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HEALTH ADVISORY FOR DIPHENYLAMINE (DPA)

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PREFACE

This report was prepared in accordance with the Memorandum of Understanding between the Department of the Army, Deputy for Environmental Safety and Occupational Health (OASA(IL&E)), and the U.S. Environmental Protection Agency (EPA), Office of Water (OW), Office of Science and Technology for the purpose of developing drinking water Health Advisories (HAs) for selected environmental contaminants, as requested by the Army.

Health Advisories provide specific advice on the levels of contaminants in drinking water at which adverse health effects would not be anticipated and which include a margin of safety so as to protect the most sensitive members of the population at risk. A Health Advisory provides health effects guidelines and analytical methods, and recommends treatment techniques on a case-by-case basis. These advisories are normally prepared for One-day, Ten-day, Longer-term, and Lifetime exposure periods where available toxicological data permit. These advisories do not condone the presence of contaminants in drinking water, nor are they legally enforceable standards. They are not issued as official regulations, and they may or may not lead to the issuance of national standards or Maximum Contaminant Levels (MCLs).

This report is the product of the foregoing process. Available toxicological data, as provided by the Army and as found in open literature sources, on the munitions chemical diphenylamine have been reviewed, and relevant findings are presented in this report in a manner so as to allow for an evaluation of the data without continued reference to the primary documents. This report has been submitted for critical internal and external review by the EPA.

I would like to thank the authors, Drs. B. Ram Das, Mary B. Deardorff, and Welford C. Roberts, who provided the extensive technical skills required for the preparation of this report. I am grateful to the members of the EPA Tox-Review Panel who took time to review this report and to provide their invaluable input, and I would like to thank Dr. Edward Ohanian, Chief, Human Risk Assessment Branch, and Ms. Margaret J. Stasikowski, Director, Health and Ecological Criteria Division, for providing me with the opportunity and encouragement to be a part of this project.

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EXECUTIVE SUMMARY

N,N-Diphenylamine or diphenylamine (DPA), also called N-phenylbenzeneamine, is a crystalline solid at room temperature and the simplest of the diarylamines. Diarylamines are aromatic organic compounds with two of the hydrogen atoms of ammonia replaced by aryl groups. Diphenylamine is practically insoluble in water (30-35.7 mg/L at 25°C) but soluble in several organic solvents. Diphenylamine is produced by self-condensation of aniline in the presence of ferrous chloride, ammonium bromide, and a small amount of a strong mineral acid. It is a chemically reactive compound. The U.S. Tariff Commission listed five domestic producers of DPA in 1975 with an annual production of 18,094 tons. Diphenylamine is also imported.

Diphenylamine is used to stabilize nitrocellulose explosives and celluloid in various gun propellant compositions. It is also used extensively as a dip spray and impregnate of paper wraps to prevent scald on apples and other fruits, and as an insecticide. Therapeutically, DPA derivatives are used to treat helminthic infections in animals and humans. Diphenylamine is used in the manufacturing of dyes, polymers, greases, and oils, in producing industrial antioxidants for rubber, and as an analytical reagent. Diphenylamine has been detected in the effluent of manufacturing plants in California, in Rhine River water, and in Norwegian rain water. It was also detected in trace amounts in the ambient air of Geismar, LA. The U.S. Food and Drug Administration's Monitoring program and Total Diet Program, and the U.S. Department of Agriculture's National Residue Program found DPA of undetermined concentration in only one fruit sample and one infant and junior food formula in several thousands of samples tested during 1970-1976.

Only a modicum of data are available on the fate of DPA in the environment. Information about potential hydrolysis of DPA and photolysis in aqueous solutions are lacking. Rapid microbial degradation of DPA occurred in a laboratory-model sewage sludge system. Based on its solubility and calculated vapor pressure, DPA is predicted to volatilize slowly. It is considered to have a medium-to-low mobility in soils.

Although no specific animal or human data on the absorption and distribution of DPA following ingestion were located, metabolic studies of DPA suggest that absorption occurs to some extent. Diphenylamine is rapidly excreted in the urine and is found in the bile of male rats intraperitoneally or intravenously injected with the compound. In human urine samples following a single oral dose

of DPA, unchanged DPA, 4-Hydroxydiphenylamine (4-HDPA), and 4,4'-Dihydroxydiphenylamine were detected (there were no adverse effects reported in this study). Glucuronic acid conjugate of 4-HDPA was the major metabolite found in the urine of rats intraperitoneally injected with a dose of either DPA, 4-HDPA, or N-hydroxydiphenylamine.

Except for some dermal studies in occupational workers, no epidemiological, clinical case histories, or experimental studies of potential human health effects to DPA exposure are available. Diphenylamine was not irritating to the skin of humans or rabbits.

Reported oral LD_{50}s for rats are 1.165 g/kg, 3.2 g/kg, and 2.0 g/kg; for mice 1.75 g/kg; and for guinea pigs 300 mg/kg. Short-term studies with Sprague-Dawley rats, Syrian hamsters, and Mongolian gerbils given 400, 600, or 800 mg DPA/kg/day in peanut oil once a day by gavage for 3 days showed that Syrian hamsters are more sensitive to DPA-related mortality and renal pathologic damage than either the rats or gerbils. These authors suggested a No-Observed-Adverse-Effect Level (NOAEL) of 400 mg/kg/day for acute renal pathologic changes (not including papillary necrosis) in rats. However, in the Syrian hamsters, this dose was a Frank-Effect-Level (FEL) because it produced 40% mortality and total renal papillary necrosis and splenomegaly in 90% of the animals. In another gavage study, observed necrosis of renal papilla and pars recta in Wistar rats given 4.1 mM (694 mg) DPA/kg for 3 or 9 days. In mice gavaged once with 600 mg DPA/kg, observed high mortality and pathological abnormalities of the intestine, kidney, and liver.

In male and female F344 rats (6/group), daily doses of DPA (111, 333, or 1,000 mg/kg/day) administered in olive oil by gavage for 28 days caused a treatment-related decrease in body weight gain and in relative organ weights (most notably the liver); depressed erythrocyte and hemoglobin levels; increased leukocytes, serum albumin, bilirubin, and potassium levels; and renal tubular degeneration and necrosis, congestion and extramedullary hematopoiesis of the spleen, bone marrow hyperplasia, and mucosal changes in the forestomach. Based on reduced liver weights and increased serum albumin levels, the Lowest-Observed-Adverse-Effect Level (LOAEL) for this study is 111 mg/kg/day, the lowest dose tested. A NOAEL was not identified.

In a 8-month study, investigated the effects of DPA on growth and the anatomy of selected internal organs in 36 female weanling albino rats fed 0.025, 0.1, 0.5, 1.0, or 1.5% DPA in the diet (corresponding to calculated doses of 27.5, 110, 550, 1,100, and 1,650 mg/kg/day, respectively) for

226 days. Dietary levels of 0.5% DPA or more caused a dose-related decrease in body weight gain, hematogenic granular pigmentation in the liver and kidneys, and focal dilatation of renal tubules with multiple cystic structures. The study suggested a NOAEL of 0.1% (calculated dose of 110 mg/kg/day) for body weight gain and renal pathological changes in female rats.

A lifetime study in which Slonaker-Addis rats (20/sex/group) were fed diets containing 0.001, 0.01, 0.1, 0.5, or 1% DPA (corresponding to calculated doses of 1.1-999 mg/kg/day for males and 0.96-812 mg/kg/day for females) for 2 years showed several DPA-related effects. Levels above 0.1% dietary DPA depressed body weight gain without decreasing food consumption, and at the 0.5 and 1% levels anemia characterized by reduced hemoglobin and erythrocyte levels occurred. Dilated cystic renal tubules accompanied by chronic interstitial nephritis occurred at the 0.1% level or more. This study suggested a NOAEL of 0.01% (calculated dose of 9.6 mg/kg/day). An unpublished report of this study indicated that levels above 0.5% dietary DPA depressed body weight gain without decreasing food consumption, and levels at or above the 1% dietary DPA level, anemia characterized by reduced hemoglobin and erythrocytes occurred. Dilated cystic renal tubules accompanied by chronic interstitial nephritis occurred at or above the 0.5% level. These results suggest a NOAEL of 0.1% (calculated dose of 100 mg/kg/day).

In a similar study, used a small number of beagle dogs fed a diet containing 0.01%, 0.1%, or 1% (2.5, 25, and 250 mg/kg/day, respectively) DPA for 2 years and observed decreased body weight gain at or above the 0.1% DPA level and a possible treatment-related decrease in hemoglobin and erythrocytes at the 0.1% level. Some pathological changes were found in the liver, spleen, kidneys, and bone marrow at the 1% level. Kidney function tests were negative. The study suggested a NOAEL of 0.01% (calculated dose of 2.5 mg/kg/day). According to an unpublished report of this study, growth inhibition, decreased blood hemoglobin and erythrocyte levels, and pathological changes occurred at the 1% level suggesting a NOAEL of 0.1% (calculated dose of 25 mg/kg/day).

Renal tubular dilatation and intrarenal cysts in rats were caused by the daily gavage of DPA at 4.1 mM (694 mg) DPA/kg daily for 3-9 days and at 1.6 mM (271 mg) DPA/kg for 1-8 weeks, and by the continuous ingestion of a diet containing DPA at 2.5% for 3-6 weeks, at 2.5% for 19 weeks, at 1.5% or 2.5% for 0.5-12 months, at 2.5% for 12 months, at 1% DPA for 18 months, and at 1% DPA for 5-20 months. Renal pathologic changes also were observed in male NMRI mice given 1.4 g DPA/kg/day doses by oral gavage for 10 weeks.

Slonaker-Addis rats of both sexes maintained on a diet containing 0.1, 0.25, or 0.5% (100, 250, and 500 mg/kg/day, respectively) DPA starting at 35 days of age were mated twice and their offspring were mated. Average litter size decreased as DPA level in the diet increased. No DPA-related effects on litter size or survival in female Wistar rats given 1,000 mg/kg/day by oral gavage during days 7-17 of gestation were found. Renal cystic dilatation of the collecting ducts and vacuolar degeneration of the proximal tubules were reported in all newborn rats (species not specified) from dams fed 200 mg DPA/kg/day by gastric tube or 2.5% dietary DPA (corresponding to a calculated dose of 2,500 mg/kg/day) during the last 6 days of gestation. In a later study, the DPA-related effects on the kidney of second generation Sprague-Dawley rats was attributed to an unidentified contaminant chromatographically isolated from DPA. This contaminant was given to dams by gastric tube at 50 µg/day in 2 mL of 70% ethanol (calculated to be 0.5 mg/kg/day) during the last 6-7 days of gestation. No significant cystic changes occurred in newborns from dams intragastrically intubated with chemically pure DPA (20 mg DPA/rat in 2 mL of ethanol; calculated to be 200 mg/kg/day).

Diphenylamine has been shown to be noncarcinogenic in Sprague-Dawley rats given a single oral dose of 300 mg DPA/kg over a 6-month period and in Slonaker-Addis rats given daily DPA in the diet at concentrations of 0.001-1% over a 2-year period. Also, no evidence of dietary DPA-induced carcinogenicity occurred in mice over a 92 week period. Negative results were obtained in a bioassay involving *in vivo* exposure of Syrian hamster fetuses to DPA and *in vitro* culture of exposed fetal cells to observe neoplastic transformation.

Diphenylamine tested negative in the Ames reverse mutation assay in *S. typhimurium* strains TA98, TA100, TA1000, TA1535, TA1537, TA1538, C3076, D3052, and G46 with and without S9 activation as well as in *E. coli* strains WP2 and WP2uvrA- with or without S9 activation. Diphenylamine also tested negative in isolated mouse lymphoma cells with S9 activation and did not cause unscheduled DNA synthesis in cultured rat hepatocytes.

No suitable study was found for the determination of the One-day HA for a 10 kg child. Therefore, the Ten-day HA of 1.0 mg/L for a 10 kg child is recommended as a conservative estimate for the One-day HA. The Ten-day HA was based on a 28-day feeding study in rats from which a LOAEL of 111 mg/kg/day was derived based on reduced weights of liver, kidney, and spleen. The Longer-term HAs of 0.3 mg/L for a 10 kg child and 0.9 mg/L for a 70 kg adult were based on a

chronic feeding study in dogs from which a NOAEL of 2.5 mg/kg/day was derived based on the absence of growth retardation and adverse hematological effects. The Lifetime HA of 0.2 mg/L was based on a drinking water equivalent level of 1.0 mg/L, which was derived from a reference dose of 0.03 mg/kg/day. The above mentioned dog study was used for the derivation of the Lifetime HA.

Diphenylamine is classified as Group D: not classifiable as to human carcinogenicity. However, chronic/lifetime bioassays in several mammalian species and the lack of mutagenicity provide more useful information to the risk assessor than a Group D classification chemical with no bioassay data.

A specific method for analyzing DPA in water was not found in the available literature. A gas chromatography method for recovering DPA in apples, and a method for determining nitrate esters, stabilizers, and plasticizers including DPA using gas-liquid chromatography have been reported. No specific DPA water treatment methods were located in the literature. The U.S. EPA carbon-adsorption isotherm for the adsorption of DPA on activated carbon is 120 mg/g.

A comparison report, *Data Deficiencies, Problem Areas, and Recommendations for Additional Database Development for Diphenylamine* (Appendix A), summarizes the scope of existing data reviewed for this HA. The report delineates the areas where additional data or a clarification of existing data would be appropriate for a revision of this HA.

I. INTRODUCTION

The Health Advisory (HA) Program, sponsored by the Office of Science and Technology (OST), provides information on the health effects, analytical methodology, and treatment technology that would be useful in dealing with the contamination of drinking water. Health Advisories describe nonregulatory concentrations of drinking water contaminants at which adverse health effects would not be anticipated to occur over specific exposure durations. Health Advisories contain a margin of safety to protect sensitive members of the population.

Health Advisories serve as informal technical guidance to assist Federal, State, and local officials responsible for protection of public health when emergency spills or contamination situations occur. They are not to be construed as legally enforceable Federal standards, and they are subject to change as new information becomes available.

Health Advisories are developed for One-day, Ten-day, Longer-term (approximately 7 years, or 10% of an individual's lifetime), and Lifetime exposures based on data describing noncarcinogenic endpoints of toxicity. Health Advisories do not quantitatively incorporate any potential carcinogenic risk from such exposure. For substances that are known or probable human carcinogens, according to the Agency classification scheme (Group A or B), Lifetime HAs are not recommended. The chemical concentration values for Group A or B carcinogens are correlated with carcinogenic risk estimates by employing a cancer potency (unit risk) value together with assumptions for lifetime exposure and the consumption of drinking water. The cancer unit risk is usually derived from the linearized multistage model with 95% upper confidence limits. This provides a low-dose estimate of cancer risk to humans that is considered unlikely to pose a carcinogenic risk in excess of the stated value. Excess cancer risk estimates may also be calculated using the one-hit, Weibull, logit, and probit models. There is no current understanding of the biological mechanisms involved in cancer to suggest that any one of these models is able to predict risk more accurately than another. Because each model is based upon differing assumptions, the estimates that are derived can differ by several orders of magnitude.

II. GENERAL INFORMATION

N,N-Diphenylamine or diphenylamine (DPA), also called N-phenylbenzeneamine, is the simplest of the diarylamines, which are aromatic organic compounds with two of the hydrogen atoms of ammonia replaced by aryl groups (Kirk-Othmer, 1978). It is manufactured by self-condensation of aniline in the presence of ferrous chloride, ammonium bromide, and a small amount of a strong mineral acid such as anhydrous hydrochloric acid, to yield aniline hydrochloride. Aniline hydrochloride is then heated with aniline to yield DPA. The overall reactions for the manufacture of DPA are presented in Figure II-1.

DPA is a crystalline solid compound at room temperature with a floral odor and is practically insoluble in water but soluble in several organic solvents. Selected physical and chemical properties of DPA are presented in Table II-1. Diphenylamine is a chemically reactive compound (Kirk-Othmer, 1978; Zbozinek, 1984). It forms salts with strong acids. The hydrogen atom attached to nitrogen can be replaced by alkali metals. For example, it reacts with metallic aluminum to form aluminum diphenylamide. With formaldehyde it forms tetraphenylmethylenediamine, and with acetone it reacts to form a variety of products depending upon experimental conditions. It can be oxidized with potassium permanganate to form tetraphenylhydrazine. It is easily dehydrogenated to form carbazole. Reaction with sulfur produces phenothiazine. Nitration usually gives rise to a trinitro derivative while reaction with nitrous acid produces diphenylnitrosamine, and halogenation yields a tetrahalo derivative.

The United States (U.S.) Tariff Commission listed five domestic producers of DPA in 1974 with an annual production growing modestly from 14,340 tons in 1966 to 18,094 tons in 1974 (Kirk-Othmer, 1978). Imports through major U.S. ports in the years 1981, 1982, and 1983 were 100,000, 550,000, and 610,000 pounds, respectively (U.S. EPA, 1985).

