FINAL

Report on Carcinogens Background Document for

1-Amino-2,4-dibromoanthraquinone

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Foreword

The Report on Carcinogens (RoC) is prepared in response to Section 301 of the Public Health Service Act as amended. The RoC contains a list of all substances (i) that either are known to be human carcinogens or may reasonably be anticipated to be human carcinogens; and (ii) to which a significant number of persons residing in the United States are exposed. The Secretary, Department of Health and Human Services (DHHS) has delegated responsibility for preparation of the RoC to the National Toxicology Program (NTP) who prepares the Report with assistance from other Federal health and regulatory agencies and non-government institutions.

Nominations for listing in or delisting from the RoC are reviewed by a formal process that includes a multi-phased, scientific peer review and multiple opportunities for public comment. The review groups evaluate each nomination according to specific RoC listing criteria. This Background Document was prepared to assist in the review of the nomination of 1-amino-2,4-dibromoanthraquinone. The scientific information in this document comes from publicly available, peer reviewed sources. Any interpretive conclusions, comments or statistical calculations, etc made by the authors of this document that are not contained in the original citation are identified in brackets [ ]. If any member(s) of the scientific peer review groups feel this Background Document does not adequately capture and present the relevant information they will be asked to write a commentary for this Background Document that will be included as an addendum to the document. In addition, a meeting summary that contains a brief discussion of the respective review group's review and recommendation for the nomination will be added to the Background Document, also as an addendum.

A detailed description of the RoC nomination review process and a list of all nominations under consideration for listing in or delisting from the RoC can be obtained by accessing the NTP Home Page at http://ntp-server.niehs.nih.gov. The most recent RoC, the 9th Edition, was published in May, 2000 and may be obtained by contacting the NIEHS Environmental Health Information Service (EHIS) at http://ehis.niehs.nih.gov (800-315-3010).

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Criteria for Listing Agents, Substances or Mixtures in the Report on Carcinogens

U.S. Department of Health and Human Services
National Toxicology Program

Known to be Human Carcinogens:

There is sufficient evidence of carcinogenicity from studies in humans, which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated to be Human Carcinogens:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible but that alternative explanations such as chance, bias or confounding factors could not adequately be excluded; or

There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species, or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well defined, structurally-related class of substances whose members are listed in a previous Report on Carcinogens as either a *known to be human carcinogen, or reasonably anticipated to be human carcinogen* or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

Executive Summary

Introduction
1-Amino-2,4-dibromoanthraquinone (ADBAQ) is an anthraquinone vat dye that is used in the textile industry. It was nominated by the National Institute of Environmental Health Sciences based on the results of the National Toxicology Program two-year feeding studies, which concluded that ADBAQ was carcinogenic in rats and mice.

Human Exposure
Use. ADBAQ is used as a dye and as a dye intermediate. Anthraquinones are widely used as starting material for the manufacture of vat dyes, which are used in fibers and textiles, typically for cotton, wool, and cellulose acetate.

Production. ADBAQ is prepared from 1-aminoanthraquinone by bromination in dilute mineral acids and is available from two vendors in the United States.

Environmental Exposure. Because ADBAQ is not found naturally in the environment, any environmental exposure will be a result of releases from facilities where ADBAQ is produced or is used as an intermediate for production of other anthraquinone dyes. A dibromoaminoanthraquinone was found in raw wastewater from a dye manufacturing plant in four of eight samples at concentrations of 92 to 170 ppb but was not observed in final effluent.

Occupational Exposure. Dermal exposure is the main source of exposure to ADBAQ. Because ADBAQ is not very volatile, inhalation exposure likely is limited to solid particulates. No specific occupational exposure information for ADBAQ or anthraquinone dyes in general was found in current literature. Epidemiological studies, however, indicate occupational exposures to anthraquinone dyes in a New Jersey dye and resin manufacturing plant.

Human Cancer Studies
Only two groups of workers exposed to anthraquinone dyes and intermediates have been investigated. All of the studies of these workers have limitations that make detection of causal relationships difficult, including small size, potential exposure to multiple agents in the workplace, and lack of quantitative exposure data. In addition, the follow-up studies lack information on nonoccupational cancer risk factors. The results of the pertinent research are not consistent. The study of Scottish workers reported small increases in esophageal and prostate cancer and found no excess of respiratory cancer (64 observed vs. 66.2 expected) or brain cancer (4/3.5). In contrast, the studies of New Jersey workers reported that work in anthraquinone dye operations was positively associated with lung cancer and with CNS tumors. In the latter investigations, the results for CNS tumors were based on a very small number of observations. The data on anthraquinone dye operations and lung cancer were more precise, and no confounding factor was identified that could explain the positive association. Thus, the increase in lung cancer in anthraquinone dye workers at the New Jersey plant may have been due to occupational

exposure. However, the specific agent(s) responsible for the excess have not been identified.

Studies in Experimental Animals
In two-year dietary studies with ADBAQ, male and female F344/N rats exhibited significantly increased incidences of neoplasms of the liver, large intestine, kidney, and urinary bladder. Significant increases in the incidence of tumors of the liver and large intestine (females only) also were observed in a 15-month stop-exposure study in rats. $B6C3F_1$ mice consuming ADBAQ-containing diets for two years had increased incidences of hepatocellular adenoma and carcinoma, squamous-cell papilloma and carcinoma of the forestomach, and alveolar/bronchiolar adenoma and carcinoma of the lung. The NTP report concluded that "under the conditions of these 2-year feed studies, there was *clear evidence for carcinogenic activity* of 1-amino-2,4-dibromoanthraquinone in male and female F344/N rats based on increased incidences of neoplasms in the liver, large intestine, kidney, and urinary bladder. There was *clear evidence for carcinogenic activity* of 1-amino-2,4-dibromoanthraquinone in male and female $B6C3F_1$ mice based on increased incidences of neoplasms in the liver, forestomach, and lung."

Genotoxicity
ADBAQ was mutagenic in *S. typhimurium* strains that revert by frameshift mutations, and the mutagenicity was decreased or eliminated in the presence of induced hamster or rat liver S9 metabolic activation. ADBAQ did not induce detectable mutations at the *tk* locus in mouse lymphoma cells; however, the concentrations used (20 or 25 μg/mL) were less than those used for the *S. typhimurium* assays (100 to 10,000 μg/mL) and slightly less than those used to assess clastogenicity (up to 100 μg/mL of ADBAQ). In CHO cells, ADBAQ induced chromosomal aberrations and SCE without metabolic activation; however, the results were inconsistent between different trials and laboratories. Forestomach and lung tumors induced by ADBAQ had a higher frequency of *ras* mutations than did spontaneous tumors. In particular, CAA to CTA transversions were common in ADBAQ-induced tumors but were absent in spontaneous tumors. The results obtained with ADBAQ must be viewed in the light of the limited solubility of this molecule and the uncertain purity of the preparations tested.

Other Relevant Data
Absorption, excretion, and metabolism. In rats, ADBAQ is rapidly absorbed from the gastrointestinal tract and distributed rapidly and widely to most soft tissues. Approximately 97% of ADBAQ is metabolized, as demonstrated by the small amount (less than 3%) of radiolabel recovered as the parent compound from either blood or urine; however, the metabolites of ADBAQ have not been identified. ADBAQ and its metabolites are excreted primarily in the feces and urine.

Potential mechanisms. ADBAQ and other anthraquinones are classified with a large number of other quinone molecules that can be derived from aromatic molecules such as benzene, naphthalene, and anthracene. Reactive oxygen species generated by metabolism of a variety of quinones may be associated with DNA damage or activation of signaling pathways involved in initiation, promotion, and progression of carcinogenesis. Four other anthraquinone vat dyes evaluated by the NTP have been listed in the RoC as *reasonably*

anticipated to be human carcinogens. In addition, a high percentage (36/80) of phenolic anthraquinones have been reported to be mutagenic in *Salmonella*.

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1 Introduction

1-Amino-2,4-dibromoanthraquinone (ADBAQ) is an anthraquinone vat dye that is used in the textile industry and is a member of a class of insoluble dyes that are impregnated into textile fibers. ADBAQ was nominated by the National Institute of Environmental Health Sciences (NIEHS) for possible listing in the Report on Carcinogens (RoC) based on a National Toxicology Program (NTP) bioassay (TR-383) reporting clear evidence of carcinogenicity in rats and mice. The NTP studied the carcinogenicity of ADBAQ as part of a class study with five other anthraquinone-derived dyes with representative and diverse structures, for the purpose of predicting the carcinogenicity of chemicals in this class. The conclusions from the NTP's two-year feeding study of ADBAQ were that there was clear evidence of carcinogenic activity in male and female F344/N rats (neoplasms in the liver, large intestine, kidney, and urinary bladder) and in male and female B6C3F_1 mice (neoplasms in the liver, forestomach, and lung).

1.1 Chemical identification

ADBAQ ($C_{14}H_7Br_2NO_2$, mol wt 381.02, CASRN 81-49-2) is a substituted anthraquinone with a single primary amine located in the 1-position and two bromine atoms located ortho and para to the amino group. Synonyms for ADBAQ include 1-amino-2,4-dibromo-9,10-anthracenedione; 1-amino-2,4-dibromanthrachinon; anthraquinone, 1-amino-2,4-dibromo-; and 2,4-dibromo-1-anthraquinonylamine. Its RTECS number is CB5500000. The structure of ADBAQ is shown in Figure 1-1.

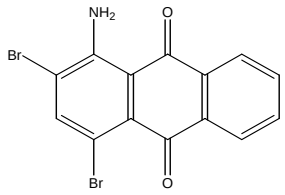


Figure 1-1. Structure of ADBAQ

1.2 Physical-chemical properties

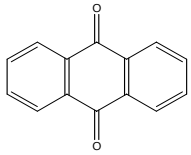
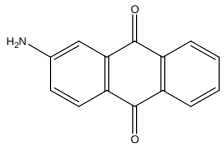
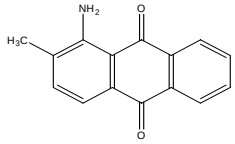
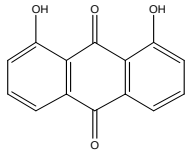
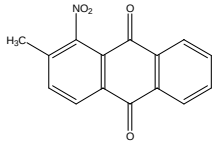
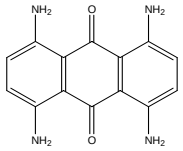
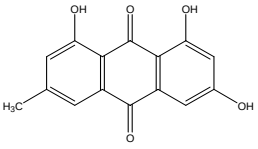
ADBAQ is a red powder at room temperature and melts at 226°C. It is sparingly soluble in ether and benzene; soluble in hot nitrobenzene, chloroform, acetic acid, pyridine, and concentrated sulfuric acid; and insoluble in water. It is sensitive to long-term air and light exposure. The physical and chemical properties of ADBAQ are summarized in Table 1-1.

Table 1-1. Physical and chemical properties of ADBAQ

Property	Information	Reference
Molecular weight	381.02	ChemFinder 2001
Color	red	ChemFinder 2001
Odor	odorless	NTP Chemical Repository 2001
Physical state	powder	ChemFinder 2001
Melting point (°C)	226	ChemFinder 2001
Flash point (°C)	> 200 (> 392°F)	NTP Chemical Repository 2001
Solubility (at 23°C):		
water	< 0.1 g/100 mL	ChemFinder 2001
acetone	< 1 mg/mL	NTP Chemical Repository 2001
DMSO	1–10 mg/mL	NTP Chemical Repository 2001
95% ethanol	< 1 mg/mL	NTP Chemical Repository 2001
toluene	< 1 mg/mL	NTP Chemical Repository 2001

Anthraquinone (TR-494) and the following six other anthraquinone-based compounds (identified in Table 1-2) have been tested in two-year bioassays by the NTP or have been listed in the RoC: 2-aminoanthraquinone (TR-144, RoC), 1-amino-2-methylanthraquinone (TR-111, RoC), 1,8-dihydroxyanthraquinone (danthron) (RoC), 2-methyl-1-nitroanthraquinone (TR-029), 1,4,5,8-tetraaminoanthraquinone (disperse blue 1) (TR-299, RoC), and emodin (TR-493). Carcinogenicity and genetic toxicology data for these seven molecules are summarized in Table 6-2. Other anthraquinone-derived compounds that are listed in the NTP database but have not been tested in two-year bioassays include the following: chrysophanic acid, CASRN 481-74-3; rhein, CASRN 478-43-3; 1,8-dihydroxy-4,5-dinitroanthraquinone, CASRN 81-55-0; C.I. disperse blue 27, CASRN 15791-78-3; C.I. disperse red 60, CASRN 17418-58-5; disperse blue 7, CASRN 3179-90-6; D&C violet no. 2, CASRN 81-48-1; D&C green 5, CASRN 4403-90-1; carminic acid, CASRN 1260-17-9; and vat blue 4, CASRN 81-77-6.

Table 1-2. Anthraquinone and some anthraquinone-based compounds

Compound name	Cas #	Chemical formula	Molecular weight	Structure
Anthraquinone	84-65-1	$C_{14}H_8O_2$	208.22	
2-Amino-anthraquinone	117-79-3	$C_{14}H_9NO_2$	223.23	
1-Amino-2-methyl-anthraquinone	82-28-0	$C_{15}H_{11}NO_2$	237.26	
1,8-Dihydroxy-anthraquinone (danthron)	117-10-2	$C_{14}H_8O_4$	240.22	
2-Methyl-1-nitro-anthraquinone	129-15-7	$C_{15}H_9NO_4$	267.24	
1,4,5,8-Tetraamino-anthraquinone (disperse blue 1)	2475-45-8	$C_{14}H_{12}N_4O_2$	268.27	
Emodin	518-82-1	$C_{15}H_{10}O_5$	270.24	

2 Human Exposure

2.1 Use

ADBAQ is used as a dye and as a dye intermediate (NTP Chemical Repository 2001). Anthraquinones are widely used as starting material for the manufacture of vat dyes. Vat dyes are a class of water-insoluble dyes that can easily be reduced to a water-soluble and usually colorless form. In this form, they can readily be impregnated into fibers and textiles. Oxidation then produces an insoluble colored form that is remarkably fast to washing, light, and chemicals. Vat dyes typically are used for cotton, wool, and cellulose acetate.

2.2 Production

ADBAQ is prepared from 1-aminoanthraquinone by bromination in dilute mineral acids (HSDB 2000). ADBAQ was found to be available from two vendors in the United States: Pfaltz and Bauer, in Connecticut, and Alfa Aesar, in Massachusetts (ChemFinder 2001). U.S. production of vat dyes totaled 14 million kilograms (30.8 million pounds) in 1991 (NTP 1996).

2.3 Analysis

ADBAQ has been analyzed in various samples by gas chromatography/flame ionization detection and gas chromatography/mass spectrometry (Games and Hites 1977). Recently, several other analytical methods have been described for detecting and quantifying anthraquinone-type dyes (liquid chromatography/nuclear magnetic resonance, liquid chromatography/mass spectrometry, and high-performance liquid chromatography [HPLC]); however, ADBAQ has not been analyzed by these methods (Preiss *et al.* 2000, Novotná *et al.* 1999).

2.4 Environmental occurrence

ADBAQ is not known to be naturally formed. Production of ADBAQ and its subsequent use as an intermediate in the production of anthraquinone dyes may result in releases into the environment (HSDB 2000). No information was found in the U.S. Environmental Protection Agency's Toxic Release Inventory regarding ADBAQ (TRI 2001).

2.5 Environmental fate

ADBAQ is sensitive to long-term exposure to light and air (NTP Chemical Repository 2001). Although ADBAQ was found in raw wastewater of a dye manufacturing plant, it was not present in the final effluent (effluent into the Cooper River, which flows into Charleston Bay of the Atlantic Ocean) (Games and Hites 1977). This may indicate that ADBAQ was either biodegraded or adsorbed to sludge during treatment (HSDB 2000).

2.5.1 Air

Based on estimated vapor pressure, ADBAQ should exist as a solid particulate in the ambient atmosphere. Particulate-phase ADBAQ would be removed from the atmosphere by dry deposition (HSDB 2000).

2.5.2 Water

ADBAQ may adsorb to suspended solids and sediment in water. The amino group in ADBAQ also may bind covalently with active sites in sediment and particulate matter. It is not expected to volatilize from water surfaces. The estimated partition coefficients and a recommended regression-derived equation were used to calculate a bioconcentration factor of 6,400 for ADBAQ. This suggests that ADBAQ will bioconcentrate in aquatic organisms (HSDB 2000).

2.5.3 Soil

ADBAQ is expected to be immobile in soil; its mobility may be limited because the amino group may bind to active sites in the soil. Volatilization is not expected to occur from either moist or dry soil surfaces (HSDB 2000).

2.6 Environmental exposure

Because ADBAQ is not found naturally in the environment, any environmental exposure will be a result of releases from facilities where ADBAQ is produced or is used as an intermediate for production of other anthraquinone dyes. A dibromoaminoanthraquinone was found in raw wastewater from a dye manufacturing plant in four of eight samples at concentrations of 92 to 170 ppb but was not observed in final effluent (HSDB 2000).

2.7 Occupational exposure

Dermal exposure is the main source of exposure to ADBAQ. Because ADBAQ is not very volatile, inhalation exposure likely is limited to solid particulates. Ikeda *et al.* (1977) found that workers exposed to dyes (as evidenced by diazo-positive metabolites in their urine) had many dye stains on their hands, wrists, and forearms. Analyses of the air in the workroom environments were not conducted.

No specific occupational exposure information for ADBAQ or anthraquinone dyes in general were found in current literature. Epidemiological studies, however, indicate potential occupational exposures to anthraquinone dyes in a New Jersey dye and resin manufacturing plant. A cohort study followed more than 3,000 workers who may have been exposed to anthraquinone dyes (Sathiakumar and Delzell 2000). Results of this study are described in Section 3.

2.8 Biological indices of exposure

No indices of ADBAQ exposure in humans or animal models were found in current literature.

2.9 Regulations

No regulations specifically for ADBAQ were identified.

3 Human Cancer Studies

No study has evaluated the relationship between human cancer and specific exposure to ADBAQ; however, exposure to anthraquinone dyes as a class has been studied. Since the late 1880s, the dye manufacturing industry has used a large number of anthraquinones and aromatic amines (arylamines) to synthesize industrial colorants and has used nitro aromatic amines to make hair dyes. Relatively little is known about the human carcinogenicity of anthraquinone dyes. The NTP classifies 2-aminoanthraquinone, 1-amino-2-methylanthraquinone, 1,8-dihydroxyanthraquinone, and 1,4,5,8-tetraaminoanthraquinone as *reasonably anticipated to be human carcinogens.* The International Agency for Research on Cancer (IARC) lists 1,8-dihydroxyanthraquinone, 2-methyl-1-nitroanthraquinone, and 1,4,5,8-tetraaminoanthraquinone as possibly carcinogenic to humans (Group 2B) and considers 2-aminoanthraquinone and 1-amino-2-methylanthraquinone not classifiable as to carcinogenicity to humans (Group 3). Neither the NTP nor IARC has evaluated the carcinogenicity of anthraquinone dyes as a chemical category or anthraquinone dye manufacturing as an exposure circumstance.

3.1 Human epidemiological studies

Data on the carcinogenicity to humans of dyes and chemicals used in synthesizing dyes come mainly from studies of workers in manufacturing plants. These groups of workers typically were exposed to many different chemicals and classes of chemicals, often making it difficult to attribute observed carcinogenic effects to a single agent. Only five epidemiologic studies of workers potentially exposed to anthraquinone dyes and anthraquinone dye intermediates are available. One of these investigations evaluated mortality among workers at a plant in Scotland that mainly made anthraquinone dyes (Gardiner *et al.* 1982). Each of the other four studies pertains to the same group of workers at a plant in New Jersey (Barbone *et al.* 1992, 1994, Delzell *et al.* 1989, Sathiakumar and Delzell 2000). The New Jersey plant's manufacturing operations were more diverse than those of the plant in Scotland and included the production of anthraquinone, anthraquinone dye intermediates and dyes, epichlorohydrin, azo dye intermediates and dyes, epoxy resins, and a variety of additives.

3.1.1 Study of Scottish anthraquinone dyestuffs workers

Gardiner *et al*. (1982) evaluated mortality from various causes among 1,975 men employed at an anthraquinone dyestuffs plant for at least six months between 1956 and the end of 1965. Personnel records were used to identify all subjects who had worked in jobs that entailed potential exposure to substituted anthraquinones. The study excluded men who had worked exclusively in clerical, accounting, and personnel management positions. Vital status and cause of death as of the end of June 1980 were determined for about 97% of the subjects through the use of plant records and data from the National Records Offices of Edinburgh and Newcastle upon Tyne. Analyses compared workers' mortality rates with those of the general male population of Scotland in 1975, adjusting for age and using the standardized mortality ratio (SMR) (ratio of observed to expected numbers of deaths, multiplied by 100) as the measure of association. An SMR of 100 implies that the mortality rate of workers was the same as that of the general population, after analytic adjustment for any difference in the age distributions of these two groups.

Gardiner *et al*. (1982) found that workers at the plant had about 23% fewer deaths from all causes combined than expected on the basis of general population rates (470 observed vs. 611.5 expected deaths, SMR = 76.9). Workers also had fewer deaths than expected from cerebrovascular disease (36/66.5, SMR = 54.1), cardiovascular disease (188/235.6, SMR = 79.8), and all cancers combined (129/149.7, SMR = 86.2). There were at least 5 observed or expected deaths for eight specific types of cancer. Among these, there were more deaths than expected for cancer of the esophagus (6/4.3) and fewer deaths than expected for cancer of the stomach (14/18.1), intestinal tract (7/11.0), pancreas (4/6.8), bladder (4/5.6), respiratory system (64/66.2), prostate (4/8.0), and rectum (6/6.8). None of these differences was statistically significant.

The mortality rate for all cancer combined was higher than expected for workers in some areas of the plant. Gardiner *et al*. (1982) did not present detailed results for specific cancers by plant area but noted statisitcally significant excesses of cancer of the esophagus in the engineering department (all deaths from this cancer were among men in this department) and cancer of the prostate in one particular process area. Workers' respiratory cancer rates were not statistically significantly greater than expected in any plant area. Work activities in the engineering department were not thoroughly described but presumably included maintenance operations. Gardiner *et al*. (1982) indicated that engineering workers constituted 45% of the study group, that they worked mainly in proximity to plant process areas, and that their plant exposures probably were similar to those of process workers. The authors could not interpret their results for prostate cancer. They stated that cadmium, a suspected cause of prostate cancer, was not used at the plant.

Gardiner *et al*. (1982) stated that their research did not provide any evidence of "an abnormal pattern of mortality" in the group of anthraquinone dye workers studied. Because of its methodologic weaknesses, the study provided little evidence either for or against the hypothesis that anthraquinones cause cancer in humans. Limitations were the small numbers of deaths for most specific types of cancer, the lack of quantitative estimates of exposure to anthraquinones or other chemicals, and the lack of data on nonoccupational factors. Moreover, the very low SMR for all causes of death combined, especially in older age groups, which probably contained a high proportion of inactive workers, suggests that ascertainment of deaths in the study group may have been incomplete or that the use of comparison rates derived from a single year (1975) was inappropriate. If so, all SMRs may have been underestimated.

3.1.2 Studies of dye and resin manufacturing workers at a plant in New Jersey

Delzell *et al*. (1989) conducted a retrospective follow-up study of mortality from 1952 through 1985 among 2,642 white men who had been employed for at least six months at a dye and resin plant in New Jersey. Barbone and colleagues carried out nested case-control studies of lung cancer (Barbone *et al.* 1992) and central nervous system (CNS) tumors (Barbone *et al.* 1994) occurring among these workers. In a later investigation, Sathiakumar and Delzell (2000) expanded the original retrospective follow-up study (Delzell *et al.* 1989) to include 3,266 male and female workers of all races and evaluated their mortality patterns during the period 1952 through 1995.

The manufacturing operations of the New Jersey plant took place in three major areas. Operations in the "south dye area" (anthraquinone dye/epichlorohydrin area) included anthraquinone production (1952 to 1983), anthraquinone dye production (1952 to 1983), anthraquinone dye intermediates production (1952 to 1983), formulation of anthraquinone dyes (1952 to late 1980s), and epichlorohydrin production (1961 to 1965). The "north dye area" produced azo dyes and intermediates from 1959 to the late 1980s. The plastics and additives area made epoxy resins, fabric softeners, water-repellent agents, optical brighteners, and various additives from 1959 to the late 1980s. Raw materials and intermediates used in each area included many chemicals, some of which are known carcinogens (e.g., benzene, benzidine, ethylene oxide) or suspected carcinogens (e.g., epichlorohydrin, formaldehyde, dimethyl sulfate). Chemicals used in the anthraquinone dye/epichlorohydrin (AQ/ECH) area included anthracene, anthraquinones, anthraquinone sulfonates, anthraquinone intermediates, aniline, substituted aniline, benzene, nitrobenzene, chlorobenzenes, chlorotoluenes, pyridine, tetrachloroethylene, chlorine, ammonia, arsenic acid, methanol, sulfuric acid, and mercury.

3.1.2.1 Original retrospective follow-up study

The first investigation of workers at the New Jersey plant used company personnel records to identify all white men with at least six months of employment as of the end of 1984 and to develop information on each subject's jobs at the facility (Delzell *et al.* 1989). Plant records and linkages with several national and state databases established the vital status of 96% of the 2,642 eligible subjects as of the end of 1985. Death certificates provided information on deceased subjects' causes of death. Comparisons of workers' mortality rates with those for the white male general population of the United States yielded observed and expected numbers of deaths for the overall study group and subgroups of hourly workers classified by the process area(s) in which they had worked for at least one year.

The overall study group had 357 deaths from all causes combined, compared with 440 expected deaths (SMR = 81, 95% confidence interval [CI] = 73 to 90), and 106 cancer deaths, compared with 97 expected cancer deaths (SMR = 109, 95% CI = 89 to 132). There were more deaths from CNS cancer than expected (5/3.3), not including the deaths of two employees from this cancer while living in Europe, and there were 3 deaths each from bladder cancer (expected = 2.3) and kidney cancer (expected = 2.5). The paper did not include results for other specific types of cancer for the overall study group.

The study included 588 hourly employees who had worked in the AQ/ECH area for at least one year. These men had fewer deaths than expected from all causes combined (65/97) and from cancer (20/22). For all specific types of cancer, observed and expected numbers of deaths were under 10 and were too small to yield statistically stable results. Hourly AQ/ECH workers had 6 lung cancer deaths (expected = 8). Although only 44 workers in this area had been employed in epichlorohydrin operations, and most of these also had worked longer in anthraquinone dye operations, this group had statistically significantly more lung cancer deaths than expected (4/0.91, $P = 0.03$).

Hourly maintenance workers (N = 514), many of whom were employed throughout the plant, had more deaths than expected from all cancers combined (37/25, SMR = 148, 95% CI = 103 to 202), from lung cancer (18/9.2, SMR = 196, 95% CI = 116 to 309), and from liver cancer (3/0.46).

The investigators concluded that the excess of lung cancer among maintenance workers could have been due to an unidentified occupational exposure. They noted that the association they observed between epichlorohydrin and lung cancer had not been found in research on other, larger groups of workers exposed to this chemical.

The study was limited by the small number of subjects and of deaths in the AQ/ECH work group. Other weaknesses were the absence of quantitative exposure estimates or data on nonoccupational risk factors. However, even if such data had been available, the study would have had little power to assess dose response, confounding, or interaction for specific forms of cancer.

3.1.2.2 Case-control study of lung cancer.

Barbone *et al*. (1992) conducted a nested case-control study of lung cancer that included as cases 51 white male employees of the New Jersey plant who had died of or developed primary lung cancer by October 1988. Controls were 102 individually matched employees chosen randomly from among other white male employees who were born in the same year and were alive as of the case subject's death or diagnosis date. Plant job records identified each process area and building in which a subject had carried out production-related work. The AQ/ECH area consisted of five buildings with the following operations: (1) anthraquinone production, (2) production of anthraquinone dye intermediates, (3) anthraquinone dye synthesis, (4) anthraquinone dye standardization, and (5) epichlorohydrin production. Interviews with long-term employees (not subjects) provided more detailed work history information on building-specific work, as well as an ordinal score for subjects' potential exposure to asbestos and epichlorohydrin. Interviews with subjects or family members furnished data on smoking and on jobs and exposures outside the New Jersey plant and were completed for 69% of cases and 74% of controls. Plant medical records, available for 67% of cases and 68% of controls, supplied information on smoking and exposures to chemicals that had been reported to the plant medical department. The odds ratio (OR) (the ratio of the rates of lung cancer in subjects exposed and not exposed to a given factor) was used as the measure of association.

As expected, smoking was positively and consistently associated with lung cancer. Lung cancer also was positively associated with ever having worked in the AQ/ECH area (OR = 2.4, 95% CI = 1.1 to 5.2), based on 21 exposed cases and 24 exposed controls. This association was limited to subjects who had worked in the area for at least 10 years (smoking-adjusted OR = 4.6, 95% CI = 0.9 to 23), but there was no trend of increasing OR with increasing time spent in the area. Lung cancer was positively associated with employment in each of the five building-specific operations in the area, but the association was statistically significant only for anthraquinone production (OR = 12, 95% CI = 1.4 to 99) and was of borderline statistical significance for anthraquinone dye standardization (OR = 3.3, 95% CI = 1.0 to 11). The positive association between work in the AQ/ECH area and lung cancer did not appear to be due to exposure to asbestos or

epichlorohydrin or to smoking. Results for other process areas were not striking or statistically significant.

Workers in the AQ/ECH area potentially were exposed to chlorine and to epichlorohydrin, in addition to anthraquinone dyes and intermediates. The study reported a positive association between acute exposure to chlorine and lung cancer (OR = 5.7, 95% CI = 1.1 to 30). Adjustment for smoking raised the OR for chlorine to 27, but these results were based on only 6 exposed cases and 3 exposed controls. Chlorine was encountered in the AQ/ECH area by 3 of the 6 chlorine-exposed case subjects and 2 of the 3 chlorine-exposed control subjects. A positive [but not statistically significant] association between lung cancer and exposure to epichlorohydrin (OR = 1.7, 95% CI = 0.7 to 4.1) was reported, based on 12 exposed cases and 18 exposed controls. The latter association was restricted to workers with short-term and relatively low exposure to epichlorohydrin. Only 3 of the 21 case subjects in the AQ/ECH area had spent any appreciable time in epichlorohydrin operations.

Barbone *et al*. (1992) noted that the positive association between lung cancer and anthraquinone dye operations could have been due to occupational exposure (to anthraquinone or other chemicals used in the area) or to chance. The study lacked data on cumulative exposure to anthraquinones. If anthraquinones cause lung cancer, the lack of an association between duration of employment in the various anthraquinone areas and lung cancer in this study could have been due to poor correlation between duration of work in the areas and amount of exposure. The study also did not have quantitative or comprehensive data on exposure to chlorine and had only crude estimates of epichlorohydrin exposure. Thus, the impact of these agents on the association between anthraquinone work areas and lung cancer may not have been accurately assessed. Barbone *et al*. (1992) noted that the only other data available on human exposure to anthraquinones, the study by Gardiner *et al*. (1982), did not find an excess of lung cancer among workers with potential exposure.

