 30 minutes to 21 days, depending on the study. The protocol for administration of DPPD in these studies is not always specific and there are no toxicological evaluations independent of the narrow focus on inflammatory response (i.e., no monitoring of body weight, food consumption, or clinical signs and gross or microscopic pathology outside of the endpoints relevant to mitigation of an artificially induced inflammatory condition). Levy and Kerley (1974) hypothesized that the inhibitory effects of DPPD on inflammatory response are due to an inhibition of prostaglandin synthesis or fatty acid peroxide formation that could also explain the adverse effects of DPPD on the uterus during the birth process.

The effects of DPPD on estrone-induced uterine growth were investigated in young mice in an attempt to explain previously observed effects of DPPD on gestation and pregnancy in laboratory rodents (Sonnen et al., 1962). Groups of young 28-day-old Swiss-Webster mice (8 to 23 mice/group) were injected subcutaneously once each day for 3 consecutive days with (1) estrone alone at doses ranging from 0.00321 to 3.21 μg per mouse; (2) DPPD alone by gavage at doses ranging from 0.03 to 9.6 mg/mouse; or (3) estrone injections (0.321 μg per mouse) in combination with orally administered DPPD at doses ranging from 0.0005 mg to 9.6 mg/mouse. A group of control mice received only injections of the carrier solvent (sesame oil) for 3 days. All mice were sacrificed on the day after the last treatment. Body and uterine weights were recorded. Estrone injections promoted an increase (greater than double the control value) in uterine weight. DPPD alone had no effect on either body or uterine weight in comparison with controls. However, DPPD antagonized estrone-induced increases in uterine weight in a dose-related manner at doses of 0.004 mg/mouse (35.5% mean inhibition) up to the highest dose tested of 9.6 mg/mouse (83.4%). Sonnen et al. (1962) suggested that because DPPD was devoid of progestational[6], antiprogestational, androgenic, antiandrogenic, and estrogenic activity in other studies conducted in their laboratory (not discussed further; no data shown), the observed results were likely due to a "unique pharmacologic property, independent of hormonal activity."

[6]"progestational" and "antiprogestational" refer to progesterone agonism and antagonism, respectively.

Genotoxicity

DPPD gave positive results for mutagenicity in *Salmonella typhimurium* TA98 and TA100 with hamster liver S9—but not with rat liver S9 or in the absence of an activating system (Zeiger et al., 1992). In another study, positive results were reported in *Salmonella typhimurium* TA98 and TA1538 in the presence—but not in the absence—of rat liver S9 (Rannug et al., 1984). DPPD produced positive results in an assay for point mutations in cultured Chinese hamster V79 cells in the absence, but not in the presence, of an unspecified S9 preparation (Donner et al., 1983). Similarly, DPPD was mutagenic in the L5178Y TK+/- lymphoma assay only when tested without rat liver S9 and not in the presence of this activating system (Microbiological Associates, Inc., 1987).

Results obtained in chromosomal aberration assays in cultured Chinese hamster ovary cells are not consistent from study to study: negative results are reported with and without rat liver S9 by Sofuni et al. (1990), there are equivocal positive results in the presence or absence of rat liver S9 by Microbiological Associates, Inc. (1988), and there are positive results in the absence of S9 by Sofuni et al. (1991). Use of longer treatment times appear to produce positive results (Microbiological Associates, Inc., 1988; Sofuni et al., 1991). Positive results were obtained for chromosomal aberrations in cultured Chinese hamster lung cells only without S9 (Sofuni et al., 1990, 1991).

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR N,N-1,4-DIPHENYL-1,4-BENZENEDIAMINE (DPPD)

The available data clearly indicated high maternal mortality and marked increase in stillborn pups at a dose (2.5 mg/kg-day) that is about 8 times higher than the NOAEL (0.3 mg/kg-day) reported in a series of studies conducted by Draper et al. (1956, 1958). Therefore, no provisional RfDs for either subchronic or chronic durations are developed. However, the Appendix of this document contains a screening value that may be useful in certain instances. Please see the attached Appendix for details.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR N,N-1,4-DIPHENYL-1,4-BENZENEDIAMINE (DPPD)

A p-RfC cannot be derived for DPPD because inhalation toxicity data for humans and animals are lacking. Furthermore, without pharmacokinetic data and information to rule out portal-of-entry effects, there is no basis to support a route-to-route extrapolation from the oral RfD.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR N,N-1,4-DIPHENYL-1,4-BENZENEDIAMINE (DPPD)

Weight-of-Evidence Descriptor

Under the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), the available evidence data provide "*Inadequate Information to Assess* [*the*] *Carcinogenic Potential*" *of DPPD*. No data regarding the potential carcinogenicity of DPPD in humans are available. In the only available chronic animal study (Hasegawa et al., 1989), DPPD in the diet was not carcinogenic to rats. DPPD gave mixed results in a small number of genotoxicity studies. DPPD was not mutagenic in *Salmonella typhimurium* without activation, but it was mutagenic with microsomal activation in some studies. DPPD increased point mutations in cultured mammalian cells without activation, but it gave negative results in two out of three tests with activation. Assays for chromosomal aberrations in cultured mammalian cell systems resulted in inconsistent findings both with and without activation; positive findings were more common with longer treatment times.