DPA is used in the manufacture of dyes, in stabilizing nitrocellulose explosives and celluloid, in various gun propellant compositions, in the production of industrial antioxidants for rubber, polymers, greases and oils, and as an analytical reagent in the detection and determination of nitrate, chlorate and other oxidizing ions. DPA is extensively used as a dip spray or impregnate of paper wraps to prevent storage scald in apples and other fruits and as an insecticide for preservation of

Figure II-1. Self Condensation Reactions of Aniline for Manufacture of Diphenylamine

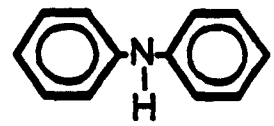
I. $C_6H_5NH_2 + HCl \rightarrow C_6H_5NH_2HCl$

II. $C_6H_5NH_2HCl + C_6H_5NH_2 \xrightarrow{300°C} (C_6H_5)_2NH + NH_4Cl$

III. $NH_4Cl + C_6H_5NH_2 \rightarrow C_6H_5NH_2HCl + NH_3$

SOURCE: Adapted from Kirk-Othmer (1978).

Table II-1. General Chemical and Physical Properties of Diphenylamine

Property	Value/Description
CAS Number	122-39-4
Synonyms	N,N-Diphenylamine, N-Phenylaniline, N-Phenylphenate, Phenylbenzeneamine, Anilinobenzene, Big Dipper, Scaldip, No Scald
Molecular Weight	169.23
Empirical Formula	$C_{12}H_{11}N$
Structure	
Physical State	Crystalline monoclinic leaflets, Colorless to grayish solid
Melting Point	52.8-55°C
Boiling point	302°C
Specific gravity (25°C)	1.159
pK_a	0.78; 0.8
Heat of combustion (kJ/M)	6409.5 at 25°C
Vapor pressure (mm Hg)	1 at 108.3°C
Octanol/water partition coefficient (K_{ow})	3.50; 3.42; 3.37
Solubility characteristics	
Water	30-35.7 mg/L at 25°C
Organic solvents	Ethyl alcohol 44 g/L; Methyl alcohol 57.5 g/L; Very soluble in acetone and benzene

SOURCE: Adapted from Budavari, 1989; Zbozinek, 1984; U.S. EPA, 1985; Sweet, 1987; Kirk-Othmer, 1978; Clayton and Clayton, 1981; Perry and Chilton, 1973; Eadsforth and Moser, 1983; Rao and Hayon, 1975.

flowers and vegetables. Diphenylamine and some of its complex derivatives are used to treat animals and humans with parasites such as several species of Schistosomes, intestinal Nematodes, and Filariae (Budavari, 1989; Kirk-Othmer, 1978; Crawford *et al.*, 1983; Doshi *et al.*, 1977; Shapiro *et al.*, 1986; Singh *et al.*, 1981; Vaidya *et al.*, 1977; Liu *et al.*, 1983; Minggang, 1985; Gang and Quan, 1986).

III. SOURCES OF EXPOSURE

The U.S. EPA STORET database contains gross analysis data from 152 observations for DPA, which ranged from 3.2-10.0 µg/L with a mean concentration of 5 µg/L (U.S. EPA, 1985). Diphenylamine was detected in the effluent from a Lake Tahoe plant at a level of 1.54 ppt and in the effluent from a Ponoma, CA, plant at 9.77 ppt (Pahren and Melton, 1979 cited in U.S. EPA, 1985). Diphenylamine was also detected in raw Rhine river water at 1 ppb (Piet and Morra, 1983 cited in U.S. EPA, 1985) and in rain water in Norway, which indicates the existence of DPA in ambient air of Norway (Lunde *et al.*, 1977 cited in U.S. EPA, 1985). Only trace but not quantified amounts of DPA were detected in a few samples of ambient air in Geismar, LA, area (Pellizzari, 1978 cited in U.S. EPA, 1985).

Pesticide residue levels monitored by Federal Programs in American Foods were reported (Duggan *et al.*, 1983). Examination of 33,000 domestic and >18,000 imported samples by the U.S. Food and Drug Administration's (FDA) Monitoring Program for raw agriculture commodities, >15,000 red meats and 11,000 poultry samples by the U.S. Department of Agriculture's National Residue Program, and an unspecified number of samples of ready-to-eat foods by FDA's the Total Diet Program revealed DPA of undetermined concentration in only one fruit sample, and only one infant and junior food formula during 1970-1976.

IV. ENVIRONMENTAL FATE

Very little information is found in the published literature on the environmental fate of DPA in water, soil, or air. The transport of DPA in the aquatic environment may be considered to depend on its solubility, volatility, adsorption to particulate matter and sediment, and bioaccumulation by aquatic organisms. Atmospheric chemical removal of DPA may be possible but pertinent data are not available. Cupitt (1980) calculated the dissolution half-life in the atmosphere of about 1.3 years for DPA based on an assumed annual rain fall of 0.75 m and a homogeneous atmosphere of 8 km height.

A. PHOTOLYSIS

DPA discolors when exposed to day light and air, becoming tan and then blue to purple, suggestive of the formation of N-Phenylbenzoquinoneimine, indophenol, possibly indoaniline, and Benzidine Blue and its N,N'-derivatives (Chemley Products Company cited in Zbozinek, 1984). Direct photolysis of DPA in a variety of organic compounds such as ketones, aromatic hydrocarbons, and several dyes has been demonstrated, but information on photolysis in aqueous solutions with environmental photosensitizers such as humic and fulvic acids is not available (U.S. EPA, 1985).

B. BIOTRANSFORMATION

Microbial degradation in a laboratory model sewage sludge system produced approximately 96% degradation of DPA (65% within the first 6 hours) whereas controls using sterilized activated sludge produced no significant degradation (U.S. EPA, 1985). Metabolites of degradation were identified by gas chromatography and mass spectrometry, and they consisted of 4-hydroxyphenylamine and one of its isomers, aniline, and indole. Ten of 25 species of microorganisms obtained from sources such as lake water, garden soil, sewage, cow manure, and stock cultures were found to generate N-nitrosodiphenylamine after 2-10 days of incubation with DPA in a mineral salt medium (Ayanaba and Alexander, 1973). Although acidic conditions favor nitrosation, many reports indicate that nitrosamines can be formed at neutral pH in the presence of microorganisms (U.S. EPA, 1985; Ayanaba and Alexander 1973; Hawksworth and Hill, 1971).

Some investigators have shown that DPA moderately bioaccumulates in fathead minnows and other fish (U.S. EPA, 1985).

C. HYDROLYSIS

No specific data on the hydrolysis of DPA in the environment were available in the published literature.

D. SORPTION ON SEDIMENT AND SOIL

A slow environmental volatilization was predicted for DPA based on its solubility and a calculated vapor pressure (U.S. EPA, 1985). No experimental aqueous evaporation studies are available for DPA. Based on experimental determinations, soil organic matter-water distribution (K_{om}) and soil organic carbon-water distribution (K_{oc}) values for DPA in four loam soils (organic matter content of 1.09-4.25%) were about 350 and >1,000, respectively (Briggs, 1981). DPA was thus considered to have a medium-to-low mobility in soils which means that some partitioning between the water and sediment matrices of the aquatic environment may occur although the quantitative significance of adsorption is difficult to ascertain.

V. TOXICOKINETICS

A. ABSORPTION

Specific data relating to the absorption of DPA following oral administration in experimental animals or in humans were not found in the published literature. However, metabolic studies with DPA in rats, rabbits, and humans following oral or intraperitoneal doses suggest that absorption does occur to a certain extent (Alexander *et al.*, 1964, 1965).

B. DISTRIBUTION

Data on the distribution of DPA or its metabolites following oral or other routes of administration in animals or in humans were not found in the available literature.

C. METABOLISM

Ether extracts of the urine samples of two human individuals orally administered with a single dose of 100 mg DPA contained unchanged DPA, 4-Hydroxydiphenylamine (4-HDPA) and 4,4'-Dihydroxydiphenylamine (DHDPA); 2-Hydroxydiphenylamine (2-HDPA) was not detected either before or after hydrolysis of the urine. DPA could be extracted from the urine with ether prior to but not after acid hydrolysis, and therefore, it is believed to have been excreted in the urine in a non-conjugated form (Alexander *et al.*, 1965).

In an unpublished report of a feeding study (Booth, 1963) weanling rats (20/sex/group, species not identified) were maintained on dietary levels of DPA at 0.025, 0.1, 0.5, and 1.5% for 226 days, the following metabolites were identified in the urine and feces: 4-HDPA, its sulfate and glucuronide conjugates, and DHDPA. DHDPA was also formed following *in vitro* incubation of rat liver extract with DPA.

A glucuronic acid conjugate of 4-HDPA was identified as the major metabolite in the urine of rats administered intrapertoneally with single 5 mg doses of DPA, 4-HDPA, or N-hydroxydiphenylamine (Alexander, 1964). Neither N-hydroxydiphenylamine, 2-HDPA, nor unchanged DPA were detected in the hydrolyzed urines. However, in a more detailed publication, the presence of 4-HDPA

and DHDPA was confirmed in acid or beta-glucuronidase hydrolysed rat urine (Alexander *et al.*, 1965).

Alexander *et al.* (1964) identified 4-HDPA and trace amounts of 2-HDPA in the ether extract of the unhydrolyzed as well as β-glucuronidase hydrolyzed urine of a male rabbit orally dosed with a total of 5 g of DPA in 1 g doses over 9 days. Additionally, the potassium salt of the sulfate ester of 4-HDPA was identified indicating direct hyroxylation of the aromatic ring followed by conjugation in this species. Only in the rabbit urine sample, the ether extracted urine, and the ether extract of aqueous residue treated with β-glucuronidase but not acid, contained 2-HDPA as a minor component (Alexander *et al.*, 1965). The two crystalline compounds identified in the urine of rabbits were diphenylamine 4-methyltri-O-acetylglucuronidate and potassium diphenylamine 4-sulfate. The isolation of these conjugates suggests that N-hydroxylation occurs in the rabbit. Further details of the metabolites of DPA in rat, rabbit, and man are presented in Table V-1.

An unpublished report (Booth, 1963) identified 4-HDPA, its sulfate and N-glucosiduronide, and DHDPA in beagle dogs (2/sex) placed on diets containing 0.01, 0.1, or 1.0% (2.5, 25, and 250 mg/kg/day, respectively) DPA in the diet for 2 years. 4-HDPA also was found in dog bile after acid hydrolysis.

Formation of diphenylnitrosamine, a carcinogen (NCI, 1979), was demonstrated in stomach contents of rats orally administered with a single 5 mg dose of DPA combined with 10 mg of sodium nitrite (Galea *et al.*, 1975) or with a solution of sodium nitrite (Sander *et al.*, 1968).

D. EXCRETION

Rapid excretion of DPA was indicated in male rats following administration with one intra-peritoneal injection of ^{14}C-DPA (Alexander *et al.*, 1965). About 75% of the administered radioactivity was recovered in the urine samples collected at 24-hour intervals, for 48 hours.

Administration of ^{14}C-DPA at 5 mg/kg by slow intravenous injection of a 50% aqueous ethanol solution (2 mg/mL) to male rats with cannulated bile ducts resulted in excretion of 25% of the radioactivity in bile samples collected at 1-hour intervals over 6 hours (Alexander *et al.*, 1965).

Table V-1. Metabolites of Diphenylamine in Rat, Rabbit, and Man[a]

METABOLITE	RABBIT			RAT				MAN	
	A	B	C	D	E	F	G	A	B
Diphenylamine	+	-	-	-	-	-	-	+	-
2-Hydroxydiphenylamine	+	-	+	-	-	-	-	-	-
4-Hydroxydiphenylamine	+	+	+	+	+	+	+	-	+
4,4'-Dihydroxydiphenylamine	+	+	+	+	+	+	+	-	+

A— urinary ether extract; B— ether extract of acid hydrolysed aqueous residue from A; C— ether extract of aqueous residue from A after treatment with β-glucuronidase; D— ether extract of acid hydrolysed urine; E— ether extract of β-glucuronidase-treated urine; F— ether extract of acid hydrolysed bile; G— ether extract of β-glucuronidase-treated bile.
[a]Identified by thin-layer chromatography

SOURCE: Adapted from Zbozinek (1984).

VI. HEALTH EFFECTS

A. HUMANS

No epidemiological, clinical case histories, or experimental studies dealing with the adverse health effects of exposure to DPA were located in the published literature. Three reports investigated possible dermal effects of DPA but none were found (Calnan, 1978; Bazin *et al.*, 1980; Slovak, 1980).

The effect of DPA on human skin was investigated in occupational workers working in a quality control laboratory of a chemical firm in Leics, England (Slovak, 1980). None of the 16 men subjected to a patch test with 1% DPA developed an immediate or delayed sensitivity reaction.

Bazin *et al.* (1980) conducted a patch test on an occupational worker, a 44-year old woman who worked in a circuit breaker factory and handled metals, plastics, and greases that contained DPA. Patch tests (details of testing not given) were negative for 1% DPA. Routine patch testing of 1,012 eczema patients with 1% DPA did not reveal appreciable dermatitic hazard as reported by Calnan (1978) but three positive reactions were attributed to a cross reaction with paraphenylenediamine (PPDA). The positive reactions were in three patients allergic to PPDA. Closed-patch skin testing with 1% DPA in petrolatum produced no irritation in human subjects (Epstein, 1976 cited in Opdyke, 1978).

B. ANIMAL EXPERIMENTS

1. Short-term Exposure

a. Acute

Various investigators reported acute oral LD50s for rats to be 1.165 g/kg (Levenstein, 1976 cited in Opdyke, 1978), 3.2 g/kg (Epstein *et al.*, 1967 and Volodchenko, 1975 cited in Opdyke, 1978), and 2.0 g/kg (Korolev *et al.*, 1976); for mice to be 1.75 g/kg (Korolev *et al.*, 1976); and for guinea pigs to be 300 mg/kg (Sweet, 1987). Premortem effects consisted of central nervous system effects and cyanosis in both mice and rats. An oral dose of 300 mg DPA/rat was lethal to 2/20 rats in 30 days

(Griswold *et al.*, 1966 cited in Opdyke, 1978). However, 7,500 mg DPA/kg was the lowest dose reported as toxic to pregnant rats (NIOSH, 1976, cited in Opdyke, 1978).

Kronevi and Holmberg (1979) gave 600 mg DPA/kg once by oral gavage to 150 female mice and observed 31% mortality within 5 days of treatment. Another nine animals died within 10 days of the treatment. However, 95 animals survived 1 month without apparent symptoms of DPA induced injury. Microscopic examinations of selected organs revealed abnormalities in intestine, kidney, and liver, but it was not clear when the animals were necropsied. No further details were provided.

Formation of methemoglobin in considerable amounts was reported in a cat orally administered with a single dose of an aqueous suspension of DPA at 169 mg/kg (Alexander *et al.*, 1965 cited in Opdyke, 1978).

b. Primary Irritation, Dermal Sensitization, and Ophthalmologic Effects

Diphenylamine was not irritating when applied full strength to intact or abraded skin of rabbits (Levenstein, 1976 cited in Opdyke, 1978). Details of the study were not provided.

c. Subacute

In a 3-day study, Lenz and Carlton (1990) administered daily, single-doses of DPA (chemical purity not indicated) in peanut oil by gavage to male Sprague-Dawley rats (10/group) at 400, 600, or 800 mg/kg/day. A control group (10 rats) received only peanut oil. Moribund animals were sacrificed and necropsied during the study period, and all surviving animals were sacrificed and immediately necropsied 24 hours following the third dose. None of the rats in any dose group became listless and moribund during the 3-day study. Bilateral renal cortical pallor was observed at necropsy in one rat at the 600 mg/kg/day dose. Two of ten rats at the 800 mg/kg/day DPA treatment level developed renal papillary lesions consisting of focal, apical, subepithelial rarefaction of the interstitial matrix, pyknosis of interstitial cells and endothelial cells, and occasional eosinophilic ghosts of cells. However, these lesions were not associated with the induction of renal papillary necrosis or necrosis of the pars recta. A gavage needle caused a single gastric perforation in the glandular fundus of another rat in the 600 mg/kg/day treatment group. In this study, a No-Observed-

Adverse-Effect Level (NOAEL) of 400 mg/kg/day is suggested for renal pathologic changes. However, the actual NOAEL may be higher because of the small number of animals in each group and the low incidence of renal effects at the 600 mg/kg/day level (1/10) and the 800 mg/kg/day level (2/10). In contrast to these findings in Sprague-Dawley rats, Powell *et al.* (1985) found renal papillary necrosis and necrosis of the pars recta in Wistar rats.