3.1.2.3 Case-control study of central nervous system tumors

The case-control study of CNS tumors included as cases 11 white male employees who were known to have died of or developed a primary CNS tumor by October 1988 (7 with astrocytoma or glioblastoma, 2 with meningioma, and 2 with a benign tumor) (Barbone *et al.* 1994). Controls were 44 individually matched subjects chosen randomly from among other white male employees who were born in the same year and were alive as of the case subject's death or diagnosis date. Procedures for obtaining and classifying work history data, estimating exposure to epichlorohydrin, conducting interviews, and obtaining data from plant medical records were similar to those used in the case-control study of lung cancer (Barbone *et al.* 1992). Interviews, completed for 73% of cases and 84% of controls, asked about non-plant jobs and occupational exposures, smoking habits, and history of head trauma, irradiation to the head, epilepsy, and use of antiepileptic drugs.

The occurrence of CNS tumors was statistically significantly and positively associated with anthraquinone dye intermediate production, but only 3 case subjects and no controls had worked in this process area. Positive associations also were found with work in the epoxy resin process area and in the azo dye area and with estimated exposure to

epichlorohydrin. Barbone *et al*. (1994) reported that the association with anthraquinone dye intermediate production was "nonindependent" of the association with exposure to epichlorohydrin and concluded that the small study size precluded firm conclusions about the causes of CNS tumors in the plant workforce. All of the results were based on small numbers of exposed subjects and were quite imprecise. The study also did not include quantitative estimates of exposure to anthraquinone or other chemicals and was not able to evaluate anthraquinone dose response or to adequately assess the possibility that the association between anthraquinone dye intermediate production and CNS tumors was confounded by the other positive associations observed, or vice versa.

3.1.2.4 Retrospective follow-up study update

Sathiakumar and Delzell (2000) expanded and updated the original study by Delzell *et al*. (1989), adding women and nonwhite men to the study group and extending follow-up through the end of 1995. The expanded study compared the mortality rates for 3,266 employees with the age-, race-, gender-, and time-period-specific rates for the general population of New Jersey.

The total study group had 728 observed and 809.8 expected deaths from all causes combined (SMR = 90, 95% CI = 83 to 97), 225 observed and 231.7 expected total cancer deaths (SMR = 97, 95% CI = 85 to 111), 89 observed and 73.2 expected lung cancer deaths (SMR = 122, 95% CI = 98 to 150), and 8 observed and 6.0 expected CNS cancer deaths (SMR = 134, 95% CI = 58 to 264). White male employees who had worked in the AQ/ECH area (N = 842) had an excess of lung cancer, based on 32 observed and 19.1 expected deaths (SMR = 168, 95% CI = 115 to 237), and had 3 deaths from CNS cancer (1.5 expected, SMR = 205, 95% CI = 42 to 598). Maintenance workers at the plant also had more deaths from lung cancer than expected (40/26.2, SMR = 153, 95% CI = 109 to 208).

The lung cancer excess among workers in the AQ/ECH area was not consistently larger in subgroups with long duration of employment (5 years or more) or long potential induction time in the area (20 years or more); however, small numbers limited the conclusions that could be drawn from these subgroup analyses. In analyses that compared the lung cancer mortality rate of AQ/ECH area workers with that of other plant employees, adjusting for age, calendar time, and employment in maintenance operations, the OR was 1.7 (95% CI = 1.1 to 2.6) for the AQ/ECH area, confirming the increased lung cancer mortality for this area seen in the SMR analysis.

This study, like the study of the same workforce by Barbone *et al*. (1992), found a positive association between employment in anthraquinone dye operations and lung cancer. There was no discernible trend of increasing lung cancer SMR or OR with increasing length of employment in anthraquinone dye operations, but this could be explained by poor correlation between duration of employment and cumulative exposure to anthraquinones or other chemicals used in the area. Sathiakumar and Delzell (2000) also found an increase in CNS cancer among workers potentially exposed to anthraquinones, based on small numbers, consistent with the report by Barbone *et al*. (1994).

Compared with the previous follow-up study of this group of workers, the update had 72% more person-years and twice as many observed deaths. The median length of follow-up was 27 years, and 22% of the workers were deceased. However, numbers of deaths from specific types of cancer were small in the work-area-specific analyses, and the update lacked direct exposure estimates and data on nonoccupational risk factors. These limitations impede the detection of causal associations.

3.2 Summary

Only two groups of workers exposed to anthraquinone dyes and intermediates have been investigated. All of the studies of these workers have limitations that make detection of causal relationships difficult, including small size, potential exposure to multiple agents in the workplace, and lack of quantitative exposure data. In addition, the follow-up studies lack information on nonoccupational cancer risk factors. The results of the pertinent research are not consistent. The study of Scottish workers reported small increases in esophageal and prostate cancer and found no excess of respiratory cancer (64 observed vs. 66.2 expected) or brain cancer (4/3.5). In contrast, the studies of New Jersey workers reported that work in anthraquinone dye operations was positively associated with lung cancer and with CNS tumors. In the latter investigations, the results for CNS tumors were based on a very small number of observations. The data on anthraquinone dye operations and lung cancer were more precise, and no confounding factor was identified that could explain the positive association. Thus, the increase in lung cancer in anthraquinone dye workers at the New Jersey plant may have been due to occupational exposure. However, the specific agent(s) responsible for the excess have not been identified. Table 3-1 summarizes the human cancer studies on anthraquinone dye workers.

Table 3-1. Human cancer studies on anthraquinone dye workers

Reference	Study design	Population	Exposure	Effects	Comments
Gardiner *et al.* 1982 Scotland	Cohort study	1,975 men employed at anthraquinone dyestuff plant for at least 6 months from 1956–1965, followed up until June 1980	Potential exposure to substituted anthraquinones was assessed from personnel records.	SMR (observed/expected deaths) all deaths 76.9 (470/611.5) all cancers 86.2 (129/149.7) esophagus 139.5 (6/4.3) stomach 77.3 (14/18.1) intestinal tract 63.6 (7/11.0) pancreas 58.8 (4/6.8) bladder 71.4 (4/5.6) respiratory 96.7 (64/66.2) prostate 50.0 (4/8.0) rectum 88.2 (6/6.8)	Smoking and alcohol consumption were not ascertained.
Delzell *et al.* 1989 New Jersey, USA	Retrospective cohort study	2,642 white men employed at a dye and resin plant for at least 6 months from 1952–1985 588 hourly workers employed in the AQ/ECH area for at least one year	Exposure was asssessed from personnel records. Three manufacturing areas: *AQ/ECH area:* anthraquinone, AQ dye and AQ dye intermediates, and ECH production and formulation of AQ dyes *North dye area:* azo dyes and intermediates *Plastics and additive area*	Observed/expected *AQ/ECH area workers:* all deaths 65/97 all cancers 20/22 lung cancer 6/8 *Epichlorohydrin workers:* (1961–1965) (N = 44) lung cancer 4/0.91; $P = 0.03$	Workers in the AQ/ECH area also were exposed to other chemicals, including aniline- and benzene-related compounds, tetrachloroethylene, chlorine, sulfuric acid, and mercury. Nonoccupational risk factors were not acertained.

Reference	Study design	Population	Exposure	Effects	Comments
Barbone *et al.* 1992 New Jersey, USA	Nested case-control study (Delzell *et al.* 1989) Lung cancer	*Cases:* 51 white male employees who died of or developed lung cancer by 10/1988 *Controls:* 102 employees randomly selected and matched for age and alive as of case's death or diagnosis date	The AQ/ECH area consisted of 5 buildings: (1) AQ production (2) production of AQ intermediates (3) AQ dye synthesis (4) AQ dye standardization (5) ECH production	OR (95% CI); no. of exposed cases *Worked in AQ/ECH area:* ever 2.4 (1.1–5.2); 21 > 10 years 4.6 (0.9–23) *Building in AQ/ECH area:* AQ production 12 (1.4–99); 6 AQ dye/standardization 3.3 (1.0–11); 8 ECH production: 3 exposed cases, 0 exposed controls; OR not calculated	ORs were computed with and without adjustment for smoking and other job exposures. Only smoking proved to be a confounder and was kept in analyses if it modified the strength or direction of an association. Lung cancer was positively associated with chlorine exposure.
Barbone *et al.* 1994 New Jersey, USA	Nested case-control study (Delzell *et al.* 1989) CNS tumors	*Cases:* 11 white males who died of or developed a primary CNS tumor by 10/1988 *Controls:* 44 employees matched for age and alive as of the case's death or diagnosis date	Same as Delzell *et al.* (1989) and Barbone *et al.* (1992)	OR (95% CI); no. of exposed cases *AQ dye intermediates:* production ∞ (1.7–∞); 3 routine potential exposure to ECH 4.2 (0.7–26); 4	Three of four case subjects with potential exposure to ECH also worked in either the AQ dye intermediates or azo dyes areas.
Sathiakumar and Delzell 2000 New Jersey, USA	Cohort study Expansion of Delzell *et al.* (1989)	3,266 employees (men and women) at a dye and resin plant, followed through 1995 728 deaths	Same as Delzell *et al.* (1989)	SMR (95% CI); observed/expected *Entire cohort:* all deaths 90 (83–97); 728/809.8 all cancers 97 (85–111); 225/231.7 *South dye area, white males* (N = 842): lung cancer 168 (115–237); 32/19.1	Nonoccupational risk factors (including smoking) were not ascertained.

4 Studies of Cancer in Experimental Animals

The NTP (1996) carried out studies in male and female rats and mice in which ADBAQ was administered in the diet continuously for either 13 weeks or two years. In addition, the NTP conducted a 15-month high-dose stop-exposure study in rats (9 months of exposure to ADBAQ followed by 6 months on a control diet). Data from the 13-week studies in rats and mice were reported by Fleischman *et al.* (1986), and results from the two-year studies in rats were reported by Harada *et al.* (1989) and Maronpot *et al.* (1989a). The results of the NTP studies and additional experiments by Maronpot *et al.* (1989b) are summarized below.

The NTP obtained two lots of ADBAQ for the studies and confirmed the identity and purity of each lot by chemical analysis. Fleischman *et al*. (1986) reported that the study material was technical-grade ADBAQ of 80% to 85% purity, determined by HPLC. The major impurities were identified as anthraquinone (9.3%) and either 2-aminodibromoanthraquinone or a different 1-aminodibromoanthraquinone (4.7%). The same material was used for the first 2 months of the two-year studies. The study material used for the remaining 22 months of the two-year studies was found to be approximately 97% pure, with six unidentified impurities.

Little information is available on the potential carcinogenicity of the three contaminants identified in the initial ADBAQ sample. Anthraquinone has not been reviewed in an IARC monograph. It has been the subject of an NTP rodent bioassay (TR-494), which concluded that there was some evidence of carcinogenic activity in male F344/N rats (kidney and urinary bladder), clear evidence of carcinogenic activity in female F344/N rats (liver, kidney, and urinary bladder), and clear evidence of carcinogenic activity in male and female B6C3F_1 mice (liver). All of the reported sites for anthraquinone-induced neoplastic lesions also are target sites for ADBAQ. No information was available on the potential carcinogenicity of the two other contaminants.

4.1 Rats

The NTP (1996) conducted three studies of ADBAQ in rats: a 13-week study, a two-year (104-week) study, and a 15-month stop-exposure study. Male and female F344/N rats were administered ADBAQ in their diet at concentrations ranging from 1,000 to 50,000 ppm.

4.1.1 Thirteen-week study

Groups of F344/N rats (10 of each sex) were fed diets containing ADBAQ at a concentration of 0, 2,500, 5,000, 10,000, 25,000, or 50,000 ppm for 13 weeks. After the 13-week exposure, necropsies were performed, in which tissues were collected and processed for histologic examination (a complete list of tissues and organs examined is included in Appendix B). Survival to study termination and mean final body weight (b.w.) as a percent of mean control body weight are shown in Table 4-1. The nonneoplastic lesions of the liver included basophilic foci, clear-cell foci, eosinophilic foci, cytomegaly, bile duct hyperplasia, inflammation, fibrosis, necrotizing cholangitis, vacuolar degeneration, and pigmentation. The incidence of hepatocellular cytomegaly

(hypertrophy) was significantly increased ($P < 0.01$, Fisher's exact test) in both male and female rats at 25,000 and 50,000 ppm and in female rats at 10,000 ppm. The incidences of inflammation around bile ducts and bile duct hyperplasia also were significantly increased ($P < 0.01$, Fisher's exact test) in male and female rats in the 25,000- and 50,000-ppm groups. The kidney lesions included renal tubule pigmentation (in both males and females in all exposure groups) and hyaline droplet accumulation (in males only in the 2,500-, 5,000-, and 10,000-ppm groups). Data for other exposure-related lesions in all groups are summarized in the NTP report (Appendix A, p. A-26, Table 4).

Table 4-1. Survival and body weight in F344/N rats following dietary exposure to ADBAQ for 13 weeks

	Exposure concentration (ppm)					
End point	**0**	**2,500**	**5,000**	**10,000**	**25,000**	**50,000**
Males						
Survival to study termination	0	10/10	9/10	10/10	10/10	7/10
Final mean b.w. ± SD[a] (g)	358 ± 3	325 ± 3	328 ± 3	310 ± 3	232 ± 3	164 ± 6
% of control mean b.w.	100	91**	92**	86**	65**	46**
Females						
Survival to study termination	10/10	10/10	10/10	10/10	10/10	9/10
Final mean b.w. ± SD (g)	211 ± 3	197 ± 3	188 ± 3	185 ± 2	159 ± 2	130 ± 4
% of control mean b.w.	100	93**	89**	88**	75**	61**

[a]SD = standard deviation.

**Significantly different ($P \leq 0.01$) from the control group by Williams's or Dunnett's test.

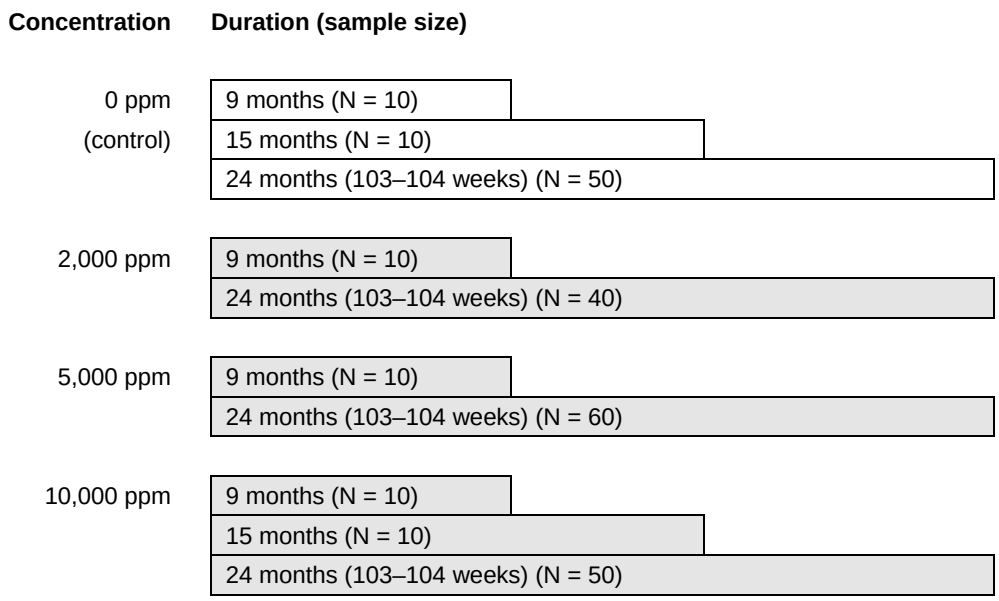
Fleischman *et al*. (1986) reported results from the NTP's 13-week toxicology studies of ADBAQ in Fischer 344/N rats. Both male and female rats in the 25,000- and 50,000-ppm groups were emaciated and lethargic, and their body weights were significantly lower ($P < 0.01$, Williams's or Dunnett's test) than those of controls. Pathological findings of nonneoplastic lesions of the liver and kidney were the same as those reported in NTP (1996). The uterus was atrophied in females fed ADBAQ at a concentration of 10,000 ppm or higher, and the thymus was atrophied in males at 5,000 ppm or higher and in females at 25,000 ppm or higher. A consistent finding in both sexes was anemia, with a significant reduction ($P < 0.01$, Student's *t* test) in the number of red blood cells at all concentrations tested. The authors concluded that ADBAQ was "markedly toxic in rats."

4.1.2 Two-year study

Dietary concentrations of ADBAQ for the two-year study in rats were based on the results of the 13-week study. Exposure-related lesions of the liver, kidney, and spleen were present mainly in the 25,000- and 50,000-ppm groups in the 13-week studies. Exposure levels of 0, 2,000, 5,000, and 10,000 ppm were selected for the two-year study. Although the 10,000-ppm exposure level could be considered slightly high, based on body-weight effects (Table 4-1), this dietary concentration was predicted not to adversely affect the health or survival of the animals because it did not cause liver toxicity in the

13-week study. A higher exposure level (20,000 ppm) was chosen for the stop-exposure study.

Male and female F344/N rats were obtained at approximately six weeks of age and quarantined for 12 to 14 days (males) or 9 days (females) before the study began. Groups of rats (70 of each sex) received ADBAQ in the diet at a concentration of 0, 5,000, or 10,000 ppm, and 50 rats of each sex received ADBAQ at 2,000 ppm. A subset from each exposure level (10 of each sex) was designated for an interim evaluation after 9 months. Additional subsets from the control and 10,000-ppm groups (10 of each sex) were evaluated after 15 months. Major tissues were collected for histological examination. The exposure protocol is summarized in Figure 4-1.



Shaded bars indicate ADBAQ-containing diet, and open bars indicate control diet. The total length of each bar indicates the time to study termination and the sacrifice of animals in that group.

Figure 4-1. Exposure protocol for the two-year study of ADBAQ in F344/N rats

Survival to study termination, final mean body weight as a percentage of mean control body weight, and neoplastic lesion incidence at two years are summarized in Table 4-2. Neoplastic lesions were observed in the liver, large intestine, kidney, urinary bladder, and forestomach. Neoplasms of the liver included both single and multiple adenomas and single and multiple carcinomas. The large intestine contained single and multiple adenomatous polyps and single and multiple carcinomas. Renal lesions included renal tubule adenoma and carcinoma, and urinary bladder lesions included transitional-cell papilloma and carcinoma. Lesions of the forestomach included squamous-cell papilloma and carcinoma, but the increased incidences did not reach statistical significance. Significant increases were seen at all exposure levels for tumors of the liver, large intestine, and kidney. The incidence of urinary bladder tumors also was significantly increased in high-dose males and mid- and high-dose females.

Table 4-2. Survival, body weight, and tumor incidence in F344/N rats following dietary exposure to ADBAQ for two years

	Exposure concentration (ppm)			
End point	**0**	**2,000**	**5,000**	**10,000**
Males				
Survival to study termination	26/50	24/40	21/60	10/50***
Final mean b.w. (g) (% of control mean b.w.)	406 (100)	349 (86)	341 (84)	283 (70)
Neoplastic lesions (two-year data only)				
Liver	N = 50	N = 40	N = 59	N = 50
Adenoma	1	20***	40***	34***
Carcinoma	1	12***	55***	46***
Adenoma or carcinoma	2	25***	57***	47***
Large intestine	N = 50	N = 40	N = 59	N = 50
Adenomatous polyp	0	13**	51**	40**
Carcinoma	0	1	11**	17**
Kidney (renal tubule)	N = 50	N = 40	N = 59	N = 50
Adenoma	2	10**	11*	14***
Carcinoma	0	0	2	1
Adenoma or carcinoma	2	10**	13**	15***
Urinary bladder (transitional cell)	N = 50	N = 38	N = 58	N = 50
Papilloma	0	1	2	8**
Carcinoma	0	0	1	4*
Papilloma or carcinoma	0	1	3	12***
Females				
Survival to study termination	38/50	32/40	38/60	12/50***
Final mean b.w. (% of control mean b.w.)	362 (100)	290 (80)	234 (65)	194 (54)
Neoplastic lesions (two-year data only)				
Liver	N = 50	N = 40	N = 60	N = 48
Adenoma	0	28***	47***	29***
Carcinoma	0	12***	57***	45***
Adenoma or carcinoma	0	33***	59***	47***
Large intestine	N = 50	N = 40	N = 60	N = 49
Adenomatous polyp	0	28**	53**	43**
Carcinoma	0	2	21**	8**
Kidney (renal tubule)	N = 50	N = 40	N = 60	N = 48
Adenoma	0	3*	16***	16***
Carcinoma	0	0	0	2
Adenoma or carcinoma	0	3*	16***	16***
Urinary bladder (transitional cell)	N = 50	N = 40	N = 60	N = 46
Papilloma	0	2	7*	9**

	Exposure concentration (ppm)			
End point	**0**	**2,000**	**5,000**	**10,000**
Carcinoma	0	0	8**	16***
Papilloma or carcinoma	0	2	17***	26***

*Significantly different ($P \leq 0.05$) from the control group by the logistic regression test.

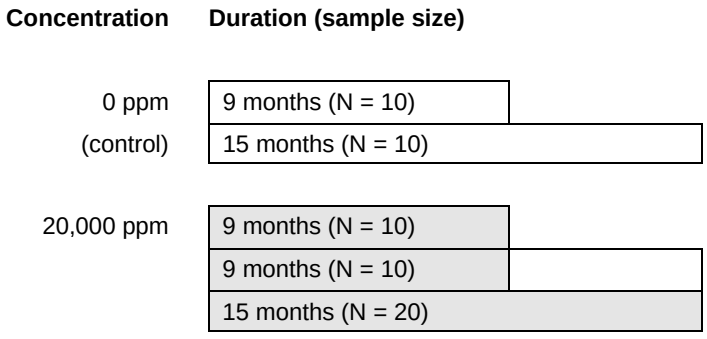
**Significantly different ($P \leq 0.01$) from the control group by the logistic regression test.

***Significantly different ($P \leq 0.001$) from the control group by lifetable pairwise comparisons (survival data) or the logistic regression test (tumor data).

Hepatocellular, large intestine, kidney, and bladder tumors also were found in rats that received ADBAQ at 10,000 ppm for 15 months (interim sacrifice). The incidences of hepatocellular adenoma and carcinoma in 10/10 male and 9/10 female rats were significantly greater ($P < 0.01$, Fisher's exact test) than in controls. A similar incidence of liver tumors was seen in the 15-month stop-exposure study (discussed below).

4.1.3 Fifteen-month stop-exposure study

The NTP conducted a stop-exposure study to evaluate the potential for progression or regression of ADBAQ-induced lesions. Male and female F344/N rats were obtained at approximately six weeks of age and quarantined for 12 to 14 days (males) or 9 days (females) before the study began. Groups of rats (10 of each sex) were fed a diet containing ADBAQ at a concentration of 20,000 ppm and were sacrificed after 9 months (9-month exposure group). Additional groups (10 of each sex) were fed a diet containing ADBAQ at 20,000 ppm for 9 months, followed by the control diet for 6 months (9-month stop-exposure group). The final group (20 of each sex) received only the diet containing ADBAQ at 20,000 ppm for 15 months (15-month exposure group). The exposure protocol for the stop-exposure studies is summarized in Figure 4-2.



Shaded bars indicate ADBAQ-containing diet, and open bars indicate control diet. The total length of each bar indicates the time to study termination and the sacrifice of animals in that group.

Figure 4-2. 15 Month stop exposure study

Survival to study termination, final mean body weight as a percentage of mean control body weight, and neoplastic lesion incidence are summarized in Table 4-3. Neoplastic lesions were found in the liver of male and female rats at the 15-month evaluation in both the 9-month stop-exposure group and the 15-month exposure group. The large intestines contained adenomatous polyps of the rectum in exposed males and females; however, the tumor incidence was statistically significantly increased ($P < 0.05$, Fisher's exact test) only in males in the 15-month exposure group and females in the 9-month stop-exposure group. The incidences of tumors of the kidney and urinary bladder were not statistically significantly increased, but the numerical increases in these tumors are concordant with those observed in the two-year bioassay (see Table 4-2).

Table 4-3. Survival, body weight, and tumor incidences in F344/N rats in the 15-month stop-exposure study of ADBAQ (20,000 ppm)

	9-month evaluation		15-month evaluation		
End point	**Control[a]**	**9-mo. exposure**	**Control[a]**	**9-mo. stop-exposure**	**15-mo. exposure**
Males					
Survival to study termination	70/70	30/30	57/60	7/10	17/20
Final mean b.w. (g)	453	364	484	388	364
% of control mean b.w.	100[b]	80	100	80[b]	75
Neoplastic lesions					
	N = 10	N = 10	N = 10	N = 10	N = 20
Liver (hepatocellular adenoma or carcinoma)	0	2	0	9**	20**
Large intestine, rectum (adenomatous polyp)	0	0[c]	0	3	7*
Kidney (renal tubule adenoma)	0	0	0	3	2
Urinary bladder:					
squamous-cell carcinoma	0	0[d]	0	0	1[e]
transitional epithelial papilloma	0	1[d]	0	0	3[e]
transitional epithelial carcinoma	0	0[d]	0	0	1[e]
Females					
Survival to study termination	69/70	29/30	59/60	10/10	12/19
Final mean b.w. (g)	258	211	328	258	220
% of mean control b.w.	100[b]	82	100	79[b]	67
Neoplastic lesions					
	N = 10	N = 10	N = 10	N = 10	N = 18
Liver (hepatocellular adenoma or carcinoma)	0	2	0	8**	16**
Large intestine, rectum (adenomatous polyp)	0	0	0	5*	3[f]
Kidney (renal tubule adenoma)	0	0	0	3	2[f]
Urinary bladder:					
squamous-cell carcinoma	0	0	0	1	4
transitional epithelial papilloma	0	0	0	0	1[g]
transitional epithelial carcinoma	0	0	0	0	1

[a]Control animals are those from the two-year study.

[b]Mean body weights are for all animals at 37 weeks in control and 15-month exposure groups.

[c]A single adenomatous polyp of the colon was observed in this group.

[d]N = 9; [e]N = 19; [f]N = 17.

[g]A single squamous-cell papilloma of the urinary bladder also was observed in this group.

*Significantly different ($P \leq 0.05$) from the control group by Fisher's exact test.

**Significantly different ($P \leq 0.01$) from the control group by Fisher's exact test.

Maronpot *et al.* (1989a) analyzed the incidence of hepatic foci that either stained positive for γ-glutamyl transpeptidase (GGT) or were identified as altered hepatocellular foci (AHF) in hematoxylin and eosin–stained sections of livers from female F344/N rats fed ADBAQ at a concentration of 10,000 or 20,000 ppm for 15 months (in the NTP two-year and stop-exposure studies). The number of foci per cubic centimeter and the focus volume fraction (percent of liver volume occupied by foci) were significantly increased in both the low- and high-dose groups, and the mean focus volume was significantly increased in the high-dose group (Table 4-4). The authors concluded, however, that increases in AHF in the two-year carcinogenicity study were insufficient evidence of hepatocarcinogenicity in the absence of a liver tumor response.

Table 4-4. Occurrence of altered hepatocellular foci in female F344/N rats following dietary exposure to ADBAQ for 15 months

Exposure concentration (ppm) (N = 10)	Foci/cm^3 (mean ± SE)	Volume fraction (mean % ± SE)	Focus volume (mean mm^3 ± SE)
0	61.27 ± 37.6	0.19 ± 0.16	0.010 ± 0.006
10,000	1,005.76 ± 254.7**	2.70 ± 0.57*	0.024 ± 0.007
20,000	1,217.53 ± 287.0[b]**	5.88 ± 0.79***	0.072 ± 0.021**

Source: Maronpot *et al.* 1989a.

*Significantly different ($P < 0.05$) from the control group (test not specified).

**Significantly different ($P < 0.01$) from the control group (test not specified).

***Significantly different ($P < 0.001$) from the control group (test not specified).

Harada *et al.* (1989) performed qualitative and quantitative assessment of altered hepatocellular foci in histological material from the NTP two-year and 15-month stop-exposure studies of ADBAQ in rats. Liver samples collected at 9-, 15-, and 24-month intervals were included in the analysis. The authors separated both basophilic and eosinophilic AHF into common and atypical types. No exposure-related changes in number, size, or volume fraction were found for commonly occurring AHF. However, these parameters were significantly increased for atypical AHF and were correlated with the level and duration of exposure to ADBAQ in both male and female rats. The numbers of AHF in the two higher-dose groups (10,000 and 20,000 ppm) tended to decrease over time, and this trend correlated with the occurrence of liver tumors in the animals selected for AHF analysis.

4.2 Mice

The NTP (1996) conducted two studies of ADBAQ in male and female B6C3F$_1$ mice: a 13-week study and a two-year (104-week) study.

4.2.1 Thirteen-week study

Groups of B6C3F$_1$ mice (10 of each sex) were fed diets containing ADBAQ at a concentration of 0, 2,500, 5,000, 10,000, 25,000, or 50,000 ppm for 13 weeks. After the 13-week exposure, necropsies were performed, in which tissues of all animals were collected, weighed, and processed for histologic examination (a complete list of tissues

and organs examined is included in Appendix B). Survival to study termination and final mean body weight as percent of control mean body weight are summarized in Table 4-5. Nonneoplastic lesions of the liver were observed, including pigmentation in males (5/10 at 2,500 ppm and 8/10 to 10/10 at 10,000 ppm or higher) and females (1/10 at 25,000 and 50,000 ppm) and centrilobular hypertrophy in males only (8/10 to 10/10 at 10,000 ppm or higher). Data for exposure-related nonneoplastic lesions in all groups are summarized in the NTP report (Appendix A, p. A-57, Table 20).

Table 4-5. Survival and body weight in B6C3F$_1$ mice following dietary exposure to ADBAQ for 13 weeks

	Exposure concentration (ppm)					
End point	**0**	**2,500**	**5,000**	**10,000**	**25,000**	**50,000**
Males						
Survival to study termination	10/10	10/10	9/10	10/10	9/10	10/10
Final mean b.w. ± SD (g)	30.5 ± 0.6	30.6 ± 0.6	30.7 ± 0.7	32.1 ± 0.4	30.9 ± 0.6	31.5 ± 0.4
% of control mean b.w.	100	100	101	105	101	103
Females						
Survival to study termination	10/10	10/10	10/10	9/10	10/10	10/10
Final mean b.w. ± SD (g)	24.0 ± 0.2	24.7 ± 0.6	25.0 ± 0.6	25.0 ± 0.4	23.6 ± 0.3	24.7 ± 0.4
% of control mean b.w.	100	103	104	104	98	103

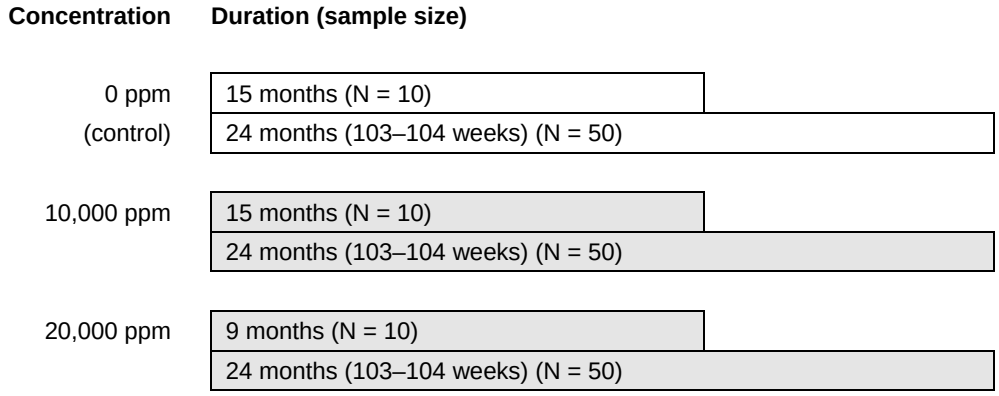
Fleischman *et al*. (1986) also reported the results from the NTP's 13-week study of ADBAQ in B6C3F$_1$ mice. The effects of exposure to ADBAQ for 13 weeks were less pronounced in mice than in rats. Body weight was not affected in either sex at any exposure level (Table 4-5). Dose-related lesions of the liver were observed in male mice and included centrilobular glycogen depletion at 10,000 ppm or higher and pigmentation at all exposure levels. The authors concluded that "at comparable doses, ADBAQ was considered to be markedly toxic in rats and of minimal, non-life-threatening toxicity in mice."