Quantitative Estimates of Carcinogenic Risk

Lack of data precludes derivation of quantitative estimates of cancer risk (i.e., p-OSF and p-IUR) for DPPD.

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APPENDIX A. DERIVATION OF SCREENING SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR N,N-1,4-DIPHENYL-1,4-BENZENEDIAMINE (CASRN 74-31-7)

For reasons noted in the main PPRTV document, it is inappropriate to derive provisional toxicity values for N,N-1,4-diphenyl-1,4-benzenediamine. However, information is available for this chemical which, although insufficient to support derivation of a provisional toxicity value, under current guidelines, may be of limited use to risk assessors. In such cases, the Superfund Health Risk Technical Support Center summarizes available information in an Appendix and develops a "screening value." Appendices receive the same level of internal and external scientific peer review as the PPRTV documents to ensure their appropriateness within the limitations detailed in the document. Users of screening toxicity values in an appendix to a PPRTV assessment should understand that there is considerably more uncertainty associated with the derivation of an appendix screening toxicity value than for a value presented in the body of the assessment. Questions or concerns about the appropriate use of screening values should be directed to the Superfund Health Risk Technical Support Center.

The toxicological database for DPPD is very limited. Apart from one comprehensive chronic toxicity study (Hasegawa et al., 1989), there are a number of limited reproductive toxicity studies conducted during the 1950s and 1960s. Table A-1 summarizes the studies of DPPD that provided information to define effect levels. The chronic study identifies a LOAEL of 194–259 mg/kg-day for nephrocalcinosis in male rats and decreased body weight in female rats. The reproductive toxicity studies identify effects at lower doses. However, most of these studies examined very few endpoints, and all are limited by incomplete reporting. Many of the studies were designed to determine whether DPPD administration could counteract the adverse effects of vitamin E deficiency on reproduction. Several studies used vitamin E-deficient diets, confounding the reproductive toxicity findings of DPPD exposure. In addition, there is evidence from one study (i.e., Draper et al., 1956) that consumption of vitamin E deficient diets by laboratory animals may have been lower than other diets, making the dose estimates for studies administering DPPD in these diets uncertain.

Despite their limitations, these studies do provide consistent evidence, regardless of diet, that DPPD exposure prior to and through gestation results in prolonged gestation and increased incidences of uterine hemorrhage, maternal mortality during parturition, and stillbirths. In addition, the series of studies conducted by Draper et al. (1958)—and shown in Tables 5 and 6—demonstrate that the reproductive effects of vitamin E deficiency (increased resorptions) differ from those observed with DPPD exposure. One possible mechanism, proposed by Ames et al. (1956), for the effects of DPPD on survival of dams and pups during delivery is that prolonged gestation leads to larger fetuses that cause difficulties during parturition. Some studies (Bionetics Research Laboratories Inc., 1968; Oser and Oser, 1956) have reported increased fetal weight or visibly larger fetuses after DPPD exposure.

Table A-1. Oral Dose-Response Data for DPPD

Species, Strain and Route (n/sex/group)	Exposure	NOAEL (mg/kg-day)	Effect Level (mg/kg-day)	Responses at the LOAEL	Reference
Rat, Chronic, Dietary, possibly purified/semipurified, vitamin E status unknown (50/sex/group)	0, 0.5, or 2% DPPD (0, 194 or 857 mg/kg-day in males and 0, 259, and 1024 mg/kg-day in females) for 104 weeks followed by 8 weeks observation	None	194 (M) (LOAEL) 259 (F) (LOAEL)	Increased incidence of nephrocalcinosis in males and decreased body weight in females.	Hasegawa et al. (1989)
Reproduction Studies Conducted with Stock Diets Containing Sufficient Vitamin E					
Mouse, C57BL6 Strain , Gavage (12 F/group)	464 mg/kg in 50% honey and water on Days 6–15 of gestation	464	None	Dams sacrificed prior to delivery (when DPPD effects typically observed).	Bionetics Research Laboratories Inc. (1968)
Rat, Wistar Strain, Diet (20 F/group)	300 or 1000 ppm (31 or 103 mg/kg-d through mating, pregnancy and lactation; males were exposed during mating only.	None	31 (FEL)	Maternal and fetal mortality during parturition	Ashe et al. (1956)
Rat, Strain not reported, Diet (10F/group)	0, 250, 1000, 4000, or 16000 ppm (0, 22, 88, 350, or 1400 mg/kg-d for 2 weeks prior to mating (to untreated males), and through gestation, parturition and lactation	None	22 (FEL)	Maternal and fetal mortality during parturition	Oser and Oser (1956)
Rat, Strain not reported, Diet (10–17 F/group)	0, 125, 625, 3130, and 15,500 ppm (0, 11, 55, 275, and 1360 mg/kg-day)10 days prior to mating, through mating, gestation and early lactation	None	11 (FEL)	Markedly increased pup mortality (≥95%)	Ames et al. (1956)