In attempts primarily to delineate the histopathology of kidney lesions, Powell *et al.* (1985) gave male Wistar rats daily suspensions of 4.1 mMol DPA/kg in 1.25% methyl cellulose, methyl cellulose alone (vehicle control), or distilled water by gastric gavage for 3 or 9 days. This dose corresponds to 694 mg DPA/kg/day. After three doses, 18-hour urines were obtained on four rats per group before they were sacrificed for renal histological examinations. After urine was collected from an additional four rats per group following the ninth dose, the rats were sacrificed, and their kidneys were examined. No significant change in body weight gain was observed in treated animals after three doses, but body weight gain compared to controls was depressed ($p < 0.05$) after nine days. Although urinary volume and osmolality tended to be low, they were not significantly different from controls after 3 or 9 days of treatment. The authors noted, however, that control animals also demonstrated poor urine concentrating abilities (normal osmolarity considered to be 1,500-2,000 mOsm/kg) but without any observed morphological abnormalities. The significantly elevated protein excretion in urine observed in treated animals at both time periods was attributed by the authors to excretion of cellular debris rather than glomerular leakage. Compared to control rats, treated rats excreted significantly more chloride at the end of both treatment periods and more sodium and potassium after nine days. Histological kidney examinations revealed wide spread necrosis after both 3 and 9 days of DPA treatment. After three doses, necrosis occurred in the pars recta of the proximal tubules with extensions into the mid to outer cortex, papilla, limbs of Henle, capillaries, and interstitial cells. Tubular lumens frequently contained amorphous casts of debris. After nine days, frank papillary necrosis was evident in some animals and proximal tubular necrosis was still present but with areas of regeneration. These results contrast with those of Lenz and Carlton's (1990) who found no renal papillary necrosis or necrosis of the pars recta in Sprague-Dawley rats given as much as 800 mg DPA/kg/day by gavage for 3 days. This 3 or 9 day study using a single dose level of DPA is not useful for the derivation of HA values, although some quantitative estimate of the histology of kidney lesions is presented.

Using the same experimental protocol as described previously, Lenz and Carlton (1990) observed more severe effects in male Syrian hamsters (10/group) than in Sprague-Dawley rats. Within 12 hours after the second dose, all animals in the 600 and 800 mg/kg/day treatment groups were listless and moribund (Table VI-1). Four hamsters at the 400 mg/kg/day level became listless and moribund before terminal sacrifice. Four hamsters at 400 mg/kg/day, seven at 600 mg/kg/day, and six at 800 mg/kg/day showed total renal papillary necrosis, which included all elements of the renal papilla—interstitial cells, vasa recta, thin limbs of Henle, and collecting tubules. Two hamsters at the 600 mg/kg/day dose developed focal intermediate renal papillary necrosis involving the distal, lateral, and apical subepithelial regions of the papilla. On gross pathologic examination, DPA had induced a diffuse, dull-brown discoloration of the kidney extending from the capsular surface through the cortex and outer medulla, and a yellow-brown discoloration of the renal papilla in the 600 and 800 mg/kg/day treatment groups. Other gross pathologic lesions were found in the stomach and spleen. One hamster at 600 mg/kg/day showed one or more gastric ulcers, visible as punctate 2-4 mm diameter black foci. Splenomegaly was observed in nine of ten hamsters but only at the 400 mg/kg/day dose. Although adverse kidney effects occurred at the lowest dose tested, 400 mg/kg/day, this DPA dose also produced 40% mortality; therefore, a LOAEL or NOAEL is not identified. The 400 mg/kg/day dose is a Frank-Effect-Level (FEL) for this study.

Following the same protocol as for rats and hamster, Lenz and Carlton (1990) administered DPA (purity not reported) to male Mongolian gerbils (10/group) at 400, 600, or 800 mg/kg/day for 3 days. A control group (10 gerbils) received only peanut oil. All animals survived for the duration of the study and were sacrificed and necropsied 24 hours following the third dose. No gross pathologic lesions or discolorations of the kidney were found. In striking contrast to DPA effects in Syrian hamsters, histological examinations of the kidney showed no renal lesions, papillary or cortical, in treated or control gerbils. The results suggest a NOAEL of 800 mg/kg/day in Mongolian gerbils.

Korolev *et al.* (1976) gave single daily doses of DPA to albino rats at 16, 80, or 400 mg/kg/day (corresponding to 1/125, 1/25, or 1/5, respectively, of the LD_{50} [2 g/kg]) for 25 days. For the LD_{50} studies the investigators perorally administered the DPA in vegetable oil and presumably followed the same procedure as in this study. They did not report species or number of animals used in this study. The authors measured erythrocyte, leukocyte, reticulocyte, hemoglobin, and methemoglobin levels; blood cholinesterase, aldonase, and peroxidase activities; protein fractions in blood serum;

Table VI-1. Mortality and Pathologic Effects in Syrian Hamsters Given Oral Doses of DPA[a]

	Dose Group (mg/kg/day)			
Lesion	400	600	800	Control[b]
Mortality	4/10[c]	10/10	10/10	0/10
Total Renal Papillary Necrosis	4/10	7/10	6/10	0/10
Intermediate Renal Papillary Necrosis	0/10	2/10	0/10	0/10
Pars Recta Necrosis	0/10	0/10	0/10	0/10
Brown Kidneys	0/10	2/10	3/10	0/10
Yellow-Brown Papilla	0/10	6/10	5/10	0/10
Pale Renal Cortex	0/10	0/10	0/10	0/10
Gastric Ulcers	0/10	1/10	0/10	0/10
Splenomegaly	9/10	0/10	0/10	0/10

[a]Diphenylamine (DPA) dissolved in peanut oil vehicle.
[b]Vehicle control.
[c]Incidence/number of animals in dose group.

SOURCE: Adapted from Lenz and Carlton (1990)

intensity of urine staining due to DPA products; and at necropsy, the weights of internal organs and hepatic cholinesterase activity. They reported that statistically significant ($p < 0.05$-0.01) changes occurred in a considerable number of these measurements at the highest dose (400 mg/kg/day) compared to controls, but no details were provided. None of the treated animals died. The authors suggested a LOAEL of 80 mg/kg/day for erythrocyte count and peroxidase activity, and a NOAEL of 16 mg/kg/day for all clinical chemistry and organ weight measures.

Yoshida *et al.* (1989) gave single daily doses of DPA by gavage to male and female F344 rats (6/sex/dose) for 28 days. DPA (98% pure) was dissolved in olive oil and administered at doses of 111, 333, or 1,000 mg/kg/day. A control group received only the vehicle. Body weight and food consumption were periodically measured during the study period. On the last treatment day, a number of serum chemistry and hematology parameters (see below) were determined before the animals were subjected to necropsy. Recovery was studied in two additional groups (6 rats/group) gavaged with 1,000 mg/kg/day or olive oil (vehicle control) for 28 days after which treatment was stopped and the animals observed for an additional 14 days. Although food consumption in the two 1,000 mg DPA/kg/day groups was lower than controls, the difference was not significant. Nevertheless, the high-dose males and females showed a decrease in body weight gain during the treatment period, and the effect persisted for 14 days after treatment ceased in the high-dose recovery group. No treatment-related mortality occurred in any of the animals.

Among the hematology parameters measured in this study (Yoshida *et al.*, 1989), the authors reported a significant reduction ($p < 0.05$) in erythrocyte count and hemoglobin in females of the 1,000 mg/kg/day dose group. Hemoglobin was also depressed in high-dose males. Leukocyte count was elevated in males and females of the high-dose group, and was also elevated in males of the 333 mg/kg/day group. Mean cell volume (MCV) was elevated in the high-dose females. However, only the DPA effect on hemoglobin and MCV persisted during the 14-day no-treatment period. Treatment-related effects of DPA on serum chemistry measured in the male rats included an increase in albumin in all treatment groups, increased bilirubin in the mid- and high-dose groups, and increased potassium levels and reduced chlorine levels in the high-dose group. Only the potassium level remained elevated 14 days after treatment was stopped. Females treated with 1,000 mg DPA/kg/day showed a tendency toward increased total bilirubin.

Treatment-related changes in absolute and relative organ weight, most notably of the liver, occurred in male and female rats. The weights of liver, kidney, and spleen were reduced in males at or above 111 mg DPA/kg/day and females at or above 333 mg/kg/day. Weight reductions in thymus, testes, and ovaries were found in animals treated at the 1,000 mg/kg/day dose. After 14 days of a post-treatment recovery period, only the thymus weight had recovered.

The major histopathologic findings are summarized in Table VI-2. In all rats of the 1,000 mg/kg/day group, slight to moderate renal tubular degeneration and necrosis in the corticomedullary junction as well as congestion and extramedullary hematopoiesis of the spleen were found on histopathological examination. Mild bone marrow hyperplasia was also found in 100% of the animals in this group. Less frequent findings at this dose level included necrosis of the renal papilla in one male and one female, slight to mild renal tubular dilatation with or without protein casts in two animals of each sex, and mucosal erosion and hyperplasia of the stomach. None of the changes persisted after treatment was stopped. The above histopathologic effects were absent at lower doses except for renal tubular degeneration in 17% of the males administered 333 mg/kg/day. The authors suggested a NOAEL of 111 mg/kg/day and a LOAEL of 333 mg/kg/day for a 28-day period. However, in view of reduced liver weights in males at 111 mg/kg/day and increased serum albumin levels, this dose is a LOAEL.

Eknoyan *et al.* (1976) fed finely ground DPA to female Sprague-Dawley rats for 3-6 weeks to study the early effects of DPA-induced experimental cystic disease. DPA (purity not specified) in the feed constituted 2.5% by weight of the dietary intake of the animals. Based on Lehman (1959) assumptions, this concentration corresponds to a calculated dose of 2,500 mg/kg/day for a young rat. The number of rats was not reported. After a minimal period of 2 weeks, treated rats showed a pronounced decrease in their ability to concentrate urine; average reduction was 50% compared to controls. No significant changes occurred in whole-kidney glomerular filtration rate (GFR), single-nephron GFR, end-proximal tubular fluid to plasma inulin (TF/P_{inulin}) ratio, glucose and bicarbonate reabsorption, free water clearance, and free water reabsorption. Histological examination showed dilatation and flattening of the tubular epithelium in the medullary collecting ducts of all treated animals. In addition, local areas of dilatation in cortical collecting ducts and distal tubules were

Table VI-2. Histopathological Findings in F334 Rats Treated with DPA in Olive Oil for 28 Days[a]

Findings	Sex	28-Day Dose Groups				Recovery Test	
		1000[b]	333	111	0	1000	0
KIDNEY							
Tubular degeneration and/or necrosis	M	100%[c]	17%	0	0	0	0
	F	100%	0	0	0	0	0
Tubular dilatation with or without protein casts	M	40%	0	0	0	0	0
	F	33%	0	0	0	0	0
BONE MARROW							
Hyperplasia	M	100%	0	0	0	0	0
	F	100%	0	0	0	0	0
SPLEEN							
Congestion and extra-medullary hematopoiesis	M	100%	0	0	0	0	0
	F	100%	0	0	0	0	0
FORESTOMACH							
Mucosal hyperplasia	M	60%	0	0	0	0	0
	F	50%	0	0	0	0	0
Mucosal erosion	M	20%	0	0	0	0	0
	F	17%	0	0	0	0	0

[a]LOAEL 111 mg/kg/day.
[b]Dose (mg/kg/day).
[c]Effected animals

SOURCE: Adapted from Yoshida *et al.* (1989).

noted, especially in animals fed DPA for over 4 weeks. Gross cysts were found in less than 10% of all the kidneys examined. Proteinaceous casts were found within the dilated tubules in some animals. The data indicated to the authors that the earliest lesion of the kidney in DPA-induced experimental cystic disease develops in the terminal portion of the collecting duct. Although the study presents useful information on the microscopic pathology of the kidney, it is not useful for the determination of HAs because only one dose level of DPA was used.

In a longer-term study with male Wistar rats, Powell *et al.* (1983) administered daily doses of 1.6 mM DPA/kg (corresponding to 270 mg/kg/day) by oral gavage for 1-8 weeks. Two rats were sacrificed at weekly intervals during the 8-week period. The remaining animals (number not specified) were sacrificed 4 weeks after treatment was stopped. No significant differences between control and treated rats occurred in food and water intake or body weight gain. Marked proteinuria developed in DPA treated rats at 4 and 8 weeks, but returned to control levels after 4 weeks of no treatment. There was no clear DPA effect on urinary volume or osmolality. Macroscopic examinations showed marked splenomegaly in treated rats as well as extramedullary hemopoiesis and increased hemosiderin in the spleens of most treated rats. After the no-treatment period, the splenomegaly had reversed, but the extramedullary hemopoiesis had only partially regressed. Histological examinations of the kidneys revealed focal necrosis of the papillary interstitium, loops of Henle and capillaries, and focal proximal tubular necrosis with active regeneration. These lesions first appeared at 1 or 2 weeks of treatment and became more severe as treatment progressed. No improvement was observed at the end of the no-treatment period.

In a 133-day study, Darmady *et al.* (1970) fed rats (species, age, and number not specified) a diet containing 2.5% DPA and performed conventional histological and microdissection studies of the treated rats at various time intervals over the 19-week period. Based on the Lehman (1959) assumptions, 2.5% DPA in the diet corresponds to a calculated dose of 2,500 mg/kg/day in young rats. The earliest observed DPA-related kidney effect was a flattening of the epithelium of the distal convoluted and collecting tubules at about 6 weeks. Four weeks later, the lumen of the collecting tubules had increased in diameter but without cast formation or obstruction. By week 15, defects were evident in both the distal and collecting tubule and possibly higher up the nephron. Many more nephrons had degenerated at 15 weeks than at early observation periods, and many tubules had also become cystic. By the 19th week, the kidney changes were essentially the same qualitatively but were more extensive.

Woodhouse *et al.* (1965) fed male weanling rats *ad libitum* on a diet to which 2.5% DPA by weight had been added for 133 days and performed microscopic studies of the kidneys. This DPA concentration corresponds to a calculated dose of 2,500 mg/kg/day in young rats (Lehman, 1959). Species and number of experimental animals were not reported. The earliest DPA-related effect observed under electron microscopy was degeneration of the renal epithelial cells of the proximal tubule characterized by a marked increase in electron dense bodies. An increase in the size and number of electron dense bodies along with swollen mitochondria, vacuolization, and fibroblast infiltration were also found in the collecting and distal tubules. Glomerular changes were either absent or minimal.

Twenty male NMRI mice receiving DPA by oral gavage in single doses of 1.4 g/kg once a week for 10 weeks were necropsied 3-4 months after treatment began (Kronevi and Holmberg, 1979). No control groups were tested. Although five deaths occurred within 2 days of the onset of dosing, ten animals survived the study period without apparent DPA-related symptoms. Among those mice that died before terminal sacrifice, severe renal changes were found all over both kidneys. Particularly evident, were the irregularly narrowed and scarred renal cortices with loss of normal architecture and dilated (cystic) or atrophic proximal, distal, and collecting tubules. In the liver, activated reticulo-endothelial cells were found that contained yellowish-brown, iron-negative pigment in their cytoplasm. Experimental details for short-term studies are summarized in Table VI-3.

2. Longer-term Exposure

a. Chronic Studies

A number of studies mentioned in the following paragraphs described histopathology of kidney lesions in rats fed DPA in the diet for 5-18 months. These studies are not amenable to determine HAs because only one high dose (1,000-2,500 mg/kg/day) was used. The primary aim of these studies, however, was the delineation of kidney pathology.

Gardner *et al.* (1976) conducted functional and anatomical studies of the kidney in male Sprague-Dawley rats fed 1% DPA by feed weight in their diet for 5-11 months or 12-20 months.

Table VI-3. Summary of Studies: Short-term Exposure of Animals to Diphenylamine

Source	Species	Dose (mg/kg/day)[a]	Route	Duration (Days)[a]
Opdyke, 1978	Rat	1,165; 2,000; 3,200	Oral	1
Korolev *et al.*, 1976	Mouse	1,750	Oral	1
Sweet, 1987	Guinea pig	300	Oral	1
Kronevi and Holmberg, 1979	Mouse	600	Gavage	1
Opdyke, 1978	Cat	169	Oral	1
Lenz and Carlton, 1990	Rat/Hamster/ Gerbil	400; 600; 800	Gavage	3
Powell *et al.*, 1985	Rat	694	Gavage	3 or 9
Korolev *et al.*, 1976	Rat	16; 80; 400	Oral	25
Yoshida *et al.*, 1989	Rat	111; 330; 1,000	Gavage	28
Powell *et al.*, 1983	Rat	270	Gavage	1 to 8 weeks
Ekoyan *et al.*, 1976	Rat	2,500	Diet	3 to 6 weeks
Kronevi and Holmberg, 1979	Mouse	1,400 mg, once/ week	Gavage	10 weeks

[a]Unless otherwise stated

The concentration corresponds to a calculated dose of 1,000 mg/kg/day for a young rat (Lehman, 1959). The authors apparently used about 8-20 rats per test, but the exact number was not reported. At the end of the two feeding periods, neither body weights nor kidney/body weight ratios differed from controls. The kidney lesions of treated rats were heterogeneous. Dilation and frank cyst formation occurred in 5-30% of surface nephrons. Elevated intraluminal hydrostatic pressures occurred in dilated but not nondilated nephrons. No increase in glomerular filtration or decreased net water reabsorption were recorded in the dilated nephrons of either treatment group, indicating that intraluminal pressures were elevated in dilated nephrons. Structural studies revealed some intrarenal cysts, and adjacent to the cysts, tubules often appeared compressed. Dilated collecting tubules typically contained debris that appeared to partially occlude the lumen. Narrowing of the proximal convoluted tubules were observed although none of the studied nephrons was completely occluded. The results suggested to the authors that partial or intermittent downstream occlusion in surface nephrons of the rat kidney may be widespread and progressive and is responsible for the elevated pressures in the dilated nephrons of DPA-treated rats.

