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HEALTH AND ENVIRONMENTAL EFFECTS PROFILE FOR N,N-DIPHENYLAMINE

Prepared for

OFFICE OF SOLID WASTE
AND EMERGENCY RESPONSE

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NOTICE

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Diphenylamine was not oncogenic in a 2-year dietary study in rats when tested at levels ≤1.0% (Thomas et al., 1967a), or after a single gavage dose of 300 mg/rat in female rats observed for 6 months (Griswold et al., 1966). No increased tumor incidence was observed in mice treated at dietary levels ≤250 ppm (≤0.025%) for 92 weeks (Coulston et al., 1971).

Negative results were obtained for reverse mutation in several strains of Salmonella typhimurium and Escherichia coli with and without S-9, for forward mutation in cultured mouse lymphoma cells with and without S-9 and for unscheduled DNA synthesis in cultured rat hepatocytes (see Table 5-5).

Increased incidences and severity of cystic renal lesions were observed in the offspring of female rats treated with aged commercial grade diphenylamine at 20 mg/day by gavage or at 2.5% in the diet during the last 7 days of gestation (Crocker et al., 1972). Similar results were obtained when contaminants of commercial grade diphenylamine at 20 mg/day were administered; however, purified diphenylamine administration at 20 mg/day resulted in greatly reduced incidences and severity of renal lesions.

Clegg et al. (1981) and Safe et al. (1977) also suggested that toxic impurities acquired during manufacture or from heating or aging contribute to the biological effects of diphenylamine.

Dose-related decreased litter sizes were observed in a two-generation feeding study in rats treated at dietary levels of 0.1-0.5% (1000-5000 ppm) (Thomas et al., 1967a); however, the decrease was believed to be secondary to reduced food intake by the parents.

Several studies of chronic dietary administration of diphenylamine have been performed in rats, mice and dogs (see Table 5-8). Thomas et al. (1967a) found that dietary administration to rats at ≥0.1% (1000 ppm)

resulted in increased incidences and severity of kidney lesions (cystic dilatation and chronic nephritis) compared with controls. In addition, an increased incidence of hyperplasia of the bladder of males was observed at 1% (10,000 ppm). Growth was arrested at 0.5 (5000 ppm) and 1% (10,000 ppm) and females treated at 0.1% (1000 ppm) had significantly reduced body weights. Food consumption was decreased at 0.5 (5000 ppm) and 1% (10,000 ppm). No effects were observed at ≤0.01% (100 ppm).

In other studies (Safouh et al., 1970; Eknoyan et al., 1976; Evan and Gardner, 1976; Gardner et al., 1976; Evan et al., 1978; Kime et al., 1962; Woodhouse et al., 1965; Philbert et al., 1979), relatively high dietary levels of diphenylamine (1-4%) were administered to rats to study the time-course of appearance and increasing severity of cystic kidney lesions. An abstract of a study by Philbert et al. (1979), in which rats and guinea pigs were treated at dietary levels of 2 or 4% (20,000 or 40,000 ppm) for an unspecified duration, reported increased mortality, liver necrosis and lung congestion, edema and inflammatory lesions in addition to renal lesions.

Mice treated with diphenylamine at dietary levels of 50-250 ppm had dose-related increases in Heinz body formation in red blood cells but no changes in hematologic parameters (Coulston et al., 1971). At 250 ppm, spleen and liver weights were increased, and there was increased hemosiderosis of the spleen. No increased incidence of histologic changes in the kidney or any other tissue were observed.

Thomas et al. (1967b) observed

No histologic changes ~~were observed~~ in the kidneys of dogs fed diets containing 0.01-1.0% (100-10,000 ppm) diphenylamine, but fatty changes in the liver, hemosiderosis in the spleen, bone marrow and kidneys, increased kidney weight, arrested growth, pronounced anemia and impaired liver function occurred at 10,000 ppm. At 1000 ppm there was arrested growth and mild anemia. No effects were observed at 100 ppm.