4.2.2 Two-year study

Dietary concentrations of ADBAQ for the two-year study in mice were based on the results of the 13-week study, specifically, on the frequency and severity of centrilobular hypertrophy of the liver observed in male mice. Because only lesions of mild severity were observed in groups exposed to ADBAQ at 10,000 or 25,000 ppm, and lesions of moderate severity were observed in the 50,000-ppm group, exposure concentrations for the two-year study were set at 0, 10,000, and 20,000 ppm. Other considerations included consistency among males and females (as females could have been given higher exposure concentrations) and correspondence to the concentrations selected for the stop-exposure study in rats (see Appendix A, p. A-57).

Male and female B6C3F$_1$ mice were obtained at five weeks of age and quarantined for 15 days before the study began. Groups of mice (60 of each sex) received diets containing

ADBAQ at a concentration of 0, 10,000, or 20,000 ppm. A subset of 10 mice from each group was sacrificed at 15 months, and the remaining 50 mice were sacrificed at 24 months. The exposure protocol is shown in Figure 4-3.



Shaded bars indicate ADBAQ-containing diet, and open bars indicate control diet. The total length of each bar indicates the time to study termination and the sacrifice of animals in that group.

Figure 4-3. Exposure protocol for the two-year study of ADBAQ in B6C3F$_1$ mice

The results of the two-year study are summarized in Table 4-6. Feed consumption by exposed mice was generally similar to that of controls; however, final mean body weight was lower for exposed mice than for controls. Survival of exposed male mice was significantly lower than that of controls, but survival of exposed females was similar to that of controls. Hepatocellular adenoma or carcinoma, forestomach squamous-cell papilloma, and alveolar/bronchiolar adenoma were observed in exposed mice but not in controls at the 15-month interim sacrifice. Incidences of hepatocellular adenoma or carcinoma in high-dose males and females, squamous-cell papilloma in high-dose males and low-dose females, and alveolar/bronchiolar adenoma in high-dose and low-dose males were significantly higher than in controls. At the end of the two-year study, significant increases were observed in the incidences of heptatocellular multiple adenomas and multiple carcinomas in both male and female mice at both exposure levels. In addition, squamous-cell papilloma and carcinoma of the forestomach were significantly increased in both males and females at both exposure levels. The incidence of alveolar/bronchiolar adenoma or carcinoma of the lung also was significantly increased in both males and females.

Table 4-6. Survival, body weight, and tumor incidence in B6C3F$_1$ mice following dietary exposure to ADBAQ for two years

	Exposure concentration (ppm)				
End point	**0**	**10,000**		**20,000**	
Males					
Survival to study termination	40/50	22/51***		23/50***	
Final mean b.w. (g) (% of control mean b.w.)	43.6 (100)	37.0 (85)		36.3 (83)	
		15-mo.	2-year	15-mo.	2-year
Liver (hepatocellular tumors)	N = 50	N = 9	N = 51	N = 10	N = 50
Adenoma	10	2	38***	4*	39***
Carcinoma	9	1	18*	0	21**
Adenoma or carcinoma	18	3	43***	4*	42***
Hepatoblastoma	0	0	3	0	5*
Forestomach (squamous-cell tumors)	N = 50	N = 9	N = 51	N = 10	N = 50
Papilloma	0	0	13***	5*	16***
Carcinoma	0	0	12***	0	13***
Papilloma or carcinoma	0	0	19***	5*	27***
Lung (alveolar/bronchiolar tumors)	N = 50	N = 9	N = 51	N = 10	N = 50
Adenoma	7	3	26***	5*	24***
Carcinoma	3	0	4	0	1
Adenoma or carcinoma	10	3	28***	5*	25**
Females					
Survival to study termination	39/50	34/50		33/50	
Final mean b.w. (g) (% of control mean b.w.)	39.2 (100)	33.6 (86)		31.9 (81)	
		15-mo.	2-year	15-mo.	2-year
Liver (hepatocellular tumors)	N = 50	N = 10	N = 50	N = 10	N = 50
Adenoma	6	2	45***	7**	49***
Carcinoma	0	0	23***	1	27***
Adenoma or carcinoma	6	2	46***	8**	50***
Hepatoblastoma	0	0	0	0	2
Forestomach (squamous-cell tumors)	N = 50	N = 10	N = 50	N = 10	N = 50
Papilloma	2	4*	16***	2	27***
Carcinoma	0	0	12***	0	11***
Papilloma or carcinoma	2	4*	25***	2	34***
Lung (alveolar/bronchiolar tumors)	N = 50	N = 10	N = 50	N = 10	N = 49
Adenoma	4	3	17***	2	13*
Carcinoma	0	0	0	0	2
Adenoma or carcinoma	4	3	17***	2	15**

*Significantly different ($P < 0.05$) from the control group by logistic regression test.

**Significantly different ($P < 0.01$) from the control group by the logistic regression test.

*** Significantly different ($P < 0.001$) from the control group by lifetable pairwise comparison (survival data) or the logistic regression test (tumor data).

4.3 Summary

ADBAQ was toxic to male and female F344/N rats at dietary concentrations of 2,500 to 50,000 ppm consumed over a 13-week period, as evidenced by decreased body weight, nonneoplastic lesions in the liver and kidney, and anemia. Exposure of male and female $B6C3F_1$ mice to ADBAQ at the same dietary concentrations resulted in lower apparent toxicity, as mice gained weight at all ADBAQ exposure levels, and fewer types of nonneoplastic lesions of the liver and kidney were observed.

In two-year dietary studies with ADBAQ, male and female F344/N rats exhibited significantly increased incidences of neoplasms of the liver, large intestine, kidney, and urinary bladder. Significant increases in the incidences of tumors of the liver and large intestinge (females only) also were observed in a 15-month stop-exposure study in rats. $B6C3F_1$ mice consuming ADBAQ-containing diets for two years had increased incidences of hepatocellular adenoma and carcinoma, squamous-cell papilloma and carcinoma of the forestomach, and alveolar/bronchiolar adenoma and carcinoma of the lung. The NTP report concluded that “under the conditions of these 2-year feed studies, there was *clear evidence for carcinogenic activity* of 1-amino-2,4-dibromoanthraquinone in male and female F344/N rats based on increased incidences of neoplasms in the liver, large intestine, kidney, and urinary bladder. There was *clear evidence for carcinogenic activity* of 1-amino-2,4-dibromoanthraquinone in male and female $B6C3F_1$ mice based on increased incidences of neoplasms in the liver, forestomach, and lung.”

5 Genotoxicity

Limited data are available on the genotoxicity of ADBAQ. The few studies that have attempted to ascertain the mutagenicity of ADBAQ have encountered solubility problems that make the assays difficult to execute and that complicate interpretation of the results.

5.1 Prokaryotic systems: *Salmonella typhimurium*

Salmonella typhimurium is the only prokaryote in which the mutagenicity of ADBAQ has been assessed. Several strains, including those that revert by base pair substitutions (TA100 and TA1535) and those that revert by frameshift mutations (TA98, TA1537, and TA1538), were used in the ADBAQ mutagenicity tests.

Haworth *et al*. (1983) showed that without rat or hamster liver microsomal (S9) metabolic activation, ADBAQ induced reverse mutation in strains TA98 and TA1537 (Table 5-1). In the presence of S9, reverse mutation was not induced in strain TA98, and strain TA1537 gave a variable response. ADBAQ was weakly mutagenic in strain TA100 (with or without S9 activation) and nonmutagenic in strain TA1535. The ADBAQ preparation used in these studies had a labeled purity of 91.7% and an analyzed purity of 79.7%. The researchers reported the presence of precipitate when ADBAQ concentrations were in the range of 333 to 10,000 μg/plate (the highest concentration tested). The NTP (1996) noted that the positive or weakly positive mutagenic responses in the Haworth *et al*. (1983) study were observed in strains in which reversion occurs by frameshift mutations.

Table 5-1. Mutagenicity of ADBAQ in *S. typhimurium*[a]

	Without S9		Hamster S9		Rat S9	
Strain	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
TA100	+ (weak)	–/+	+ (weak)	–/+	–/+	–
TA1535	–	–	–	–	–	–
TA1537	+	+	+	–	+	–/+
TA98	+	+	–/+	–	–	–

Data were presented by Haworth *et al*. (1983) and NTP (1996). Trial summaries were obtained from Table G1 of NTP (1996).

[a]+ = positive (a reproducible dose-related increase); – = negative; –/+ = equivocal (positive or weakly positive responses that were not replicated in a second trial).

5.2 Mammalian systems

Rodent cells were used to determine the mutagenicity and DNA-damaging potential of ADBAQ. No reports of *in vivo* mammalian genotoxicity assays were found in the literature.

5.2.1 Mouse lymphoma L5178YTK$^{+/-}$ cells

Harrington-Brock *et al*. (1991) carried out a limited evaluation of the ability of ADBAQ to induce mutations at the thymidine kinase (*tk*) locus, but they were unable to test for

micronucleus formation because of ADBAQ's limited solubility. ADBAQ was not mutagenic in the absence or presence of induced-rat liver S9 at the highest testable concentrations (25 μg/mL without S9 and 20 μg/mL with S9). The authors reported that the sample of ADBAQ was from the same batch evaluated in the NTP rodent carcinogenesis assay, and that the purity of the sample was 79.7%.

5.2.2 Chromosomal aberrations

Chinese hamster ovary (CHO) cells were exposed to various concentrations of ADBAQ and then analyzed for chromosomal aberrations (Loveday *et al.* 1990). The authors reported the presence of a precipitate in the flasks containing ADBAQ at 50 μg/mL (the highest concentration tested). Two separate trials without S9 metabolic activation gave equivocal results (Table 5-2). In one trial, exposure to ADBAQ at 3.02 or 10.10 μg/mL significantly increased the percentage of cells with chromosomal aberrations (Dunnett's test, $P < 0.05$). In the second trial, the percentage of cells with chromosomal aberrations was not significantly increased by ADBAQ at concentrations of 1.0, 3.0, 10.0, or 30.0 μg/mL. In the presence of S9, the percentage of cells with chromosomal aberrations was not significantly increased by exposure to ADBAQ at 3.02, 10.10, or 30.20 μg/mL.

The NTP (1996) reported additional results obtained in two trials performed by Environmental Health Research and Testing, Inc. (Research Triangle Park, NC). In the absence of S9, the percentage of cells with chromosomal aberrations was significantly increased by exposure to ADBAQ at 50 μg/mL in the first trial, but increases observed at 16, 30, or 50 μg/mL in the second trial were not statistically significant. In the presence of S9, the percentage of cells with chromosomal aberrations was not significantly increased by ADBAQ at 16, 50, or 100 μg/mL. Based on these studies, the NTP (1996) concluded that ADBAQ induced chromosomal aberrations only in the absence of S9.

5.2.3 Sister chromatid exchange

Loveday *et al*. (1990) examined the effect of ADBAQ on the induction of sister chromatid exchange (SCE) in CHO cells. The chemical purity of ADBAQ was reported as greater than 79.7%, but the impurities were not identified. A trend test was used to describe the strength of evidence for SCE induction. Results were designated as negative, questionable, weakly positive, or positive, based on the trend test and the number of exposure levels resulting in at least a 20% increase over controls. Three exposure levels were used for each trial, and at least two had to produce a 20% increase for the result to be considered positive. In the presence or absence of induced-rat liver S9, ADBAQ induced SCE in a dose-dependent manner. In the absence of S9, ADBAQ induced at least a 20% increase in the number of SCEs at a concentration of 10 μg/mL, but not at 5 or 2.5 μg/mL. In the presence of S9, ADBAQ induced at least a 20% increase in the number of SCEs at 3.01, 10.1, or 30.1 μg/mL in trial 1 and at the highest concentration (15 μg/mL) in trial 2. The authors classified ADBAQ as weakly positive for SCE induction in the absence of S9 and positive (trial 1) or weakly positive (trial 2) in the presence of S9 (Table 5-2).

The NTP (1996) also reported results from the Environmental Health Research and Testing Laboratory, which found that ADBAQ tested at concentrations ranging from 5 to

50 µg/mL induced SCE in the absence of S9 (results were positive in one trial and weakly positive in another). In the presence of S9, ADBAQ at 5, 16, 50, or 100 µg/mL (one trial) did not induce SCE. The NTP (1996) noted that the discrepancy in SCE induction in the presence of S9 could not be explained by differences between the ADBAQ concentrations used by Loveday *et al.* (1990) and the NTP (1996).

Table 5-2. Induction of chromosomal aberrations and SCE in CHO cells by ADBAQ[a]

Metabolic activation		Chromosomal aberrations		Sister chromatid exchanges	
		Loveday *et al.* 1990	NTP 1996[b]	Loveday *et al.* 1990	NTP 1996[b]
-S9	Trial 1	+	+ (weak)*	+ (weak)	+
	Trial 2	–	+ (weak)	NR	+ (weak)
+S9	Trial 1	–	–	+	–
	Trial 2	NR	NR	+ (weak)	NR

[a]+ = positive; – = negative; NR = not reported.

[b]Reported by the NTP (1996) as results from the Environmental Health Research and Testing Laboratory.

*$P < 0.05$ for 50 µg/mL concentration.

5.2.4 Ras *mutations in tumors*

Hayashi *et al.* (2001) examined 8 forestomach squamous-cell papillomas, 24 squamous-cell carcinomas, 19 alveolar/bronchiolar adenomas, and 4 alveolar/bronchiolar carcinomas for mutations in the H-*ras* and/or K-*ras* genes. These tumors were obtained from male and female $B6C3F_1$ mice that were fed diets containing ADBAQ at a concentration of 10,000 or 20,000 ppm for two years (NTP 1996). *Ras* codons 12, 13, and 61, which are hot spots for mutations in human and rodent tumors, were examined in tumors from exposed mice, in spontaneous tumors from control mice, and in histologically normal forestomach and lung tissues. The ADBAQ-induced tumors had a higher frequency of *ras* mutations than did spontaneous tumors, and the mutations were found in both benign and malignant tumors (Table 5-3). The mutation spectra for the low- and high-dose groups were the same. Mutations in *ras* were not detected in any of the normal tissues. The predominant mutations in ADBAQ-induced tumors were CAA to CTA transversions and CAA to CGA transitions in codon 61 of K-*ras* (lung tumors) or H-*ras* (forestomach tumors). No A to T transversions at the second base were detected in spontaneous tumors. Mutations observed in codons 12 or 13 generally occurred in both ADBAQ-induced and spontaneous tumors. The authors concluded that alterations in the *ras* gene might be an early event in ADBAQ-induced carcinogenesis.

Table 5-3. Frequency and spectra of *ras* mutations in forestomach and lung tumors from B6C3F$_1$ mice exposed to ADBAQ for two years[a]

Group	Total activated *ras* (%)	K-*ras* codons				H-*ras* codon		
		12	13	61		61		
		Total	Total	A→T	A→G	A→T	A→G	C→A
Forestomach tumors								
Control[b]	4/11 (36)	3	1	0	0	0	1	0
ADBAQ	23/32 (72)	5	3	0	0	14	6	3
SCP	5/8 (63)	2	0	0	0	4	0	1
SCC	18/24 (75)	3	3	0	0	10	6	2
Lung tumors								
Control[c]	26/86 (30)	16	3	0	2	NT	NT	NT
ADBAQ	16/23 (70)	7	1	6	5	NT	NT	NT
ABA	14/19 (74)	7	1	6	3	NT	NT	NT
ABC	2/4 (50)	0	0	0	2	NT	NT	NT

[a]SCP = squamous-cell papilloma; SCC = squamous-cell carcinoma; ABA = alveolar/bronchiolar adenoma; ABC = alveolar/bronchiolar carcinoma; NT = not tested.

[b]Spontaneous forestomach tumors in B6C3F$_1$ mice.

[c]Study controls (N = 4) combined with historical spontaneous lung tumors (N = 82) of B6C3F$_1$ mice.

5.3 Summary

ADBAQ was mutagenic in *S. typhimurium* strains that revert by frameshift mutations, and the mutagenicity was decreased or eliminated in the presence of induced hamster or rat liver S9 metabolic activation. ADBAQ did not induce detectable mutations at the *tk* locus in mouse lymphoma cells; however, the concentrations used (20 or 25 μg/mL) were less than those used for the *S. typhimurium* assays (100 to 10,000 μg/mL) and slightly less than those used to assess clastogenicity (up to 100 μg/mL of ADBAQ). In CHO cells, ADBAQ induced chromosomal aberrations and SCE without metabolic activation; however, the results were inconsistent between different trials and laboratories. Forestomach and lung tumors induced by ADBAQ had a higher frequency of *ras* mutations than did spontaneous tumors. In particular, CAA to CTA transversions were common in ADBAQ-induced tumors but were absent in spontaneous tumors. The results obtained with ADBAQ must be viewed in the light of the limited solubility of this molecule and the uncertain purity of the preparations tested.

6 Other Relevant Data

6.1 Absorption, distribution, metabolism, and excretion

The NTP (1996) reported studies on the absorption, distribution, metabolism, and excretion of ADBAQ. Male F344/N rats were administered [^{14}C]ADBAQ orally or intravenously (i.v.), and disposition and elimination of radioactivity (parent compound and/or metabolites) was monitored for up to 72 hours. The specific location of the radioactive carbon atom(s) in the ADBAQ molecule was not identified.

6.1.1 Absorption

ADBAQ was readily absorbed from the gastrointestinal tract. The percentage of the dose absorbed was inversely proportional to dose level. When rats were administered single oral doses of 2, 23, 118, 814, or 1,473 mg/kg b.w., 90% of the lowest dose (2 mg/kg) was absorbed, but only 2% of the 814-mg/kg dose (NTP 1996).

6.1.2 Distribution and metabolism

ADBAQ was rapidly and widely distributed after i.v. administration. Radioactivity in blood was located primarily in plasma, as reflected by a whole blood:plasma ratio of 0.5. Fifteen minutes after i.v. administration (0.4 mg/kg b.w.), radioactivity was detected in most soft tissues, with the highest concentrations in excretory organs, lung, kidney, small intestine, liver, adipose tissue, and adrenal glands. At 15 minutes, tissue:blood ratios of radioactivity for these tissues were 3.0 or greater. Tissue:blood ratios declined during the 72 hours after administration, remaining greater than 1.0 only in liver and kidney at 72 hours. No quantitative data on tissue distribution were reported (NTP 1996).

Two hours after administration, only 3% of circulating radioactivity was in the form of unchanged parent compound, indicating that ADBAQ was quickly metabolized. Unchanged ADBAQ was deposited in adipose tissue, where it remained largely unmetabolized. As late as 24 hours after administration, the radioactivity remaining in adipose tissue was still unchanged ADBAQ, whereas that in liver, muscle, and skin was primarily ADBAQ metabolites (which were not identified) (NTP 1996). The metabolites of ADBAQ are unknown. Other anthraquinone-based dyes (Section 1, Table 1-2) were examined by IARC (1982) and the NTP (1978a, 1978b, 1978c, 1986), but their metabolites also are unknown. The NTP (1996) study did find that very little (less than 3%) of [^{14}C]ADBAQ was excreted as unmetabolized ADBAQ.

6.1.3 Excretion

In the three days after administration of [^{14}C]ADBAQ, approximately 50% of ADBAQ-associated radioactivity was recovered from feces, 15% from urine, and 6% from expired air. Consistent with extensive metabolism, less than 3% of total radioactivity excreted represented unchanged ADBAQ. Terminal half-lives of administered radioactivity in liver and kidney were estimated to be 40 and 90 hours, respectively, and the elimination half-life from adipose tissue was 11 hours.

6.2 Liver focus model

Maronpot *et al.* (1989b) tested ADBAQ in a neonatal rat liver focus model for its potential both as an initiator and as a promoter. In the test for initiation potential, 0.07 mg/g b.w. of ADBAQ was injected intraperitoneally (i.p.) within 24 hours after birth, and phenobarbital (500 ppm) was used as the promoting agent over the succeeding 75 or 300 days. The authors found no evidence of initiating activity for ADBAQ.

In the test for promotion potential, each neonatal rat was injected with a "subcarcinogenic" dose (4 μg/g b.w.) of the initiator, diethylnitrosamine (DEN), within 24 hours of birth. After weaning, rats received ADBAQ at either 5,000 ppm or 10,000 ppm in the diet for either 75 or 300 days prior to sacrifice. Livers were examined microscopically and graded with respect to number of atypical hepatocellular foci, mean focus volume, and tumor incidence. The qualitative results are summarized in Table 6-1. Equivocal results were reported for number of foci and the mean focus volume. The authors concluded that the lack of a clear association between the equivocal stereological response and positive hepatocarcinogenicity in the two-year study (see Table 4-2) may have resulted from unpalatability of the feed containing ADBAQ. The neonatal rats initiated with DEN may not have consumed a sufficient dose of ADBAQ to produce an unequivocal AHF response.

Table 6-1. Occurrence of altered hepatocellular foci neonatal rats following dietary exposure to ADBAQ for 75 or 300 days[a]

Exposure concentration (ppm)	Mean foci/cm^3	Mean focus volume	Tumor incidence
75 days			
Males			
5,000	NS	↑ P< 0.01	—
10,000	NS	NS	NR
Females			
5,000	↓ P < 0.05	NS	NR
10,000	↓ P < 0.05	NS	NR
300 days			
Males			
5,000	NS	NS	NS
10,000	NS	↑ P < 0.01	NS
Females			
5,000	↑ P < 0 .01	↑ P < 0.01	NS
10,000	NS	NS	NS

Source: Maronpot *et al.* 1989b

[a]NS: no significant difference from control; ↑ = significantly higher than control; ↓ = significantly lower than control; NR = not reported.

6.3 Carcinogenicity and mutagenicity of other quinones and anthraquinones

ADBAQ and other anthraquinones belong to a general class of quinone molecules that can be derived from aromatic molecules such as benzene, naphthalene, and anthracene. Quinone molecules can be reduced to a relatively stable hydroquinone, which usually is not associated with oxidative stress, or they may be reduced in a one-electron reduction to semiquinone free radicals that give rise to superoxide anions, hydrogen peroxide, and other reactive oxygen species (Parkinson 1996). Bolton *et al.* (2000) recently reviewed the role of quinones in toxicology, including carcinogenesis. Quinones may be produced from benzene, polycyclic aromatic hydrocarbons, estrogens, and catecholamines and give rise to reactive oxygen species that can damage DNA and other cellular macromolecules and activate signaling pathways. These molecular events may be associated with the initiation, promotion, and progression of carcinogenesis.

The benzoquinone metabolite of benzene has been proposed as a proximate carcinogenic product (Golding and Watson 1999); however, the IARC (1999) overall evaluation was that 1,4-benzoquinone is not classifiable as to its carcinogenicity to humans (Group 3). In a review of the role of quinoids in estrogen carcinogenesis, Bolton *et al.* (1998) suggested that the catechol metabolites of estrogens that are implicated in the carcinogenic effects of these steroids could be metabolized to electrophilic and redox-active quinoids that could damage DNA. Their conclusion, however, was that the ultimate carcinogenic metabolites of estrogens have not been unequivocally established.

Anthraquinones comprise a large class of diverse molecules which may be naturally occurring or synthetic in origin. Sendelbach (1989) reviewed the toxicity and carcinogenicity of anthraquinone derivatives with phenolic, amino, or nitro substitutions to the anthraquinone ring structure. The author's general conclusion concerning amino substitutions was that addition of an amino group at either the 1- or 2-position of the anthraquinone ring appeared to consistently produce renal lesions in rats. The relevance of this conclusion to ADBAQ, which has both amino and halogen substitutions to the anthraquinone ring, is not clear. However, anthraquinone also produces renal tumors. Renal tubule adenomas and carcinomas (combined) were significantly increased ($P < 0.05$) by dietary exposure to ADBAQ at 5,000 ppm in female rats and at 10,000 ppm in both male and female rats (see Table 4-2).

A number of anthraquinone-derived vat dyes have been reviewed by both IARC and the NTP. ADBAQ produces tumors at some of the same sites as other anthraquinones. Table 6-2 summarizes the IARC and NTP evaluations of several anthraquinone derivatives. Information on a number of other anthraquinone derivatives at various stages of testing by the NTP can be accessed through the NTP Web site (http://ntp-server.niehs.nih.gov/main_pages/NTP_ALL_STDY_PG.html).

As described in Section 5.2.1, Harrington-Brock *et al.* (1991) carried out a limited evaluation of the ability of ADBAQ to induce mutations at the thymidine kinase (*tk*) locus of L5178/TK$^{+/-}$mouse lymphoma cells, but they were unable to test for micronucleus formation because of ADBAQ's limited solubility. An additional 15 anthraquinones were tested in the same assays. Testing of 8 of these substances was incomplete because of their limited solubility. For these 15 compounds, Table 6-3

summarizes the results of tests of mutagenicity at the L5178/TK$^{+/-}$ locus in two or three experiments and micronucleus analysis from one experiment per compound. Table 6-3 also summarizes the mutagenicity of these compounds in *S. typhimurium,* as reviewed by Harrington-Brock *et al.* (1991).

Brown (1980) reviewed the genetic effects of approximately 80 phenolic anthraquinones and reported that a high percentage of these hydroxyanthraquinones (36, or 45%) were mutagenic in *S. typhimurium* strains TA1537, TA1538, and TA98, which are sensitive to mutagens that cause frameshifts. A small portion (~10%) of the hydroxyanthraquinones contained either amino or halogen (chlorine or bromine) substitutions; however, the relevance of these findings to the possible mutagenicity of ADBAQ is not clear.

Table 6-2. Carcinogenicity and mutagenicity of anthraquinone and some anthraquinone-derived dyes (data from NTP and IARC)

Property	Anthraquinone	2-Amino-anthraquinone	1-Amino-2-methyl-anthraquinone	1,8-Dihydroxy-anthraquinone (danthron)	2-Methyl-1-nitro-anthraquinone	1,4,5,8-Tetraamino-anthraquinone (disperse blue 1)	Emodin
Long-term carcinogenicity in mice	Liver neoplasms in male and female $B6C3F_1$ mice	Hepatocellular carcinomas in male and female $B6C3F_1$ mice (NTP) Malignant hematopoietic lymphomas in high dose female mice (NTP)	Negative in male mice (NTP) Hepatocellular carcinomas and neoplastic liver nodules in female $B6C3F_1$ mice (NTP)	Hepatocellular adenomas and carcinomas and adenomatous hyperplasia of the cecum in C3H/HeN male mice (IARC)	Hemangiosarcomas in male and female $B6C3F_1$ mice (NTP)	Hepatocellular adenomas and carcinomas (combined) in male $B6C3F_1$ mice (NTP) Alveolar/bronchiolar adenomas and carcinomas (combined) in male $B6C3F_1$ mice (NTP)	Uncommon renal tubule neoplasms in male $B6C3F_1$ mice
Long-term carcinogenicity in rats	Renal tubule neoplasms in male and female F344/N rats Transitional epithelial papillomas of the urinary bladder in male F344/N rats	Hepatocellular carcinomas in male F344 rats (NTP) Inadequate study in female F344 rats (poor survival) (NTP)	Hepatocellular carcinomas in male and female F344 rats (NTP)	Adenocarcinomas of the colon in male ACI rats (IARC)	Hepatocellular carcinomas in male F344 rats (NTP) Subcutaenous fibromas in male and female F344 rats (NTP)	Transitional-cell neoplasms of the urinary bladder in male and female F344 rats (NTP) Squamous-cell neoplasms in male F344 rats (NTP) Leiomyomas and leiomyosarcomas (combined) of the urinary bladder in female F344 rats (NTP)	Zymbal's gland carcinomas in female F344/N rats

Property	Anthraquinone	2-Amino-anthraquinone	1-Amino-2-methyl-anthraquinone	1,8-Dihydroxy-anthraquinone (danthron)	2-Methyl-1-nitro-anthraquinone	1,4,5,8-Tetraamino-anthraquinone (disperse blue 1)	Emodin
Genetic toxicology	Positive for mutagenicity in *S. typhimurium* TA98 and TA100 with and without S9 Positive for micronucleated normochromatic erythrocytes in male and female mice (14-week dietary study) Negative for micronuclei in mouse bone marrow (i.p. injection)	Positive for chromosomal aberrations Positive for SCE Positive for mutagenicity in *S. typhimurium* (NTP)	Positive for chromosomal aberrations Positive for SCE Positive for mutagenicity in *S. typhimurium* (NTP)	Positive for chromosomal aberrations Not tested for SCE Negative for micronuclei [cells not specified] Weakly positive for mutagenicity in *S. typhimurium* (NTP)	Positive for chromosomal aberrations Positive for SCE Negative for reciprocal translocation in *Drosophila* Positive for mutagenicity in *S. typhimurium*	Positive for chromosomal aberrations Positive for SCE Positive for mutagenicity in mouse lymphoma cells Negative for micronuclei [cells not specified] Positive for mutagencity in *S. typhimurium*	Positive for mutagenicity in *S. typhimurium* (TA100) with S9; negative in TA98 with or without S9 Positive for chromosal aberrations Negative for micronuclei in mouse bone marrow (i.p. injection) Weakly postive for micronuclei in female mice (14-week dietary study) [cells not specified]

Property	Anthraquinone	2-Amino-anthraquinone	1-Amino-2-methyl-anthraquinone	1,8-Dihydroxy-anthraquinone (danthron)	2-Methyl-1-nitro-anthraquinone	1,4,5,8-Tetraamino-anthraquinone (disperse blue 1)	Emodin
RoC listing or NTP evaluation	NTP: some evidence of carcinogenic activity in male F344/N rats; clear evidence of carcinogenic activity in female F344/N rats; clear evidence of carcinogenic activity in male and female $B6C3F_1$ mice	RoC: reasonably anticipated to be a human carcinogen	RoC: reasonably anticipated to be a human carcinogen	RoC: reasonably anticipated to be a human carcinogen	NTP: found to be carcinogenic in male and female rats and mice	RoC: reasonably anticipated to be a human carcinogen	NTP: equivocal evidence of carcinogenic activity in female F344/N rats and $B6C3F_1$ mice
IARC evaluation[a]	Not listed	Group 3 (IARC 1987)	Group 3 (IARC 1987)	Group 2B (IARC 1990)	Group 2B (IARC 1987)	Group 2B (IARC 1990)	Not listed

[a]Group 2B = possibly carcinogenic to humans, based on limited evidence of carcinogenicity in humans, less than sufficient evidence of carcinogenicity in animals. Group 3 = not classifiable as to its carcinogenicity in humans, based on inadequate or limited evidence of carcinogenicity in animals and inadequate evidence of carcinogenicity in humans.