Safouth *et al.* (1970) conducted renal histological studies on Sprague-Dawley rats fed a diet containing 1.5% DPA by weight (40 rats) for 0.5-10 months or 2.5% DPA by weight (60 rats) for 2-12 months. Dietary concentrations of 1.5% and 2.5% DPA correspond to a calculated dose of 1,500 and 2,500 mg/kg/day, respectively, for a young rat (Lehman, 1959). A direct relationship between dose and degree of cystic changes occurred in treated rats at both dose levels, although factors other than DPA appeared to play a role in cystic development. Six of eight rats fed 2.5% DPA evidenced dilation and early cystic changes of the renal collecting tubules after 2 months of treatment. As the duration of exposure increased, the lesions became more extensive (although glomeruli remained normal), and brown pigmentation thought to be hematogenic in origin occurred with increasing frequency in the cytoplasm of tubular cells. The urinary concentrating capacity of the kidneys was significantly depressed from controls in the high-dose group after 5 weeks of DPA treatment and remained depressed after 7 months of DPA exposure.

Kime *et al.* (1962) studied DPA-induced polycystic renal disease in weanling and adult Sprague-Dawley rats exposed to 2.5% DPA in the diet for periods up to 12 months. Eight weanling rats of both sexes and an unspecified number of male adult rats were similarly treated with DPA in the diet. The dietary concentration of 2.5% DPA corresponds to a calculated dose of 2,500 mg/kg/day in a young rat and 1,250 mg/kg/day in an older rat (Lehman, 1959). Structural alterations of the kidney,

which varied from tubular dilatation to overt cyst formation, developed within 47 days in all rats (weanling and adult) fed 2.5% DPA in the diet. The severity of the lesions was roughly proportional to the duration of exposure. In addition, after 1 year, the weanling rats showed decreased creatinine clearance values and capacity to concentrate urine, which were considered by the authors to be roughly proportional to the degree of renal morphological changes. The study demonstrated that prolonged oral administration of 2.5% DPA in the diet will result in the development of an acquired form of polycystic disease in both weanling and adult Sprague-Dawley rats.

Based on kidney examinations of three Sprague-Dawley rats fed 1% DPA by weight in the diet (calculated dose of 1,000 mg/kg/day; Lehman, 1959) for 12-18 months, Evan *et al.* (1976) found that in the terminal stages of renal disease, few cellular or gross morphological differences exist between DPA-induced polycystic disease and human medullary cystic disease. Diphenylamine-induced renal lesions more closely resemble human polycystic disease.

In another study using 1% DPA in the diet (calculated dose of 1,000 mg/kg/day; Lehman, 1959) of Sprague-Dawley rats, Evan *et al.* (1978) followed the development of DPA-induced functional and structural changes in the kidney for up to 18 months. Five rats each from the control and experimental groups were paired and their renal concentrating ability measured at 2, 4, 5, 10, and 20 weeks. Additional animals were subjected to morphological examinations on 2, 5, 10, 15, 20, 25, 52, and 78 days of the study. After an initial weight loss, body weights and growth rates in DPA-treated rats paralleled those of control rats. Urine flow increased and the capability to concentrate urine (osmolality value) decreased in the treatment group within 2 weeks but did not achieve statistical significance until 6 weeks. Creatinine clearance, a measure of glomerular filtration, was not significantly different from control values. The first apparent morphologic changes occurred at 5 weeks and involved cellular hyperplasia described as multilayering of cells, increased numbers of nuclei, and ^{3}H-thymidine-labeling of nuclei in the medullary collecting ducts. By 10 weeks, some collecting ducts of treated animals showed dilation with focal areas of cellular necrosis and some cast material in the ducts. By 15-20 weeks numerous collecting ducts were dilated and contained cast material. By 24 weeks, frank cysts in the cortex and medulla and necrotic cells along the collecting ducts were found. The severity of the damage greatly increased by 1-2 years with evidence of frank cysts in every segment of the nephron along with a loss of nephrons and areas of chronic inflammation. However, most of these anomalies had been observed to a lesser degree at 6 months of treatment. Proximal tubule, loop of Henle, and distal tubule were essentially normal.

Twelve chickens fed DPA in their feed for 120 days developed renal lesions (Sorrentino *et al.*, 1978). The investigators were in determining whether DPA nephrotoxicity, which had been demonstrated in rodents, was common to a variety of animal species. The birds' feed contained 2% DPA, and the birds were allowed to eat and drink *ad libitum*. Food consumption was not reported. Nine of the chickens died between the 90th and 120th day. The three remaining birds were sacrificed on the 120th day. All the treated chickens weighed 10% less than control birds. Microscopic examination of the kidneys revealed serious degeneration of the renal tubular epithelium without cyst formation. More extensive renal degeneration was observed in those chickens that died before terminal sacrifice. Kidney degeneration was the only DPA-related effect observed.

In a 226-day feeding study with 36 female weanling albino rats (species not specified), Thomas *et al.* (1957) evaluated DPA-related effects on body weight gain and gross and histopathology of the kidney, liver, spleen, adrenal, and heart. The rats (6/dose) were fed 0.025, 0.1, 0.5, 1.0, or 1.5% DPA in their diet (corresponding to a calculated dose of 27.5, 110, 550, 1,100, or 1,650 mg/kg/day based on food consumption of 11 g/rat/day reported by the authors and an assumed rat weight of 0.1 kg). Food consumption was similar (10.37-11.45 g/rat/day) throughout the study period for all treatment and control groups. However, a dose-related inhibition of body weight gain occurred at dietary levels of 0.5-1.5% DPA. All, but one animal that was discarded on the 156th day because of a respiratory infection, survived the experimental period. At necropsy, brown granular pigmentation, considered by the authors to be hematogenic in origin, was found in Kupffer cells of the liver and in tubular epithelial cells of the kidneys of all animals fed 0.5% DPA, or more. The quantity of pigmentation appeared to be dose-related. The only other DPA-related changes reported involved the kidney where DPA levels at or above 0.5% resulted in focal dilatation of renal tubules and multiple cystic structures. These lesions were most conspicuous at the corticomedullary junction of the kidney. A NOAEL of 0.1% DPA (110 mg/kg/day) in the diet is suggested by this study for body weight gain and renal pathological changes.

Korolev *et al.* (1976) conducted chronic experiments on male albino rats using DPA doses of 0.05, 0.5, and 5 mg/kg. Although the authors did not specify route of exposure or vehicle, they presumably used peroral dosing with DPA dissolved in vegetable oil as they had done in related experiments reported in the same paper. No further information was provided on animal species and number, frequency of dosing, or study duration. Several clinical chemistry parameters (also measured in the subacute studies, see section VI.B.1.c), were evaluated. In addition, the chronic

study included tests of conditioned reflex activity; serum transaminase and phosphatase activities; and ceruloplasmin, β-lipoprotein, and SH-group levels in blood. At the end of the dosing period, serum bilirubin and cholic acid quantities, internal organ weights, and pathomorphological studies were evaluated. Although the results were not presented in detail, the high dose provoked changes in conditioned reflex activity, hepatic excretory function, peroxidase activity, and levels of ceruloplasmin and SH-groups in blood. The study found a NOAEL of 0.5 mg/kg/day.

Experimental details for long-term studies of DPA toxicity are summarized in Table VI-6.

b. Lifetime Studies

In a 2-year dietary study, Thomas *et al.* (1967a) administered 99.9% chemically pure DPA at concentrations of 0.001, 0.01, 0.1, 0.5, or 1% by weight in the feed to weanling Slonaker-Addis rats (20/sex/group) for 734 days. Based on food consumption data reported by the authors for the first 240 days and an assumed body weight of 0.1 kg for young rats (Lehman, 1959), these levels correspond to calculated doses of 1.1, 11.7, 118.2, 543, 999 mg/kg/day, respectively, in males; and 0.96, 9.6, 92.5, 438.5, 812 mg/kg/day, respectively, in females. Average food consumption of DPA-treated rats at levels of 0.001% and 0.1% during the first 240 days (11.5-11.8 g/rat/day for males; 9.3-9.6 g/rat/day for females) was similar to that of controls. However, at DPA levels of 0.5% and 1%, food consumption was significantly reduced ($p < 0.01$) in males (10-10.9 g/rat/day) and females (8.1-8.8 g/rat/day). Body weight gain in males and females over the first 240 days of treatment was significantly less ($p < 0.01$) than controls at the 0.5 and 1% DPA levels, but food consumption was also depressed. Females on the 0.1% DPA diet, however, showed a significantly decreased ($p < 0.01$) growth rate without decreased food consumption. The authors suggested that a similar effect on males may have been obscured by the greater range of body weights in males than in either females or controls at the outset of the study. They also noted that growth rates for the remainder of the 2-year treatment period revealed no additional pertinent information.

The number of animals surviving in the control group males at 640 days and 734 days was 13 and 14/20, respectively, whereas in treated animals, these numbers varied from 10-15/20 with no indication of any dose relationship. Survival of female control rats was 14 and 11/20 at 640 and 734 days, respectively, while survival in treated groups ranged from 11-18/20 and no specific dose response could be ascertained. Hematological findings showed that while leukocyte counts remained

normal in all groups through the 463rd day, rats in the 0.5 and 1% treatment groups showed moderately depressed hemoglobin and erythrocyte levels. Two-year ingestion of DPA caused chronic nephritis and cystic dilatation of renal tubules with accompanying interstitial inflammation at DPA dietary levels of 0.1%, or more, in males and females. Also, in some rats at these dose levels, pigment and protein accumulated in the renal pelvis or bladder and were accompanied by mild epithelial hyperplasia or squamous metaplasia of the epithelial lining. Glomeruli, however, were not altered, and glycosuria did not develop. The study suggested a NOAEL of 0.01% dietary DPA (calculated dose, 9.6 mg/kg/day) for body weight changes, hematology, and renal pathology.

An unpublished report of the same study (Thomas *et al.*, 1967a) indicated similar results but a slightly different NOAEL (Booth, 1963). In the study reported by Booth (1963) the survival, growth, hematological, and histopathological effects of DPA were investigated in weanling albino rats (20/sex/group) given 0.001, 0.01, 0.1, 0.5, or 1% DPA in their feed for 2 years. The DPA was stated to be 100% pure, as determined by cryoscopy. Based on Lehman (1959), these concentrations correspond to calculated doses of 1, 10, 100, 500, or 1,000 mg/kg/day, respectively, for a young rat. The animal species and food consumption rates were not reported. Although some animals apparently died before the end of the study, there was no significant mortality in any of the treatment groups. No impact on body weight gain occurred in either sex during the first 240 days at dietary levels of 0.1% DPA, or less, but a dietary level of 0.5% DPA inhibited growth without reducing food consumption. At the highest DPA concentration, the food consumption rate was reduced more than 10%. A moderate degree of anemia (reduction in hemoglobin and erythrocyte levels and increased numbers of circulating normoblasts) developed in the 1% DPA treatment group during the hematological test period (126-463 days). In another group of rats studied separately, cessation of DPA feeding after 106 days at the high treatment level led to rapid restoration of hemoglobin values, most notably in females. Growth inhibition also recovered, though more slowly. Animals sacrificed after 640 days of treatment at the 0.5% and 1% levels evidenced dilated cystic renal tubules accompanied by chronic interstitial nephritis. The study suggested a NOAEL of 0.1% DPA (calculated dose, 100 mg/kg/day) for renal pathology, body weight gain, and anemia.

Thomas *et al.* (1967b) mixed 0.01%, 0.1%, or 1% DPA into the feed of 8-month old beagle dogs (two/sex/group) for 2 years. These concentrations correspond to a calculated dose of 2.5, 25, or 250 mg/kg/day (Lehman, 1959). Chemical purity of DPA was reported to be 99.9%. An outbreak of distemper, which occurred on day 414, caused the death of one female in the 0.1% DPA group on

day 434 and the next day necessitated the sacrifice of two males (one each from the 0.01% and 0.1% treatment groups; all three dogs were from one litter). All four dogs in the 1% DPA group rejected minor portions of their feed during the first 6 days of treatment, but overall, food consumption was normal in all the groups throughout the study. Treatment-related decreases in body weight gain occurred in males and females during the 266-400 day period at 0.1 and 1% levels. The authors calculated that at 400 days, the high-dose males had ingested a total of 86 g DPA/kg and the females, 99 g DPA/kg. By the 87th day of treatment, all dogs on the 1% DPA diet had developed obvious anemia, manifested by decreased hemoglobin and erythrocyte levels. The data indicated a treatment-related decrease in hemoglobin and erythrocytes in dogs at the 0.1% DPA level, although the conclusions are based on a few survivors. After 2 years, erythrocytes in the dogs at the 1% level showed decreased fragility. Leukocyte counts remained within normal limits in all groups. Sulfobromophthalein tests conducted from days 618-627 indicated moderate damage to liver function in the 1% dietary DPA. Blood glucose levels were normal. Kidney function tests, which included phenolsulfonephthalein, urinary albumin and glucose levels, excretion rates (between days 692 and 732), and urine specific gravities, in treated animals were not different from controls. Pathologic changes occurred in all the dogs (two of each sex) fed the 1% DPA diet although the dogs were reported to be in good health, high spirited, and active after 2 years of DPA treatment. The most conspicuous of the lesions in these dogs was a peripherolobular fatty change in the liver accompanied by a marked increase in liver weight and ether-extractable lipids. Other less severe pathologic changes included mild hemosiderosis of the spleen, kidneys, and bone marrow, a mild increase in kidney weight, and a slight increase in hepatic intracellular bilirubin. This study suggests a NOAEL of 0.01% dietary DPA (calculated dose, 2.5 mg/kg/day) for the absence of decreased body weight gain, hematology, liver function, and pathological effects. The results of this study should be considered with some reservation because of the small number of animals used. The observed effects suggest that the dog may be a more sensitive species than the rat (Thomas *et al.*, 1967a) to DPA induced effects; however, the occurrence of distemper may have weakened the dogs. The DPA effects in dogs are summarized in Table VI-4.

In an unpublished report of the same study (Thomas *et al.*, 1967b), DPA concentrations of 0.01, 0.1, or 1% (corresponding to a calculated dose of 2.5, 25, or 250 mg/kg/day (Lehman, 1959) were given in the diet to groups of two beagle dogs of each sex for 2 years (Booth, 1963). Severe growth inhibition occurred before the 400th day in the 1% treatment group, but growth in the other treatment groups was the same as the control group. Leukocyte and differential cell counts in all treatment

Table VI-4. Effects Produced in Dogs fed Diphenylamine (DPA) in the Diet

Level of DPA in the Diet (mg/kg/day)	Effects
2.5	None
25	Decreased body weight during 262-400 days of feeding. Decreased hemoglobin and erythrocytes.
250	Decreased body weight during 262-400 days of feeding. Obvious anemia by 87th day indicated by decreased hemoglobin and erythrocyte levels. Fragile erythrocytes after two years of treatment. Moderate liver function damage. Histopathological liver changes included increased peripheral lobular fatty changes and slight increase in hepatic intracellular bilirubin. Hemosiderosis of the spleen, kidneys and bone marrow.

SOURCE: Adapted from Thomas *et al.*, 1976b

groups were similar to controls, but hemoglobin and erythrocyte levels decreased at 724-731 days in the 1% treatment group. Additional tests confirmed the fragility of the erythrocytes in dogs at this level. Liver and kidney function tests performed after the 600th day were not conclusive though they indicated possible sulphobromophthalein retention in dogs on 1% dietary DPA and no obvious renal impairment. Increased liver weight, with marked fatty changes, and moderate amounts of intracellular bilirubin were found at the 1% dietary DPA level. Some hemosiderosis of spleen, kidney, and bone marrow also occurred at this DPA level. This study suggests a NOAEL of 0.1% dietary DPA (calculated dose, 25 mg/kg/day), which is one order of magnitude higher than that determined by Thomas *et al.* (1967b) in beagle dogs. Longer-term studies are summarized in Table VI-5.

3. Reproductive Effects

In a two-generation reproduction study, Thomas *et al.* (1967a) mixed DPA (99.9% pure) concentrations of 0.1, 0.25, or 0.5% in the diet of 12 female and 3 male Slonaker-Addis rats starting at 35 days of age. These concentrations correspond to calculated doses of 100, 250, or 500 mg/kg/day, respectively, in young rats (Lehman, 1959). Food consumption was not measured. Mating took place when the rats were 100 days old. After the litters were weaned, the parents were remated as were the offspring of the first mating. Average litter size from the first mating of dams on the 0.5% DPA diet was significantly less ($p < 0.05$) than control levels; after the second mating, the mean litter size was significantly ($p < 0.05$ to 0.01) less at all tested dose levels (Table VI-6). Average litter size of the remated dams decreased as the dietary level of DPA increased. However, among litters in the second generation, only those from mothers on 0.1% dietary DPA were smaller in size compared to controls. Body weight gain data were inconclusive. A NOAEL was not determined.

