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DIPHENYLAMINE

REVIEW AND EVALUATION OF ADI

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LIST OF ABBREVIATIONS

ADI	Acceptable daily intake
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
ppm	Parts per million
TLV	Threshold limit value

1. REVIEW AND EVALUATION OF ADI

1.1. REVIEW OF ADI

The Environ, Inc. (1984) ADI for diphenylamine is 0.05 mg/kg/day (uncertainty factor=100) and was derived from a rat subchronic to chronic oral NOEL from an unspecified study in which higher doses caused renal cysts. The ADI was taken from the U.S. EPA (1984) list of ADIs that cited an earlier U.S. EPA (1982) document in which an ADI of 3.6 mg/day for a 70 kg human (0.051 mg/kg/day) was derived from the TLV, however, the U.S. EPA (1985) list of ADIs reports only an interim ADI of 0.031 mg/kg/day. This ADI is based on a rat chronic oral NOEL of 3.1 mg/kg/day where higher doses caused renal cysts. The NOEL was reported to be 3.1 mg/kg/day by U.S. EPA (1985) and was attributed to a study by Thomas et al. (1967a). An uncertainty factor of 100 was applied. In this study, groups of 40 weanling albino rats (Slonaker-Addis strain, 20/sex) were fed diets containing 0, 0.001, 0.01, 0.1, 0.5 and 1.0% diphenylamine for 2 years. Depressed growth attributed to significantly lower food consumption occurred at the two highest doses. A slight inhibition of growth apparently caused by diphenylamine and not by lower food consumption, occurred in females at the 0.1% level. Rats receiving $\geq$ 0.1% diphenylamine exhibited kidney lesions, (i.e., cystic dilatation of renal tubules with interstitial inflammation). Selected hematological parameters, measured in control and high dose rats at intervals up to day 463 of exposure, were not affected by treatment and treatment appeared to have no effect on survival. No adverse effects were observed in rats receiving dietary concentrations of $\geq$ 0.01% diphenylamine. At this dietary level, U.S. EPA (1985) appears to have calculated a dose of 3.1 mg/kg/day for rats from body weight and food consumption data provided by the investigators.

1.2. OTHER RELEVANT INFORMATION

Diphenylamine is a fungicide which causes effects similar to those of clinical polycystic kidney disease in mammals. As described by Smith et al. (1985), several studies reported toxic effects when diphenylamine was administered in the diet of rats at concentrations of $\geq$ 1% Since these studies will not affect the risk assessment, they will not be discussed; however, studies that reported toxic effects and NOAELs at lower doses will be discussed.

Thomas et al. (1957) reported toxic effects (growth inhibition, renal tubule dilatation) in rats exposed to dietary concentrations of $\geq$ 0.5% diphenylamine These effects were not observed in rats receiving 0.025 or 0.1% diphenylamine.

Coulston et al. (1971) fed mice diets containing 0, 50, 100 and 250 ppm for up to 92 weeks. They observed increased spleen weights, increased hemosiderosis in spleens, and increased numbers of blood reticulocytes, evidence of increased erythrocytopoiesis in the highest dose group. Dose related increases in Heinz bodies in red blood cells also occurred at concentrations of $\geq$ 50 ppm; however, the biological significance of this effect in the absence of hematological changes is uncertain (Smith et al., 1985).

Thomas et al. (1967b) conducted an experiment in which groups of four beagle dogs were fed diets containing 0, 0.01, 0.1 or 1.0% diphenylamine for 2 years. In dogs receiving the highest dose, adverse effects included fatty changes in the liver, hemosiderosis in the spleen, bone marrow and kidneys, and an increase in kidney weight. Dogs fed 0.1 or 1.0% diphenylamine exhibited depressed growth and anemia; however, no toxic effects were observed at 0.01%. The authors reported that at the end of the study, the two female

dogs receiving 0.01% diphenylamine had each consumed 14 g diphenylamine or 1.54 g/kg. Dividing this value by 730 days, the duration of the study, results in an ADI of 2.11 mg/kg/day.

There is no evidence available that indicates that diphenylamine is carcinogenic. No increase in tumor incidence occurred in the chronic feeding studies in rats (Thomas et al., 1967a) or mice (Coulston et al., 1971. A combined in vivo/in vitro bioassay for neoplastic transformation in hamsters yielded negative results for diphenylamine but positive results for known carcinogens. Diphenylamine was also negative in several mutagenicity assays using bacteria and cultured mammalian cells, as summarized by Smith et al. (1985). Diphenylamine, however, may be converted to carcinogenic nitrosamines in the stomach by reaction with nitrite. N-nitrosodiphenylamine has been found to cause urinary bladder carcinomas in rats. A common impurity of diphenylamine, 4-aminobiphenyl, is also considered a carcinogen (IARC, 1972). Diphenylamine has not been scheduled for testing by the National Toxicology Program (NTP 1985).

There is little information available concerning the possible teratogenic effects of diphenylamine. Pregnant rats received diphenylamine in the diet (1.5 or 2.5%) or by gavage for the last 7 days of gestation (Crocker et al., 1972). Although dams were unaffected, cystic lesions occurred in the proximal nephrons of offspring. The severity of the lesions decreased greatly when purified diphenylamine was used, and a purified contaminant of commercial diphenylamine produced similar lesions. Therefore, the authors concluded that this contaminant rather than diphenylamine might be the nephrotoxic component of commercial diphenylamine.

ACGIH (1984) lists a TLV for diphenylamine in the atmosphere of 10 mg/m^3.

1.3. EVALUATION OF ADI

The ADI of 0.05 mg/kg/day listed by Environ, Inc. (1984) for diphenylamine should be rejected because Environ, Inc. (1984) cited the U.S. EPA (1984) list of ADIs which erroneously reported that the ADI was based on the Thomas et al. (1967a) study in rats rather than on the human TLV. The U.S. EPA (1985) reported an ADI of 0.031 mg/kg/day based on the rat NOEL of 3.1 mg/kg/day (Thomas et al., 1967a) with an uncertainty factor 100. Apparently, the U.S. EPA (1985) calculated the dose of 3.1 mg/kg/day from body weight and food consumption data provided by the investigators. Since long-term data in mice (Coulston et al., 1971) and dogs (Thomas et al., 1967b) support the NOEL in the rat study, the ADI of 0.031 mg/kg/day (2.17 mg/day for a 70 kg human) calculated by the U.S. EPA (1985) is recommended as the provisional ADI for diphenylamine.

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