Table 6-3. Genotoxicity of some anthraquinone dyes[a]

Anthraquinone	CASRN	Purity (%)	Mutagenicity in mouse lymphoma cells	Micronuclei in mouse lymphoma cells	Reverse Mutation in *S. typhimurium*
1-Amino-2-methylanthraquinone	82-28-0	99	+ w/o S9	+ w/o S9	+
2-Aminoanthraquinone	117-79-3	86.9	+ w/o S9	+ w/o S9	+
D&C green 5	4403-90-1	60	limited solubility	limited solubility	–/+
Disperse blue 1	2475-45-8	50	limited solubility	limited solubility	+
Disperse blue 3	2475-46-9	42	+ w/o S9	+ w/o S9	+
Disperse blue 7	3179-90-6	60	+ w/o S9	+	+
Disperse red 11	2872-48-2	95	+ w/o S9	+ w/o S9	+
Disperse red 60	17418-58-5	92	limited solubility	limited solubility	NR
1-Nitro-2-methylanthraquinone	129-15-7	95.9	limited solubility (–/+ w S9)	limited solubility (–/+ w S9)	+
Reactive blue 19	2580-78-1	36	+ (weak) w/o S9	increased from 9/1,000 to 19/1,000 cells w/o S9	NR
Vat blue 4	81-77-6	48	limited solubility	limited solubility	NR
Vat blue 20	116-71-2	26	limited solubility	limited solubility	NR
Vat brown 1	2475-33-4	56	limited solubility	limited solubility	NR
Vat brown 3	131-92-0	85	limited solubility	limited solubility	–
Vat yellow 4	128-66-5	18.2	+ w S9	slight increase w S9	–

Adapted from Harrington-Brock *et al.* 1991 and references therein.

[a]+ = positive; – = negative; –/+ equivocal; NR = not reported.

6.4 Summary

In rats, ADBAQ is rapidly absorbed from the gastrointestinal tract and distributed rapidly and widely to most soft tissues. Approximately 97% of ADBAQ is metabolized, as demonstrated by the small amount (less than 3%) of radiolabel recovered as the parent compound from either blood or urine; however, the metabolites of ADBAQ have not been identified. ADBAQ and its metabolites are excreted primarily in the feces and urine.

ADBAQ and other anthraquinones are classified with a large number of other quinone molecules that can be derived from aromatic molecules such as benzene, naphthalene, and anthracene. Reactive oxygen species generated by metabolism of a variety of quinones may be associated with DNA damage or activation of signaling pathways involved in initiation, promotion, and progression of carcinogenesis. Four other anthraquinone vat dyes evaluated by the NTP have been listed in the RoC as *reasonably anticipated to be human carcinogens.* In addition, a high percentage (36/80) of phenolic anthraquinones have been reported to be mutagenic in *Salmonella*.

7 References

1. Barbone, F., E. Delzell, H. Austin, and P. Cole. 1992. A case-control study of lung cancer at a dye and resin manufacturing plant. *Am J Ind Med* 22:835-849.

2. Barbone, F., E. Delzell, H. Austin, and P. Cole. 1994. Exposure to epichlorohydrin and central nervous system neoplasms at a resin and dye manufacturing plant. *Arch Environ Health* 49:355-358.

3. Bolton, J.L., E. Pisha, F. Zhang, and S. Qiu. 1998. Role of quinoids in estrogen carcinogenesis. *Chem Res Toxicol* 11:1113-1127.

4. Bolton, J.L., M.A. Trush, T.M. Penning, G. Dryhurst, and T.J. Monks. 2000. Role of quinones in toxicology. *Chem Res Toxicol* 13:135-160.

5. Brown, J.P. 1980. A review of the genetic effects of naturally occurring flavonoids, anthraquinones and related compounds. *Mutat Res* 75:243-277.

6. ChemFinder. 2001. 1-Amino-2,4-Dibromoanthraquinone. Available at http://www.chemfinder.camsoft.com (and search 81-49-2).

7. Delzell, E., M. Macaluso, and P. Cole. 1989. A follow-up study of workers at a dye and resin manufacturing plant. *J Occup Med* 31:273-278.

8. Fleischman, R.W., H.J. Esber, M. Hagopian, H.S. Lilja, and J. Huff. 1986. Thirteen-week toxicology studies of 1-amino-2,4-dibromoanthraquinone in Fischer 344/N rats and B6C3F_1 mice. *Toxicol Appl Pharmacol* 82:389-404.

9. Games, L.M. and R.A. Hites. 1977. Composition, treatment efficiency, and environmental significance of dye manufacturing plant effluents. *Anal Chem* 49:1433-1440.

10. Gardiner, J.S., S.A. Walker, and A.J. MacLean. 1982. A retrospective mortality study of substituted anthraquinone dyestuffs workers. *Br J Ind Med* 39:355-360.

11. Golding, B.T. and W.P. Watson. 1999. Possible mechanisms of carcinogenesis after exposure to benzene. *IARC Sci Publ* 150:75-88.

12. Harada, T., R.R. Maronpot, R.W. Morris, and G.A. Boorman. 1989. Observations on altered hepatocellular foci in National Toxicology Program two-year carcinogenicity studies in rats. *Toxicol Pathol* 17:690-706; discussion 707-708.

13. Harrington-Brock, K., L. Parker, C. Doerr, M.C. Cimino, and M.M. Moore. 1991. Analysis of the genotoxicity of anthraquinone dyes in the mouse lymphoma assay. *Mutagenesis* 6:35-46.

14. Haworth, S., T. Lawlor, K. Mortelmans, W. Speck, and E. Zeiger. 1983. Salmonella mutagenicity test results for 250 chemicals. *Environ Mutagen* 5:1-142.

15. Hayashi, S., H.H. Hong, K. Toyoda, T.V. Ton, T.R. Devereux, R.R. Maronpot, J. Huff, and R.C. Sills. 2001. High frequency of *ras* mutations in forestomach and lung tumors of B6C3F1 mice exposed to 1-amino-2,4-dibromoanthraquinone for 2 years. *Toxicol Pathol* 29:422-429.

16. HSDB. 2000. Hazardous Substance Data Bank. Revised February 2, 2000. Last reviewed January 31, 1996. National Library of Medicine. Available at http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB (and search CAS No. 81-49-2).

17. IARC. 1982. Some Aromatic Amines, Anthraquinones and Nitroso Compounds, and Inorganic Fluorides Used in Drinking Water and Dental Preparations, Vol. 27. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. World Health Organization, International Agency for Research on Cancer, Lyon, France.

18. IARC. 1987. Overall Evaluations of Carcinogenicity: An-Updating of IARC Monographs Volumes 1 to 42, Suppl. 7. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. World Health Organization, International Agency for Research on Cancer, Lyon, France.

19. IARC. 1990. Some Flame Retardants and Textile Chemicals, and Exposures in the Textile Manufacturing Industry, Vol. 48. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. World Health Organization, International Agency for Research on Cancer, Lyon, France.

20. IARC. 1999. Re-evaluation of some organic chemical, hydrazine and hydrogen peroxide (three parts), Vol. 71. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. World Health Organization, International Agency for Research on Cancer, Lyon, France.

21. Ikeda, M., T. Watanabe, I. Hara, T. Tabuchi, S.I. Nakamura, H. Kosaka, M. Minami, and Y. Sakurai. 1977. A field survey on the health status of workers in dye-producing factories. *Int Arch Occup Environ Health* 39:219-235.

22. Loveday, K.S., B.E. Anderson, M.A. Resnick, and E. Zeiger. 1990. Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro. V: Results with 46 chemicals. *Environ Mol Mutagen* 16:272-303.

23. Maronpot, R.R., T. Harada, A.S. Murthy, and G.A. Boorman. 1989a. Documenting foci of hepatocellular alteration in two-year carcinogenicity studies: current practices of the National Toxicology Program. *Toxicol Pathol* 17:675-683; discussion 683-674.

24. Maronpot, R.R., H.C. Pitot, and C. Peraino. 1989b. Use of rat liver altered focus models for testing chemicals that have completed two-year carcinogenicity studies. *Toxicol Pathol* 17:651-662.

25. Novotná, P., V. Pacáková, Z. Bosáková, and K. Štulík. 1999. High-performance liquid chromatographic determination of some anthraquinone and naphthoquinone dyes occurring in historical textiles. *J Chromatogr A* 863:235-241.

26. NTP. 1978a. Bioassay of 2-Methyl-1-nitroanthraquinone for Possible Carcinogenicity. National Toxicology Program Technical Report Series TR-29.

27. NTP. 1978b. Bioassay of 1-Amino-2-methylanthraquinone for Possible Carcinogenicity. National Toxicology Program Technical Report Series TR-111.

28. NTP. 1978c. Bioassay of 2-Aminoanthraquinone for Possible Carcinogenicity. National Toxicology Program Technical Report Series TR-144.

29. NTP. 1986. Toxicology and Carcinogenesis Studies of C.I. Disperse Blue 1 (A Commercial Dye Containing Approximately 50% 1,4,5,8-Tetraaminoanthraquinone, and 20% Water) (CAS No. 2475-45-8) in F344/N Rats and B6C3F1 Mice (Feed Studies). National Toxicology Program Technical Report Series No 299.

30. NTP. 1996. Toxicology and carcinogenesis studies of 1-Amino-2,4-Dibromoanthraquinone in F344/N rats and B6C3F$_1$ mice (feed studies). National Toxicology Program Technical Report Series No 383.

31. NTP Chemical Repository. 2001. 1-amino-2,4-dibromoanthraquinone. Revised August 13, 2001. National Toxicology Program. NTP Chemical Repository. Available at http://ntp-server.niehs.nih.gov/cgi/iH_Indexes/ALL_SRCH/iH_ALL_SRCH_Frames.html and search 81-49-2.

32. Parkinson, A. 1996. Biotransformation of Xenobiotics. In Casarett and Doull's Toxicology: The Basic Science of Poisons, Fifth Edition. Klaassen, C.D., M.O. Amdur and J. Doull, eds. McGraw-Hill, New York. pp. 113-186.

33. Preiss, A., U. Sänger, N. Karfich, K. Levsen, and C. Mugge. 2000. Characterization of dyes and other pollutants in the effluent of a textile company by LC/NMR and LC/MS. *Anal Chem* 72:992-998.

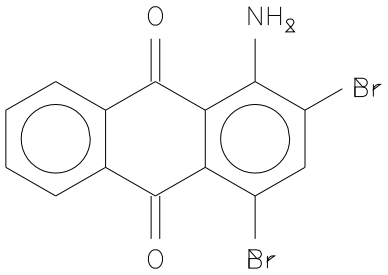
34. Sathiakumar, N. and E. Delzell. 2000. An updated mortality study of workers at a dye and resin manufacturing plant. *J Occup Environ Med* 42:762-771.

35. Sendelbach, L.E. 1989. A review of the toxicity and carcinogenicity of anthraquinone derivatives. *Toxicology* 57:227-240.

36. TRI. 2001. Toxic Release Inventory. Revised 2001. Environmental Protection Agency. Available at (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?TRI).

Appendix A: NTP TR 383 (1996). Toxicology and Carcinogenesis Studies of 1-Amino-2,4-Dibromoanthraquinone in F344/N Rats and B6C3F1 Mice (Feed Studies). PP A-1 – A-86.

ABSTRACT



1-AMINO-2,4-DIBROMOANTHRAQUINONE

CAS No. 81-49-2

Chemical Formula: $C_{14}H_7Br_2NO_2$ Molecular Weight: 381.04

Synonym: ADBAQ

1-Amino-2,4-dibromoanthraquinone is an anthraquinone-derived vat dye, a member of a class of insoluble dyes that are impregnated into textile fibers. Five anthraquinone-derived dyes with representative and diverse structures, as well as the parent chemical, anthraquinone, were selected for toxicology and carcinogenesis evaluation. Similar to the benzidine dye initiative, the rationale for selecting these vat dyes was to generate sufficient toxicologic data to permit more reliable predictions of carcinogenicity to be made on other chemicals in this class, thereby eliminating or reducing the need to study every anthraquinone dye. 1-Amino-2,4-dibromoanthraquinone is the last anthraquinone-derived dye in this group to be studied.

Groups of male and female F344/N rats and B6C3F$_1$ mice were exposed to 1-amino-2,4-dibromoanthraquinone (87% to 97% pure) for 13 weeks or for 9, 15, or 24 months. Because 1-amino-2,4-dibromoanthraquinone was predicted to be carcinogenic, these studies were designed to evaluate the potential for tumor progression and regression. Absorption and excretion studies were carried out in male F344/N rats. Genetic toxicity was determined *in vitro* using *Salmonella typhimurium* and cultured Chinese hamster ovary cells. Extensive chemical analyses were performed to identify and characterize impurities of the 1-amino-2,4-dibromoanthraquinone used in these studies.

13-WEEK STUDY IN RATS

Groups of 10 male and 10 female rats were given 0, 2,500, 5,000, 10,000, 25,000, or 50,000 ppm 1-amino-2,4-dibromoanthraquinone in feed for 13 weeks. These levels correspond to approximately 150 to 3,200 mg 1-amino-2,4-dibromoanthraquinone/kg body weight per day for males and to approximately 170 to 3,200 mg/kg for females. Chemical-related mortality was limited to one male and one female in the 50,000 ppm groups. Final mean body weights and body weight gains of all exposed groups of rats were significantly lower than those of the controls. Feed consumption by all exposed groups was less than that by the controls throughout the study and generally decreased with increasing exposure concentration. Pink-red staining of the fur and tail was observed in all exposed groups. Absolute and relative liver weights of all exposed groups were generally significantly greater than those of the controls.

Chemical-related lesions were present in the liver, kidney, and spleen of male and female rats. Nonneoplastic lesions in the liver included foci of hepatocellular alteration, diffuse hepatocellular hypertrophy (cytomegaly), hepatocellular cytoplasmic vacuolation, bile duct hyperplasia, inflammation, and pigmentation. These differences were observed primarily in the 25,000 and 50,000 ppm groups of males and females; the spectrum of proliferative lesions of the bile ducts (hyperplasia, fibrosis, and necrotizing cholangitis) in the 25,000 and 50,000 ppm groups was morphologically consistent with the lesion described as cholangiofibrosis. Pigmentation was present in the renal tubule epithelium of all groups of exposed rats; nuclear enlargement (karyomegaly) was also present in the renal tubule epithelium in some of the exposed rats. Accumulation of hyaline droplets in the cytoplasm of the renal tubule epithelium and tubule lumina was present in 2,500, 5,000, 10,000, and 25,000 ppm males. Incidences of hematopoiesis of the spleen in exposed groups of males and females were increased compared to those in the controls.

13-Week Study in Mice

Groups of 10 male and 10 female mice were given 0, 2,500, 5,000, 10,000, 25,000, or 50,000 ppm 1-amino-2,4-dibromoanthraquinone in feed for 13 weeks. These levels correspond to approximately 500 to 10,600 mg 1-amino-2,4-dibromoanthraquinone/kg body weight per day for males and approximately 660 to 11,700 mg/kg per day for females. There was no chemical-related mortality. Feed consumption and final mean body weights of exposed groups were similar to those of the controls. Red staining of the fur was observed in all exposed groups. Absolute and relative liver weights of the exposed groups were greater than those of the controls except for the absolute liver weight of 2,500 ppm males. Absolute and relative kidney weights of 25,000 and 50,000 ppm males were lower than those of the controls.

Chemical-related lesions were limited to the livers of males and consisted of pigmentation of hepatocytes at all exposure concentrations and centrilobular hepatocellular hypertrophy at 10,000, 25,000, and 50,000 ppm. Minimal pigment was present in the liver of one female in the 25,000 ppm group and in one female in the 50,000 ppm group.

2-Year Study in Rats

Groups of 70 male and 70 female rats were given 0, 5,000, or 10,000 ppm 1-amino-2,4-dibromoanthraquinone in feed for 103 weeks. In addition, groups of 50 male and 50 female rats were given 2,000 ppm 1-amino-2,4-dibromoanthraquinone in feed for 104 weeks. These exposure concentrations were approximately equal to 90, 240, or 490 mg 1-amino-2,4-dibromoanthraquinone/kg body weight for males and 110, 285, or 600 mg/kg for females. Ten animals from each group were evaluated for histopathology at 9 months. Additional groups of 10 animals from the 0 and 10,000 ppm groups were evaluated for histopathology at 15 months.

Survival, Body Weights, Feed Consumption, and Clinical Findings

In the 2-year study, survival of the 10,000 ppm males and females was significantly lower than that of the controls. Survival of the 2,000 and 5,000 ppm groups was similar to that of the controls. During the last year of the study, the mean body weights of exposed males were 80% to 91% those of controls, and the mean body weights of exposed females were 67% to 84% those of controls. Feed consumption among exposed groups was generally similar, but was less than that by controls. The fur and urine of all exposed male and female groups were discolored.

Pathology Findings

In the 2-year study, 1-amino-2,4-dibromoanthraquinone was associated with significant chemical-related increases in the incidences of benign and malignant neoplasms in the liver, large intestine, kidney, and urinary bladder of males and females. Chemical-related nonneoplastic proliferative and degenerative lesions occurred in the liver, kidney, urinary bladder, and forestomach of males and females.

The incidences of foci of hepatocellular alteration and pigmentation in the liver of males and females were increased at the 9-month interim evaluation, and a hepatocellular adenoma was present in one 5,000 ppm male. At the 15-month interim evaluation, hepatocellular adenoma or carcinoma (combined) occurred in all males and nine females in the 10,000 ppm groups. By the end of the 2-year study, hepatocellular adenoma, carcinoma, cholangioma, or cholangiocarcinoma were observed in males and

females in the 5,000 and 10,000 ppm groups. In the 2,000 ppm groups, similar liver neoplasms were present in 63% of the males and in 83% of the females. Of the hepatocellular carcinomas in the 5,000 and 10,000 ppm groups of males and females, 31% to 49% were metastatic to the lungs or other sites. Increases in the incidences of foci of hepatocellular alteration (basophilic, eosinophilic, and clear cell) and pigmentation of the liver were also observed in exposed groups of males and females.

Adenomatous polyps (adenoma) of the large intestine were present in six 10,000 ppm males at the 15-month interim evaluation. Incidences of adenomatous polyp (adenoma) and carcinoma of the large intestine were significantly increased in exposed groups of males and females after 2 years; multiple benign and malignant intestinal neoplasms were observed in many of these rats.

In the kidney, incidences of renal tubule adenoma and carcinoma were significantly increased in exposed groups of males and females after 2 years. Renal tubule adenomas were present in two 10,000 ppm males at 15 months. There were also chemical-related increases in the incidences and severities of renal tubule epithelial hyperplasia, pigmentation, and transitional cell hyperplasia in the kidney of males and females. Hyaline droplet accumulation was present in all exposed male rats at 9 months.

Incidences of transitional cell papilloma and carcinoma of the urinary bladder were increased at 2 years in males and females in the 10,000 ppm groups. Transitional cell hyperplasia was observed in exposed males and females at the 15-month interim evaluation. Other nonneoplastic lesions observed in the urinary bladder at 2 years included metaplasia of the transitional epithelium and submucosal stromal tissue.

In the forestomach, the incidences and severities of inflammation, ulceration, hyperkeratosis, and hyperplasia of the squamous mucosa were increased in all exposed groups of males and females at 2 years, but not at the 9- or 15-month interim evaluations.

In exposed males and females, the incidences of mononuclear cell leukemia were significantly decreased. The incidences of atrophy of the seminal vesicle were increased in exposed male rats in the 2-year study.

Stop-Exposure Evaluation in Rats

Groups of 40 male and 40 female rats were given 20,000 ppm 1-amino-2,4-dibromoanthraquinone in feed for 9 or 15 months. At 9 months, 10 males and 10 females were evaluated for histopathology (9-month interim evaluation groups). After 9 months of exposure, an additional 10 males and 10 females were fed control diet until the end of the 15-month evaluation (9-month stop-exposure groups), and 20 males and 20 females continued to receive 20,000 ppm 1-amino-2,4-dibromoanthraquinone until the end of the evaluation (15-month exposure groups). The approximate daily consumption of 1-amino-2,4-dibromoanthraquinone was 1,335 mg/kg for males and 1,790 mg/kg for females in the 9-month stop-exposure groups and 1,115 mg/kg for males and 1,435 mg/kg for females in the 15-month exposure groups.

Survival was similar among groups except for the females in the 15-month exposure group; the survival of this group was lower than that of the controls. Lower mean body weights were related to increased exposure duration. The mean body weights of exposed males were 76% to 82% that of controls, and the mean body weights of exposed females were 73% to 84% that of controls.

For the stop-exposure evaluation, similar chemical-related neoplasms and nonneoplastic lesions were observed in the same sites as in the 2-year study: liver, large intestine, kidney, urinary bladder, and forestomach.

After 9 months of dietary exposure to a concentration of 20,000 ppm 1-amino-2,4-dibromoanthraquinone, hepatocellular adenoma and carcinoma occurred in males and females. Nonneoplastic chemical-related lesions in the liver of exposed rats included pigmentation, focal hepatocellular alteration, and bile duct hyperplasia. Neoplasms at other sites in males included one adenomatous polyp (adenoma) in the large intestine and one transitional cell papilloma in the urinary bladder. Hyaline droplet accumulation was present in the kidney of exposed males at 9 months.

In the stop-exposure groups examined at 15 months, hepatocellular adenoma and carcinoma were present in most males and females. Adenomatous polyp (adenoma) of the colon, renal tubule cell adenoma, and urinary bladder transitional cell papilloma and

carcinoma also occurred in males and females. Nonneoplastic chemical-related lesions included foci of hepatocellular alteration in the liver and hyperplasia of the renal tubule epithelium and urinary bladder transitional epithelium. Hyperplasia, hyperkeratosis, inflammation, and ulceration were observed in the forestomachs of some male and female rats continuously exposed for 15 months.

2-YEAR STUDY IN MICE

Groups of 60 male and 60 female mice were given 0, 10,000, or 20,000 ppm 1-amino-2,4-dibromoanthraquinone in feed for 104 weeks. The daily compound consumption was approximately 1,690 or 3,470 mg 1-amino-2,4-dibromoanthraquinone/kg body weight for males and 1,950 or 4,350 mg/kg for females. Ten animals from each group were evaluated for histopathology at 15 months.

Survival, Body Weights, Feed Consumption, and Clinical Findings

In the 2-year study, survival of exposed males was significantly lower than that of the controls. Survival of exposed females was similar to that of the controls. The final mean body weights of exposed males were 83% to 85% that of controls, and the final mean body weights of exposed females were 81% to 86% that of controls. Feed consumption by exposed groups was generally similar to that by controls. Discoloration of the fur, urine, and feces was observed in all exposed groups.

Pathology Findings

In the 2-year study, 1-amino-2,4-dibromoanthraquinone was associated with significant chemical-related increases in the incidences of benign and malignant neoplasms in the liver, forestomach, and lung of males and females.

Incidences of hepatocellular adenoma and carcinoma were increased in exposed groups at the 15-month interim evaluation and at 2 years. At 2 years, there were significant increases in the incidences of multiple hepatocellular adenoma and carcinoma in males and females and in the incidences of hepatoblastoma in males. Centrilobular hypertrophy of hepatocytes in males and foci of hepatocellular alteration and pigmentation in the liver of males and females were also chemical-related changes.

Squamous cell papilloma of the forestomach mucosa occurred in 10,000 ppm females and 20,000 ppm males and females at the 15-month interim evaluation, and the incidences of squamous cell papilloma and carcinoma were significantly increased in exposed groups of males and females at 2 years. Chemical-related hyperplasia of forestomach epithelium was also present at 15 months and at 2 years.

Alveolar/bronchiolar adenomas were present only in the exposed groups of males and females at 15 months, and the incidences of alveolar/bronchiolar adenoma were significantly increased in exposed males and females at 2 years. The incidences of multiple alveolar/bronchiolar adenoma were also increased in exposed males.

In the kidney, pigmentation was present in the renal tubules of most mice after 2 years of exposure.

DISPOSITION AND METABOLISM STUDIES

Adult male F344/N rats were given [^{14}C]-labeled 1-amino-2,4-dibromoanthraquinone as a single intravenous dose of 0.4 mg/kg body weight or as a single oral dose of 2, 23, 118, 814, or 1,473 mg/kg. A 6-hour bile cannulation study was also performed. From day 0 through day 3 after intravenous administration, about 50% of the ^{14}C was excreted in the feces, 15% in the urine, and 6% in expired air. Unmetabolized 1-amino-2,4-dibromoanthraquinone accounted for less than 3% of the excreted ^{14}C after intravenous administration. For oral doses administered, the amount of the dose that was absorbed fit the equation: $absorbed\ dose = 6.6 \log(dose)$. After intravenous administration, the metabolites of 1-amino-2,4-dibromoanthraquinone in blood were primarily in the plasma fraction (blood:plasma ratio of approximately 0.5:1). The highest concentrations of ^{14}C in tissues 15 minutes after intravenous dosing were in excretory organs, lung, kidney, small intestine, liver, adipose tissue, and adrenal gland.

GENETIC TOXICOLOGY

1-Amino-2,4-dibromoanthraquinone was mutagenic in *Salmonella typhimurium* strains TA98 and TA1537 in the absence of S9; with S9, an equivocal response was observed in TA1537. 1-Amino-2,4-dibromoanthraquinone resulted in an equivocal response in

strain TA100 with and without S9, and no mutagenic activity was detected with strain TA1535. In cultured Chinese hamster ovary cells, 1-amino-2,4-dibromoanthraquinone induced sister chromatid exchanges with and without S9; chromosomal aberrations were induced only in the absence of S9.

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *clear evidence of carcinogenic activity** of 1-amino-2,4-dibromoanthraquinone in male and female F344/N rats based on increased incidences of neoplasms in the liver, large intestine, kidney, and urinary bladder. There was *clear evidence of carcinogenic activity* of 1-amino-2,4-dibromoanthraquinone in male and female $B6C3F_1$ mice based on increased incidences of neoplasms in the liver, forestomach, and lung.

Exposure of male and female rats to 1-amino-2,4-dibromoanthraquinone for 2 years was associated with basophilic focus (males only), clear cell focus, eosinophilic focus, and pigmentation in the liver; renal tubule hyperplasia, renal tubule pigmentation, and transitional cell hyperplasia in the kidney; transitional cell hyperplasia, squamous metaplasia, and stromal metaplasia (females only) in the urinary bladder; squamous hyperplasia, hyperkeratosis, ulceration, and inflammation of the forestomach mucosa; and seminal vesicle atrophy. Exposure of male and female mice to 1-amino-2,4-dibromoanthraquinone for 2 years was associated with centrilobular hepatocellular hypertrophy (males only), basophilic focus, clear cell focus (females only), eosinophilic focus, and pigmentation in the liver; pigmentation in the kidney; and hyperplasia, basal cell hyperplasia, hyperkeratosis, and inflammation of the forestomach mucosa.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 13. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 15.

Font Size:11pt**Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of 1-Amino-2,4-dibromoanthraquinone**

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses	0, 2,000, 5,000, or 10,000 ppm [approximately 90, 240, or 490 mg/kg/day]	0, 2,000, 5,000, or 10,000 ppm [approximately 110, 285, or 600 mg/kg/day]	0, 10,000, or 20,000 ppm [approximately 1,690 or 3,470 mg/kg/day]	0, 10,000, or 20,000 ppm [approximately 1,950 or 4,350 mg/kg/day]
Body weights	Exposed groups lower than controls	Exposed groups lower than controls	Exposed groups lower than controls	Exposed groups lower than controls
2-Year survival rates	26/50, 24/40, 21/60, 10/50	38/50, 32/40, 38/60, 12/49	40/50, 22/51, 23/50	39/50, 34/50, 33/50
Nonneoplastic effects	Liver: basophilic focus (9/50, 12/40, 24/59, 22/50); clear cell focus (3/50, 26/40, 39/59, 27/50); eosinophilic focus (1/50, 13/40, 14/59, 6/50); pigmentation (3/50, 19/40, 48/59, 39/50) Kidney: renal tubule hyperplasia (9/50, 30/40, 25/59, 19/50); renal tubule pigmentation (5/50, 40/40, 58/59, 49/50); transitional cell hyperplasia (30/50, 40/40, 51/59, 35/50) Urinary bladder: transitional cell hyperplasia (1/50, 5/38, 17/58, 30/50); squamous metaplasia (0/50, 0/38, 0/58, 3/50)	Liver: clear cell focus (3/50, 28/40, 39/60, 17/48); eosinophilic focus (7/50, 23/40, 12/60, 1/48); pigmentation (1/50, 19/40, 51/60, 45/48) Kidney: renal tubule hyperplasia (1/50, 12/40, 23/60, 27/48); renal tubule pigmentation (0/50, 40/40, 60/60, 48/48); transitional cell hyperplasia (10/50, 16/40, 44/60, 21/48) Urinary bladder: transitional cell hyperplasia (1/50, 2/40, 41/60, 41/46); squamous metaplasia (0/50, 1/40, 4/60, 8/46); stromal metaplasia (0/50, 0/40, 4/60, 2/46)	Liver: centrilobular hepatocyte hypertrophy (0/50, 17/51, 13/50); basophilic focus (0/50, 4/51, 3/50); eosinophilic focus (0/50, 6/51, 1/50); pigmentation (1/50, 50/51, 47/50) Kidney: renal tubule pigmentation (0/50, 42/51, 43/50) Forestomach: hyperplasia (1/50, 9/50, 4/50); basal cell hyperplasia (0/50, 0/50, 2/50); hyperkeratosis (1/50, 7/50, 6/50); inflammation (2/50, 6/50, 13/50)	Liver: basophilic focus (0/50, 4/50, 5/50); clear cell focus (0/50, 10/50, 9/50); eosinophilic focus (0/50, 4/50, 2/50); pigmentation (0/50, 44/50, 49/50) Kidney: renal tubule pigmentation (0/50, 43/50, 43/50) Forestomach: hyperplasia (9/48, 15/50, 19/50); basal cell hyperplasia (0/48, 7/50, 3/50); hyperkeratosis (10/48, 14/50, 17/50); inflammation (7/48, 10/50, 21/50)

(continued)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of 1-Amino-2,4-dibromoanthraquinone (continued)

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Nonneoplastic effects (continued)	Forestomach: squamous hyperplasia (3/49, 19/39, 25/59, 26/49); hyperkeratosis (5/49, 18/39, 21/59, 20/49); ulcer (3/49, 10/39, 15/59, 16/49); inflammation (3/49, 12/39, 11/59, 11/49); Seminal vesicle: atrophy (1/49, 30/40, 35/59, 23/50)	Forestomach: squamous hyperplasia (2/49, 7/40, 26/60, 33/47); hyperkeratosis (2/49, 7/40, 23/60, 28/47); ulcer (1/49, 2/40, 7/60, 17/47); inflammation (0/49, 1/40, 13/60, 10/47)		
Neoplastic effects	Liver: hepatocellular adenoma (1/50, 20/40, 40/59, 34/50); hepatocellular carcinoma (1/50, 12/40, 55/59, 46/50); hepatocholangio-carcinoma (0/50, 0/40, 6/59, 2/50) Large intestine (all sites): adenomatous polyp (adenoma) (0/50, 13/40, 51/59, 40/50); carcinoma (0/50, 1/40, 11/59, 17/50) Kidney (renal tubule): adenoma (2/50, 10/40, 11/59, 14/50); carcinoma (0/50, 0/40, 2/59, 1/50) Urinary bladder: transitional cell papilloma (0/50, 1/38, 2/58, 8/50); transitional cell carcinoma (0/50, 0/38, 1/58, 4/50)	Liver: hepatocellular adenoma (0/50, 28/40, 47/60, 29/48); hepatocellular carcinoma (0/50, 12/40, 57/60, 45/48); hepatocholangio-carcinoma (0/50, 0/40, 11/60, 13/48) Large intestine (all sites): adenomatous polyp (adenoma) (0/50, 28/40, 53/60, 43/49); carcinoma (0/50, 2/40, 21/60, 8/49) Kidney (renal tubule): adenoma (0/50, 3/40, 16/60, 16/48); carcinoma (0/50, 0/40, 0/60, 2/48) Urinary bladder: transitional cell papilloma (0/50, 2/40, 7/60, 9/46); transitional cell carcinoma (0/50, 0/40, 8/60, 16/46)	Liver: hepatocellular adenoma (10/50, 38/51, 39/50); hepatocellular carcinoma (9/50, 18/51, 21/50); hepatoblastoma (0/50, 3/51, 5/50) Forestomach: squamous cell papilloma (0/50, 13/51, 16/50); squamous cell carcinoma (0/50, 12/51, 13/50) Lung: alveolar/ bronchiolar adenoma (7/50, 26/51, 24/50)	Liver: hepatocellular adenoma (6/50, 45/50, 49/50); hepatocellular carcinoma (0/50, 23/50, 27/50) Forestomach: squamous cell papilloma (2/50, 16/50, 27/50); squamous cell carcinoma (0/50, 12/50, 11/50) Lung: alveolar/ bronchiolar adenoma (4/50, 17/50, 13/49)

(continued)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of 1-Amino-2,4-dibromoanthraquinone (continued)

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Level of evidence of carcinogenic activity	Clear evidence	Clear evidence	Clear evidence	Clear evidence
Genetic toxicology				
Salmonella typhimurium gene mutation:	Equivocal in strain TA100 with and without S9; negative in strain TA1535 with and without S9; positive in strain TA1537 without S9, equivocal with S9; positive in strain TA98 without S9, negative with S9			
Sister chromatid exchanges				
Cultured Chinese hamster ovary cells *in vitro*:	Positive with and without S9 (combined results from testing in two laboratories)			
Chromosomal aberrations				
Cultured Chinese hamster ovary cells *in vitro*:	Weakly positive without S9, negative with S9			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on 1-amino-2,4-dibromoanthraquinone on June 21, 1994, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On June 21, 1994, the draft Technical Report on the toxicology and carcinogenesis studies of 1-amino--2,4-dibromoanthraquinone received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.E. Huff, NIEHS, introduced the toxicology and carcinogenesis studies of 1-amino-2,4-dibromoanthraquinone by discussing the uses and rationale for study, including it being a part of a class study of anthraquinone derivatives. He described the experimental design, reported on survival and body weight effects, and commented on chemical-related neoplasms and nonneoplastic lesions in male and female rats and mice. The proposed conclusions for the studies were *clear evidence of carcinogenic activity* in male and female F344/N rats and in male and female B6C3F$_1$ mice.