In what appears to be an unpublished report of this same two-generation study (Thomas *et al.*, 1967a), albino rats (species not reported) were given 0.1, 0.25, or 0.5% DPA in the diet for an unspecified time period (Booth, 1963). These concentrations correspond to calculated doses of 100, 250, or 500 mg/kg/day, respectively, in young rats (Lehman, 1959). A control group was also studied. Apparently, DPA had no effect on the mortality of the offspring or number of litters born to treated dams although there was some indication that the 0.5% dietary level reduced litter size and body weight. No further details were provided.

Table VI-5. Summary Studies: Longer-term Exposure of Animals to Diphenylamine

Source	Species	Dose (mg/kg/day)	Route	Duration
Darmady *et al.*, 1970	Rat	2,500	Diet	6 to 19 weeks
Woodhouse *et al.*, 1965	Rat	2,500	Diet	19 weeks
Gardner *et al.*, 1976	Rat	1,000	Diet	5 to 20 months
Safouth *et al.*, 1970	Rat	1,500 or 2,500	Diet	2 to 12 months
Kime *et al.*, 1962	Rat	1,250 or 2,500	Diet	12 months
Evan *et al.*, 1978	Rat	1,000	Diet	Up to 18 months
Thomas *et al.*, 1957	Rat	27.5; 110; 550; 1,100; 1,650	Diet	226 days
Thomas *et al.*, 1967[a]	Rat	1.1; 11.7; 118.2; 543; 999	Diet	2 years
Thomas *et al.*, 1967[b]	Dog	2.5; 25; 50	Diet	2 years
Sorrentino *et al.*, 1978	Chicken	2% in the feed[a]	Diet	120 days

[a]Exact food consumption was not given and therefore, dose was not calculated.

Table VI-6. Reproduction Performance of Slonaker-Addis Rats fed DPA

DPA Level in Diet (%)	Number of Females	Born		Weaned	
		Number of Litters	Mean Number of Litters	Number of Litters	Mean Number of Litters
		First Mating			
Control	12	12	8.3	10	7.5
0.10	12	10	9.0	10	8.3
0.25	12	10	6.8	9	7.1
0.50	12	10	6.3[a]	8	6.3
		Second Mating			
Control	12	9	9.6	9	9.3
0.10	12	12	7.3[a]	11	7.1
0.25	12	12	7.3[a]	12	7.3
0.50	12	11	6.6[b]	11	6.6
		Second Generation			
Control	12	10	8.6	10	8.5
0.10	11[c]	11	5.8[b]	11	5.7
0.25	12	12	7.3	12	7.0
0.50	12	11	7.0	11	6.6

[a]Significant at $p < 0.05$ level.
[b]Significant at $p < 0.01$ level.
[c]One died during first week.

SOURCE: Adapted from Thomas *et al.* (1967a).

Korolev *et al.* (1976) (study described in section VI.B.1.c) found that in male albino rats (species and number not reported) that were dosed perorally with DPA dissolved in vegetable oil, the maximum tested dose of 5 mg/kg had no effect on chromosomal reconstruction in bone marrow cells and no gonadotropic effects. No further details were provided.

In a validation study of the Chernoff-Kavlock assay in Wistar rats, Wickramaratne (1987) assayed DPA as well as 25 other chemicals and found no fetotoxic effects (litter size or survival) in 15 females given 1,000 mg DPA/kg/day by oral gavage during days 7-17 of gestation.

4. Developmental Toxicity

Diphenylamine induced polycystic renal disease in newborn rats (species not specified) born of dams tube fed daily with single doses of 2 mL of 1% DPA in alcohol (20 mg/rat, corresponding to a calculated dose of 200 mg/kg/day for a 0.1 kg rat) or born of dams provided a diet containing 2.5% DPA (corresponding to a calculated dose of 2,500 mg/kg/day [Lehman, 1959]) during the last 6 days of pregnancy (Crocker and Vernier, 1970 [abstract]). Control groups were either tube fed with 2 mL alcohol or fed a diet without DPA. All 70 newborns from dams in the experimental groups evidenced cystic dilatation of the collecting ducts and vacuolar degeneration of the proximal tubules. A NOAEL was not determined.

Based on oral studies with DPA in pregnant Sprague-Dawley rats, Crocker *et al.* (1972) concluded that a contaminant of commercial DPA, rather than chromatographically pure DPA, was responsible for *in utero* cystic tubular lesions in the kidneys of newborn rats. These authors investigated the effects of commercial DPA from two chemical companies as well as chromatographically pure DPA and three contaminants (E, F, and G), which were isolated from commercial DPA by thin-layer chromatography but not characterized in this study (see Safe *et al.*, 1977, in last paragraph of this section for further details on the contaminants). From 14 day's gestation to term, pregnant rats were given single daily amounts of the different compounds either by gastric tube at 0.05 or 20 mg/rat in 2 mL of 70% ethanol (corresponding to calculated doses 0.5 or 200 mg/kg/day, respectively) or by ingestion in the diet at concentrations of 1.5 or 2.5% commercial DPA (corresponding to calculated doses of 1,500 or 2,500 mg/kg/day, respectively, in young rats [Lehman, 1959]). Control groups received 70% ethanol or a normal diet *ad libitum*. The experimental details are presented in Table VI-7. Although cannibalism of offspring was high and contributed to the

Table VI-7. Cystic Renal Disease in Neonatal Rats of Dams Exposed to DPA *In Utero* for 7 Days

Chemical	Daily Dose or Concent.	Number of Pregnant Rats		Number of Newborn Rats with Histological Changes			
		Delivered	Failed[a]	Negative	Mild	Mod.	Severe
Daily Gavage in 2 mL of 70% EtOH							
DPA-K[b], aged 2 years	20 mg	18	3	8	6	21	4
DPA-E[c], purified	20 mg	20	0	26	6	0	0
Contaminant[d] E	50 µg	36	8	18	4	30	0
Contaminant F	50 µg	11	2	18	0	0	0
Contaminant G	50 µg	6	1	12	8	0	0
Control—70% alcohol	—	11	3	24	0	0	0
Dietary Exposure							
DPA-K, aged 2 years	1.5%	8	4	4	4	0	0
DPA-K, aged 2 years	2.5%	27	10	0	13	31	6
DPA-K, aged 3 months	2.5%	5	0	10	6	0	0
DPA-B, aged 2 years	2.5%	6	2	20	10	4	0
Control—powdered chow *ad libitum*	—	7	2	12	0	0	0

[a]Pregnant rats failed to deliver viable young due to maternal death, cannibalism of newborns, or still borns.
[b]Commercially supplied DPA: DPA-K from Eastman Chemical Co., DPA-B from Baker Chemical Co.
[c]DPA isolated from contaminants of aged DPA-K by thin-layer chromatography.
[d]Isolated bands from thin-layer chromatography of DPA.

SOURCE: Adapted from Crocker *et al.* (1972).

wide range in the number of offspring observed per litter (3-17), no statistical analysis was provided of its incidence. Diphenylamine from Eastman Chemical Co. (DPA-K) aged for 2 years induced the most severe renal effects in newborns from dams of both gastric tube and dietary groups, while chemically pure DPA produced no significant effects. No DPA-related effects were observed in any of the dams of any treatment group during the experiment. Though not discussed by the authors, it should be noted that ethanol itself can cause significant toxicity and may have contributed (*e.g.*, combined effects with DPA) to adverse outcomes.

Contaminant E (50 µg/day) and 20 mg/day of aged DPA-K frequently caused moderate cystic changes in the proximal tubules of newborns of dams treated by stomach tube, even though the dose level was substantially different. No significant cystic changes were reported in the 20 mg/day chromatographically pure DPA newborns or in those born of dams treated with contaminant fractions, F or G. While newborns in the 70% ethanol control group did not develop cystic tubular dilatation, their proximal tubules were minimally abnormal. In the dietary treatment groups, 2.5% aged DPA-K regularly induced cystic changes in the proximal tubules of newborns. Only infrequently were cysts found in the collecting duct system, and occasionally the medullary collecting duct was slightly dilated. Dietary levels of 1.5% DPA-K aged 2 years, 2.5% DPA-K aged 3 months, or 2.5% DPA-B (from Baker Chemical Co.) aged 2 years produced less common and less severe renal lesions. This study indicates that chemically pure DPA is not nephrotoxic in newborn rats.

In a later study, Safe *et al.* (1977) used gas chromatography and mass spectrometry to analyze impurities in six commercial brands of DPA as well as the contaminants that Crocker *et al.* (1972) used in their investigations of developmental effects in rats. One impurity (*o*-cyclohexylaniline) was found in all of the DPA samples and was determined to be contaminant F used in the Crocker *et al.* (1972) study. Contaminant G was identified as *p*-cyclohexylaniline. Contaminant E corresponded to the first peak (band 1), the least polar band of the chromatographic analysis. Infrared spectrum of band 1 revealed two broad bands indicating the presence of more than one impurity, and nuclear magnetic resonance spectrum showed a series of unresolved multiplets. Thus, contaminant E was not fully characterized. Four of the commercial DPA samples contained the carcinogen, *p*-biphenylamine (Safe *et al.*, 1977).

5. Carcinogenicity

Diphenylamine, which produces renal cysts in rats (Thomas *et al.*, 1957), was tested along with 50 other chemicals for carcinogenicity in female Sprague-Dawley rats (Griswold *et al.*, 1966). The animals were administered by single intragastric intubation DPA in sesame oil at 300 mg/rat (20 rats/dose; the dose was determined in a separate experiment to be the maximum tolerated dose for DPA) and observed for the appearance of mammary tumors or other grossly apparent tumors over a 6-month period. Animals that died during the study and those that survived until the end of the observation period were subjected to necropsy, including gross and histologic examinations of most major organs. Neoplastic changes suggestive of carcinogenicity were not reported in any tested organs by this rapid tumor induction method using DPA although several inherently carcinogenic compounds such as polynuclear aromatic hydrocarbon compounds tested positive by this test. Mortality for DPA-treated animals at 6 months was 2/20 and for controls 5/89.

Groups of mice (Strain unspecified; 200/sex/group) maintained on diets containing DPA at 50, 100, or 250 ppm (0.005, 0.01, and 0.025%, respectively; corresponding to calculated doses of 7.5, 15, and 37.5 mg/kg/day, respectively [Lehman, 1959]) for 92 weeks revealed no increase in the incidence or rate of tumor formation from those of controls (an unpublished study cited in U.S. EPA, 1985 and attributed to Coulston *et al.*, 1972). In this study, interim kills were performed on an unspecified number of mice at 6, 12, and 18 months of treatment and unspecified organs were examined histologically for the induction of neoplastic changes. At 250 ppm, spleen and liver weights were increased, splenic hemosiderosis was observed, but no other incidences of histologic lesions in kidney or other tissues were found.

In a chronic feeding study by Thomas *et al.* (1967a), Slonaker-Addis rats, 20/sex/group, were maintained on dietary levels of DPA at 0.001, 0.005, 0.01, 0.10, 0.50, or 1.0% (1, 10, 100, 500, or 1,000 mg/kg/day, respectively (Lehman, 1959]) for a period of 734 days. The number of animals surviving in the control group males at 640 days and 734 days was 13/20, whereas in treated animals these numbers varied from 10-15/20 with no indication of any particular dose-relationship. Survival of females in controls was 14 and 11/20 at 640 and 734 days, respectively, while survival in treated groups ranged from 11-18/20 and no specific dose-response could be ascertained. Animals that died prior to the 640th day of treatment, usually of respiratory infections, were not subjected to necropsy. Additional experimental details and results on the noncarcinogenic adverse health effects of this

study are presented in section VI.B.2.b. Neither the benign tumor incidences observed, such as adenomatous hyperplasia of adrenals, mammary adenofibroma, and adenoma of pituitary gland, nor the malignant tumor incidences of adrenal medullary adenocarcinoma were relatively increased in the treated animals as compared to controls. Other benign tumors seen in controls or in one of the treated groups were: abdominal lipoma, thyroid adenoma, uterus leiomyoma, and ovary granulosa cell. Similarly, some malignant tumors appeared in controls or in one of the DPA-treated groups involving liver, lung, pancreas, pituitary, or vulva. Selected information on sites and types of tumors found in this study is presented in Table VI-8. The authors attributed the tumor incidences not to the treatment but to the aging and senility of animals. Thus, in this study on carcinogenesis, dietary ingestion of DPA for over 2-years did not result in carcinogenesis in Slonaker-Addis rats.

Thomas *et al.* (1976b) fed 8-month old beagle dogs (two/sex/group) DPA in the diet at calculated levels of 2.5, 25, or 250 mg/kg/day for 2 years. Death of one female in the mid-dose group occurred on day 434 due to distemper. This necessitated the killing of two males, one each from low- and mid-dose groups (these three dogs were from one litter). After 2 years, the adverse effects observed were limited to dogs fed the high dose. These consisted of decreased erythrocyte fragility; moderate liver function damage;slight increase in intracellular bilirubin in liver; hepatic peripherolobular fatty changes; less severe hemosiderosis of the spleen, kidneys, and bone marrow; and a mild increase in kidney weight. Compound-related carcinogenic effects due to dietary DPA feeding were not found (non-carcinogenic effects are presented in detail in section VI.B.2). The limited number of animals used in the study must be borne in mind for any definite conclusions to be drawn. Nevertheless, when this information is considered along with the observed noncarcinogenicity in rats (Thomas *et al.*, 1976a) tested using 6 different dose levels, the existence of cancer potential for DPA becomes doubtful.

Negative results were obtained in a combined *in vitro* and *in vivo* assay wherein fetuses of pregnant Syrian golden hamsters were exposed *in utero* to an intraperitoneal injection of 5-20 mg/kg DPA. The fetuses were subsequently excised and the fetal cells were cultured *in vitro* and observed for the presence of transformed cells (DiPaolo *et al.*, 1973). In this bioassay, no neoplastic transformation was observed.

Table VI-8. Selected Information on Sites and Types of Tumors in Rats Fed DPA up to 734 Days[a]

Tumor	Dietary Levels of Diphenylamine											
	Controls		0.001%		0.01%		0.1%		0.5%		1%	
	M	F	M	F	M	F	M	F	M	F	M	F
Benign Tumors												
Adrenal medulla adenomatous hyperplasia	8	5	0	3	8	2	5	6	4	1	5	3
Mammary adenofibroma	1	2	2	5	1	6	0	5	0	2	0	0
Pituitary adenoma	1	-	-	1	-	3	-	1	-	1	-	1
Adrenal pheochromocytoma	-	-	1	-	1	-	-	-	-	-	-	-
Malignant Tumors												
Adrenal medulla adenocarcinoma	-	1	1	-	1	-	-	-	-	-	-	-
Adrenal cortical adenocarcinoma	-	-	1	-	-	-	-	-	-	-	-	-

[a]Animals that died before 640 days were not necropsied. A few were necropsied between 640 and 734 days, but the majority were necropsied at 734 days.

SOURCE: Adapted from Thomas *et al.* (1967a).

Experimental production of N-nitrosodiphenylamine (NNDPA) in the stomach of rats (species unspecified) fed a diet containing 0.02% DPA and 0.04% sodium nitrite has been demonstrated (IARC, 1975). Dietary administration of NNDPA resulted in an increased incidence of transitional-cell carcinomas of the urinary bladder in F344 male and female rats over controls (IARC, 1982). Dietary feeding of NNDPA for 101 weeks produced epithelial hyperplasia of urinary bladder mucosa in $B6C3F_1$ mice whereas this lesion was absent in controls (IARC, 1982). However, with regard to incidences and types of other tumors, they were similar in treated and control animals. In another experiment, one-week-old $(C57BL/6XAnf)F_1$ or $(C57BL/6XAKR)F_1$ mice were given NNDPA in dimethyl sulfoxide for the first 3 weeks followed by dietary feeding until 79 weeks of age (IARC, 1972). No increased incidences of tumors were observed over controls in these mice.

6. Genotoxicity

DPA tested negative by Ames reverse mutation assay with or without S9 metabolic activation in *Salmonella typhimurium* strains TA98, TA100, TA1000, TA1535, TA1537, TA1538, C3076, D3052, and G46 (Ferretti *et al.*, 1976; Florin *et al.*, 1980; Probst *et al.*, 1981), and in *Escherichia coli* strains WP2 and WP2uvrA- (Probst *et al.*, 1981) in the presence or absence of the S9 factor.

DPA was also negative for forward mutation in the thymidine kinase locus of cultured mouse lymphoma cells in the presence of S9 factor (Amacher *et al.*, 1980), and for unscheduled DNA synthesis in cultured hepatocytes prepared from Fischer 344 rats (Probst *et al.*, 1981).

DPA was tested for co-mutagenicity with norharman, a component of cigarette smoke, in *S. typhimurium* TA98 (U.S. EPA, 1985). DPA was more mutagenic in the presence of S9 factor and norharman, than NNDPA suggesting that NNDPA may metabolically yield DPA. Negative results were obtained when NNDPA was tested for genotoxicity by several short-term assays in prokaryotes and eukaryotes (IARC, 1982).