The dietary level of 100 ppm is a NOEL in rats and dogs. The rats ingesting the 0.1 % diphenylamine diet for two years, at which time the males averaged 400 g in weight and had consumed an average of 0.9 g of diphenylamine or 2.25g/kg (Thomas et al., 1967 a). The female dogs fed the 0.01 % diphenylamine diet for two years averaged 9.09 kg in weight, each having consumed 14 g or 1.54g/kg of the compound (Thomas et al., 1967 b). By dividing the above intakes by 730 days, the NOELs for rats and dogs are respectively equivalent to 3.1 or 2.1 mg/kg/day. In the mouse study, 50 ppm could be considered a MOAEL, but the suitability of this study for deriving an ADI is questionable, since the study by Coulston et al. (1971) is unpublished, was received only briefly by FAO/WHO (1976) and the biological significance of increased Heinz body formation in the absence of hematologic changes is uncertain. The next highest dietary level in the studies in rats and dogs is 1000 ppm; at which rats had renal lesions and dogs had arrested growth and anemia. Based on authors' estimate of unaffected food consumption, 1000 ppm is equal to 31 mg/kg/day for rats and 21 mg/kg/day for dogs. Therefore, the LOAEL for chronic dietary exposure to diphenylamine is 21 mg/kg/day, and the most suitable NOEL, below which no adverse effects were observed, is 3.1 mg/kg/day from the rat study. Using an uncertainity factor of 100 (10 for interspecies extrapolation and 10 to protect the most sensitive individuals of the human population), an ADI of 0.031 mg/kg/day or 2.17 mg/day for a 70 kg man is derived.

9. REPORTABLE QUANTITIES

9.1. REPORTABLE QUANTITY (RQ) RANKING BASED ON CHRONIC TOXICITY

Several chronic studies describing the effects of oral exposure to diphenylamine were summarized in Table 5-8. In addition, effects on the offspring of female rats treated during gestation were described in Section 5.3., and a reproduction study was summarized in Section 5.4. Data used to calculate CSs are summarized in Table 9-1.

The reproduction study of Thomas et al. (1967a) is not included in Table 9-1 because the apparent reduction in litter size with increasing dietary concentration was secondary to reduced food intake of the parents. Several chronic feeding studies (Safouh et al., 1970; Eknoyan et al., 1976; Evan and Gardner, 1976; Gardner et al., 1976; Evan et al., 1978; Kime et al., 1962; Woodhouse et al., 1965) are not included in Table 9-1 because the relatively high dietary concentrations (1.5-2.5% or 15,000-25,000 ppm) in these studies were administered to rats to study the time-course of appearance and increasing severity of polycystic kidney disease, an effect also observed at lower concentrations ($\geq$0.10% or 1000 ppm in the diet) (Thomas et al., 1967a). Data from the studies in which diphenylamine was administered in the diet to pregnant rats (Crocker et al., 1972; Crocker and Vernier, 1970) are not included in Table 9-1 because the equivalent doses in mg/kg/day are higher than that used in the portion of the Crocker et al. (1972) study, whereby daily gavage dosing of rats during gestation resulted in polycystic kidney disease in the offspring. Finally, data from the Philbert et al. (1979) study are not included because the rats and guinea pigs were dying within the first 6 months of dietary exposure to 2 or 4% (20,000-40,000 ppm).

For the studies included in Table 9-1, dosages in mg/kg/day were calculated from the dietary levels, assuming that the amount of food consumed/day