Dr. Huff reviewed the carcinogenic responses in other anthraquinone derivatives that had been studied, noting that the liver seemed to be a major site and that 1-amino-2,4-dibromoanthraquinone was the most active as far as the number of sites. Interpretive conclusions that could be drawn from the cumulative National Toxicology Program studies on this class of insoluble dyes were that anthraquinones are typically mutagenic and clastogenic, they are carcinogenic to male and female rats and mice, and they are predicted to represent likely carcinogenic hazards to humans exposed to these agents, especially occupationally. Dr. J.R. Bucher, NIEHS, reported that the toxicology and carcinogenesis studies on anthraquinone were in progress.

Dr. van Zwieten, a principal reviewer, agreed with the proposed conclusions. He thought there should be more discussion of the findings from the stop-exposure groups of rats. (Stop-exposure groups were evaluated at 9 and 15 months as part of an attempt to gain insight into the progression or regression of chemical-induced lesions.) Dr. van Zwieten noted the high impurity levels in the first lot of the chemical used for the 13-week studies and for the first 2 months of the 2-year studies and said that a statement indicating that this did not affect the integrity of the studies might be helpful. Dr. Huff responded that the impurities had been characterized (page 20; Arneson *et al.*, 1996).

Dr. Ward, the second principal reviewer, agreed with the proposed conclusions. He commented that no hyaline droplets were reported in the kidney of rats after 9 months, and since 1-amino-2,4-dibromoanthraquinone might cause accumulation of $\alpha_{2\mu}$-globulin, the report should indicate that droplets were searched for but not found or found but not reported (page 83). Dr. Ward objected to characterizing cholangiofibrosis found in the liver of rats in a 13-week study as "premalignant." He stated that this lesion is usually induced by liver carcinogens but does not typically progress to bile duct neoplasms. Dr. M.R. Elwell, NIEHS, said this interpretation was from the literature and the wording on neoplastic potential would be revised to also reflect Dr. Ward's experience.

Dr. Reddy, the third principal reviewer, also agreed with the proposed conclusions. He said it would have been useful to characterize the chemical nature of the pigment that accumulated in the liver, kidney, and other organs, as well as in the fur and tail. Dr. Huff responded that, logically, the pigment was either the chemical or one of its metabolites, but the feasibility of going back and defining it better would have to be determined.

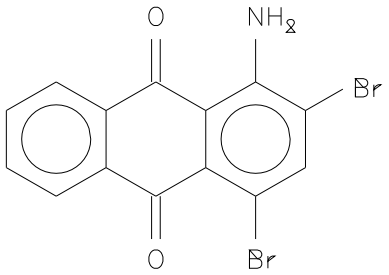
Dr. Russo had observed evidence of chronic inflammation in one of the plates and wondered whether the liver lesions were associated with hepatitis. Dr. Karol asked if there was inflammation of the eosinophilic foci, which would suggest a hypersensitivity-type reaction. Dr. Elwell said there was some inflammation with the cholangiofibrosis, but this was really limited to the focal lesions where there was fibrosis and to cystic bile ducts, and there was not an eosinophilic inflammation; the term "eosinophilic foci" referred to a focal cellular alteration of hepatocytes.

Dr. Bailey cited a statement from the use, production, and human exposure sections that "no individualized information was located regarding amounts produced

or specific uses of 1-amino-2,4-dibromoanthraquinone," leading him to wonder if this chemical is currently used. Dr. Huff said this was a valid question for 1-amino-2,4-dibromoanthraquinone and the other anthraquinone derivatives. He said proprietary information was difficult to obtain, although he was hopeful that a request to the American Pharmaceutical Association concerning anthraquinone dyes in over-the-counter or prescription items might yield some data on human exposure. There was some discussion that primary exposure to these dyes would be from topical application or exposure.

Dr. van Zwieten moved that the Technical Report on 1-amino-2,4-dibromoanthraquinone be accepted with the revisions discussed and the conclusions as written for male and female rats and mice, *clear evidence of carcinogenic activity.* Dr. Reddy seconded the motion, which was accepted unanimously with eleven votes.

INTRODUCTION



1-AMINO-2,4-DIBROMOANTHRAQUINONE

CAS No. 81-49-2

Chemical Formula: $C_{14}H_7Br_2NO_2$ Molecular Weight: 381.04

Synonym: ADBAQ

CHEMICAL AND PHYSICAL PROPERTIES

1-Amino-2,4-dibromoanthraquinone, a reddish brown to orange powder, is an anthraquinone-derived vat dye. Anthraquinone (9,10-anthraquinone: CAS No. 84-65-1), which does not occur naturally, was first synthesized by Laurent in 1835 as an oxidation product of anthracene and nitric acid (Chung, 1978). "Anthra" comes from the Greek word for coal, from which anthracene was originally obtained.

USE, PRODUCTION, AND HUMAN EXPOSURE

Anthraquinone is an important and widely used starting material for the manufacture of vat dyes (*Merck Index*, 1989). Class homologues of anthraquinone comprise a greater number of dyes having outstanding "fastness" properties than any other group of commercial dyes (Chung, 1978; Chung and Farris, 1979). No information was located regarding amounts produced or specific uses of 1-amino-2,4-dibromoanthraquinone. The 2-alkyl derivatives of anthraquinone with alkyl chains ranging from one to five carbons are most often used in the dye industry (Chung, 1978).

Vat dyes are a class of water-insoluble dyes that can be easily reduced (i.e., vatted) to a water-soluble and usually colorless leuco form in which they can readily impregnate fibers and textiles. Subsequent oxidation then produces the insoluble colored form that is remarkably "fast" to washing, light, and chemicals. The reducing agents are usually alkaline solutions of sodium hydrosulfite; oxidation takes place in the presence of air, perborate, dichromate, and other agents (Hawley, 1981). Vat dyes are used typically for cotton, wool, and cellulose acetate. Production of vat dyes in the United States totaled 14,000,000 kg (30.8 million pounds) in 1991 (USITC, 1993); these figures do not account for the "large" amounts extracted from botanical species containing naturally occurring anthraquinones used therapeutically and for other purposes.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

No information on the absorption, distribution, metabolism, and excretion of 1-amino-2,4-dibromoanthraquinone in experimental animals or in humans was found in a search of the available literature.

TOXICITY

No information on the toxicity of 1-amino-2,4-dibromoanthraquinone in experimental animals or in humans was found in a search of the available literature.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

No information on the reproductive and developmental toxicity of 1-amino-2,4-dibromoanthraquinone in experimental animals or in humans was found in a search of the available literature.

CARCINOGENICITY

Experimental Animals

Chemicals belonging to the anthraquinone class of dyes are carcinogenic to rodents (IARC, 1987; Sendelbach, 1989) and consistently induce neoplasms of the liver (Huff *et al.*, 1991). However, each anthraquinone derivative appears to induce cancer in other organs or tissue sites as well (Huff *et al.*, 1991).

For the five anthraquinones evaluated and reported by NCI/NTP, the 2-year exposure concentrations in the feed varied from a low of 300 ppm (0.03%) for 2-methyl-1-nitroanthraquinone to a high of 20,000 ppm (2%) for 1-amino-2,4-dibromoanthraquinone (Table 1).

Humans

No information on the potential carcinogenicity of 1-amino-2,4-dibromoanthraquinone in humans was found in a search of the available literature.

GENETIC TOXICITY

All five anthraquinones evaluated and reported by NCI/NTP induced mutations in *Salmonella typhimurium* (Brown and Brown, 1976; Haworth *et al.*, 1983; Dunkel *et al.*, 1985; Zeiger *et al.*, 1988). Each also caused sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells (Anderson *et al.*, 1990; Loveday *et al.*, 1990; NTP, unpublished). S9 activation was not required for 1-amino-2,4-dibromoanthraquinone to produce these effects. The parent compound, anthraquinone, is also mutagenic in *S. typhimurium*; significant increases in mutant colonies were observed with strains TA98 and TA100 with and without S9 (Zeiger *et al.*, 1988). In addition, anthraquinone and 1-aminoanthraquinone (250 mg/kg) were reported to induce DNA strand breaks in liver and kidney tissue of male Swiss (CD-1®) mice following intraperitoneal injection (Cesarone *et al.*, 1982).

STUDY RATIONALE

The NCI selected and evaluated several of the anthraquinone-derived dyes for a class study to determine whether these dyes have any inherent potential for carcinogenicity in laboratory rodents and, if so, in humans as well. The first three studies were conducted with 2-aminoanthraquinone (NCI, 1978a), 1-amino-2-methylanthraquinone (NCI, 1978b), and 2-methyl-1-nitroanthraquinone (NCI, 1978c). A fourth substance, 1,4,5,8-tetraaminoanthraquinone (C.I. Disperse Blue 1) was selected and evaluated for carcinogenicity by the NTP (NTP, 1986a). This Technical Report addresses the fifth chemical in this class, 1-amino-2,4-dibromoanthraquinone. In addition, the parent chemical, anthraquinone, has been selected for study to complete the overall effort on these dyes.

Anthraquinone and the five substituted anthraquinones (Figure 1), representative of a large group of amino-, alkyl-, and nitro-, or halogen-containing anthraquinones, were chosen for toxicologic characterization and to establish some predictive structure-activity relationships that could be used on other dyes in this category rather than testing each and every one. Other chemical classes that have been likewise evaluated by the NCI/NTP to reduce the need for "one-by-one" testing include benzidine-based dyes (Morgan *et al.,* 1994), phthalates (Kluwe *et al.*, 1982; Huff and Kluwe, 1984; Kluwe *et al.*, 1985), benzene and metabolites (Huff, 1992), dioxins (Huff, 1992), anilines (Weisburger *et al.*, 1984; Lamb *et al.*, 1986), naturally occurring "gums" (Melnick *et al.*, 1983), chlorinated paraffins (Bucher *et al.*, 1987), 1,3-butadiene and derivatives (Melnick and Huff, 1992), pesticides (Yang *et al.*, 1989; Huff and Haseman, 1991), and penicillins and tetracyclines (Dunnick *et al.*, 1989; Dietz *et al.*, 1991).

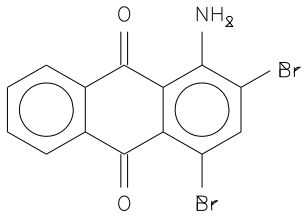
The bases for selection of anthraquinones (and other chemical classes as well) centered mainly on four criteria: 1) lack of available or adequate data on carcinogenicity, 2) magnitude of production and use

TABLE 1
Exposure Concentrations in the NCI/NTP 2-Year Feed Studies of Anthraquinone Derivatives[a]

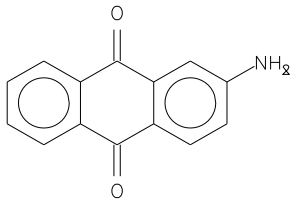
Anthraquinone Derivative	Low Dose (ppm)	High Dose (ppm)	Carcinogenic Response
Rats			
Male			
2-Aminoanthraquinone[b]	3,500	6,900	liver
1-Amino-2,4-dibromoanthraquinone	2,000	10,000	liver, large intestine, kidney, urinary bladder
1-Amino-2-methylanthraquinone[b]	1,000	2,000	liver, kidney
2-Methyl-1-nitroanthraquinone	600	1,200	liver, skin
1,4,5,8-Tetraaminoanthraquinone	1,250	5,000	urinary bladder, pancreas
Female			
2-Aminoanthraquinone[c]	2,000		
1-Amino-2,4-dibromoanthraquinone	2,000	10,000	liver, large intestine, kidney, urinary bladder
1-Amino-2-methylanthraquinone	1,000	2,000	liver
2-Methyl-1-nitroanthraquinone	600	1,200	skin
1,4,5,8-Tetraaminoanthraquinone	1,250	5,000	urinary bladder
Mice			
Male			
2-Aminoanthraquinone	5,000	10,000	liver
1-Amino-2,4-dibromoanthraquinone	10,000	20,000	liver, forestomach, lung
1-Amino-2-methylanthraquinone	600	–[d]	
2-Methyl-1-nitroanthraquinone	300	600	hemangiosarcoma
1,4,5,8-Tetraaminoanthraquinone	600	2,500	liver, lung[e]
Female			
2-Aminoanthraquinone	5,000	10,000	liver, lymphoma
1-Amino-2,4-dibromoanthraquinone	10,000	20,000	liver, forestomach, lung
1-Amino-2-methylanthraquinone	600	–[d]	liver
2-Methyl-1-nitroanthraquinone	300	600	hemangiosarcoma
1,4,5,8-Tetraaminoanthraquinone	600	2,500	

[a] Data from NCI, 1978a, 1978b, 1978c; NTP, 1986a
[b] Exposure concentrations in this study were time-weighted averages.
[c] Inadequate study
[d] Two dosage regimens were used, but the time-weighted average concentrations were the same.
[e] "Equivocal evidence" for both organs

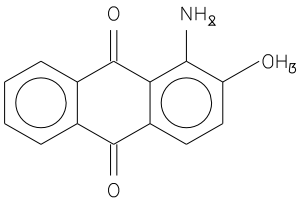
patterns, 3) awareness of potential and actual human exposure, and 4) representation of as broad a spec-trum of structural diversity within this class as possible. 1-Amino-2,4-dibromoanthraquinone was selected from a group of 36 environmentally significant aryl bromides. Because every other anthraquinone derivative tested so far for carcinogenic activity had been shown to be carcinogenic in rodents, 1-amino-2,4--dibromoanthraquinone was also expected to be carcinogenic in laboratory animals. Thus, the experimental design, while being consistent with a "core protocol" (Huff *et al.*, 1988), contains several modifications such as "stop-exposure" groups to better characterize this chemical. Additionally, chemical disposition studies were accomplished prior to the 2-year exposures to permit optimal selection of exposure concentrations for this water-insoluble dye. Because these chemicals may and often do contain considerable quantities of the parent chemical and other anthraquinone derivatives, an extensive chemical analysis was undertaken on these five chemicals to quantitate their purity and to identify the major impurities of 1-amino-2,4-dibromoanthraquinone (Arneson *et al.*, 1996).



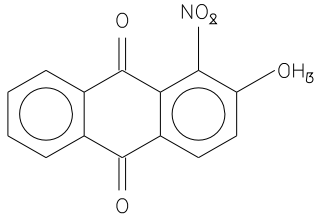
1-Amino-2,4-dibromoanthraquinone



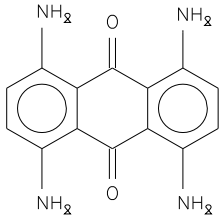
2-Aminoanthraquinone



1-Amino-2-methylanthraquinone



2-Methyl-1-nitroanthraquinone



1,4,5,8-Tetraaminoanthraquinone

FIGURE 1
Five Anthraquinone Derivatives Evaluated by the NCI/NTP

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF 1-AMINO-2,4-DIBROMOANTHRAQUINONE

1-Amino-2,4-dibromoanthraquinone was obtained from American Color and Chemical Corporation (Charlotte, NC; lot 1076-C) and Mobay Corporation (Pittsburgh, PA). The second lot was procured from Mobay Corporation since American Color and Chemical Corporation had stopped production. Lot 1076-C was used in the 13-week studies and for 2 months of the 2-year studies. The lot from Mobay Corporation was assigned lot number M061583 and was used throughout the remainder of the 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO) (Appendix I). Reports on analyses performed in support of the 1-amino-2,4-dibromoanthraquinone studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

The two lots of the chemical, a reddish brown to orange powder, were identified as 1-amino-2,4-dibromoanthraquinone by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. The purity of each lot was determined by elemental analyses, Karl Fischer water analysis, thin-layer chromatography, and high-performance liquid chromatography.

For lot 1076-C, elemental analyses for carbon, hydrogen, nitrogen, and bromine were in general agreement with theoretical values for 1-amino-2,4-dibromoanthraquinone. Karl Fischer water analysis indicated approximately 0.21% water. Thin-layer and high-performance liquid chromatography indicated a major peak and eight impurities. Five of the impurities had peak areas of less than 0.3%. The three major impurities were identified as anthraquinone, 1-amino-2-bromoanthraquinone, and 2-amino-1,3-dibromoanthraquinone. By high-performance liquid chromatography, anthraquinone was found to be present at a concentration of approximately 5.0%. 1-Amino-2-bromoanthraquinone and 2-amino-1,3-dibromoanthraquinone were found to be present at concentrations of approximately 4.3% and 2.2%, respectively. The overall purity of lot 1076-C was determined to be approximately 87%.

For lot M061583, elemental analyses for carbon, hydrogen, nitrogen, and bromine were in general agreement with theoretical values for 1-amino-2,4-dibromoanthraquinone. Karl Fischer water analysis indicated approximately 0.32% water. Thin-layer and high-performance liquid chromatography indicated a major peak and six impurities with the same retention times as found for lot 1076-C. A total impurity area of 3% of the total chromatographic peak area was found. The overall purity of lot M061583 was determined to be approximately 97%.

Stability studies performed using high-performance liquid chromatography indicated that 1-amino-2,4-dibromoanthraquinone, when stored protected from light, was stable as a bulk chemical for at least 2 weeks at temperatures up to 60° C. To ensure stability, the bulk chemical was stored in the dark at 4° ± 3° C throughout the studies. During the 2-year studies, the stability of the bulk chemical was monitored periodically by the study laboratory using high-performance liquid chromatography; no degradation of 1-amino-2,4-dibromoanthraquinone was observed throughout the studies.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared weekly by mixing 1-amino-2,4-dibromoanthraquinone with feed (Table I1). Homogeneity and at least 2-week stability at 25° C were confirmed by the analytical chemistry laboratory using spectrophotometry and high-performance liquid chromatography, respectively. During the 13-week and 2-year feed studies, the dose formulations were stored in the dark for no longer than 2 weeks.

The study laboratory conducted periodic analyses of the 1-amino-2,4-dibromoanthraquinone dose formulations using a spectrophotometric method. For the 13-week feed studies, dose formulations were

analyzed at the beginning, midpoint, and end of the studies (Table I2). During the 2-year feed studies, the dose formulations were analyzed every 6 to 10 weeks (Table I3). All dose formulations for rats and mice were within 10% of the target concentrations during the 13-week and 2-year studies. Results of periodic referee analyses performed by the analytical chemistry laboratory indicated good agreement with the results obtained by the study laboratory (Table I4).

13-WEEK STUDIES

The 13-week studies were conducted to evaluate the cumulative toxic effects of repeated exposure to 1-amino-2,4-dibromoanthraquinone and to determine the appropriate exposure concentrations to be used in the 2-year studies.

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories (Portage, MI). Upon receipt, the animals were 5 weeks old. The rats and mice were quarantined for 15 days before the studies began.

Groups of 10 male and 10 female rats and 10 male and 10 female mice received 0, 2,500, 5,000, 10,000, 25,000, or 50,000 ppm 1-amino-2,4-dibromoanthraquinone in feed for 13 weeks. Males and females were housed five per cage; water and feed were available *ad libitum*, and feed consumption was measured weekly. Clinical findings were recorded twice daily. Animals were weighed at study initiation, weekly, and at the end of the studies. Further details of study design and animal maintenance are summarized in Table 2.

A necropsy was performed on all animals. The brain, heart, right kidney, liver, lungs, right testis, and thymus of all animals were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. A complete histopathologic examination was performed on all animals that died prior to the end of the studies, control animals, and animals administered 50,000 ppm. Table 2 lists the tissues and organs examined.

2-YEAR STUDIES

Study Design

Groups of 70 male and 70 female rats received 0, 5,000, or 10,000 ppm 1-amino-2,4-dibromoanthraquinone in feed, and a group of 50 male and 50 female rats received 2,000 ppm 1-amino-2,4-dibromoanthraquinone in feed for 104 weeks. Ten male and 10 female rats from the 0, 2,000, 5,000, and 10,000 ppm groups were designated for an interim evaluation after 9 months. Ten male and 10 female rats from the 0 and 10,000 ppm groups were designated for an interim evaluation after 15 months. Groups of 60 male and 60 female mice received 0, 10,000, or 20,000 ppm 1-amino-2,4-dibromoanthraquinone in feed for 104 weeks. Ten male and 10 female mice per group were evaluated after 15 months.

Stop-Exposure Evaluation

Groups of 40 male and 40 female rats received 20,000 ppm 1-amino-2,4-dibromoanthraquinone in feed for 9 months, when 10 males and 10 females were evaluated. At 9 months, the dosed feed was replaced with a control diet for 10 male and 10 female rats, which were then necropsied and evaluated at 15 months. Twenty male and 20 female rats continued to receive 20,000 ppm 1-amino-2,4-dibromoanthraquinone in feed and were also evaluated at 15 months.

Source and Specification of Animals

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Frederick Cancer Research Facility (Frederick, MD). Rats were quarantined 12 to 14 days (males) or 9 days (females) and mice were quarantined 12 days (males) or 15 days (females) before the beginning of the studies. Five male and five female rats and mice were selected and evaluated for evidence of parasites and gross observation of disease. Serology samples were collected for viral screening. Rats and mice were approximately 6 weeks old at the beginning of the 2-year studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix L).

Animal Maintenance

Males and females were housed five per cage. Feed and water were available *ad libitum*. Feed consumption was measured monthly (Appendix J). Cages and

racks were rotated every 2 weeks during the studies. Further details of animal maintenance are given in Table 2. Information on feed composition and contaminants is provided in Appendix K.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded weekly for 14 weeks then monthly until the end of the studies. Animals were weighed at study initiation, weekly for 14 weeks, and monthly thereafter.

Animals were killed with CO_2, and a complete necropsy was performed on all animals. The right kidney and liver of rats and mice were weighed at the interim evaluations (Appendix H). At necropsy, all organs and tissues were examined for gross lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for microscopic examination. Histopathologic examinations were performed on all tissues with grossly visible lesions. For all paired organs (i.e., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined are listed in Table 2.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The microscope slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet-tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent pathology quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated by the quality assessment laboratory. The quality assessment pathologist microscopically reviewed selected neoplasms or nonneoplastic lesions.

The quality assessment report and slides were submitted to the NTP Pathology Working Group (PWG) chair, who reviewed the selected slides and any other tissues for which a disagreement in diagnosis between the laboratory and quality assessment pathologists existed. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the chair to the PWG for review. Tissues examined included the adrenal cortex (female rats), ear (rats), kidney (rats), large intestine (rats), liver, lung (mice), skin (rats), forestomach, thyroid gland (rats), and urinary bladder (rats). The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of exposure groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Thus, the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analysis of pathology data, the diagnosed lesions for each tissue type were evaluated separately or combined according to the guidelines of McConnell *et al.* (1986).

STATISTICAL METHODS

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes or missexed animals were censored from the survival analyses; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions as presented in Tables A1, A5, B1, B5, C1, C5, D1, D5, E1, E3, F1, and F3 are given as the number of animals bearing such lesions at a specific anatomic site and the number of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A3, B3, C3, D3, E2a, E2b, F2a, and F2b) and of all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., harderian gland, intestine, mammary gland, and skin) before microscopic evaluation or when neoplasms

had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A3, B3, C3, D3, E2a, E2b, F2a, and F2b also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm, i.e., the Kaplan-Meier estimate of the neoplasm incidence that would have been observed at the end of the study in the absence of mortality from all other competing risks (Kaplan and Meier, 1958).

Analysis of Neoplasm Incidence

The majority of neoplasms in these studies were considered to be incidental to the cause of death or not rapidly lethal. Thus, the primary statistical method used was logistic regression analysis, which assumed that the diagnosed neoplasms were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, neoplasm prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if the fit of the model was not significantly enhanced. The neoplasm incidences of exposed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986). When neoplasms are incidental, this comparison of the time-specific neoplasm prevalences also provides a comparison of the time-specific neoplasm incidences (McKnight and Crowley, 1984).

In addition to logistic regression, other methods of statistical analysis were used, and the results of these tests are summarized in the appendixes. These methods include the life table test (Cox, 1972; Tarone, 1975), appropriate for rapidly lethal neoplasms, and the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.*, 1979), procedures based on the overall proportion of lesion-bearing animals.

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-related trend. Continuity-corrected tests were used in the analysis of neoplasm incidence, and reported P values are one sided. The procedures described in the preceding paragraphs were also used to evaluate selected nonneoplastic lesions. For further discussion of these statistical methods, refer to Haseman (1984).

Analysis of Nonneoplastic Lesion Incidences

Because all nonneoplastic lesions in this study were considered to be incidental to the cause of death or not rapidly lethal, the primary statistical analysis used was a logistic regression analysis in which nonneoplastic lesion prevalence was modeled as a logistic function of chemical exposure and time. For lesions detected at the interim evaluation, the Fisher exact test, a procedure based on the overall proportion of affected animals, was used.

Analysis of Continuous Variables

Organ and body weight data, which have approximately normal distributions, were analyzed using the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's test). Prior to analysis, extreme values identified by the outlier test of Dixon and Massey (1951) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance using the Mann-Whitney U test (Hollander and Wolfe, 1973).

Historical Control Data

Although the concurrent control group is always the first and most appropriate control group used for evaluation, historical control data can be helpful in the overall assessment of lesion incidence in certain instances. Consequently, neoplasm incidences from the NTP historical control database (Haseman *et al.*, 1984, 1985) are included in the NTP reports for neoplasms appearing to show compound-related effects.

Quality Assurance Methods

The 13-week and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, these

studies were audited retrospectively by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report were conducted. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, so all discrepancies had been resolved or were otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of 1-amino-2,4-dibromoanthraquinone was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* cells and sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells. The protocols for these studies and the results are given in Appendix G.

The genetic toxicity studies of 1-amino-2,4-dibromoanthraquinone are part of a larger effort by the NTP to develop a database that would permit the evaluation of carcinogenicity in experimental animals from the structure and responses of the chemical in short-term *in vitro* and *in vivo* genetic toxicity tests. These genetic toxicity tests were originally developed to study mechanisms of chemically induced DNA damage and to predict carcinogenicity in animals, based on the electrophilic theory of chemical carcinogenesis and the somatic mutation theory (Miller and Miller, 1977; Straus, 1981; Crawford, 1985).

There is a strong correlation between a chemical's potential electrophilicity (structural alert to DNA reactivity), mutagenicity in *Salmonella*, and carcinogenicity in rodents. The combination of electrophilicity and *Salmonella* mutagenicity is highly correlated with the induction of carcinogenicity in rats and mice and/or at multiple tissue sites (Ashby and Tennant, 1991). Other in vitro genetic toxicity tests do not correlate well with rodent carcinogenicity (Tennant *et al.,* 1987; Zeiger et al., 1990), although these other tests can provide information on the types of DNA and chromosome effects that can be induced by the chemical being investigated. Data from NTP studies show that a positive response in *Salmonella* is currently the most predictive in vitro test for rodent carcinogenicity (89% of the *Salmonella* mutagens were rodent carcinogens), and that there is no complementarity among the *in vitro* genetic toxicity tests. That is, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone.