7. Other Effects

Hong *et al.* (1974) investigated the effect of DPA on sodium and water transport in isolated toad (*Bufo marinus*) skin and urinary bladder. A pair of symmetrical abdominal skin samples was obtained from each of seven toads for control and treatment measurements. Similarly, pairs of

hemibladder samples was excised for the bladder studies. Reagent grade DPA obtained from Baker Chemical Co. was prepared by dissolving 5 mg DPA in 50 µL ethanol and 100 mL Ringer solution. Ethanol at this concentration had no effect on sodium or water transport in the controls. DPA in the outside bathing medium of the skin caused the short-circuit current and electrical potential difference to rapidly decrease, reaching a plateau in 40-50 minutes. Short-circuit current was reduced 70% when measured at 60 minutes of exposure. Recovery was no more than 50%, 60 minutes after washout. Adding DPA to the inside surface of the skin caused a much less rapid effect with a 30% reduction at 60 minutes exposure. Skin pretreated with DPA had no effect on the usual effect of an antidiuretic hormone. The reduction of short-circuit current was accompanied by a proportional reduction in net sodium influx without any change in net chloride fluxes. In the urinary bladder experiments, the mucosal side was bathed with 1:5 choline chloride-Ringer and the serosal side with full-strength sodium chloride Ringer. Osmotic water transfer induced by antidiuretic hormone was significantly reduced in the presence of DPA on either the serosal or mucosal side of the bladder, indicating that DPA interferes with the water permeability of the urinary bladder. The authors concluded that DPA inhibits both the active transport of sodium across the toad skin and the antidiuretic hormone-induced passive transport of water across the toad urinary bladder.

VII. HEALTH ADVISORY DEVELOPMENT

A. SUMMARY OF HEALTH EFFECTS DATA

Diphenylamine is used to stabilize nitrocellulose explosives and celluloid in various gun propellant compositions. It is also used extensively as a dip spray and impregnate of paper wraps to prevent scald on apples and other fruits, and as an insecticide. Therapeutically, DPA derivatives are used to treat helminthic infections. Diphenylamine is used in the manufacturing of dyes, polymers, greases, and oils, in producing industrial antioxidants for rubber, and as an analytical reagent (Budavari, 1989). Diphenylamine has been detected in the effluent of manufacturing plants in California, in Rhine River water, and in Norwegian rain water (U.S. EPA, 1985). It was also detected in trace amounts in the ambient air of Geismar, LA (U.S. EPA, 1985). The U.S. Food and Drug Administration's Monitoring program and Total Diet Program, and the U.S. Department of Agriculture's National Residue Program found DPA of undetermined concentration in only one fruit sample and one infant and junior food formula in several thousands of samples tested during 1970-1976.

Only a modicum of data are available on the fate of DPA in the environment. Information about potential hydrolysis of DPA and photolysis in aqueous solutions are lacking. Rapid microbial degradation of DPA occurred in a laboratory-model sewage sludge system (U.S. EPA, 1985). Based on its solubility and calculated vapor pressure, DPA is predicted to volatilize slowly (U.S. EPA, 1985). It is considered to have a medium-to-low mobility in soils (Briggs, 1981).

No specific animal or human data on the absorption and distribution of DPA following ingestion were located. However, metabolic studies of DPA suggest that absorption does occur. Diphenylamine is rapidly excreted in the urine and is found in the bile of male rats intraperitoneally or intravenously injected with the compound (Alexander *et al.*, 1965). In human urine samples following a single oral dose of DPA, unchanged DPA, 4-Hydroxydiphenylamine (4-HDPA), and 4,4'-Dihydroxydiphenylamine were detected (Alexander *et al.*, 1965). Glucuronic acid conjugate of 4-HDPA was the major metabolite found in the urine of rats intraperitoneally injected with a dose of either DPA, 4-HDPA, or N-hydroxydiphenylamine (Alexander *et al.*, 1965).

Except for some dermal studies in occupational workers, no epidemiological, clinical case histories, or experimental studies of potential human health effects to DPA exposure are available. Diphenylamine was not irritating to the skin of humans or rabbits (Slovak, 1980; Calnan, 1978; Epstein, 1976 cited in Opdyke, 1978; Levenstein, 1976 cited in Opdyke, 1978).

Reported oral LD_{50}s for rats are 1.165 g/kg (Levenstein, 1976 cited in Opdyke, 1978), 3.2 g/kg (Epstein *et al.*, 1967 and Volodchenko, 1975 cited in Opdyke, 1978), and 2.0 g/kg (Korolev *et al.*, 1976); for mice 1.75 g/kg (Korolev *et al.*, 1976); and for guinea pigs 300 mg/kg (Sweet, 1987). Short-term studies with Sprague-Dawley rats, Syrian hamsters, and Mongolian gerbils given 400, 600, or 800 mg DPA/kg/day in peanut oil once a day by gavage for 3 days showed that Syrian hamsters are more sensitive to DPA-related mortality and renal pathologic damage than either the rats or gerbils (Lenz and Carlton, 1990). These authors suggested a No Observed Adverse Effect Level (NOAEL) of 400 mg/kg/day for acute renal pathologic changes (not including papillary necrosis) in rats. However, in the Syrian hamsters, this dose produced 40% mortality and total renal papillary necrosis and splenomegaly in 90% of the animals. In another gavage study, Powell *et al.* (1985) observed necrosis of renal papilla and pars recta in Wistar rats given 4.1 mM (694 mg) DPA/kg once a day by gavage for 3 or 9 days. In mice gavaged once with 600 mg DPA/kg, Kronevi and Holmberg (1979) observed high mortality and pathological abnormalities of the intestine, kidney, and liver.

In male and female F344 rats (6/group), daily doses of DPA (111, 333, or 1,000 mg/kg/day) administered in olive oil by gavage for 28 days caused a treatment-related decrease in body weight gain and in relative organ weights (most notably the liver); depressed erythrocyte and hemoglobin levels; increased leukocytes, serum albumin, bilirubin, and potassium levels; and renal tubular degeneration and necrosis, congestion and extramedullary hematopoiesis of the spleen, bone marrow hyperplasia, and mucosal changes in the forestomach (Yoshida *et al.*, 1989). The Lowest-Observed-Adverse-Effect Level (LOAEL) in this study is 111 mg/kg/day, the lowest dose tested, based on liver weight changes in male rats. There is no NOAEL for this study. The 25-day oral study in rats by Korolev *et al.* (1976) suggested a NOAEL of 16 mg DPA/kg/day but presented no details of the results.

In an 8-month study, Thomas *et al.* (1957) investigated the effects of DPA on growth and the anatomy of selected internal organs in 36 female weanling albino rats fed 0.025, 0.1, 0.5, 1.0, or

1.5% DPA in the diet (corresponding to calculated doses of 27.5, 110, 550, 1,100, and 1,650 mg/kg/day, respectively) for 226 days. Dietary levels of at or above 0.5% DPA caused a dose-related decrease in body weight gain, hematogenic granular pigmentation in the liver and kidneys, and focal dilatation of renal tubules with multiple cystic structures. The study suggested a NOAEL of 0.1% (calculated dose of 110 mg/kg/day) for body weight gain and renal pathological changes in female rats. A chronic study by Korolev *et al.* (1976) suggested a NOAEL of 0.5 mg/kg/day in male albino rats based on the absence of several clinical chemistry parameters, reflex activity, and morphological studies, but the authors did not present any details regarding animal species and number, dosing frequency, study duration, or results.

A lifetime study in which Slonaker-Addis rats (20/sex/group) were fed diets containing 0.001, 0.01, 0.1, 0.5, or 1% DPA (corresponding to calculated doses of 1.1-999 mg/kg/day for males and 0.96-812 mg/kg/day for females) for 2 years showed several DPA-related effects (Thomas *et al.*, 1967a). Levels above 0.1% dietary DPA depressed body weight gain without decreasing food consumption, and at the 0.5 and 1% levels anemia characterized by reduced hemoglobin and erythrocyte levels occurred. Dilated cystic renal tubules accompanied by chronic interstitial nephritis occurred at or above the 0.1% level. This study suggested a NOAEL of 0.01% (calculated dose of 9.6 mg/kg/day). An unpublished report of this study (Booth, 1963) indicated that levels above 0.5% dietary DPA depressed body weight gain without decreasing food consumption, and at or above the 1% dietary DPA level, anemia characterized by reduced blood hemoglobin and erythrocytes occurred. Dilated cystic renal tubules accompanied by chronic interstitial nephritis occurred at or above the 0.5% level. These results suggest a NOAEL of 0.1% (calculated dose of 100 mg/kg/day).

In a similar study, Thomas *et al.* (1967b) fed a small number of beagle dogs a diet containing 0.01%, 0.1%, or 1% DPA for 2 years and observed decreased body weight gain at or above the 0.1% DPA level and a possible treatment-related decrease in hemoglobin and erythrocytes at the 0.1% level. Some pathological changes were found in the liver, spleen, kidneys, and bone marrow at the 1% level. Kidney function tests were negative. The study suggested a NOAEL of 0.01% (calculated dose of 2.5 mg/kg/day based on Lehman [1959]). According to an unpublished report of this study (Booth, 1963), growth inhibition, decreased blood hemoglobin and erythrocyte levels, and pathological changes occurred at the 1% level suggesting a NOAEL of 0.1% (calculated dose of 25 mg/kg/day based on Lehman [1959]).

Renal tubular dilatation and intrarenal cysts in rats have been caused by daily gavage of DPA at 4.1 mM (694 mg) DPA/kg daily for 3-9 days (Powell *et al.*, 1985) and at 1.6 mM (271 mg) DPA/kg for 1-8 weeks (Powell *et al.*, 1983), and by the continuous ingestion of a diet containing DPA at 2.5% for 3-6 weeks (Eknoyan *et al.*, 1976), at 2.5% for 19 weeks (Woodhouse *et al.*, 1965; Darmady *et al.*, 1970), at 1.5% or 2.5% for 0.5-12 months (Safouth *et al.*, 1970), at 2.5% for 12 months (Kime *et al.*, 1962), at 1% DPA for 18 months (Evan *et al.*, 1976), and at 1% DPA for 5-20 months (Gardner *et al.*, 1976). Renal pathologic changes also have been observed in male NMRI mice given 1.4 g DPA/kg/day doses by oral gavage for 10 weeks (Kronevi and Holmberg, 1979).

Slonaker-Addis rats of both sexes maintained on a diet containing 0.1, 0.25, or 0.5% DPA starting at 35 days of age were mated twice and their offspring were mated (Thomas *et al.*, 1967a). Average litter size decreased as DPA level in the diet increased. Wickramaratne (1987) found no DPA-related effects on litter size or survival in female Wistar rats given 1,000 mg/kg/day by oral gavage during days 7-17 of gestation. Crocker and Vernier (1970, abstract) reported renal cystic dilatation of the collecting ducts and vacuolar degeneration of the proximal tubules in all newborn rats (species not specified) from dams fed 200 mg DPA/kg/day by gastric tube or 2.5% dietary DPA (corresponding to a calculated dose of 2,500 mg/kg/day) during the last 6 days of gestation. In a later study (Crocker *et al.*, 1972), the DPA-related effects on the kidney of second generation Sprague-Dawley rats was attributed to an unidentified contaminant chromatographically isolated from DPA. This contaminant was given to dams by gastric tube at 50 µg/day in 2 mL of 70% ethanol (calculated to be 0.5 mg/kg/day) during the last 6-7 days of gestation. No significant cystic changes occurred in newborns from dams intragastrically intubated with chemically pure DPA (20 mg DPA/ rat in 2 mL of ethanol; calculated to be 200 mg/kg/day).

Diphenylamine has been shown to be noncarcinogenic in Sprague-Dawley rats given a single oral dose of 300 mg DPA/kg over a 6-month period (Griswold *et al.*, 1966) and in Slonaker-Addis rats given daily DPA in the diet at concentrations of 0.001-1% over a 2-year period (Thomas *et al.*, 1967a). Also, no evidence of dietary DPA-induced carcinogenicity occurred in mice over a 92 week period (U.S. EPA, 1985). Negative results were obtained in a bioassay involving *in vivo* exposure of Syrian hamster fetuses to DPA and *in vitro* culture of exposed fetal cells to observe neoplastic transformation, if any (DiPaolo *et al.*, 1973).

Diphenylamine tested negative in the Ames reverse mutation assay in *S. typhimurium* strains TA98, TA100, TA1000, TA1535, TA1537, TA1538, C3076, D3052, and G46 with and without S9 activation (Ferretti *et al.*, 1976; Florin *et al.*, 1980; Probst *et al.*, 1981) as well as in *E. coli* strains WP2 and WP2uvrA- with or without S9 activation (Probst *et al.*, 1981). Diphenylamine also tested negative in isolated mouse lymphoma cells with S9 activation (Amacher *et al.*, 1980) and did not cause unscheduled DNA synthesis in cultured rat hepatocytes (Probst *et al.*, 1981).

B. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

Health Advisories are generally determined for One-day, Ten-day, Longer-term (approximately 7 years), and Lifetime exposures if adequate data are available that identify a sensitive noncarcinogenic endpoint of toxicity. The HAs for noncarcinogenic toxicants are derived using the following formula:

$$HA = \frac{(NOAEL \text{ or } LOAEL)\ (BW)}{(UF[s])\ (___\ L/day)} = ___\ mg/L$$

where:

NOAEL or LOAEL = No- or Lowest- Observed Adverse Effect Level (in mg/kg bw/day).

BW = assumed body weight of a child (10 kg) or an adult (70 kg).

UF[s] = uncertainty factors (10; 100; 1,000; or 10,000) in accordance with NAS/EPA guidelines.

___ L/day = assumed daily water consumption of a child (1 L/day) or an adult (2 L/day).

1. One-day Health Advisory

No suitable study was found that could be used to derive the One-day HA. Among the studies considered, Kronevi and Holmberg (1979) used one high dose of 600 mg DPA/kg in mice and observed 31% mortality within 5 days of treatment. The 3-day oral gavage study in Sprague-Dawley rats, Syrian hamsters, or gerbils by Lenz and Carlton (1990) indicated that the lowest tested dose of 400 mg/kg/day produced significant lethality (hamsters only) and/or other adverse effects. Powell *et al.* (1985) observed adverse renal effects in gavaged Wistar rats with only one high dose of 4.1 mM

(694 mg) DPA/kg daily for 3 or 9 days. Thus, the Ten-day HA for a child of 1.0 mg/L is suggested as a conservative estimate for a One-day HA.

2. Ten-day Health Advisory

The 28-day oral study by Yoshida *et al.* (1989) was considered the best available study for the determination of a Ten-day HA. In this study, rats were administered DPA daily by single oral gavage at 111, 333, or 1,000 mg/kg/day. Data on food consumption, growth rate, detailed clinical chemistries, gross and microscopic pathology were presented. This study suggested a LOAEL of 111 mg/kg/day for reduced weights of liver, kidney, and spleen. The 25-day oral study in rats by Korolev *et al.* (1976) suggested a NOAEL of 16 mg DPA/kg/day but presented no details with regard to the species, sex, and number of animals used nor any histopathological studies.

Using the LOAEL of 111 mg/kg/day, the Ten-day HA for the 10-kg child is calculated as follows:

$$\text{Ten-day HA} = \frac{(111\ \text{mg/kg/day})\ (10\ \text{kg})}{(1000)\ (1\ \text{L/day})} = 1.11\ \text{mg/L} = (\text{rounded to } 1.0\ \text{mg/L or } 1{,}000\ \mu\text{g/L})$$

where:

111 mg/kg/day = LOAEL, based on reduced weights of liver, kidney, and spleen in rats following 28 days of daily oral exposure.

10 kg = assumed body weight of a child.

1000 = uncertainty factor (UF), chosen in accordance with NAS/EPA guidelines for use of a LOAEL from an animal study. This UF includes a factor of 10 for interspecies variability, a factor of 10 for intraspecies variability, and a factor of 10 for use of a LOAEL in the absence of a NOAEL.

1 L/day = assumed daily water consumption of a child.

3. Longer-term Health Advisory

The feeding study in beagle dogs for over 400 days was considered most suitable for the determination of the Longer-term HA. In this study, 8-month old beagle dogs (two/sex/group) were

fed chemically pure (99.9%) DPA in the diet at 0.01%, 0.1%, and 1% levels (2.5, 25, 250 mg/kg/day, respectively). An outbreak of distemper caused the death of one female (0.1% DPA) on day 434 of treatment and also necessitated the sacrifice of two males. The overall food consumption was normal in all groups. Dose-related growth retardation and adverse blood effects occurred during the 266-400 day period at 0.1% and 1% levels (more details of adverse effects are presented in section VI.B.2.b). This study suggested a NOAEL of 0.01% dietary DPA (calculated dose of 2.5 mg/kg/day) for the absence of depressed body weight gain, hematological, and renal pathological effects.