Table A-1. Oral Dose-Response Data for DPPD					
Species, Strain and Route (n/sex/group)	**Exposure**	**NOAEL (mg/kg-day)**	**Effect Level (mg/kg-day)**	**Responses at the LOAEL**	**Reference**
Reproduction Studies Conducted Using Vitamin E-Deficient Diets with or without Supplementation					
Rat, SD, Diet Multiple experiments (5–25 F/group)	0, 6, 50, 250, or 1000 ppm (0, 0.3, 2.5, 12.5, or 50 mg/kg-day) from weaning through mating and lactation, for multiple reproductive cycles and generations	0.3	2.5 (FEL)	Markedly increased stillbirths (≥81%)	Draper et al. (1956; 1958)
Rat, Strain not reported, Diet (9 F)	2000 ppm (100 mg/kg-day) 7 days before mating, through gestation and lactation.	None	100 (FEL)	Maternal and pup mortality	Ames et al. (1956)
Rat, Strain not reported, Diet (10–17F/group)	0, 125, 625, 3130, and 15,500 ppm (0, 6.3, 31, 157, and 775 mg/kg-day) from the day of insemination through gestation and lactation.	None	6.3 (FEL)	Markedly increased pup mortality (≥42%)	Ames et al. (1956)

The reproductive studies observed frank effects (consisting of maternal mortality during parturition or pronounced pup mortality and/or stillbirths) at the lowest dose tested in most of the studies (as low as 2.5 mg/kg-day) (Table A-1). A NOAEL is identified in one study (i.e., Draper et al., 1956) and supported in a second study by the same study authors giving the same NOAEL (Draper et al., 1958). Because frank effects were observed at the lowest doses producing effects in the reproductive toxicity studies, benchmark dose modeling of the critical effects would not be appropriate. Furthermore, BMD analysis was also performed using data from Ames et al. (1956) and Oser and Oser (1956). The analysis did not yield $BMDL_{10}$ values lower than the NOAEL, thus it was not used. The NOAEL from the reproductive/developmental study of 0.3 mg/kg-day identified in the Draper et al. (1956, 1958) studies was used to calculate **a screening subchronic and chronic p-RfD for N,N-diphenyl-1,4-benzenediamine** as follows:

$$\begin{aligned} \textbf{Screening Subchronic and Chronic p-RfD} &= \text{NOAEL} \div \text{UF} \\ &= 0.3 \text{ mg/kg-day} \div 1000 \\ &= \mathbf{0.0003} \text{ or } \mathbf{3 \times 10^{-4}} \text{ mg/kg-day} \end{aligned}$$

The composite UF of 1000 is composed of the following:

- UF_H: A factor of 10 is applied for extrapolation to a potentially susceptible human subpopulation because data for evaluating susceptible human response are incomplete.
- UF_A: A factor of 10 is applied for animal-to-human extrapolation because data for evaluating relative interspecies sensitivity are incomplete.
- UF_D: A factor of 10 is applied for database limitations. The toxicological database for DPPD includes one incompletely reported chronic toxicity study in rats and a number of limited reproductive toxicity studies in rats and one in mice. The available reproductive toxicity studies are limited by inadequate reporting, limited evaluations, use of vitamin E-deficient diets (in some cases), and failure to identify a LOAEL that did not produce frank effects. The database lacks a comprehensive multigeneration reproductive toxicity study (including exposure and evaluation of males) and studies evaluating potential teratogenicity.
- UF_S: A factor of 1 is applied to derive the chronic RfD because further adjustments for duration of exposure is not warranted when reproductive toxicity data are used.

Confidence in the principal studies (Draper et al., 1956, 1958) are low. While these studies employed a long exposure period (from weaning through parturition) and, in some experiments, over two to four reproductive cycles (mating, gestation and lactation) in more than one generation, they suffer from a number of limitations. These include lack of detail in the reporting of methods and results, limited evaluations of effects, failure to identify a LOAEL that did not produce frank effects, and use of a specialized diet (purified, vitamin E-deficient) that may have confounded the reproductive toxicity findings. However, the findings in these studies are corroborated at higher doses by results in studies using stock diets that provided adequate vitamin E. Despite the corroborating studies, confidence in the database is low because of the lack of a subchronic or chronic study in a second species, the lack of comprehensive multigeneration reproductive toxicity and developmental toxicity studies, and because of the failure of the available studies to identify a LOAEL that did not produce frank effects. Accordingly, confidence in the subchronic and chronic screening p-RfD is low.