TABLE 2
Experimental Design and Materials and Methods in the Feed Studies of 1-Amino-2,4-dibromoanthraquinone

13-Week Studies	2-Year Studies	Stop-Exposure Evaluation
Study Laboratory EG&G Mason Research Institute (Worcester, MA)	Same as 13-week studies	Same as 13-week studies
Strain and Species Rats: F344/N Mice: $B6C3F_1$	Rats: F344/N Mice: $B6C3F_1$	Rats: F344/N
Animal Source Charles River Breeding Laboratories (Portage, MI)	Frederick Cancer Research Facility (Frederick, MD)	Same as 2-year studies
Time Held Before Studies 15 days	Rats: 12-14 days (males) or 9 days (females) Mice: 12 days (males) or 15 days (females)	12-14 days (males) or 9 days (females)
Average Age When Studies Began 7 weeks	6 weeks	6 weeks
Date of First Dose Rats: 22 April (males) or 29 April (females) 1982 Mice: 6 May (males) or 13 May (females) 1982	Rats: 13 July (males) or 4 August (females) 1983 Mice: 20 June (males) or 30 June (females) 1983	13 July (males) or 4 August (females) 1983
Duration of Dosing 13 weeks	104 weeks	9-Month stop-exposure group: 39 weeks (males) or 40 weeks (females) followed by control feed for remainder of study 15-Month exposure group: 66 weeks
Date of Last Dose Rats: 21-23 July (males) or 28-30 July (females) 1982 Mice: 4-6 August (males) or 11-13 August (females) 1982	Rats: 3 July (males) or 25 July (females) 1985 Mice: 10 June (males) or 20 June (females) 1985	9-Month stop-exposure group: 10-13 April (males) or 8-10 May (females) 1984 15-Month exposure group: 10-12 October (males) or 7-9 November (females) 1984

TABLE 2
Experimental Design and Materials and Methods in the Feed Studies of 1-Amino-2,4-dibromoanthraquinone (continued)

13-Week Studies	2-Year Studies	Stop-Exposure Evaluation
Necropsy Dates		
Rats: 21-23 July (males) or 28-30 July (females) 1982 Mice: 4-6 August (males) or 11-13 August (females) 1982	Rats: 9-Month interim evaluation: 10-13 April (males) or 8-10 May (females) 1984 15-Month interim evaluation: 10-12 October (males) or 7-9 November (females) 1984 Terminal: 10-16 July (males) or 1-8 August (females) 1985 Mice: 15-Month interim evaluation: 19-21 September (males) or 26-28 September (females) 1984 Terminal: 17-20 June (males) or 27 June - 2 July (females) 1985	9-Month interim evaluation: 10-13 April (males) or 8-10 May (females) 1984 15-Month terminal: 10-12 October (males) or 7-9 November (females) 1984
Average Age at Necropsy		
20 weeks	9-Month interim evaluation: 45-46 weeks 15-Month interim evaluation: 72 weeks Terminal: 110-112 weeks	9-Month interim evaluation: 45-46 weeks 15-Month terminal: 72 weeks
Size of Study Groups		
10 males and 10 females	Rats: 70 males and 70 females in the 0, 5,000, and 10,000 ppm groups; 50 males and 50 females in the 2,000 ppm group Mice: 60 males and 60 females	40 males and 40 females
Method of Animal Distribution		
Animals were caged by 1-gram weight classes and then distributed into treatment groups such that within a given sex, all cage weights were approximately equal (± 2 g).	Animals were distributed randomly into groups of approximately equal initial mean body weight.	Same as 2-year studies
Animals per Cage		
5	5	5
Method of Animal Identification		
Ear punch	Ear punch	Ear punch

TABLE 2
Experimental Design and Materials and Methods in the Feed Studies of 1-Amino-2,4-dibromoanthraquinone (continued)

13-Week Studies	2-Year Studies	Stop-Exposure Evaluation
Diet		
NIH-07 open formula meal diet (Zeigler Brothers, Inc., Gardners, PA), Available *ad libitum*, changed weekly	Same as 13-week studies	Same as 13-week studies
Water		
Tap water (City of Worcester) available *ad libitum* via automatic watering system (Edstrom Industries, Inc., Waterford, WI)	Same as 13-week studies	Same as 13-week studies
Cages		
Polycarbonate cage (Lab Products, Inc., Rochelle Park, NJ), changed twice weekly	Same as 13-week studies	Same as 13-week studies
Bedding		
Aspen Bed® heat-treated hardwood chips (American Excelsior, Baltimore, MD), changed twice weekly	Same as 13-week studies; BetaChips® hardwood chips (Northeastern Products, Warrensburg, NY) were used if necessary.	Same as 2-year studies
Cage Filters		
Nonwoven fiber filters (Lab Products, Rochelle Park, NJ; or Snow Filtration, Cincinnati, OH); changed every 2 weeks	Nonwoven fiber filters (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks	Same as 2-year studies
Racks		
Stainless steel racks (Lab Products, Inc., Maywood, NY), changed every 2 weeks	Same as 13-week studies	Same as 13-week studies
Animal Room Environment		
Average temperature: 22° to 26° C Relative humidity: 24% to 66% (rats), 28% to 66% (mice) Fluorescent light: 12 hours/day Room air: 12 to 15 changes/hour	Average temperature: 19° to 26° C Relative humidity: 16% to 76% Fluorescent light: 12 hours/day Room air: 12 to 15 changes/hour	Same as 2-year studies

TABLE 2
Experimental Design and Materials and Methods in the Feed Studies of 1-Amino-2,4-dibromoanthraquinone (continued)

13-Week Studies	2-Year Studies	Stop-Exposure Evaluation
Doses		
0, 2,500, 5,000, 10,000, 25,000, or 50,000 ppm in feed, available *ad libitum*	Rats: 0, 2,000, 5,000, or 10,000 ppm in feed, available *ad libitum* Mice: 0, 10,000, or 20,000 ppm in feed, available *ad libitum*	20,000 ppm in feed, available *ad libitum*
Type and Frequency of Observation		
Observed twice daily; animals weighed initially, weekly, and at end of studies; clinical observations recorded twice daily; feed consumption measured weekly	Observed twice daily; animals weighed initially, weekly for 14 weeks, and monthly thereafter; clinical observations recorded weekly for 14 weeks, then monthly until end of the studies; feed consumption measured monthly	Same as 2-year studies
Method of Sacrifice		
CO_2 asphyxiation	CO_2 asphyxiation	CO_2 asphyxiation
Necropsy		
Necropsy performed on all animals. Organs weighed were brain, heart, right kidney, liver, lungs, right testis, and thymus.	Necropsy was performed on all animals. Organs weighed at the 9- and 15-month interim evaluations were right kidney and liver.	Necropsy was performed on all animals. Organs weighed at 9 months and 15 months were right kidney and liver.
Histopathology		
Complete histopathologic examinations were performed on all animals that died during the study, control animals, and 50,000 ppm animals. In addition to tissue masses, gross lesions, and associated lymph nodes, the tissues examined included: adrenal gland, bone and marrow, brain, clitoral gland, epididymis, esophagus, gallbladder (mice), heart, kidney, large intestine (cecum, colon, rectum), liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, small intestine (duodenum, jejunum, ileum), spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, and uterus. The kidney, liver, spleen (rats), thymus (rats), and uterus (rats) of all other exposed animals were examined.	Complete histopathologic examinations were performed on all animals. In addition to tissue masses, gross lesions, and associated lymph nodes, the tissues examined included: adrenal gland, bone and marrow, brain, clitoral gland, epididymis, esophagus, gallbladder (mice), heart, kidney, large intestine (cecum, colon, rectum), liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, small intestine (duodenum, jejunum, ileum), spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, and uterus.	Complete histopathologic examinations were performed on all animals. In addition to tissue masses, gross lesions, and associated lymph nodes, the tissues examined included: adrenal gland, bone and marrow, brain, clitoral gland, epididymis, esophagus, heart, kidney, large intestine (cecum, colon, rectum), liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, small intestine (duodenum, jejunum, ileum), spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, and uterus.

RESULTS

RATS

13-WEEK STUDY

One male (week 13) and one female (week 8) in the 50,000 ppm groups died during the study (Table 3). The deaths of one 5,000 ppm male (week 4) and two additional 50,000 ppm males (week 13) were not chemical related. The final mean body weights and body weight gains of all exposed rat groups were significantly lower than those of the controls. Feed consumption by all exposed groups was less than that by the controls throughout the study and generally decreased with increasing exposure concentration (Table 3). The greatest differences in feed consumption from that by the controls occurred in the 25,000 and 50,000 ppm males and females. Feed consumption by these groups ranged from 45% to 79% that by the controls at week 1 and from 64% to 82% that by the controls at week 13. Dietary levels of 2,500, 5,000, 10,000, 25,000, and 50,000 ppm delivered daily doses of approximately 150, 300, 620, 1,600, and 3,200 mg 1-amino-2,4-dibromoanthraquinone/kg body

TABLE 3
Survival, Mean Body Weights, and Feed Consumption of Rats in the 13-Week Feed Study of 1-Amino-2,4-dibromoanthraquinone

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 13
Male							
0	10/10	180 ± 3	358 ± 3	179 ± 3		14.9	18.1
2,500	10/10	180 ± 3	325 ± 3**	145 ± 3**	91	14.3	16.7
5,000	9/10[d]	180 ± 3	328 ± 3**	148 ± 6**	92	13.9	17.1
10,000	10/10	181 ± 3	310 ± 3**	129 ± 3**	86	13.5	17.0
25,000	10/10	181 ± 3	232 ± 3**	52 ± 4**	65	11.7	14.9
50,000	7/10[e]	180 ± 3	164 ± 6**	-17 ± 6**	46	10.3	11.6
Female							
0	10/10	140 ± 2	211 ± 3	71 ± 2		13.0	15.7
2,500	10/10	140 ± 2	197 ± 3**	57 ± 3**	93	10.5	12.7
5,000	10/10	140 ± 2	188 ± 3**	47 ± 3**	89	10.2	12.1
10,000	10/10	140 ± 2	185 ± 2**	45 ± 2**	88	9.6	11.9
25,000	10/10	140 ± 2	159 ± 2**	19 ± 2**	75	7.0	10.6
50,000	9/10[f]	140 ± 2	130 ± 4**	-10 ± 4**	61	5.9	11.5

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] Feed consumption is expressed as grams of feed consumed per animal per day.
[d] Week of death: 4
[e] Week of death: 13, 13, 13 (2 were accidental deaths)
[f] Week of death: 8

weight to males and 170, 340, 660, 1,500, and 3,200 mg/kg to females. Pink-red staining of the fur and tail was observed in all exposed groups of rats. The bedding of all exposed groups except the 2,500 ppm groups was stained pink-red from day 2 of the study. Lethargy and emaciation were noted in all 50,000 ppm males. Female rats in the 25,000 and 50,000 ppm groups were lethargic and staggered, and 50,000 ppm females exhibited hunched posture.

The relative liver weights of exposed groups of males and the absolute and relative liver weights of exposed groups of females were significantly greater than those of the controls (Table H1). The absolute and relative thymus weights of exposed males and females were significantly lower than those of controls. The lower absolute brain, heart, kidney, lung, and testis weights of exposed male and female rats were probably related to the lower final mean body weights of these groups.

Observations at necropsy included red or pink staining of the gastrointestinal tract contents and/or mucosa, kidneys, and urine. In addition, regional lymph nodes and livers were dark in color, and capsular surfaces of the livers were granular in appearance. These findings were most common in the 25,000 and 50,000 ppm groups.

Chemical-related lesions were present in the liver, kidney, and spleen of male and female rats. In the liver, a spectrum of nonneoplastic degenerative and proliferative lesions occurred in males and females in the 25,000 and 50,000 ppm groups (Table 4). Hepatocellular cytomegaly (hypertrophy) was present in all rats in the 25,000 and 50,000 ppm groups and in most females in the 10,000 ppm group. This lesion consisted of enlarged hepatocytes with eosinophilic cytoplasm and marked variation in nuclear size. In the centrilobular areas of a few rats from exposed groups, there was a minimal to mild cytoplasmic vacuolation (vacuolar degeneration). The incidence of vacuolar degeneration was not dose-related, but at the higher exposure concentrations, minimal hepatocellular necrosis was sometimes associated with vacuolar change. Focal hepatocellular alterations including basophilic, eosinophilic, or clear cell foci were also present in the 25,000 and 50,000 ppm groups. In the periportal region of the hepatic lobules, there was an increased number of inflammatory cells around the bile ducts. Bile duct hyperplasia consisted of proliferation of oval cells in the periportal area as well as proliferation of larger bile ducts lined by hyperchromatic, pleomorphic biliary epithelium. Focal necrosis of biliary epithelium and acute inflammation (necrotizing cholangitis) in some hyperplastic bile ducts were associated with periportal fibrosis. The spectrum of proliferative bile duct lesions (hyperplasia, necrotizing cholangitis, and fibrosis) was morphologically consistent with the lesion described as cholangiofibrosis. A brown pigment was present in the cytoplasm of hepatocytes. The pigment was negative for iron, PAS, bile, and acid-fast staining; did not polarize light or fluoresce; and was considered to representl-amino-2,4-dibromoanthraquinone and/or its metabolites.

In the kidney of exposed groups of males and females, there were chemical-related increases in the incidences of a brown, granular pigment in the tubule epithelium (Table 4). This brown pigment had the same staining features as the pigment that was present in the liver. In both males and females, there were renal tubule cells with enlarged nuclei. In males, there was a hyaline droplet nephropathy characterized by an increase in eosinophilic protein droplets (hyaline droplet accumulation) in the cytoplasm of the renal tubule epithelium as well as in the lumen of the tubules. There was no evidence of increased severity of tubule regeneration in males or females.

Chemical-related effects in the spleen of all exposed groups of males and females consisted of a slight increase in the amount of hematopoiesis relative to that normally present in controls.

Other nonspecific changes included lymphoid depletion in the thymus and decreased uterus size. These findings were attributed to the markedly lower body weight gain in rats from the higher exposure groups.

Dose Selection Rationale: Based on chemical disposition studies, mean body weights, and chemical-related lesions of the liver, kidney, and spleen present mainly in the 25,000 and 50,000 ppm groups, exposure concentrations selected for the 2-year feed study of 1-amino-2,4-dibromoanthraquinone in rats were 0, 2,000, 5,000, and 10,000 ppm. Much of the differences in mean body weights recorded for the 13-week studies were more likely due to decreased feed palatability than to any overt toxicity. Nonetheless, if

TABLE 4
Incidences of Selected Nonneoplastic Lesions in Rats in the 13-Week Feed Study of 1-Amino-2,4-dibromoanthraquinone

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	25,000 ppm	50,000 ppm
Male						
Liver[a]	10	10	10	10	10	10
Basophilic Focus[b]	0	0	0	0	4* (1.5)[d]	9** (2.3)
Clear Cell Focus	0	0	0	0	6** (1.0)	0
Eosinophilic Focus	0	0	0	0	4* (1.5)	0
Cytomegaly	0	0	0	0	10** (3.2)	10** (4.0)
Bile Duct Hyperplasia	0	0	0	0	8** (2.3)	10** (3.1)
Inflammation	0	0	0	0	10** (2.1)	10** (3.0)
Fibrosis[c]	0	0	0	0	10** (1.9)	10** (3.1)
Necrotizing Cholangitis[c]	0	0	0	0	7** (1.3)	10** (2.8)
Vacuolar Degeneration[c]	0	0	4* (1.5)	5* (1.8)	3 (1.3)	4* (1.5)
Pigmentation	0	0	0	0	10** (1.5)	9** (1.0)
Kidney	10	10	10	10	10	10
Renal Tubule Pigmentation	0	10** (1.0)	9** (1.0)	10** (1.1)	10** (2.2)	10** (1.8)
Hyaline Droplet Accumulation	0	10** (1.7)	9** (1.7)	10** (2.0)	2 (1.0)	0
Female						
Liver	10	10	10	10	10	10
Basophilic Focus	0	0	0	0	1 (1.0)	9** (2.6)
Eosinophilic Focus	0	0	0	0	1 (1.0)	0
Cytomegaly	0	0	0	8** (1.0)	10** (2.2)	10** (4.0)
Bile Duct Hyperplasia	0	0	0	4* (1.0)	9** (1.6)	10** (2.4)
Inflammation	0	0	0	0	10** (1.6)	9** (2.3)
Fibrosis[c]	0	0	0	0	2 (2.0)	9** (2.7)
Necrotizing Cholangitis[c]	0	0	0	0	0	9** (2.6)
Vacuolar Degeneration[c]	0	0	0	1 (1.0)	2 (2.0)	8** (1.4)
Pigmentation	0	0	1 (1.0)	7** (1.0)	9** (1.2)	10** (1.5)
Kidney	10	10	10	10	10	10
Renal Tubule Pigmentation	0	10** (1.0)	9** (1.0)	10** (1.2)	10** (1.7)	10** (1.9)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with organ examined microscopically
[b] Number of animals with lesion
[c] Data from Fleischman *et al.*, 1986
[d] Average severity of lesions in affected rats: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked

exposure selection were based on mean body weights alone for male rats, the 10,000 ppm exposure concentration could have been considered slightly high. Moreover, considering the lack of liver toxicity at exposures of 10,000 ppm and below, this exposure concentration was predicted not to adversely affect the health or survival of these animals. Higher exposure concentrations (20,000 ppm) were chosen for the start-stop, progression/regression experiments (stop-exposure evaluation).

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 5 and in the Kaplan-Meier survival curves in Figure 2. Survival of male and female rats in the 10,000 ppm groups was significantly lower than that of the controls.

Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of exposed male and female rats were lower than those of the controls after week 2 (Tables 6 and 7, Figure 3). Final mean body weights of exposed males were 14% to 30% lower than that of the controls; final mean body weights of exposed females were 20% to 46% lower than that of the controls. Feed consumption by exposed males and females was similar among exposed groups and was slightly lower than that by the controls (Tables J3 and J4). Dietary levels of 2,000, 5,000, and 10,000 ppm delivered average daily doses of approximately 90, 240, and 490 mg 1-amino-2,4-dibromoanthraquinone/kg body weight to males and 110, 285, and 600 mg/kg to females. Discoloration of the fur and urine was evident in all exposed groups as early as day 8 and was observed throughout the study. Emaciation occurred in a dose-related manner in male and female rats and occurred in over 50% of the rats exposed to 10,000 ppm.

TABLE 5
Survival of Rats in the 2-Year Feed Study of 1-Amino-2,4-dibromoanthraquinone

	0 ppm	2,000 ppm	5,000 ppm	10,000 ppm
Male				
Animals initially in study	70	50	70	70
9-Month interim evaluation[a]	10	10	10	10
15-Month interim evaluation[a]	10	0	0	10
Moribund	19	15	34	33
Natural deaths	5	1	5	7
Animals surviving to study termination	26[e]	24	21	10
Percent probability of survival at end of study[b]	53	60	35	20
Mean survival (days)[c]	586	618	615	547
Survival analyses[d]	P<0.001	P=0.467N	P=0.141	P<0.001
Female				
Animals initially in study	70	50	70	70
9-Month interim evaluation[a]	10	10	10	10
15-Month interim evaluation[a]	10	0	0	10
Missexed[a]	0	0	0	1
Moribund	8	5	15	29
Natural deaths	4	3	7	8
Animals surviving to study termination	38[f]	32	38	12
Percent probability of survival at end of study	76	80	63	25
Mean survival (days)	610	626	643	569
Survival analyses	P<0.001	P=0.857N	P=0.216	P<0.001

[a] Censored from survival analyses
[b] Kaplan-Meier determinations based on the number of animals alive on first day of terminal sacrifice
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[d] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed columns. A lower mortality in an exposure group is indicated by N.
[e] Includes three males that died during the last week of the study.
[f] Includes one female that died during the last week of the study.

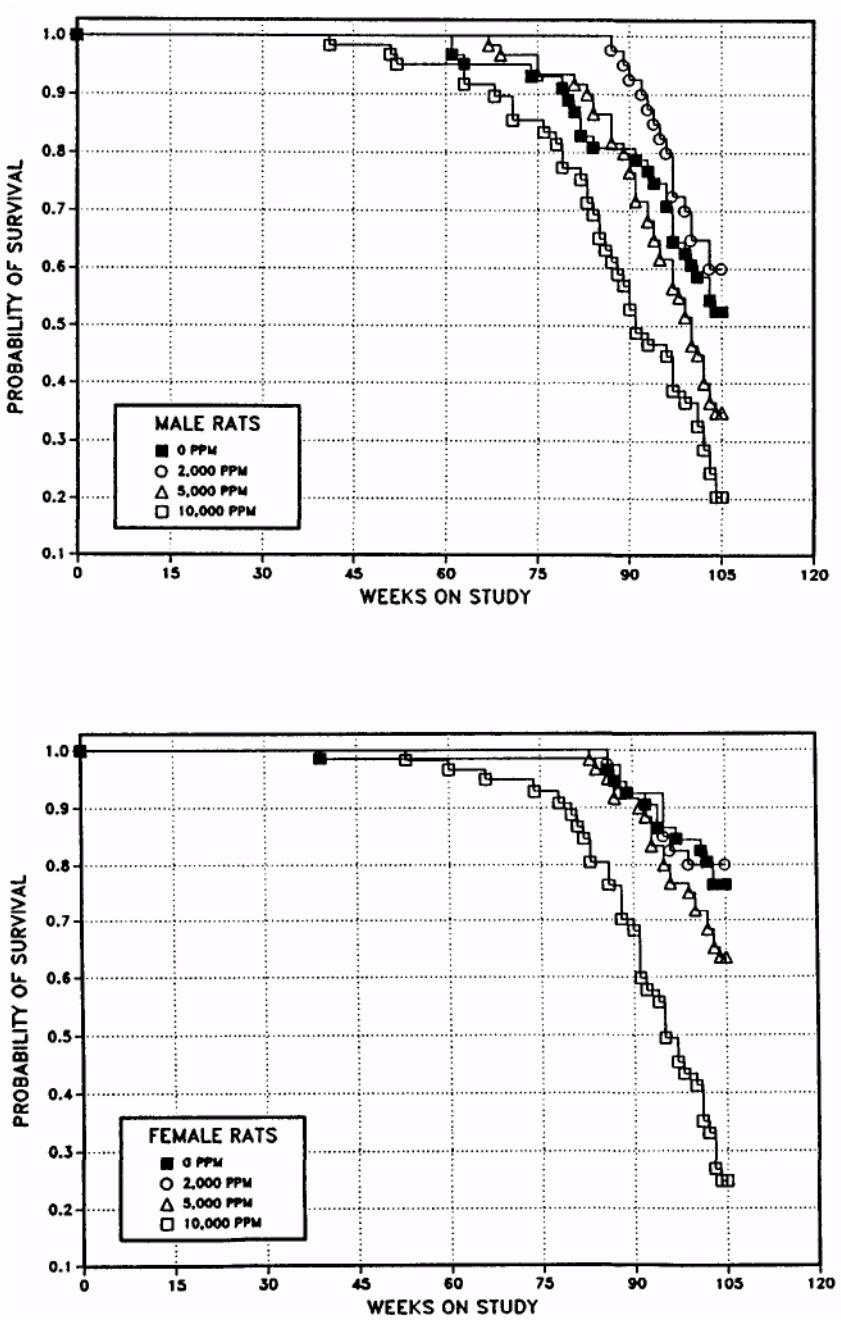


FIGURE 2
Kaplan-Meier Survival Curves for Rats Administered l-Amino-2,4-dibromoanthraquinone in Feed for 2 Years

TABLE 6
Mean Body Weights and Survival of Male Rats in the 2-Year Feed Study of 1-Amino-2,4-dibromoanthraquinone

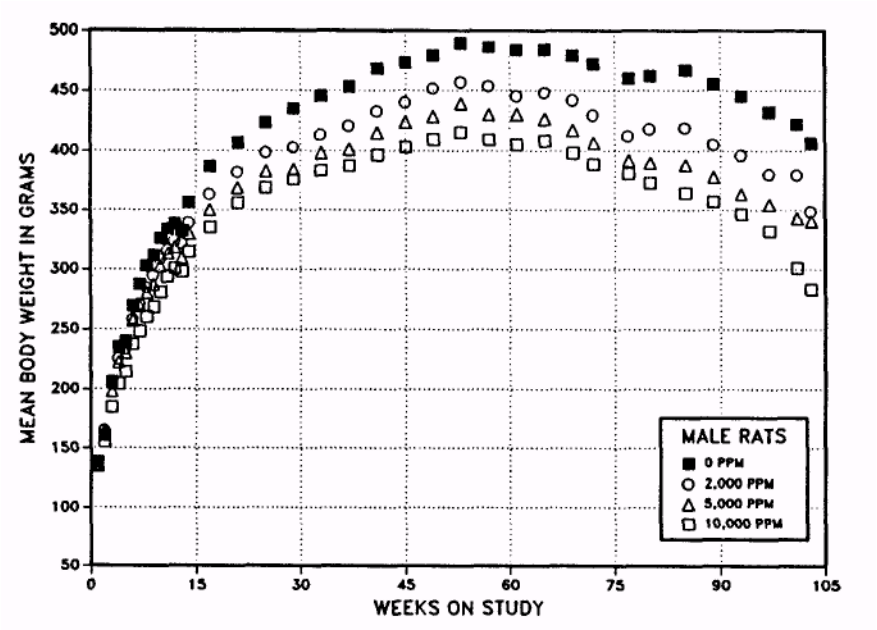
Weeks on Study	0 ppm		2,000 ppm			5,000 ppm			10,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	139	70	136	98	50	136	98	70	134	97	70
2	161	70	165	103	50	164	102	70	155	97	70
3	206	70	203	99	50	198	96	70	184	89	70
4	235	70	225	96	50	222	95	70	204	87	70
5	240	70	233	97	50	230	96	70	215	90	70
6	269	70	258	96	50	257	96	70	237	88	70
7	287	70	271	94	50	269	94	70	248	86	70
8	302	70	285	94	50	280	93	70	260	86	70
9	312	70	295	95	50	287	92	70	268	86	70
10	325	70	308	95	50	303	93	70	281	86	70
11	333	70	316	95	50	313	94	70	293	88	70
12	338	70	323	96	50	318	94	70	300	89	70
13	332	70	321	97	50	309	93	70	298	90	70
14	356	70	339	95	50	330	93	70	314	88	70
17	387	70	363	94	50	350	91	70	335	87	70
21	406	70	382	94	50	368	91	70	356	88	70
25	423	70	398	94	50	383	90	70	369	87	70
29	435	70	403	93	50	384	88	70	376	86	70
33	445	70	413	93	50	398	89	70	383	86	70
37	453	70	420	93	50	401	89	70	388	86	70
41[a]	468	60	433	92	40	415	89	60	397	85	59
45	473	60	440	93	40	424	90	60	403	85	59
49	479	60	452	94	40	428	89	60	409	84	57
53	489	60	457	93	40	439	90	60	415	85	57
57	486	60	453	93	40	430	88	60	409	84	57
61	484	59	445	92	40	430	89	60	405	84	57
65	484	57	448	93	40	426	88	60	408	84	55
69[a]	479	47	442	92	40	417	87	59	398	83	44
72	472	46	429	91	40	407	86	58	389	82	42
77	460	46	412	90	40	392	85	56	381	83	41
80	462	44	418	91	40	390	84	56	373	81	38
85	467	40	419	90	40	388	83	52	365	78	34
89	455	40	405	89	39	378	83	49	357	79	29
93	445	39	396	89	36	363	82	43	347	78	24
97	432	35	380	88	32	355	82	37	332	77	22
101	422	30	380	90	26	343	81	28	301	71	18
103	406	29	349	86	24	341	84	22	283	70	13
Mean for weeks											
1-13	268		257	96		253	94		237	89	
14-52		433		404	93		388	90		373	86
53-103	460		417	91		393	85		369	80	

[a] Interim evaluations occurred during week 39 for all groups and week 66 for the 0 and 10,000 ppm groups.

TABLE 7
Mean Body Weights and Survival of Female Rats in the 2-Year Feed Study of 1-Amino-2,4-dibromoanthraquinone

Weeks on Study	0 ppm		2,000 ppm			5,000 ppm			10,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	93	70	93	100	50	94	101	70	94	101	70
2	114	70	110	96	50	107	94	70	102	90	70
3	133	70	128	96	50	121	91	70	116	87	70
4	147	70	141	96	50	136	93	70	128	87	70
5	157	70	151	96	50	146	93	70	137	87	70
6	166	70	159	96	50	152	91	70	145	87	70
7	173	70	169	97	50	161	93	70	150	87	69
8	180	70	174	97	50	167	93	70	158	88	69
9	186	70	178	96	50	173	93	70	167	90	69
10	191	70	185	97	50	179	94	70	173	90	69
11	196	70	189	96	50	184	94	70	177	90	69
12	203	70	194	95	50	188	93	70	181	89	69
13	208	70	195	94	50	192	93	70	185	89	69
14	212	70	201	95	50	196	93	70	189	89	69
17	222	70	209	94	50	205	92	70	200	90	69
21	228	70	216	95	50	213	93	70	205	90	69
25	237	70	219	93	50	216	91	70	208	88	69
29	246	70	223	91	50	219	89	70	212	86	69
33	251	70	227	90	50	222	88	70	213	85	69
37	258	70	233	90	50	230	89	70	218	85	69
41[a]	265	59	233	88	40	227	86	60	217	82	59
45	272	59	239	88	40	231	85	60	221	81	59
49	284	59	246	87	40	237	84	60	227	80	59
53	299	59	257	86	40	245	82	60	232	78	59
57	311	59	264	85	40	249	80	60	238	77	58
61	315	59	267	85	40	251	80	60	238	76	57
65	328	59	275	84	40	257	78	60	242	74	57
68[a]	333	49	277	83	40	257	77	60	241	72	47
73	343	49	286	83	40	263	77	60	245	71	46
77	347	49	289	83	40	269	78	60	243	70	45
81	351	49	296	84	40	270	77	60	239	68	43
85	354	49	295	83	40	268	76	58	234	66	39
89	354	47	298	84	37	262	74	55	229	65	34
93	356	45	299	84	37	251	71	53	224	63	28
97	358	43	298	83	33	250	70	46	213	60	23
100	361	42	293	81	32	243	67	43	202	56	20
103	362	38	290	80	32	234	65	39	194	54	13
Mean for weeks											
1-13	165		159	96		154	93		147	89	
14-52	248		225	91		220	89		211	85	
53-103	341		285	84		255	75		230	67	

[a] Interim evaluations occurred during week 40 for all groups and week 66 for the 0 and 10,000 ppm groups.



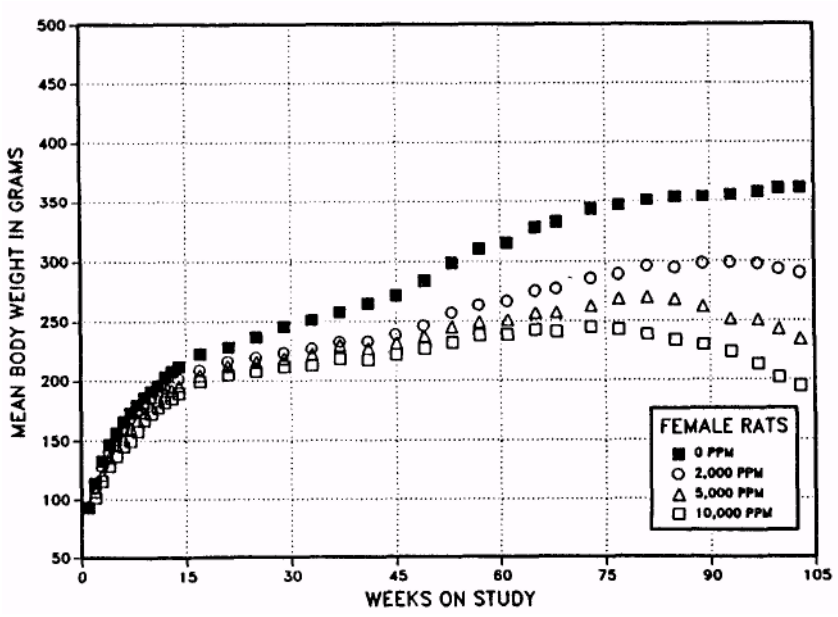


FIGURE 3
Growth Curves for Rats Administered 1-Amino-2,4-dibromoanthraquinone in Feed for 2 Years

Pathology and Statistical Evaluation

This section describes the statistically significant or biologically noteworthy changes in the incidences of mononuclear cell leukemia; neoplasms of the liver, large intestine, kidney, urinary bladder, and other organs; and nonneoplastic lesions of the liver, kidney, urinary bladder, forestomach, and seminal vesicles of rats. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one exposure group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Liver: At the 9-month interim evaluation, the absolute and relative liver weights of exposed groups of males and females were significantly greater than those of the controls (Table H2). One hepatocellular adenoma was observed in a 5,000 ppm male at 9 months (Tables 8 and A1). Incidences of foci of hepatocellular alteration were increased in males in the 10,000 ppm group, and a minimal accumulation of pigment in hepatocytes was present in males and females from the 10,000 ppm groups and in females from the 5,000 ppm group (Tables 8, A5, and B5). At the 15-month interim evaluation, the absolute and relative liver weights of exposed groups of females were significantly greater than those of the controls (Table H3). Incidences of single and multiple hepatocellular adenomas and carcinomas were increased at 15 months in 10,000 ppm males and females (Tables 8, A1, and B1). Incidences of foci of hepatocellular alteration and accumulation of pigment in hepatocytes were also increased in exposed groups of males and females (Tables 8, A5, and B5).

At the end of the 2-year study, the incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) were significantly increased in all exposed groups of males and females (Tables 8, A3, and B3).