TABLE 9-1

Composite Scores for Toxicity of Diphenylamine

Species	Dose/Exposure	Chronic Human MED (mg/day)	RV_d	Effects	RV_e	CS	RQ	Reference
Rat	50 mg/kg/day (1000 ppm in diet for 2 years)	598	1.3	Increased incidence and severity of polycystic renal disease and chronic nephritis	7	9.1	1000	Thomas et al., 1967a
Rat	250 mg/kg/day (5000 ppm in diet for 226 days)	2992	1	Suggestion of red blood cell destruction, not further described	4	4	5000	Thomas et al., 1957
Rat	500 mg/kg/day (10,000 ppm in diet for 2 years)	5985	1	Increased incidence of hyperplasia of bladder, bladder weight not reported	3	3	5000	Thomas et al., 1957
Rat	57 mg/kg/day by gavage, last 7 days of gestation	682	1.2	Polycystic renal disease in offspring, no maternal toxicity	8	9.6	1000	Crocker et al., 1972
Mouse	6.5 mg/kg/day (50 ppm in diet, 92 weeks)	34	3.2	Increased number of Heinz bodies in red blood cells in absence of other hematologic changes	2	6.4	1000	Coulston et al., 1971
Mouse	32.5 mg/kg/day (250 ppm in diet, 92 weeks)	172	2.1	Increased weight of liver, spleen; increased hemosiderosis in spleen; increased number of reticulocytes in blood, but no changes in hematologic parameters	4	8.2	1000	Coulston et al., 1971
Dog	29 mg/kg/day (1000 ppm in diet for 2 years)	1187	1	Arrested growth with normal food consumption, slightly decreased red blood cell count	4	4	5000	Thomas et al., 1967b
Dog	290 mg/kg/day (10,000 ppm in diet for 2 years)	11,871	1	Fatty changes in the liver, increased hemosiderosis in spleen, liver and bone marrow, pronounced reduction in red blood cell count and hemoglobin concentration, increased kidney weight, impaired liver function (BSP test)	7	7	1000	Thomas et al., 1967b

is equivalent to 5% of the body weight of rats, 13% of the body weight of mice and 2.9% of the body weight of dogs. The equivalent chronic human MEDs were calculated by multiplying the animal dosage in mg/kg/day by the cube root of the ratio of the animal weight (0.35 kg for rats, 0.03 kg for mice and 14 kg for dogs) to the human weight (assumed to be 70 kg), and multiplying by 70 kg. The RV_ds associated with the MEDs range from 1-3.2. In animals, the effects observed associated with the MEDs were ranked as follows. Increased incidence and severity of polycystic kidney disease has an RV_e of 7, except when observed in the offspring of rats treated during gestation and, as a fetotoxic effect, receives an RV_e of 8. The suggestion of red cell destruction that was not further described was considered roughly equivalent to an atrophic change, hence, an RV_e of 4. Increased incidence of bladder hyperplasia without evidence of hypertrophy ranks 3. Increased number of Heinz bodies without other hematologic changes observed in mice ranks 2, while increased liver and spleen weights along with hemosiderosis of the spleen is a more serious effect, receiving an RV_e of 4. The growth inhibition in dogs is ranked 4, while the impaired liver function along with other effects has an RV_e of 7.

The CSs for these dose-effect data are obtained by multiplying the RV_d by the RV_e. The highest CS of 9.6 was obtained from the study of Crocker et al. (1972), in which offspring of rats treated by gavage with diphenylamine at 57 mg/kg/day during the last 7 days of gestation had polycystic renal lesions. The CS corresponds to an RQ of 1000 (Table 9-2).

9.2. WEIGHT OF EVIDENCE FOR CARCINOGENICITY AND DERIVATION OF POTENCY FACTOR ($F=1/ED_{10}$)

According to SRC (1983), pertinent data regarding the carcinogenicity of diphenylamine in animals were not located in the available literature; however, several negative studies were summarized in Section 5.1. of this

TABLE 9-2

Diphenylamine

Minimum Effective Dose (MED) and Reportable Quantity (RQ)

Route:	oral
Dose*:	682
Effect:	polycystic kidney disease in offspring
Reference:	Crocker et al., 1972
RV_d:	1.2
RV_e:	8
Composite Score:	9.6
RQ:	1000

*Equivalent human dose

profile (see Tables 5-1, 5-2 and 5-4). In a -year feeding study, no increased incidence of tumors was found in rats treated at dietary levels ≤1.0% (10,000 ppm) (Thomas et al., 1967a). Female rats treated by gavage with a single dose of 300 mg/rat did not develop tumors after 6 months of observation (Griswold et al., 1966). Mice maintained on diets containing diphenylamine at ≤250 ppm for 92 weeks did not have increased incidences of tumors compared with controls (Coulston et al., 1971). Data regarding the carcinogenic effect of diphenylamine in humans were not located in the available literature as cited in the Appendix.

Since positive studies of the carcinogenic potential of diphenylamine were not available, the derivation of the potency factor is precluded. This chemical should be placed in IARC Group 3, as it cannot be classified as to its carcinogenicity for humans. Also, according to the U.S. EPA Cancer Assessment Group's proposed cancer risk guidelines, this chemical would be in group D, not adequate evidence for evaluation as a human carcinogen.