The 226-day feeding study in rats by Thomas *et al.* (1957) was considered less suitable than the dog study for the determination of Longer-term HA for DPA. In this study, data on food consumption, growth rate, and histopathology of most major organs were evaluated and a NOAEL of 0.01% dietary DPA (calculated dose, 110 mg DPA/kg/day) was suggested for the absence of renal pathological changes. Another study that was considered but was not found suitable was the rat feeding study of Korolev *et al.* (1976). These authors provided no information on the species/sex/number of animals, dosing frequency, or study duration although several clinical parameters were evaluated and a NOAEL of 0.5 mg DPA/kg/day was found. Beagle dog appears to be more sensitive than rats to DPA effects based on detailed gross and histopathological findings. Thus, the NOAEL of 2.5 mg/kg/day obtained in the dog study is used as a conservative value for the calculation of the Longer-term HA.

The Longer-term HA for a 10-kg child is calculated as follows:

$$\text{Longer-term HA} = \frac{(2.5\ \text{mg/kg/day})\ (10\ \text{kg})}{(100)\ (1\ \text{L/day})} = 0.25\ \text{mg/L} \quad \text{(rounded to 0.3 mg/L or 300 } \mu\text{g/L)}$$

where:

2.5 mg/kg/day = NOAEL, based on the absence of depressed body weight gain and blood effects in beagle dogs fed dietary DPA daily for over 400 days.

10 kg = assumed weight of a child.

100 = uncertainty factor (UF), chosen in accordance with NAS/EPA guidelines for use of a NOAEL from an animal study. This UF includes a factor of 10 for interspecies variability and a factor of 10 for intraspecies variability.

1 L/day = assumed water consumption of a 10-kg child.

The Longer-term HA for a 70-kg adult is calculated as follows:

$$\text{Longer-term HA} = \frac{(2.5\ \text{mg/kg/day})\ (70\ \text{kg})}{(100)\ (2\ \text{L/day})} = 0.875\ \text{mg/L}\ \ (\text{rounded to } 1.0\ \text{mg/L or } 1{,}000\ \mu g/L)$$

where:

2.5 mg/kg/day = NOAEL, based on the absence of depressed body weight gain and blood effects in beagle dogs fed dietary DPA daily for over 400 days.

70 kg = assumed weight of an adult.

100 = uncertainty factor (UF), chosen in accordance with NAS/EPA guidelines for use of a NOAEL from an animal study. This UF includes a factor of 10 for interspecies variability and a factor of 10 for intraspecies variability.

2 L/day = assumed water consumption of a 70-kg adult.

4. Lifetime Health Advisory

The Lifetime HA represents that portion of an individual's total exposure that is attributed to drinking water and is considered protective of noncarcinogenic adverse health effects over a lifetime exposure. The Lifetime HA is derived in a three-step process. Step 1 determines the Reference Dose (RfD), formerly called the Acceptable Daily Intake (ADI). The RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious health effects during a lifetime, and is derived from the NOAEL (or LOAEL), identified from a chronic (or subchronic) study, divided by an uncertainty factor(s). From the RfD, a Drinking Water Equivalent Level (DWEL) can be determined (Step 2). A DWEL is a medium-specific (*i.e.*, drinking water) lifetime exposure level, assuming 100% exposure from that medium, at which adverse, noncarcinogenic health effects would not be expected to occur. The DWEL is derived from the multiplication of the RfD by the assumed body weight of an adult and divided by the assumed daily water consumption of an adult. The Lifetime HA in drinking water alone is determined in Step 3 by factoring in other sources of exposure, the relative source contribution (RSC). The RSC from drinking water is based on actual exposure data or, if data are not available, a value of 20% is assumed.

If the contaminant is classified as a known, probable, or possible carcinogen, according to the Agency's classification scheme of carcinogenic potential (U.S. EPA, 1986), then caution must be exercised in making a decision on how to deal with possible lifetime exposure to this substance. For

known human (A) or probable (B) human carcinogens, a Lifetime HA is not recommended. For possible (C) human carcinogens, an additional 10-fold safety factor is used in the calculation of the Lifetime HA. The risk manager must balance this assessment of carcinogenic potential and the quality of the data against the likelihood of occurrence and significance of health effects related to noncarcinogenic endpoints of toxicity.

The 2-year dog feeding study (Thomas *et al.*, 1967b) is considered most appropriate for calculation of the RfD. In this study, 0.01% dietary DPA produced no gross or histopathological indicators of adverse health effects. At 0.1% DPA, while no kidney changes were observed, there was growth reduction and mild anemia, and at 1% DPA the liver, kidney, and blood effects were significantly increased (details are presented in section VI.B.2.b). Thus, 0.01% DPA, which calculates to a dose of 2.5 mg DPA/kg/day, was considered the NOAEL, and 0.1% DPA was considered the LOAEL based on decreased weight gain and increased liver and kidney weights.

The 2-year, feeding toxicity/oncogenicity study in rats by Thomas *et al.* (1967a) also is suitable for determining the Lifetime HA. In this study, rats were fed DPA at dietary levels of 0.001, 0.01, 0.1, 0.5, or 1% for 734 days. Sufficient details regarding food consumption, growth rate, hematology, gross and histopathology were presented. A NOAEL of 0.01% dietary DPA, which calculates to a dose of 9.6 mg DPA/kg/day, was also suggested in this study. This value was considered less conservative than the value for beagle dogs, and the dogs appear to be more sensitive to DPA. Thus, the NOAEL for dogs is used for calculation of the RfD. No other studies of lifetime duration were available for consideration in determining a Lifetime HA.

Step 1: Determination of the Reference Dose (RfD)

$$\text{RfD} = \frac{2.5\ \text{mg/kg/day}}{100} = 0.025\ \text{mg/kg/day} \quad (\text{rounded to } 0.03\ \text{mg/kg/day})$$

where:

2.5 mg/kg/day = NOAEL, based on the absence of growth retardation and adverse hematological effects in dogs fed DPA in the diet daily for 2 years.

100 = uncertainty factor (UF), chosen in accordance with NAS/EPA guidelines for use with a NOAEL from an animal study. This UF includes a factor of 10 for interspecies variability and a factor of 10 for intraspecies variability.

Step 2: Determination of the Drinking Water Equivalent Level (DWEL)

$$\text{DWEL} = \frac{(0.03\ \text{mg/kg/day})\ (70\ \text{kg})}{2\ \text{L/day}} = 1.05\ \text{mg/L}\quad \text{(rounded to 1.0 mg/L or 1,000 } \mu\text{g/L)}$$

where:

0.03 mg/kg/day = RfD.

70 kg = assumed body weight of an adult.

2 L/day = assumed daily water consumption of an adult.

Step 3: Determination of the Lifetime Health Advisory

$$\text{Lifetime HA} = (1.0\ \text{mg/L})\ (0.2) = 0.2\ \text{mg/L}\quad (200\ \mu\text{g/L})$$

where:

1.0 mg/L = DWEL

0.2 = assumed relative source contribution (20%) from water.

Evaluation of Carcinogenic Potential

C. QUANTIFICATION OF CARCINOGENIC POTENTIAL

Three available carcinogenicity studies are considered to be inadequate to estimate the carcinogenic potential of DPA. Lifetime feeding studies in rats and mice and a chronic feeding study in dogs did not reveal compound-related neoplastic effects; however, either design deficiencies or lack of reported information limit the conclusions concerning the carcinogenic potential of DPA.

A lifetime feeding study in rats using traditional cancer bioassay methods did not reveal compound-related neoplastic effects, although some malignant and benign tumors involving many organs were related to aging and senility (Thomas *et al.*, 1967a). This study was adequate with regard to its duration, doses of DPA, food consumption and growth data, and gross and histopathological observations. It is limited because, with the small number of animals that were treated, the statistical power to detect cancer is reduced, and there is added uncertainty for a conclusion of no evidence of carcinogenicity.

A 2-year feeding study in beagle dogs (Thomas *et al.*, 1976b) gave no indication of carcinogenicity. Deficiencies in the study, such as, the small number of animals used, the occurrence of distemper and related mortality which further reduced the number of animals in some dose groups, and relatively short study duration, limit its conclusions.

Negative results were reported for a 92-week feeding study in mice (U.S. EPA, 1985) but the study is unpublished and has been reviewed only briefly by the Food and Agricultural Organization of the United Nations and the World Health Organization. The quality of the study and validity of the conclusions cannot be assessed because the description in the EPA document is limited to a single paragraph with little experimental details. There is no information concerning animal survival, the organs examined, histopathology, systemic effects, and statistical significance.

Though DPA is not classifiable as to human carcinogenicity, there is useful information concerning its cancer potential that can be inferred from the existing cancer and mutagenicity studies. If cancer was not detected because of the deficiencies in the studies, it is reasonable to consider that the cancer potency of DPA is nil to low. If DPA were a highly potent carcinogen, then cancer would be expected to have occurred in one or more of the chronic/lifetime studies. Also, DPA was negative in mutagenicity assays with bacterial and mammalian test systems which is supporting evidence for low or no cancer potential.

The study in rats (Thomas *et al.*, 1976a) used six dose groups rather than the two or three routinely used in NTP cancer bioassays. The high dose approached 1,000 mg/kg/day. Though there were only 20 animals/sex/dose (rather than 30-50 animals/sex/dose, which are required for adequate statistical significance), high cancer potential would be expected to be detected even with the small number of animals in the dose groups.

The overall evidence does not meet the EPA criteria to classify DPA as having no evidence of human carcinogenicity (Groups E). A Group E classification requires no evidence of carcinogenicity in at least two species and in well-conducted studies (U.S. EPA, 1986). The existing studies were not adequate according to current guidelines.

There is sufficient information to indicate the carcinogenic potential of DPA when fed in the diet or by other methods of oral administration over a lifetime. Based on this information and applying

the U.S. EPA (1986) guidelines for the assessment of carcinogenic risk, DPA is classified in Group D: not classifiable as to human carcinogenicity.

VIII. OTHER CRITERIA, GUIDANCE, AND STANDARDS

The American Conference of Governmental Industrial Hygienists has established a Threshold Limit Value of 10 mg/m^3, the time-weighted average (TWA) concentration for a normal 8-hour work day and a 40-hr work week, to which nearly all occupational workers may be repeatedly exposed daily without adverse effect (ACGIH, 1990). U.S. Department of Labor, Occupational Safety and Health Administration has established a limit for air contamination of DPA at 10 mg/m^3, TWA (OSHA, 1989). Federal Register, 1962, Title 21-Food and Drugs (Cited in U.S. EPA, 1985) lists tolerances for residues of DPA in or on apples of 10 ppm (mg/kg) to cover residues from preharvest spray or use of impregnated wraps; and 0 ppm in meat or milk. Diphenylamine is listed under the Resource Conservation and Recovery Act, Appendix IX, for groundwater monitoring (U.S. EPA, 1990). Diphenylamine is not listed under Title III of the Superfund Amendments and Reauthorization Act of 1986. No other guidelines or standards were located in the available literature.

IX. ANALYTICAL METHODS

A gas chromatography method was described by Gutenmann and Lisk (1963) for the determination of DPA in apples. Apples to which DPA had been applied as scald-control were chopped and blended with acetone. Filtered acetone samples were extracted with hexane (for instance, 1 mL of acetone and 15 mL of hexane were required for 3 ppm DPA in the fruit). The hexane extract was then directly brominated (for instance, to 1 mL of hexane extract was added 0.1 mL of carbon tetrachloride saturated with iodine crystals containing by volume 5% liquid bromine; the mixture was then heated for 10 minutes at 40-45°C) presumably to yield the ortho-, para-hexabromo derivative of DPA. Suitable aliquot of hexane extract containing brominated DPA was dried by evaporation and the bromination procedure was repeated. Finally a 5-10 µL sample of hexane extract was subjected to gas chromatography.

The experimental chromatographic conditions were: An U-shaped borosilicate glass column packed with 5% ethyl acetate fractionated high-vacuum silicone grease on 80-100 mesh acid-washed Chromosorb W; operating temperatures for column, flash heater, and detector were 235°, 275°, and 265°C, respectively; carrier gas was nitrogen, 60 mL/min; and the column was conditioned for 16 hours at 245°C before use. The recoveries of added DPA to apples (0.1-5.0 ppm) ranged from 97-118% (Table IX-1). The method was sensitive to about 0.02 ppm DPA. The complete analysis took about one hour. The standard curve for DPA gave a straight line for 0.0002-0.001 µg DPA.

Alley and Dykes (1982) described a gas-liquid chromatographic (GLC) method for the separation and estimation of diphenylamine (DPA). The authors developed this method essentially for determining nitrate esters, stabilizers and plasticizers used in a variety of nitrocellulose-based propellants and found it to be precise, accurate, and time-saving.

A gas chromatograph with dual hydrogen flame ionization detector and an electronic digital integrator was used. Dual chromatographic-grade stainless steel columns (1/8 inch X 2 feet) were packed with solid support adsorbents Gas-Chrom q, Chromosorb W-HP, or Gas-Chrom Q slurried with volatile solvent OV-101, OV-210, or OV-225. The packed columns were preconditioned at 250°C for 4-6 hours while maintaining a helium flow of 10-15 mL/min. Methylene chloride was used for the direct analysis of most of the test samples. Normal alkanes were preferred for use as internal standards because of the ease of handling. Specific GLC experimental conditions for DPA

Table IX-1. Recovery of DPA Added to Apples by Direct Bromination Gas Chromatography

Apple Variety	Amount DPA Added (ppm)	Recovery (%)
Wagener	0.1	120
	0.3	97
	0.6	101
	1.0	118
	2.0	110
	5.0	105
Cortland	1.0	108, 110
McIntosh	1.0	118

SOURCE: Adapted from Gutenmann and Lisk (1963).

were: Column temperature-programmed, from 70-220°C at 6°/min; Flame ionization detector temperature, 225°C; and the other data are presented in Table IX-2. No other analytical method specifically applicable to DPA was found in the available published literature.

Table IX-2. Gas-Liquid Chromatography Retention Data for Diphenylamine

Columns	3.8% OV-101	2.5% OV-210	1.1% OV-225
Retention Time (minutes)[a]	8.65	1567	122
Kovats Index	6.26	1730	108
Retention Temperature (°C)	11.59	2151	140

[a]Retention time for methylene chloride solvent was 0.11 min.

SOURCE: Adapted from Alley and Dykes (1982).

X. TREATMENT TECHNOLOGIES

No water treatment technologies for DPA were located in the available literature. However, the U.S. EPA has developed a carbon-adsorption isotherm of 120 mg/g for the adsorption of DPA on activated carbon (Kirk-Othmer, 1984).

XI. CONCLUSIONS

No suitable study was available for use in the calculation of a One-day HA inspite of the fact that there are several studies dealing with oral LD_{50}s as well as studies using one to three daily doses of oral DPA in a number of animal species. All of these studies used high doses of DPA which produced lethality or pronounced adverse effects. As such, no information on a LOAEL or NOAEL could be obtained from them. Therefore, the Ten-day HA for a 10 kg child of 1.0 mg/L is suggested as a conservative estimate for a One-day HA.

The Ten-day HA for a 10 kg child of 1.0 mg/L was derived using the LOAEL of 111 mg/kg/day, which was based on reduced weights of liver, kidney, and spleen, in rats administered DPA daily by oral gavage for 28 days.

Although there are a number of animal studies that investigated the long-term effects of DPA, many of them used one or two high doses primarily with the object of producing kidney lesions in the tested species to learn about the etiology of human polycystic kidney disease. These studies were not useful in identifying a NOAEL or LOAEL of DPA. However, three chronic feeding studies (two in rats and one in dogs) was found suitable for determining the Longer-term HA. The Longer-term HA for a 10 kg child of 0.3 mg/L and that for a 70 kg adult of 1.0 mg/L were based on the NOAEL of 2.5 mg/kg/day which in turn was based on the absence of depressed body weight gain and adverse blood effects in beagle dogs fed DPA in the diet for over 400 days.

The Lifetime HA of 0.2 mg/L for a 70 kg adult was based on a Drinking Water Equivalent Level of 1.0 mg/L. The DWEL is derived from a Reference Dose (RfD) of 0.03 mg/kg/day. For the calculation of RfD, the NOAEL of 2.5 mg/kg/day derived from the 2-year dog feeding study based on the absence of growth retardation and adverse hematological effects, was used. This study was also used for the derivation of Longer-term HA as mentioned previously.

Diphenylamine was not carcinogenic in three cancer bioassays and one chronic study in dogs. One of these studies was a lifetime study in rats and another one was a lifetime study in mice that was not sufficiently reviewed. Some malignant and benign tumors observed in several organs in rats was attributed to the age and senility of the animals and not treatment-related. Another carcinogenicity study in rats used a non-conventional assay in that the rats were subjected to one maximum

tolerated dose of DPA given intraperitoneally and the animals were observed for a period of six months. While known carcinogens gave positive responses by this method DPA did not produce any malignancy. The chronic feeding study in dogs also did not produce any malignancy. Thus, in at least two animal species, no evidence was found for the induction of cancer.

The carcinogenicity studies are considered to be inadequate because either design deficiencies or lack of reported information limit the conclusions concerning the carcinogenic potential of DPA. Based on this information, DPA is classified in Group D: not classifiable as to human carcinogenicity.