The incidences of multiple hepatocellular adenomas and multiple hepatocellular carcinomas in exposed male and female groups were greater than those of the controls (Tables 8, A1, and B1). Incidences of hepatocellular adenoma or carcinoma (combined) in all exposed groups of males and females exceeded the NTP historical ranges (males: 0%-10%; females: 0%-6%) for feed study controls (Tables 8, A4a, and B4a). The majority of the benign and malignant liver neoplasms consisted of well-differentiated neoplastic hepatocytes with cellular atypia and increased numbers of mitoses. Carcinomas had trabecular, glandular, or solid growth patterns (Plate 1) with areas of necrosis, cavitation, and fibrosis. Metastases were common in the lungs (Plates 2 and 3), but metastatic foci were also present in the stomach, pancreas, adrenal gland, lymph node, and spleen.

The incidences of single and multiple hepatocholangiocarcinoma were significantly increased in 5,000 ppm males and females and in 10,000 ppm females (Tables 8, A3, and B3). These neoplasms consisted of a mixture of malignant hepatocytes and well-differentiated cuboidal epithelium forming distinct ductular structures (Plate 4). Both hepatocellular and biliary components of this neoplasm were present in metastatic foci. In addition, several other benign (cholangioma and hepatocholangioma) and malignant (cholangiocarcinoma) liver neoplasms occurred only in exposed groups of males and females (Tables 8, A1, and B1).

During the 2-year study, the incidences of pigmentation and foci of hepatocellular alteration (clear cell, basophilic, and eosinophilic) were increased in exposed groups of males and females (Tables 8, A5, and B5). Cells in some foci had intensely eosinophilic cytoplasm and hepatocellular atypia similar to the appearance of cells in the hepatocellular neoplasms. The pigment was considered to be 1-amino-2,4-dibromoanthraquinone or a metabolite based on the results of the histochemical procedures performed during the 13-week study and the 15-month interim evaluation.

TABLE 8
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Rats in the 2-Year Feed Study of 1-Amino-2,4-dibromoanthraquinone

	0 ppm	2,000 ppm	5,000 ppm	10,000 ppm
Male				
9-Month Interim Evaluation				
Number Examined	10	10	10	10
Basophilic Focus[a]	0	0	1 (1.0)[b]	1 (1.0)
Clear Cell Focus	0	0	0	4* (1.0)
Eosinophilic Focus	0	0	0	1 (1.0)
Pigmentation	0	1 (1.0)	0	6** (1.0)
Hepatocellular Adenoma	0	0	1	0
15-Month Interim Evaluation				
Number Examined	10	-[c]	-	10
Basophilic Focus	1 (1.0)			5 (1.0)
Clear Cell Focus	0			7** (1.0)
Eosinophilic Focus	1 (1.0)			0
Pigmentation	0			10** (1.0)
Hepatocellular Adenoma (Multiple)	0			2
Hepatocellular Adenoma (Single or Multiple)	0			4*
Hepatocellular Carcinoma (Multiple)	0			3
Hepatocellular Carcinoma (Single or Multiple)	0			7**
Hepatocellular Adenoma or Carcinoma	0			10**
2-Year Study				
Number Examined	50	40	59	50
Basophilic Focus	9 (1.1)	12 (1.7)	24** (1.8)	22** (1.5)
Clear Cell Focus	3 (1.0)	26** (1.8)	39** (1.8)	27** (1.8)
Eosinophilic Focus	1 (1.0)	13** (2.9)	14** (2.1)	6 (2.6)
Pigmentation	3 (1.0)	19** (1.1)	48** (1.1)	39** (1.1)
Hepatocellular Adenoma (Multiple)	0	10**	23**	24**
Hepatocellular Adenoma (Single or Multiple)				
Overall rate[d]	1/50 (2%)	20/40 (50%)	40/59 (68%)	34/50 (68%)
Terminal rate[e]	1/26 (4%)	16/24 (67%)	18/21 (86%)	9/10 (90%)
Adjusted rate[f]	3.8%	71.3%	92.3%	97.0%
First incidence (days)	729 (T)	675	521	435
Logistic regression test[g]	P<0.001	P<0.001	P<0.001	P<0.001
Hepatocellular Carcinoma (Multiple)	0	1	43**	37**
Hepatocellular Carcinoma (Single or Multiple)				
Overall rate	1/50 (2%)	12/40 (30%)	55/59 (93%)	46/50 (92%)
Terminal rate	0/26 (0%)	9/24 (38%)	21/21 (100%)	10/10 (100%)
Adjusted rate	2.7%	43.5%	100.0%	100.0%
First incidence (days)	666	650	465	436
Logistic regression test	P<0.001	P<0.001	P<0.001	P<0.001

(continued)

TABLE 8
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Rats in the 2-Year Feed Study of 1-Amino-2,4-dibromoanthraquinone (continued)

	0 ppm	2,000 ppm	5,000 ppm	10,000 ppm
Male (continued)				
2-Year Study (continued)				
Hepatocellular Adenoma or Carcinoma[h]				
Overall rate	2/50 (4%)	25/40 (63%)	57/59 (97%)	47/50 (94%)
Terminal rate	1/26 (4%)	19/24 (79%)	21/21 (100%)	10/10 (100%)
Adjusted rate	6.4%	83.1%	100.0%	100.0%
First incidence (days)	666	650	465	435
Logistic regression test	P<0.001	P<0.001	P<0.001	P<0.001
Number Examined	50	40	59	50
Hepatocholangioma	0	0	1	1
Hepatocholangiocarcinoma	0	0	6*	2
Cholangioma	0	0	2	0
Cholangiocarcinoma	0	0	0	1
Female				
9-Month Interim Evaluation				
Number Examined	10	10	10	10
Basophilic Focus	1 (1.0)	0	0	1 (1.0)
Clear Cell Focus	0	0	0	1 (1.0)
Pigmentation	0	2 (1.0)	6** (1.0)	6** (1.0)
15-Month Interim Evaluation				
Number Examined	10	-	-	10
Basophilic Focus	8 (1.0)			9 (1.4)
Clear Cell Focus	0			5* (1.6)
Pigmentation	1 (1.0)			10** (1.1)
Hepatocellular Adenoma (Multiple)	0			5*
Hepatocellular Adenoma (Single or Multiple)	0			8**
Hepatocellular Carcinoma (Multiple)	0			3
Hepatocellular Carcinoma (Single or Multiple)	0			6**
Hepatocellular Adenoma or Carcinoma	0			9**
2-Year Study				
Number Examined	50	40	60	48
Basophilic Focus	39 (1.3)	15** (1.6)	22** (1.7)	16** (1.4)
Clear Cell Focus	3 (1.3)	28** (1.6)	39** (2.0)	17** (1.6)
Eosinophilic Focus	7 (1.4)	23** (2.0)	12 (2.5)	1 (4.0)
Pigmentation	1 (1.0)	19** (1.1)	51** (1.4)	45** (1.1)

(continued)

TABLE 8
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Rats in the 2-Year Feed Study of 1-Amino-2,4-dibromoanthraquinone (continued)

	0 ppm	2,000 ppm	5,000 ppm	10,000 ppm
Female (continued)				
2-Year Study (continued)				
Number Examined	50	40	60	48
Hepatocellular Adenoma (Multiple)	0	18**	39**	22**
Hepatocellular Adenoma (Single or Multiple)				
Overall rate	0/50 (0%)	28/40 (70%)	47/60 (78%)	29/48 (60%)
Terminal rate	0/38 (0%)	23/32 (72%)	29/38 (76%)	8/12 (67%)
Adjusted rate	0.0%	75.5%	83.7%	83.6%
First incidence (days)_	-[i]	600	575	418
Logistic regression test	P<0.001	P<0.001	P<0.001	P<0.001
Hepatocellular Carcinoma (Multiple)	0	7**	51**	41**
Hepatocellular Carcinoma (Single or Multiple)				
Overall rate	0/50 (0%)	12/40 (30%)	57/60 (95%)	45/48 (94%)
Terminal rate	0/38 (0%)	12/32 (38%)	37/38 (97%)	12/12 (100%)
Adjusted rate	0.0%	37.5%	98.3%	100.0%
First incidence (days)	-	729 (T)	575	460
Logistic regression test	P<0.001	P<0.001	P<0.001	P<0.001
Hepatocellular Adenoma or Carcinoma[j]				
Overall rate	0/50 (0%)	33/40 (83%)	59/60 (98%)	47/48 (98%)
Terminal rate	0/38 (0%)	28/32 (88%)	38/38 (100%)	12/12 (100%)
Adjusted rate	0.0%	89.1%	100.0%	100.0%
First incidence (days)	-	600	575	418
Logistic regression test	P<0.001	P<0.001	P<0.001	P<0.001
Hepatocholangioma	0	0	2	0
Hepatocholangiocarcinoma	0	0	11**	13**
Cholangioma	0	0	0	1

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test (9-month and 15-month interim evaluations) or the logistic regression test (2-year study)
** $P \leq 0.01$
(T)Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected rats: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked
[c] Liver not microscopically examined in this group
[d] Number of animals with neoplasm per number of animals with liver examined microscopically
[e] Observed incidence in animals surviving until the end of the study
[f] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[g] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards these lesions as nonfatal.
[h] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 45/1,350 (3.3% ± 3.6%); range, 0%-10%
[i] Not applicable; no neoplasms in animal group
[j] Historical incidence: 9/1,351 (0.7% ± 1.5%); range, 0%-6%

Large Intestine: Adenomatous polyps (adenomas) were observed in the large intestine of 10,000 ppm males and females at the 15-month interim evaluation (Tables 9, A1, and B1).

At 2 years, the incidences of adenomatous polyps (adenomas) in the rectum were significantly increased in all exposed groups of males and females (Tables 9, A3, and B3). The incidence of carcinoma of the

TABLE 9
Incidences of Neoplasms of the Large Intestine in Rats in the 2-Year Feed Study of 1-Amino-2,4-dibromoanthraquinone

	0 ppm	2,000 ppm	5,000 ppm	10,000 ppm
Male				
15-Month Interim Evaluation				
Rectum[a]	9	-[c]	-	10
Adenomatous Polyp (Adenoma)[b]	0			6**
2-Year Study				
Colon				
Adenomatous Polyp (Adenoma)				
Overall rate[d]	0/50 (0%)	1/40 (3%)	1/59 (2%)	3/50 (6%)
Terminal rate[e]	0/26 (0%)	1/24 (4%)	0/21 (0%)	1/10 (10%)
Adjusted rate[f]	0.0%	4.2%	4.3%	19.9%
First incidence (days)	-[h]	729 (T)	720	590
Logistic regression test[g]	P=0.027	P=0.484	P=0.494	P=0.081
Carcinoma[i]				
Overall rate	0/50 (0%)	0/40 (0%)	1/59 (2%)	4/50 (8%)
Terminal rate	0/26 (0%)	0/24 (0%)	1/21 (5%)	0/10 (0%)
Adjusted rate	0.0%	0.0%	4.8%	20.4%
First incidence (days)	-	-	729 (T)	590
Logistic regression test P=0.003	-	P=0.457	P=0.046	
Rectum				
Adenomatous Polyp (Adenoma)				
Overall rate	0/50 (0%)	13/40 (33%)	51/59 (86%)	40/50 (80%)
Terminal rate	0/26 (0%)	9/24 (38%)	21/21 (100%)	10/10 (100%)
Adjusted rate	0.0%	45.8%	100.0%	100.0%
First incidence (days)	-	659	478	352
Logistic regression test P<0.001	P<0.001	P<0.001	P<0.001	
Carcinoma[i]				
Overall rate	0/50 (0%)	1/40 (3%)	10/59 (17%)	15/50 (30%)
Terminal rate	0/26 (0%)	0/24 (0%)	5/21 (24%)	4/10 (40%)
Adjusted rate	0.0%	3.8%	32.4%	63.0%
First incidence (days)	-	718	608	493
Logistic regression test P<0.001	P=0.480	P=0.003	P<0.001	
Large Intestine (All Sites)	50	40	59	50
Adenomatous Polyp (Adenoma) (Multiple)	0	1	34**	32**
Adenomatous Polyp (Adenoma) (Single or Multiple)	0	13**	51**	40**
Carcinoma (Multiple)	0	0	0	3
Carcinoma (Single or Multiple)	0	1	11**	17**

(continued)

TABLE 9
Incidences of Neoplasms of the Large Intestine in Rats in the 2-Year Feed Study of 1-Amino-2,4-dibromoanthraquinone (continued)

	0 ppm	2,000 ppm	5,000 ppm	10,000 ppm
Female				
15-Month Interim Evaluation				
Rectum	10			10
Adenomatous Polyp (Adenoma)	0	-	-	2
2-Year Study				
Colon	50	40	60	49
Adenomatous Polyp (Adenoma)	0	1	2	2
Carcinoma[j]	0	1	2	1
Rectum				
Adenomatous Polyp (Adenoma)				
Overall rate	0/50 (0%)	27/40 (68%)	53/60 (88%)	43/49 (88%)
Terminal rate	0/38 (0%)	23/32 (72%)	38/38 (100%)	12/12 (100%)
Adjusted rate	0.0%	75.0%	100.0%	100.0%
First incidence (days)	-	616	582	512
Logistic regression test	P<0.001	P<0.001	P<0.001	P<0.001
Carcinoma[j]				
Overall rate	0/50 (0%)	1/40 (3%)	19/60 (32%)	7/49 (14%)
Terminal rate	0/38 (0%)	1/32 (3%)	13/38 (34%)	4/12 (33%)
Adjusted rate	0.0%	3.1%	41.7%	41.9%
First incidence (days)	-	729 (T)	606	625
Logistic regression test	P<0.001	P=0.466	P<0.001	P=0.001
Large Intestine (All Sites)	50	40	60	49
Adenomatous Polyp (Adenoma) (Multiple)	0	18**	46**	32**
Adenomatous Polyp (Adenoma) (Single or Multiple)	0	28**	53**	43**
Carcinoma (Multiple)	0	1	1	1
Carcinoma (Single or Multiple)	0	2	21**	8**

** Significantly different ($P \leq 0.01$) from the control group by the Fisher exact test (15-month interim evaluation) or the logistic regression test (2-year study)
(T)Terminal sacrifice
[a] Number of animals with large intestine examined microscopically
[b] Number of animals with lesion
[c] Large intestine not microscopically examined in this group
[d] Number of animals with neoplasm per number of animals necropsied
[e] Observed incidence in animals surviving until the end of the study
[f] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[g] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards these lesions as nonfatal.
[h] Not applicable; no neoplasms in animal group
[i] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 1/1,353 (0.1% ± 0.4%); range, 0%-2% (includes all carcinomas of the large intestine)
[j] Historical incidence: 0/1,351 (includes all carcinomas of the large intestine)

colon was significantly increased in 10,000 ppm males, and the incidences of rectal carcinoma were significantly increased in 5,000 and 10,000 ppm males and females (Tables 9, A3, and B3). The intestinal neoplasms that occurred in the distal colon and rectum of rats were morphologically similar.

Adenomatous polyps (adenomas) consisted of pedunculated, exophytic masses (Plate 5) of well-differentiated, columnar epithelium with prominent, hyperchromatic nuclei. Carcinoma (adenocarcinoma) was generally similar to adenoma, except that invasion of the stromal stalk of the neoplasm and extension into the submucosa, and occasionally into the muscular wall, were evident microscopically. In the malignant neoplasms, irregular glandular structures or cords of atypical epithelial cells were present in the submucosa (Plate 6) and were frequently associated with a scirrhous response. In males, metastatic colon carcinoma was observed in the lung and mesenteric lymph nodes, and metastatic rectal carcinoma was observed in the lumbar lymph nodes and pancreas (Table A1). Incidences of carcinoma (colon and rectum combined) in exposed groups of males and females exceeded the NTP historical ranges (males: 0%-2%; females: 0%) for feed study controls (Tables 9, A4b, and B4b).

Kidney: At the 9-month interim evaluation, the relative kidney weights of exposed groups of males and females were significantly greater than those of the controls (Table H2). Pigmentation and hyaline droplet accumulation were present in the kidneys of all exposed males (Tables 10 and A5). The sizes of some renal tubule nuclei were minimally increased in males and females from all exposure groups, and the severity of the nephropathy (tubule epithelial regeneration, mononuclear inflammation, and renal tubule dilation with protein casts) was slightly more severe than that observed in the controls (Table 10). At the 15-month interim evaluation, the relative kidney weights of 10,000 ppm males and females were significantly greater than those of the controls (Table H3). The severity of nephropathy was increased in 10,000 ppm males and females compared to controls (Table 10). This was characterized by an increase in the foci of tubule epithelial regeneration; nuclear enlargement in some tubule epithelium, similar to that in the 13-week studies and at the 9-month interim evaluation, was also present. At 15 months in the 10,000 ppm groups, pigmentation of renal tubule epithelium was present in all rats; renal tubule epithelial hyperplasia was observed in two males and three females; and adenomas were observed in two males (Tables 10, A1, A5, and B5).

At 2 years, there was a significant dose-related increase in the incidences of renal tubule adenoma in exposed groups of males and females (Tables 10, A3, and B3). Multiple adenomas were observed in all exposed groups of males and in the 5,000 and 10,000 ppm females. Adenomas were expansile lesions involving one or more adjacent tubules and were generally five or more times the diameter of the normal renal tubule. The cells within the adenomas were generally similar in morphology to those in the focal hyperplastic lesions. Carcinomas were larger than adenomas and frequently had more cellular atypia, necrosis, and local invasion. One carcinoma in a male rat metastasized to the lung, and one in a female rat metastasized to the adrenal gland. Renal tubule carcinomas occurred in two 5,000 ppm males, one 10,000 ppm male, and two 10,000 ppm females. The combined incidences of renal tubule adenoma or carcinoma were significantly increased in exposed males and females and exceeded the NTP historical ranges (males: 0%-6%; females: 0%-2%) for feed study controls (Tables 10, A4c, and B4c).

Incidences of renal tubule hyperplasia were significantly increased in exposed males and females (Tables 10, A5, and B5). Hyperplasia consisted of a tubule lined by two or more layers of renal tubule epithelium; these were most often located in the cortex or outer stripe of the outer medulla. Foci of hyperplasia were distinguished from the more basophilic foci of tubule epithelial regeneration typically associated with nephropathy. There was no clear dose-related increase in the incidence or severity of nephropathy in rats at 2 years (Table 10). The incidences and severity of transitional cell hyperplasia in the renal pelvis were increased in exposed groups of males and females (Tables 10, A5, and B5); there were no increases in the incidences of transitional cell papilloma or carcinoma of the renal pelvis (Tables 10, A1, and B1). The incidence of a reddish brown pigment within the renal tubule epithelium and lumina of exposed rats was increased at the 9- and 15-month interim evaluations and at 2 years. The pigment was characterized in the 13-week study and at the 15-month interim evaluation as PAS-negative; resistant to digestion by diastase; isotropic; and negative for melanin, hemosiderin, hematoidin, bile, lipofuscin, or ceroid staining methods. The pigment was presumed to be 1-amino-2,4-dibromoanthraquinone or one of its metabolites.

TABLE 10
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Rats in the 2-Year Feed Study of 1-Amino-2,4-dibromoanthraquinone

	0 ppm	2,000 ppm	5,000 ppm	10,000 ppm
Male				
9-Month Interim Evaluation				
Number Examined	10	10	10	10
Renal Tubule Hyaline Droplet Accumulation[a]	0	10** (2.0)[b]	10** (2.0)	10** (1.9)
Renal Tubule Pigmentation	0	10** (1.1)	10** (1.4)	10** (1.9)
15-Month Interim Evaluation				
Number Examined	10	–[c]	–	10
Nephropathy	10 (2.0)			10 (2.5)
Renal Tubule Hyperplasia	0			2 (1.0)
Renal Tubule Pigmentation	0			10** (2.0)
Transitional Cell Hyperplasia	0			4* (1.3)
Renal Tubule Adenoma	0			2
2-Year Study				
Number Examined	50	40	59	50
Nephropathy	50 (2.9)	40 (3.6)	59 (2.9)	49 (2.7)
Renal Tubule Hyperplasia	9 (2.2)	30** (2.9)	25** (2.4)	19** (2.9)
Renal Tubule Pigmentation	5 (2.0)	40** (1.9)	58** (2.0)	49** (1.9)
Transitional Cell Hyperplasia	30 (1.4)	40** (2.1)	51** (1.9)	35* (1.6)
Transitional Cell Papilloma	0	0	1	0
Renal Tubule Adenoma (Multiple)	0	4*	4	5*
Renal Tubule Adenoma (Single or Multiple)				
Overall rate[d]	2/50 (4%)	10/40 (25%)	11/59 (19%)	14/50 (28%)
Terminal rate[e]	2/26 (8%)	6/24 (25%)	4/21 (19%)	5/10 (50%)
Adjusted rate[f]	7.7%	33.6%	34.6%	68.3%
First incidence (days)	729 (T)	618	636	588
Logistic regression test[g]	P<0.001	P=0.007	P=0.014	P<0.001
Renal Tubule Carcinoma	0	0	2	1
Renal Tubule Adenoma or Carcinoma[h]				
Overall rate	2/50 (4%)	10/40 (25%)	13/59 (22%)	15/50 (30%)
Terminal rate	2/26 (8%)	6/24 (25%)	4/21 (19%)	5/10 (50%)
Adjusted rate	7.7%	33.6%	39.4%	69.1%
First incidence (days)	729 (T)	618	636	497
Logistic regression test	P<0.001	P=0.007	P=0.005	P<0.001

(continued)

TABLE 10
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Rats in the 2-Year Feed Study of 1-Amino-2,4-dibromoanthraquinone (continued)

	0 ppm	2,000 ppm	5,000 ppm	10,000 ppm
Female				
15-Month Interim Evaluation				
Number Examined	10	-	-	10
Nephropathy	10 (1.7)			10 (2.5)
Renal Tubule Hyperplasia	0			3 (1.0)
Renal Tubule Pigmentation	0			10** (2.0)
Transitional Cell Hyperplasia	3 (1.0)			4 (1.8)
2-Year Study				
Number Examined	50	40	60	48
Nephropathy	50 (1.9)	39 (2.6)	60 (2.7)	46 (2.7)
Renal Tubule Hyperplasia	1 (3.0)	12** (2.5)	23** (2.7)	27** (2.7)
Renal Tubule Pigmentation	0	40** (2.0)	60** (2.0)	48** (2.0)
Transitional Cell Hyperplasia	10 (1.2)	16* (1.8)	44** (1.5)	21** (1.6)
Transitional Cell Papilloma	0	0	0	1
Transitional Cell Carcinoma	0	1	0	0
Renal Tubule Adenoma (Multiple)	0	0	5*	5*
Renal Tubule Adenoma (Single or Multiple)				
Overall rate	0/50 (0%)	3/40 (8%)	16/60 (27%)	16/48 (33%)
Terminal rate	0/38 (0%)	1/32 (3%)	11/38 (29%)	6/12 (50%)
Adjusted rate	0.0%	8.0%	36.0%	69.7%
First incidence (days)	-[i]	600	601	625
Logistic regression test	P<0.001	P=0.049	P<0.001	P<0.001
Renal Tubule Carcinoma	0	0	0	2
Renal Tubule Adenoma or Carcinoma[j]				
Overall rate	0/50 (0%)	3/40 (8%)	16/60 (27%)	16/48 (33%)
Terminal rate	0/38 (0%)	1/32 (3%)	11/38 (29%)	6/12 (50%)
Adjusted rate	0.0%	8.0%	36.0%	69.7%
First incidence (days)	-	600	601	625
Logistic regression test	P<0.001	P=0.049	P<0.001	P<0.001

* Significantly different ($P \leq 0.05$) from the control group by the Fischer exact test (9-month and 15-month interim evaluations) or the logistic regression test (2-year study)
** $P \leq 0.01$
(T)Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected rats: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked
[c] Kidney not microscopically examined in this group
[d] Number of animals with neoplasm per number of animals with kidney examined microscopically
[e] Observed incidence in animals surviving until the end of the study
[f] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[g] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards these lesions as nonfatal.
[h] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 15/1,350 (1.1% ± 1.7%); range, 0%-6%
[i] Not applicable; no neoplasms in animal group
[j] Historical incidence: 1/1,348 (0.1% ± 0.4%); range, 0%-2%

Urinary Bladder: Hyperplasia of the transitional cell epithelium of the urinary bladder was observed in 10,000 ppm females at 9 months and in 10,000 ppm males and females at 15 months (Tables 11, A5, and B5). Transitional cell hyperplasia was present in most 5,000 and 10,000 ppm females at 2 years, and the incidences of this lesion were significantly increased in 5,000 and 10,000 ppm males. Hyperplasia consisted of a diffuse or focal increase in thickness of the transitional epithelium; minimal cellular pleomorphism and increased numbers of mitotic cells were sometimes present. Other nonneoplastic lesions that occurred only in exposed rats included squamous metaplasia of the transitional epithelium and fatty metaplasia (fat proliferation) of the stroma of the bladder wall.

Two transitional cell carcinomas and one papilloma occurred in 10,000 ppm females at the 15-month interim evaluation. At 2 years, incidences of transitional cell papilloma, carcinoma, and papilloma or carcinoma (combined) were significantly increased in 10,000 ppm males and 5,000 and 10,000 ppm females (Tables 11, A3, and B3) and exceeded the NTP historical ranges (males: 0%-2%; females: 0%-2%) for feed study controls (Tables 11, A4d, and B4d). Transitional cell papilloma consisted of a pedunculated or broad-based mass of transitional epithelium with a central fibrovascular stroma; there was squamous metaplasia of the surface epithelium in some papillomas. Transitional cell carcinoma was characterized by an exophytic or endophytic growth pattern and invasion of the lamina propria or muscularis of the bladder wall (Plates 7 and 8). There was cellular atypia and squamous or mucous metaplasia of transitional epithelium in some carcinomas.

TABLE 11
Incidences of Neoplasms and Nonneoplastic Lesions of the Urinary Bladder in Rats in the 2-Year Feed Study of 1-Amino-2,4-dibromoanthraquinone

	0 ppm	2,000 ppm	5,000 ppm	10,000 ppm
Male				
15-Month Interim Evaluation				
Number Examined	10	–[b]	–	10
Transitional Cell Hyperplasia[a]	0			3 (1.3)[c]
Metaplasia, Squamous	0			1 (2.0)
2-Year Study				
Number Examined	50	38	58	50
Transitional Cell Hyperplasia	1 (2.0)	5 (2.0)	17** (1.9)	30** (2.1)
Metaplasia, Squamous	0	0	0	3 (1.7)
Transitional Cell Papilloma				
Overall rate[d]	0/50 (0%)	1/38 (3%)	2/58 (3%)	8/50 (16%)
Terminal rate[e]	0/26 (0%)	0/22 (0%)	2/21 (10%)	2/10 (20%)
Adjusted rate[f]	0.0%	3.7%	9.5%	40.3%
First incidence (days)	–[h]	700	729 (T)	493
Logistic regression test[g]	P<0.001	P=0.459	P=0.192	P=0.004
Transitional Cell Carcinoma				
Overall rate	0/50 (0%)	0/38 (0%)	1/58 (2%)	4/50 (8%)
Terminal rate	0/26 (0%)	0/22 (0%)	0/21 (0%)	1/10 (10%)
Adjusted rate	0.0%	0.0%	4.3%	24.5%
First incidence (days)	–	–	720	674
Logistic regression test	P=0.001	–	P=0.491	P=0.022
Transitional Cell Papilloma or Carcinoma[i]				
Overall rate	0/50 (0%)	1/38 (3%)	3/58 (5%)	12/50 (24%)
Terminal rate	0/26 (0%)	0/22 (0%)	2/21 (10%)	3/10 (30%)
Adjusted rate	0.0%	3.7%	13.5%	56.2%
First incidence (days)	–	700	720	493
Logistic regression test	P<0.001	P=0.459	P=0.096	P<0.001
Female				
9-Month Interim Evaluation				
Number Examined	10	10	10	10
Transitional Cell Hyperplasia	0	0	0	2 (1.5)
15-Month Interim Evaluation				
Number Examined	10	–	–	10
Transitional Cell Hyperplasia	0			9** (2.6)
Transitional Cell Papilloma	0			1
Transitional Cell Carcinoma	0			2
Squamous Cell Carcinoma	0			2

(continued)

TABLE 11
Incidences of Neoplasms and Nonneoplastic Lesions of the Urinary Bladder in Rats in the 2-Year Feed Study of 1-Amino-2,4-dibromoanthraquinone (continued)

	0 ppm	2,000 ppm	5,000 ppm	10,000 ppm
Female (continued)				
2-Year Study				
Number Examined	50	40	60	46
Transitional Cell Hyperplasia	1 (1.0)	2 (3.0)	41** (2.0)	41** (2.3)
Metaplasia, Squamous	0	1 (1.0)	4 (2.3)	8** (2.9)
Fat Proliferation	0	0	4 (2.3)	2 (2.5)
Transitional Cell Papilloma				
Overall rate	0/50 (0%)	2/40 (5%)	7/60 (12%)	9/46 (20%)
Terminal rate	0/38 (0%)	2/32 (6%)	6/38 (16%)	1/12 (8%)
Adjusted rate	0.0%	6.3%	17.6%	39.5%
First incidence (days)	–	729 (T)	691	637
Logistic regression test	P<0.001	P=0.201	P=0.012	P=0.003
Transitional Cell Carcinoma				
Overall rate	0/50 (0%)	0/40 (0%)	8/60 (13%)	16/46 (35%)
Terminal rate	0/38 (0%)	0/32 (0%)	6/38 (16%)	4/12 (33%)
Adjusted rate	0.0%	0.0%	19.5%	55.8%
First incidence (days)	–	–	670	367
Logistic regression test	P<0.001	–	P=0.008	P<0.001
Transitional Cell Papilloma or Carcinoma[j]				
Overall rate	0/50 (0%)	2/40 (5%)	17/60 (28%)	26/46 (57%)
Terminal rate	0/38 (0%)	2/32 (6%)	14/38 (37%)	6/12 (50%)
Adjusted rate	0.0%	6.3%	40.9%	78.1%
First incidence (days)	–	729 (T)	670	367
Logistic regression test	P<0.001	P=0.201	P<0.001	P<0.001
Squamous Cell Papilloma (Single or Multiple)	0	0	1	2
Squamous Cell Carcinoma	0	0	1	0

** Significantly different ($P \leq 0.01$) from the control group by the Fisher exact test (9-month and 15-month interim evaluations) or the logistic regression test (2-year study)
(T)Terminal sacrifice
[a] Number of animals with lesion
[b] Urinary bladder not microscopically examined in this group
[c] Average severity grade of lesions in affected rats: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked
[d] Number of animals with neoplasm per number of animals necropsied or examined microscopically
[e] Observed incidence in animals surviving until the end of the study
[f] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[g] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards these lesions as nonfatal.
[h] Not applicable; no neoplasms in animal group
[i] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 3/1,329 (0.2% ± 0.6%); range, 0%-2%
[j] Historical incidence: 3/1,334 (0.2% ± 0.6%); range, 0%-2%

Forestomach: Several proliferative and degenerative lesions occurred with increased incidences in the forestomach of exposed males and females (Table 12). These mucosal lesions frequently occurred together and consisted of thickening (hyperplasia) of the squamous epithelium and an increase in the surface keratin layers (hyperkeratosis) (Tables 12, A5, and B5). Focal areas of hyperplasia were sometimes adjacent to ulceration and inflammation of the squamous mucosa. There was no significant increase in incidences of neoplasms of the forestomach (Tables 12, A1, and B1).