A specific method related to the separation and analysis of DPA in drinking water was not found in the published literature. However, two analytical methods described in the literature, one for the determination of DPA in apples and the other for the determination of nitrate esters, stabilizers, and plasticizers may be useful for the analysis of DPA in environmental samples. Both methods are based on gas chromatography.

Water treatment technologies specific for DPA were not found in the available literature.

XII. REFERENCES

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APPENDIX A

Data Deficiencies, Problem Areas, and Recommendations for Additional Database Development for Diphenylamine

DATA DEFICIENCIES, PROBLEM AREAS, AND RECOMMENDATIONS FOR ADDITIONAL DATABASE DEVELOPMENT FOR DIPHENYLAMINE

A. OBJECTIVES

The objective of this document is to provide an evaluation of data deficiencies and problem areas encountered after a careful review of the literature on diphenylamine (DPA) and to make recommendations for additional database development, if necessary. This document is presented as an independent analysis of the current data related to DPA in drinking water, and it includes a summary of the background information used in development of the Health Advisory (HA). For greater detail on the toxicology of DPA, the Health Advisory for Diphenylamine (DPA) should be consulted.

B. BACKGROUND

N,N-Diphenylamine or diphenylamine (DPA), also called N-phenylbenzeneamine, is a crystalline solid at room temperature and the simplest of the diarylamines (Kirk-Othmer, 1978). Diarylamines are aromatic organic compounds with two of the hydrogen atoms of ammonia replaced by aryl groups. Diphenylamine is practically insoluble in water (30-35.7 mg/L at 25°C) but soluble in several organic solvents. Diphenylamine is produced by self-condensation of aniline in the presence of ferrous chloride, ammonium bromide, and a small amount of a strong mineral acid. It is a chemically reactive compound. The U.S. Tariff Commission listed five domestic producers of DPA in 1975 with an annual production of 18,094 tons. Diphenylamine is also imported (U.S. EPA, 1985).

Diphenylamine is used to stabilize nitrocellulose explosives and celluloid in various gun propellant compositions. It is also used extensively as a dip spray and impregnate of paper wraps to prevent scald on apples and other fruits, and as an insecticide. Therapeutically, DPA derivatives are used to treat helminthic infections. Diphenylamine is used in the manufacturing of dyes, polymers, greases, and oils, in producing industrial antioxidants for rubber, and as an analytical reagent (Budavari *et al.*, 1989). Diphenylamine has been detected in the effluent of manufacturing plants in California, in Rhine River water, and in Norwegian rain water (U.S. EPA, 1985). It was also detected in trace amounts in the ambient air of Geismar, LA (U.S. EPA, 1985). The U.S. Food and

Drug Administration's Monitoring program and Total Diet Program, and the U.S. Department of Agriculture's National Residue Program found DPA of undetermined concentration in only one fruit sample and one infant and junior food formula in several thousands of samples tested during 1970-1976.

Only a modicum of data are available on the fate of DPA in the environment. Information about potential hydrolysis of DPA and photolysis in aqueous solutions are lacking. Rapid microbial degradation of DPA occurred in a laboratory-model sewage sludge system (U.S. EPA, 1985). Based on its solubility and calculated vapor pressure, DPA is predicted to volatilize slowly (U.S. EPA, 1985). It is considered to have a medium-to-low mobility in soils (Briggs, 1981).

Although no specific animal or human data on the absorption and distribution of DPA following ingestion were located, metabolic studies of DPA suggest that absorption occurs to some extent. Diphenylamine is rapidly excreted in the urine and is found in the bile of male rats intraperitoneally or intravenously injected with the compound (Alexander *et al.*, 1965). In human urine samples following a single oral dose of DPA, unchanged DPA, 4-Hydroxydiphenylamine (4-HDPA), and 4,4'-Dihydroxydiphenylamine were detected (Alexander *et al.*, 1965). Glucuronic acid conjugate of 4-HDPA was the major metabolite found in the urine of rats intraperitoneally injected with a dose of either DPA, 4-HDPA, or N-hydroxydiphenylamine (Alexander *et al.*, 1965).

Except for some dermal studies in occupational workers, no epidemiological, clinical case histories, or experimental studies of potential human health effects to DPA exposure are available. Diphenylamine was not irritating to the skin of humans or rabbits (Slovak, 1980; Calnan, 1978; Epstein, 1976 cited in Opdyke, 1978; Levenstein, 1976 cited in Opdyke, 1978).

Reported oral LD_{50}s for rats are 1.165 g/kg (Levenstein, 1976 cited in Opdyke, 1978), 3.2 g/kg (Epstein *et al.*, 1967 and Volodchenko, 1975 cited in Opdyke, 1978), and 2.0 g/kg (Korolev *et al.*, 1976); for mice 1.75 g/kg (Korolev *et al.*, 1976); and for guinea pigs 300 mg/kg (Sweet, 1987). Short-term studies with Sprague-Dawley rats, Syrian hamsters, and Mongolian gerbils given 400, 600, or 800 mg DPA/kg/day in peanut oil once a day by gavage for 3 days showed that Syrian hamsters are more sensitive to DPA-related mortality and renal pathologic damage than either the rats or gerbils (Lenz and Carlton, 1990). These authors suggested a No Observed Adverse Effect Level (NOAEL) of 400 mg/kg/day for acute renal pathologic changes (not including papillary

necrosis) in rats. However, in the Syrian hamsters, this dose produced 40% mortality and total renal papillary necrosis and splenomegaly in 90% of the animals. In another gavage study, Powell *et al.* (1985) observed necrosis of renal papilla and pars recta in Wistar rats given 4.1 mM (694 mg) DPA/kg for 3 or 9 days. In mice gavaged once with 600 mg DPA/kg, Kronevi and Holmberg (1979) observed high mortality and pathological abnormalities of the intestine, kidney, and liver.

In male and female F344 rats (6/group), daily doses of DPA (111, 333, or 1,000 mg/kg/day) administered in olive oil by gavage for 28 days caused a treatment-related decrease in body weight gain and in relative organ weights (most notably the liver); depressed erythrocyte and hemoglobin levels; increased leukocytes, serum albumin, bilirubin, and potassium levels; and renal tubular degeneration and necrosis, congestion and extramedullary hematopoiesis of the spleen, bone marrow hyperplasia, and mucosal changes in the forestomach (Yoshida *et al.*, 1989). The Lowest-Observed-Adverse-Effect-Level (LOAEL) is 111 mg/kg/day based on liver weight changes in male rats. The 25-day oral study in rats by Korolev *et al.* (1976) suggested a NOAEL of 16 mg DPA/kg/day but presented no details of the results.

In a 8-month study, Thomas *et al.* (1957) investigated the effects of DPA on growth and the anatomy of selected internal organs in 36 female weanling albino rats fed 0.025, 0.1, 0.5, 1.0, or 1.5% DPA in the diet (corresponding to calculated doses of 27.5, 110, 550, 1,100, and 1,650 mg/kg/day, respectively) for 226 days. Dietary levels of 0.5% DPA or more caused a dose-related decrease in body weight gain, hematogenic granular pigmentation in the liver and kidneys, and focal dilatation of renal tubules with multiple cystic structures. The study suggested a NOAEL of 0.1% (calculated dose of 110 mg/kg/day) for body weight gain and renal pathological changes in female rats. A chronic study by Korolev *et al.* (1976) suggested a NOAEL of 0.5 mg/kg/day in male albino rats based on the absence of several clinical chemistry parameters, reflex activity, and morphological studies, but the authors did not present any details regarding animal species and number, dosing frequency, study duration, or results.

A lifetime study in which Slonaker-Addis rats (20/sex/group) were fed diets containing 0.001, 0.01, 0.1, 0.5, or 1% DPA (corresponding to calculated doses of 1.1-999 mg/kg/day for males and 0.96-812 mg/kg/day for females) for 2 years showed several DPA-related effects (Thomas *et al.*, 1967a). Levels above 0.1% dietary DPA depressed body weight gain without decreasing food consumption, and at the 0.5 and 1% levels anemia characterized by reduced hemoglobin and erythro-

cyte levels occurred. Dilated cystic renal tubules accompanied by chronic interstitial nephritis occurred at the 0.1% level or more. This study suggested a NOAEL of 0.01% (calculated dose of 9.6 mg/kg/day). An unpublished report of this study (Booth, 1963) indicated that levels above 0.5% dietary DPA depressed body weight gain without decreasing food consumption, and levels at or above the 1% dietary DPA level, anemia characterized by reduced hemoglobin and erythrocytes occurred. Dilated cystic renal tubules accompanied by chronic interstitial nephritis occurred at or above the 0.5% level. These results suggest a NOAEL of 0.1% (calculated dose of 100 mg/kg/day).

In a similar study, Thomas *et al.* (1967b) used a small number of beagle dogs fed a diet containing 0.01%, 0.1%, or 1% DPA for 2 years and observed decreased body weight gain at or above the 0.1% DPA level and a possible treatment-related decrease in hemoglobin and erythrocytes at the 0.1% level. Some pathological changes were found in the liver, spleen, kidneys, and bone marrow at the 1% level. Kidney function tests were negative. The study suggested a NOAEL of 0.01% (calculated dose of 2.5 mg/kg/day based on Lehman [1959]). According to an unpublished report of this study (Booth, 1963), growth inhibition, decreased blood hemoglobin and erythrocyte levels, and pathological changes occurred at the 1% level suggesting a NOAEL of 0.1% (calculated dose of 25 mg/kg/day based on Lehman [1959]).

Renal tubular dilatation and intrarenal cysts in rats have been caused by daily gavage of DPA at 4.1 mM (694 mg) DPA/kg daily for 3-9 days (Powell *et al.*, 1985) and at 1.6 mM (271 mg) DPA/kg for 1-8 weeks (Powell *et al.*, 1983), and by the continuous ingestion of a diet containing DPA at 2.5% for 3-6 weeks (Eknoyan *et al.*, 1976), at 2.5% for 19 weeks (Woodhouse *et al.*, 1965; Darmady *et al.*, 1970), at 1.5% or 2.5% for 0.5-12 months (Safouth *et al.*, 1970), at 2.5% for 12 months (Kime *et al.*, 1962), at 1% DPA for 18 months (Evans *et al.*, 1976), and at 1% DPA for 5-20 months (Gardner *et al.*, 1976). Renal pathologic changes also have been observed in male NMRI mice given 1.4 g DPA/kg/day doses by oral gavage for 10 weeks (Kronevi and Holmberg, 1979).

Slonaker-Addis rats of both sexes maintained on a diet containing 0.1, 0.25, or 0.5% DPA starting at 35 days of age were mated twice and their offspring were mated (Thomas *et al.*, 1967a). Average litter size decreased as DPA level in the diet increased. Wickramaratne (1987) found no DPA-related effects on litter size or survival in female Wistar rats given 1,000 mg/kg/day by oral gavage during days 7-17 of gestation. Crocker and Vernier (1970, abstract) reported renal cystic dilatation of the collecting ducts and vacuolar degeneration of the proximal tubules in all newborn

rats (species not specified) from dams fed 200 mg DPA/kg/day by gastric tube or 2.5% dietary DPA (corresponding to a calculated dose of 2,500 mg/kg/day) during the last 6 days of gestation. In a later study (Crocker *et al.*, 1972), the DPA-related effects on the kidney of second generation Sprague-Dawley rats was attributed to an unidentified contaminant chromatographically isolated from DPA. This contaminant was given to dams by gastric tube at 50 µg/day in 2 mL of 70% ethanol (calculated to be 0.5 mg/kg/day) during the last 6-7 days of gestation. No significant cystic changes occurred in newborns from dams intragastrically intubated with chemically pure DPA (20 mg DPA/ rat in 2 mL of ethanol; calculated to be 200 mg/kg/day).

Diphenylamine was not carcinogenic to Sprague-Dawley rats given a single oral dose of 300 mg DPA/kg over a 6-month period (Griswold *et al.*, 1966) and in Slonaker-Addis rats given daily DPA in the diet at concentrations of 0.001-1% (1.1 to 999 mg/kg/day) over a 2-year period (Thomas *et al.*, 1967a). Also, no evidence of dietary DPA-induced carcinogenicity occurred in mice over a 92 week period (U.S. EPA, 1985). Negative results were obtained in a bioassay involving *in vivo* exposure of Syrian hamster fetuses to DPA and *in vitro* culture of exposed fetal cells to observe neoplastic transformation, if any (DiPaolo *et al.*, 1973).

Diphenylamine tested negative in the Ames reverse mutation assay in *S. typhimurium* strains TA98, TA100, TA1000, TA1535, TA1537, TA1538, C3076, D3052, and G46 with and without S9 activation (Ferretti *et al.*, 1976; Florin *et al.*, 1980; Probst *et al.*, 1981) as well as in *E. coli* strains WP2 and WP2uvrA- with or without S9 activation (Probst *et al.*, 1981). Diphenylamine also tested negative in isolated mouse lymphoma cells with S9 activation (Amacher *et al.*, 1980) and did not cause unscheduled DNA synthesis in cultured rat hepatocytes (Probst *et al.*, 1981).

C. DISCUSSION

Data specific for DPA were not found in the published literature on many aspects of environmental fate, for instance the photolysis of DPA in aqueous solutions with environmental photosensitizers such as humic and fulvic acids, environmental hydrolysis, or sorption on soil. However, there are some limited amount of data obtained under laboratory experimental conditions on the distribution half-life of DPA in the atmosphere, the direct photolysis of DPA in a variety of organic compounds such as ketones, aromatic hydrocarbons and dyes, and the microbial degradation of DPA in laboratory sludge systems.

There is no information on the absorption and distribution of ingested DPA in humans or experimental animal species. It can be assumed that absorption does occur to some extent based on metabolic studies of DPA. The urinary metabolites of DPA have been identified and characterized in humans, rats, rabbits, and dogs following oral administration of DPA.

Short- or long-term exposure studies on DPA in humans were not found in the published literature but there are a number of publications dealing with lethality, short- and long-term exposure in several mammalian animal species. Only a few published studies were found that suggest that DPA produces neither dermal irritation nor sensitization when applied topically to human or rabbit skin. No report was available on the ophthalmological effect of DPA.

One of the main interests in the toxicology of DPA during the 1960s pertained to its effect on kidneys. The toxic effects of DPA on the renal system were considered to resemble the effects observed in human polycystic kidney disease. It was thought that DPA might serve as a useful model substance for understanding the pathology of human polycystic kidney disease. Therefore, a number of studies were published delineating the histopathology of renal effects of DPA. These studies, especially in experimental rats, used one or two frankly toxic doses of DPA for short- or long-term periods and thus, are not useful in establishing a LOAEL or NOAEL for the purposes of suggesting health advisories. None the less, there are a sufficient number of well-executed toxicologic studies, a 28-day feeding study, a 226-day feeding study, and a 734-day feeding study, all in rats; a 2-year feeding study in beagle dogs; and a 92-week feeding study in mice that have provided useful toxicological information.

The only published reproduction study in rats available is dated and not useful in assessing the reproductive effects of DPA. As regards the developmental effects of DPA, there is only one published abstract and the emphasis of this study was on the renal effects. Additionally, the sample of commercial DPA used in this study may have been contaminated with other substances toxic to the renal tissue.

There are several oral carcinogenicity studies that, when considered with mutagenicity effects, provide useful information to the risk assessor, but do not allow an estimation of the carcinogenic potential of DPA. DPA tested negative for mutagenicity in several microbial assays, in one forward mutation assay in mouse lymphoma cells, and in a study of unscheduled DNA synthesis in cultured

rat hepatocytes. It would thus appear that DPA may induce genotoxic or carcinogenic effects in humans.

D. CONCLUSIONS AND RECOMMENDATIONS

- Published information pertaining to the toxicokinetics of DPA covers minimally some aspects of urinary excretion and metabolism. Therefore, further studies on absorption, distribution and metabolism of DPA in mammalian species appear warranted.

- There are a number of published toxicologic studies which are useful in understanding the adverse health effects of DPA but a well-executed subchronic study is needed . The available 2-year dog feeding study used very few number of animals. This species appears to be more sensitive to the toxic effects of DPA than rats or mice. Thus, a new subchronic dog feeding study, using a larger number of animals and several doses of DPA is recommended. This new study would help to more clearly define the LOAEL and NOAEL than the currently available study.

- The available reproductive effects study was not performed according to the stringent requirements currently required by the U.S. EPA. A new two-generation rat study for DPA is highly recommended. Such a study would not only produce valuable information on the male and female reproductive effects and developmental effects but also will provide new data for the reevaluation of One-day and Ten-day Health Advisories for DPA. The currently available information on developmental effects of DPA is not very useful.

- There is insufficient data to conclude that DPA is not a likely human carcinogen. Well conducted cancer bioassays in at least two different species are required. Although mutagenicity studies on DPA using bacterial systems are adequate, some more studies using nonmicrobial and *in vivo* systems are recommended to further substantiate the absence of genotoxic effects observed in bacterial systems.

- Since there is insufficient information on the environmental fate of DPA, additional studies pertaining to sorption on soils and sediments, hydrolysis, photolysis, and biotransformation of

DPA will provide the needed information for the future evaluation of the environmental effects of DPA.

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