TABLE 12
Incidences of Neoplasms and Nonneoplastic Lesions of the Forestomach in Rats in the 2-Year Feed Study of 1-Amino-2,4-dibromoanthraquinone

	0 ppm	2,000 ppm	5,000 ppm	10,000 ppm
Male				
Number Examined	49	39	59	49
Hyperkeratosis[a]	5 (2.4)[b]	18** (1.8)	21** (2.0)	20** (2.0)
Hyperplasia, Squamous	3 (1.7)	19** (3.0)	25** (3.2)	26** (3.0)
Inflammation, Chronic Active	3 (2.0)	12** (2.4)	11 (2.0)	11* (2.2)
Ulcer	3 (2.7)	10** (3.2)	15** (2.7)	16** (2.6)
Squamous Cell Papilloma[c]	0	2	0	1
Squamous Cell Carcinoma	0	0	0	1
Squamous Cell Papilloma or Carcinoma[d]	0	2	0	2
Female				
Number Examined	49	40	60	47
Hyperkeratosis	2 (2.0)	7* (1.4)	23** (2.1)	28** (1.9)
Hyperplasia, Squamous	2 (2.0)	7* (1.9)	26** (2.9)	33** (3.0)
Inflammation, Chronic Active	0	1 (2.0)	13** (2.2)	10** (2.2)
Ulcer	1 (2.0)	2 (2.5)	7 (1.7)	17** (2.9)
Squamous Cell Papilloma	0	0	0	1
Squamous Cell Carcinoma	0	1	1	1
Squamous Cell Papilloma or Carcinoma[e]	0	1	1	2

* Significantly different ($P \leq 0.05$) from the control group by the logistic regression test
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked
[c] Number of animals with neoplasm per number of animals necropsied
[d] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 4/1,353 (0.3% ± 0.7%); range, 0%-2%
[e] Historical incidence: 2/1,351 (0.2% ± 0.5%); range, 0%-2%

Miscellaneous Neoplasms and Nonneoplastic Lesions: In exposed males and females, incidences of mononuclear cell leukemia occurred with significant negative trends (Tables 13, A3, and B3). The incidences of pituitary gland adenoma in males and females (males: 0 ppm, 21/48; 2,000 ppm, 14/40; 5,000 ppm, 10/56; 10,000 ppm, 10/49; females: 32/50, 19/39, 32/60, 13/47; Tables A3 and B3) and the incidence of mammary gland fibroadenoma (21/50, 10/40, 9/60, 5/49; Table B3) in females also occurred with significant negative trends. These decreases may have been related to lower body weights.

TABLE 13
Incidences of Mononuclear Cell Leukemia in Rats in the 2-Year Feed Study of 1-Amino-2,4-dibromoanthraquinone

	0 ppm	2,000 ppm	5,000 ppm	10,000 ppm
Male				
15-Month Interim Evaluation				
Mononuclear Cell Leukemia[a]	0/10	–[b]	–	2/10
2-Year Study				
Mononuclear Cell Leukemia				
Overall rate[a]	25/50 (50%)	5/40 (13%)	3/59 (5%)	1/50 (2%)
Terminal rate[c]	9/26 (35%)	4/24 (17%)	2/21 (10%)	0/10 (0%)
Adjusted rate[d]	59.0%	18.8%	11.7%	2.9%
First incidence (days)	514	604	650	590
Life table test[e]	P<0.001N	P<0.001N	P<0.001N	P<0.001N
Logistic regression test	P<0.001N	P<0.001N	P<0.001N	P<0.001N
Female				
2-Year Study				
Mononuclear Cell Leukemia				
Overall rate	9/50 (18%)	1/40 (3%)	5/60 (8%)	1/49 (2%)
Terminal rate	6/38 (16%)	0/32 (0%)	1/38 (3%)	0/12 (0%)
Adjusted rate	21.5%	3.0%	10.0%	3.7%
First incidence (days)	620	689	601	662
Life table test	P=0.112N	P=0.026N	P=0.177N	P=0.162N
Logistic regression test	P=0.011N	P=0.023N	P=0.111N	P=0.024N

[a] Number of animals with neoplasm per number of animals necropsied
[b] Animals not microscopically examined in this group
[c] Observed incidence in animals surviving until the end of the study
[d] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[e] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. A negative trend or a lower incidence in an exposure group is indicated by N.

In males, there was a chemical-related increased incidence of atrophy of the seminal vesicles (1/49, 30/40, 35/59, 23/50; Table A5). This was not evident at the 9- or 15-month interim evaluation, but was present in most males at the end of the 2-year study.

Atrophy of the seminal vesicles of exposed males was characterized by a reduction in the size of the secretory epithelium from a tall columnar shape to a low cuboidal shape and by an increase in the amount of connective tissue stroma in the gland.

STOP-EXPOSURE EVALUATION

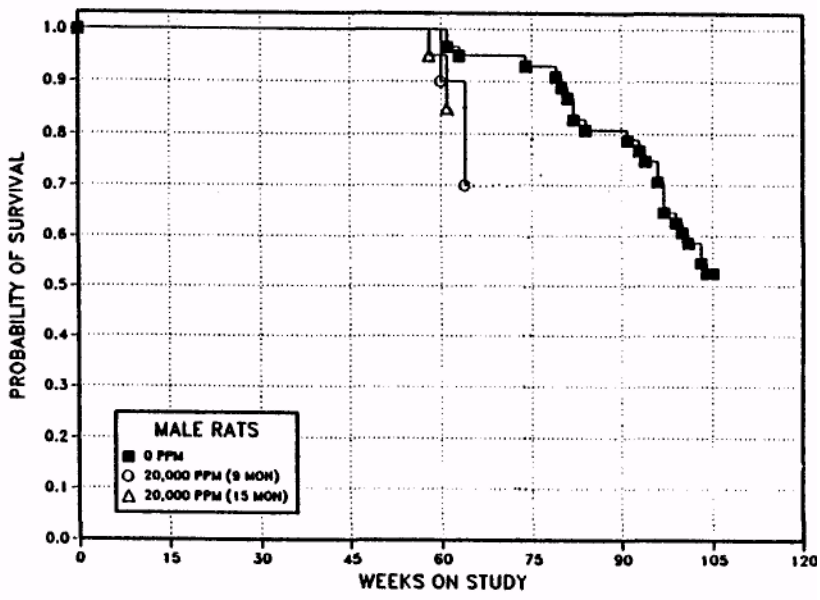
Stop-exposure groups of male and female rats were included in the NTP 2-year study to evaluate the potential for progression or regression of chemical-related liver, large intestine, kidney, urinary bladder, and forestomach lesions during a recovery period. Ten male and 10 female rats were exposed to 20,000 ppm 1-amino-2,4-dibromoanthraquinone in feed for 9 months followed by administration of undosed feed until the end of the 15-month period (9-month stop-exposure groups). In addition, 30 males and 30 females were exposed to 20,000 ppm 1-amino-2,4-dibromoanthraquinone in feed for 15 months (15-month exposure groups). Ten males and 10 females from the 15-month exposure groups were evaluated at the 9-month interim evaluation (9-month interim evaluation groups).

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in the Kaplan-Meier survival curves in Figure 4. All males survived 9 months; three males from the 9-month stop-exposure group and three males from the 15-month exposure group died before the end of the 15-month evaluation (Table 14). All females in the 9-month stop-exposure group survived until the end of the 15-month evaluation. One female from the 15-month exposure group died during the first 9 months; an additional seven females died between month 9 and the end of the 15-month evaluation (Table 15).

Body Weights and Feed and Compound Consumption

The mean body weights of male and female rats in the 9-month stop-exposure and 15-month exposure groups are compared with the controls from the 2-year core study in Tables 14 and 15, and the growth curves for exposed rats in the 15-month exposure groups are shown in Figure 5. The mean body weights of males and females in the 9-month stop-exposure groups were 20% to 22% lower than those of the controls at the 9-month interim evaluation of the 2-year core study and were 20% to 21% lower than those of the controls at the 15-month interim evaluation of the 2-year core study. The mean body weights of males and females in the 15-month exposure groups were 19% to 21% lower than those of the controls at the 9-month interim evaluation of the 2-year core study and were 25% to 33% lower than those of the controls at the 15-month interim evaluation of the 2-year core study. Feed consumption by 9-month stop-exposure and 15-month exposure males and females was generally lower than that by the controls throughout the study (Tables J1 and J2). The dietary level of 20,000 ppm delivered daily doses of approximately 1,300 mg 1-amino-2,4-dibromoanthraquinone/kg body weight to males and 1,800 mg/kg to females in the 9-month stop-exposure groups and daily doses of approximately 1,100 mg/kg to males and 1,400 mg/kg to females in the 15-month exposure groups.



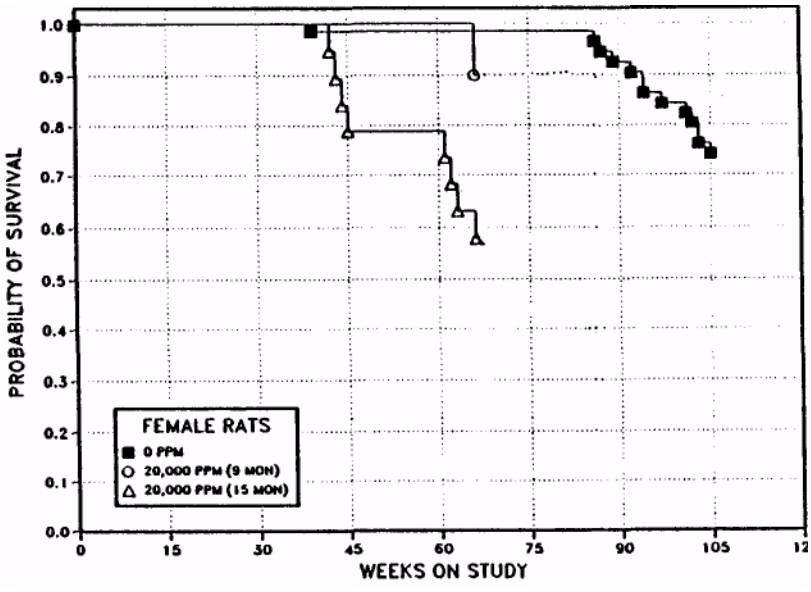
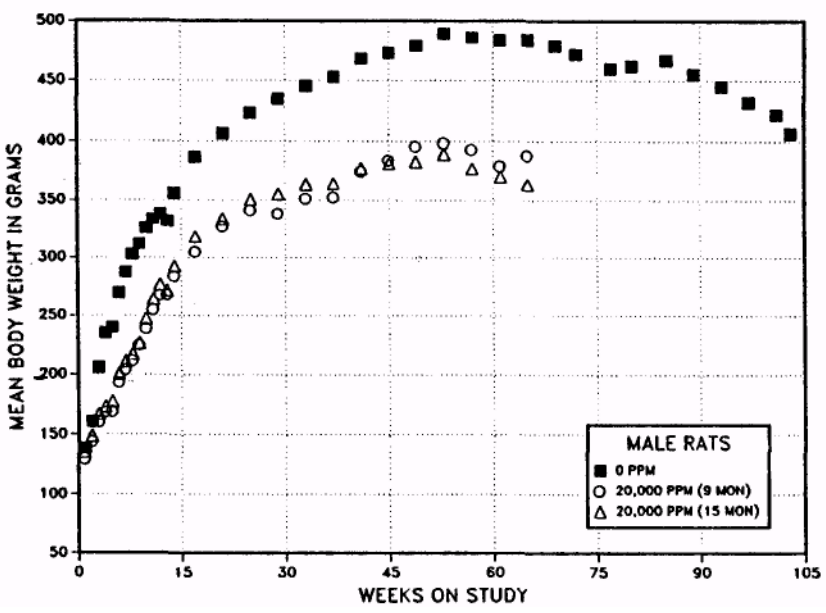


FIGURE 4
Kaplan-Meier Survival Curves for Rats Administered l-Amino-2,4-dibromoanthraqninone in Feed in the 15-Month Stop-Exposure Evaluation



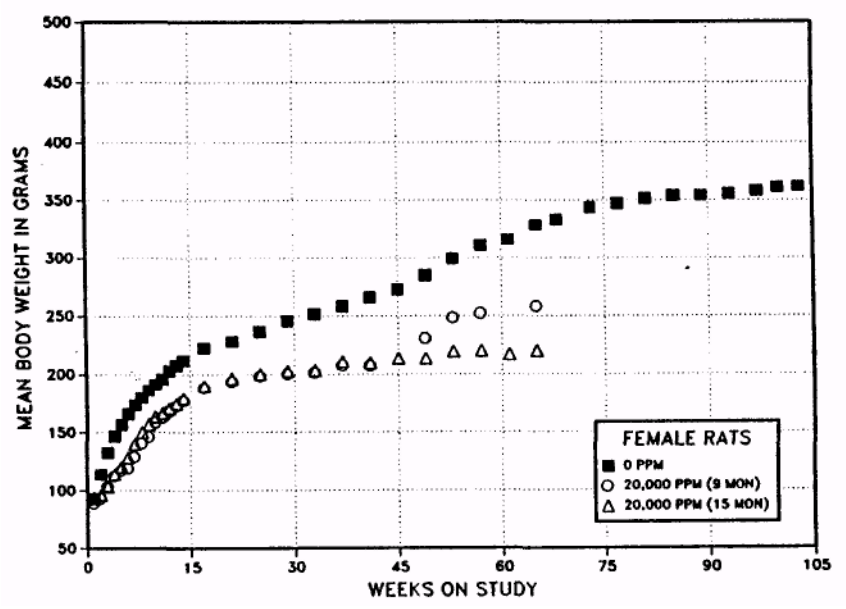


FIGURE 5
Growth Curves for Rats Administered l-Amino-2,4-dibromoanthraquinone in Feed in the 15-Month Stop-Exposure Evaluation

TABLE 14
Mean Body Weights and Survival of Male Rats in the Stop-Exposure Evaluation of 1-Amino-2,4-dibromoanthraquinone

Weeks on Study	0 ppm		20,000 ppm (9-Month Stop-Exposure)			20,000 ppm (15-Month Exposure)		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	139	70	130	94	10	136	98	30
2	161	70	144	90	10	149	93	30
3	206	70	160	78	10	167	81	30
4	235	70	169	72	10	173	74	30
5	240	70	169	71	10	177	74	30
6	269	70	193	72	10	201	75	30
7	287	70	204	71	10	211	74	30
8	302	70	211	70	10	217	72	30
9	312	70	225	72	10	226	73	30
10	325	70	239	73	10	247	76	30
11	333	70	255	76	10	264	79	30
12	338	70	267	79	10	277	82	30
13	332	70	267	81	10	271	82	30
14	356	70	284	80	10	292	82	30
17	387	70	305	79	10	318	82	30
21	406	70	327	81	10	334	82	30
25	423	70	341	81	10	351	83	30
29	435	70	338	78	10	355	82	30
33	445	70	351	79	10	364	82	30
37	453	70	352	78	10	364	80	30
41	468	60[a]	374	80	10[b]	377	81	20[a]
45	473	60	384	81	10	381	81	20
49	479	60	395	83	10	383	80	20
53	489	60	398	81	10	389	80	20
57	486	60	393	81	10	377	78	20
61	484	59	379	78	9	370	77	19
65	484	57	388	80	7	364	75	17
Mean for weeks								
1-13	268		203	76		209	78	
14-37	415		328	79		340	82	
41-65	480		387	81		377	79	

[a] Interim evaluation occurred during week 39.
[b] Animals switched to undosed feed

TABLE 15
Mean Body Weights and Survival of Female Rats in the Stop-Exposure Evaluation of 1-Amino-2,4-dibromoanthraquinone

Weeks on Study	0 ppm		20,000 ppm (9-Month Stop-Exposure)			20,000 ppm (15-Month Exposure)		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	93	70	90	96	10	94	101	30
2	114	70	93	82	10	97	85	30
3	133	70	103	78	10	104	78	30
4	147	70	113	77	10	114	78	30
5	157	70	117	75	10	121	77	30
6	166	70	119	72	10	129	78	30
7	173	70	129	75	10	141	81	30
8	180	70	141	78	10	150	84	30
9	186	70	146	79	10	157	84	30
10	191	70	159	83	10	163	85	30
11	196	70	163	83	10	166	85	29
12	203	70	168	83	10	171	84	29
13	208	70	172	83	10	175	84	29
14	212	70	177	84	10	179	85	29
17	222	70	188	85	10	190	85	29
21	228	70	194	85	10	196	86	29
25	237	70	198	84	10	200	85	29
29	246	70	200	82	10	203	83	29
33	251	70	201	80	10	203	81	29
37	258	70	207	80	10	211	82	29
41	265	59[a]	207	78	10[b]	210	79	19[a]
45	272	59				213	78	16
49	284	59	231	81	10	213	75	15
53	299	59	248	83	10	219	73	15
57	311	59	252	81	10	220	71	15
61	315	59				217	69	15
65	328	59	258	79	10	220	67	12
Mean for weeks								
1-13	165		132	80		137	83	
14-37	236		195	83		197	84	
41-65	296		239	81		216	73	

[a] Interim evaluation occurred during week 40.
[b] Animals switched to undosed feed

Pathology and Statistical Analysis of Results

Summaries of the incidences of neoplasms and nonneoplastic lesions are shown in Tables E1 and E3 for male rats and Tables F1 and F3 for female rats. For statistical analyses, the incidences in the 9-month stop-exposure groups and the 15-month exposure groups at the end of 15 months are compared with the 15-month interim evaluation controls of the 2-year core study for male rats (Table E2a) and female rats (Table F2a). The incidences in the 15-month exposure groups are compared with the 9-month stop-exposure groups after 6 months of recovery for male rats (Table E2b) and female rats (Table F2b).

Progression or Regression of Chemical-Induced Lesions

Liver: Rats in the stop-exposure study exposed to 20,000 ppm 1-amino-2,4-dibromoanthraquinone in the feed for 9 or 15 months had chemical-related effects similar to those observed in rats exposed to concentrations up to 10,000 ppm in the 2-year core study. The absolute and relative liver weights of exposed males and females were significantly greater than those of the controls at both the 9-month interim and 15-month evaluations of the 15-month exposure groups (Tables H2 and H3). With respect to both neoplasms and nonneoplastic lesions in the liver, there was no evidence of regression in the incidence or severity of chemical-related pigmentation, focal hepatocellular alteration, or hepatocellular adenoma or carcinoma when administration of 1-amino--2,4-dibromoanthraquinone was discontinued after 9 months. The incidences and severity of liver lesions after 9 months of exposure were similar with and without a 6-month recovery period. The incidences of liver lesions in the 15-month exposure groups were greater than in the 9-month stop-exposure groups but the severities were comparable (Tables 16, E2a, and F2a).

TABLE 16
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Rats in the 9-Month, 9-Month with 6-Month Recovery, and 15-Month Exposure Evaluation of 1-Amino-2,4-dibromoanthraquinone

	9-Month Evaluation[a]		15-Month Evaluation[b]		
	0 ppm	20,000 ppm (9-Month Exposure)	0 ppm	20,000 ppm (9-Month Exposure Plus 6-Month Recovery)	20,000 ppm (15-Month Exposure)
Male					
Number Examined	10	10	10	10	20
Basophilic Focus[c]	0	6** (1.5)[d]	1 (1.0)	4 (1.0)	13** (1.5)
Clear Cell Focus	0	4* (1.0)	0	6** (1.6)	13** (1.2)
Eosinophilic Focus	0	0	1 (1.0)	0	2 (1.5)
Bile Duct Hyperplasia	1 (1.0)	7** (1.3)	10 (1.1)	7 (1.0)	19 (1.3)
Chronic and Chronic Active Periportal Inflammation	(1.0)	10 (1.0)	10 (1.1)	7 (1.7)	18 (1.9)
Pigmentation	0	10**(1.0)	0	8** (1.0)	18** (1.0)
Hepatocellular Adenoma	0	2	0	7**	8*
Hepatocellular Carcinoma	0	2	0	7**	19**
Hepatocellular Adenoma or Carcinoma	0	2	0	9**	20**
Female					
Number Examined	10	10	10	10	18
Basophilic Focus	1 (1.0)	3 (1.3)	8 (1.0)	6 (1.2)	13 (1.5)
Clear Cell Focus	0	1 (1.0)	0	5* (1.0)	13** (1.2)
Eosinophilic Focus	0	0	2 (1.5)	2 (1.0)	3 (1.7)
Bile Duct Hyperplasia	1 (1.0)	9** (1.1)	7 (1.1)	10 (1.5)	18* (1.6)
Chronic and Chronic Active Periportal Inflammation	6 (1.0)	10* (1.7)	10 (1.0)	9 (1.6)	18 (1.7)
Pigmentation	0	10**(1.2)	1 (1.0)	10** (1.0)	17** (1.2)
Hepatocellular Adenoma	0	2	0	6**	10**
Hepatocellular Carcinoma	0	1	0	6**	15**
Hepatocellular Adenoma or Carcinoma	0	2	0	8**	16**

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
** $P \leq 0.01$
[a] Controls from the 9-month interim evaluation of the 2-year core study were used for statistical comparison.
[b] Controls from the 15-month interim evaluation of the 2-year core study were used for statistical comparison.
[c] Number of animals with lesion
[d] Average severity grade of lesions in affected rats: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked

Large Intestine: The stop-exposure regimen had no effect on the development of adenomatous polyps (adenomas) of the large intestine. An adenomatous polyp was observed in the colon of one exposed male at 9 months. After 15 months, the incidence of adenomatous polyps of the rectum was significantly increased in the 9-month stop-exposure females (Tables 17 and F2a). At the 15-month evaluation, adenomatous polyps were observed in the rectums of three males in the 9-month stop-exposure group, seven males in the 15-month exposure group, five females in the 9-month stop-exposure group, and three females in the 15-month exposure group. No carcinomas of the colon or rectum were observed.

Kidney: At the 9-month and 15-month evaluations, the relative kidney weights of males and females in the 15-month exposure groups were significantly greater than those of the controls (Tables H2 and H3). In the exposed males and females at 9 months, kidney changes included pigmentation and minimal enlargement of some renal tubule cell nuclei (karyomegaly). In exposed males, there was also hyaline droplet accumulation and a slight increase in the severity of nephropathy. At 15 months, renal tubule epithelial pigmentation, karyomegaly, and increased severity of nephropathy and transitional cell hyperplasia of the renal pelvis were observed in exposed groups of males and females. The severities of these lesions were similar or slightly less severe in the stop-exposure groups than in those exposed continuously for 15 months. At the 15-month evaluation, renal tubule adenomas were observed in the 9-month stop-exposure and 15-month exposure groups of males and females (Tables 17, E1, and F1). In the 9-month

TABLE 17
Incidences of Neoplasms and Nonneoplastic Lesions of the Large Intestine and Kidney in Rats in the 9-Month, 9-Month with 6-Month Recovery, and 15-Month Exposure Evaluation of 1-Amino-2,4-dibromoanthraquinone

	9-Month Evaluation[a]		15-Month Evaluation[b]		
	0 ppm	20,000 ppm (9-Month Exposure)	0 ppm	20,000 ppm (9-Month Exposure Plus 6-Month Recovery)	20,000 ppm (15-Month Exposure)
Male					
Large Intestine, Colon[c]	10	10	10	10	20
Adenomatous Polyp (Adenoma)[d]	0	1	0	0	0
Large Intestine, Rectum	10	10	9	10	20
Adenomatous Polyp (Adenoma)	0	0	0	3	7*
Kidney	10	10	10	10	20
Nephropathy	10 (1.0)[e]	10 (1.8)	10 (2.0)	10 (2.0)	20 (2.5)
Transitional Cell Hyperplasia	0	0	0	1 (1.0)	11** (1.3)
Hyaline Droplet Accumulation	0	10** (2.1)	0	0	0
Pigmentation	0	10** (2.0)	0	9** (1.2)	20** (2.0)
Renal Tubule Hyperplasia	0	0	0	1 (1.0)	1 (1.0)
Renal Tubule Adenoma	0	0	0	3	2
Female					
Large Intestine, Rectum	10	10	10	10	17
Adenomatous Polyp (Adenoma)	0	0	0	5*	3
Kidney	10	10	10	10	18
Nephropathy	4 (1.0)	7 (1.0)	10 (1.7)	10 (2.2)	18 (2.1)
Transitional Cell Hyperplasia	0	0	3 (1.0)	1 (1.0)	5 (1.6)
Pigmentation	0	10** (2.0)	0	10** (1.9)	18** (2.0)
Renal Tubule Hyperplasia	0	0	0	2 (2.0)	2 (1.5)
Renal Tubule Adenoma	0	0	0	3	2

* Significantly different ($P \le 0.05$) from the control group by the Fisher exact test
** $P \le 0.01$
[a] Controls from the 9-month interim evaluation of the 2-year core study were used for statistical comparison.
[b] Controls from the 15-month interim evaluation of the 2-year core study were used for statistical comparison.
[c] Number of animals with organ examined microscopically
[d] Number of animals with lesion
[e] Average severity grade of lesions in affected rats: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked

stop-exposure and 15-month exposure groups, renal tubule hyperplasia and adenomas were observed in males and females. At 15 months, renal tubule adenomas were observed in three males and three females in the 9-month stop-exposure groups and in two males and two females in the 15-month exposure groups. No renal tubule carcinomas were found.

Urinary Bladder: A transitional cell papilloma was present in the urinary bladder of one exposed male at 9 months (Tables 18 and E1). When exposure was discontinued at 9 months, no chemical-related nonneoplastic lesions or neoplasms were present at 15 months. With continuous treatment, transitional cell hyperplasia and neoplasms of the urinary bladder developed by 15 months. In females, a minimal to mild transitional cell hyperplasia was observed at 9 months and did not completely regress with the cessation of exposure; a squamous cell papilloma was observed in one female at 15 months (Tables 18 and F1). In contrast, continuous exposure for 15 months resulted in an increased incidence and severity of transitional cell hyperplasia, as well as squamous metaplasia of the transitional epithelium and a fatty metaplasia (fat proliferation) of the stroma of the bladder wall.

Forestomach: Chemical-related lesions of the forestomach were not present in males or females at the 9-month interim evaluation. Hyperplasia, hyperkeratosis, inflammation, and ulceration were present in approximately 20% of exposed males, but not in the controls at 15 months (Table 18). A squamous cell papilloma was present in one male from the 15-month exposure group (Table E1). In female rats, forestomach lesions were not present in the control or 9-month stop-exposure groups. Hyperplasia, hyperkeratosis, and/or ulceration were observed in a few females in the 15-month exposure group; no neoplasms were present in the forestomach.

TABLE 18
Incidences of Neoplasms and Nonneoplastic Lesions of the Urinary Bladder and Forestomach in Rats in the 9-Month, 9-Month with 6-Month Recovery, and 15-Month Exposure Evaluation of 1-Amino-2,4-dibromoanthraquinone

	9-Month Evaluation[a]		15-Month Evaluation[b]		
	0 ppm	20,000 ppm (9-Month Exposure)	0 ppm	20,000 ppm (9-Month Exposure Plus 6-Month Recovery)	20,000 ppm (15-Month Exposure)
Male					
Urinary Bladder[c]	10	9	10	10	19
Fat Proliferation[d]	0	0	0	0	1 (3.0)[e]
Transitional Epithelial Hyperplasia	0	0	0	0	9** (1.9)
Squamous Cell Carcinoma	0	0	0	0	1
Transitional Epithelial Papilloma	0	1	0	0	3
Transitional Epithelial Carcinoma	0	0	0	0	1
Forestomach	10	10	10	10	20
Hyperkeratosis	0	0	0	2 (2.5)	1 (1.0)
Hyperplasia	0	0	0	2 (2.0)	3 (1.3)
Inflammation	0	0	0	1 (3.0)	1 (3.0)
Ulceration	0	0	0	2 (2.5)	0
Female					
Urinary Bladder	10	10	10	10	18
Fat Proliferation	0	0	0	0	2 (3.0)
Transitional Epithelial Hyperplasia	0	4* (1.5)	0	4* (1.8)	17** (2.6)
Transitional Epithelial Squamous Metaplasia	0	0	0	0	3 (2.7)
Squamous Cell Papilloma	0	0	0	0	1
Squamous Cell Carcinoma	0	0	0	1	4
Transitional Epithelial Papilloma	0	0	0	0	1
Transitional Epithelial Carcinoma	0	0	0	0	1
Forestomach	10	10	10	10	18
Hyperkeratosis	0	0	0	0	1 (1.0)
Hyperplasia	0	0	0	0	6* (1.0)
Ulceration	0	0	0	0	1 (3.0)

* Significantly different ($P \le 0.05$) from the control group by the Fisher exact test
** $P \le 0.01$
[a] Controls from the 9-month interim evaluation of the 2-year core study were used for statistical comparison.
[b] Controls from the 15-month interim evaluation of the 2-year core study were used for statistical comparison.
[c] Number of animals with organ examined microscopically
[d] Number of animals with lesion
[e] Average severity grade of lesions in affected rats: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked

MICE

13-WEEK STUDY

One 25,000 ppm male (week 11) and one 5,000 ppm male (week 13) died during the study (Table 19). Neither death was chemical related. One 10,000 ppm female was accidently killed. Final mean body weights of exposed groups of male and female mice were similar to those of the controls. Mean body weight gains of exposed groups of males and females were generally greater than those of the controls. Feed consumption by exposed mice was similar to that by the controls. Dietary levels of 2,500, 5,000, 10,000, 25,000, and 50,000 ppm delivered average daily doses of approximately 500, 1,080, 1,850, 6,200, and 10,600 mg 1-amino-2,4-dibromoanthraquinone/kg body weight to males and approximately 660, 1,150, 2,600, 5,900, and 11,700 mg/kg to females. Reddened fur was observed in 10,000, 25,000, and 50,000 ppm mice as early as day 4 in males and day 5 in females and was observed throughout the study. No other clinical observations were attributed to 1-amino-2,4-dibromoanthraquinone.

TABLE 19
Survival, Mean Body Weights, and Feed Consumption of Mice in the 13-Week Feed Study of 1-Amino-2,4-dibromoanthraquinone

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 13
Male							
0	10/10	23.7 ± 0.4	30.5 ± 0.6	6.9 ± 0.7		5.8	6.0
2,500	10/10	23.5 ± 0.3	30.6 ± 0.6	7.1 ± 0.6	100	5.3	5.6
5,000	9/10[d]	23.5 ± 0.4	30.7 ± 0.7	7.3 ± 0.5	101	6.1	5.6
10,000	10/10	23.7 ± 0.5	32.1 ± 0.4	8.4 ± 0.4	105	5.5	4.8
25,000	9/10[e]	23.4 ± 0.3	30.9 ± 0.6	7.5 ± 0.5	101	6.3	7.1
50,000	10/10	23.4 ± 0.3	31.5 ± 0.4	8.1 ± 0.4	103	6.1	5.5
Female							
0	10/10	18.2 ± 0.3	24.0 ± 0.2	5.8 ± 0.2		4.8	6.2
2,500	10/10	18.1 ± 0.3	24.7 ± 0.6	6.6 ± 0.4	103	5.0	6.4
5,000	10/10	18.4 ± 0.3	25.0 ± 0.6	6.5 ± 0.4	104	4.3	5.7
10,000	9/10[f]	18.3 ± 0.3	25.0 ± 0.4	6.5 ± 0.3	104	4.5	6.7
25,000	10/10	18.2 ± 0.2	23.6 ± 0.3	5.4 ± 0.3	98	3.8	6.1
50,000	10/10	18.4 ± 0.3	24.7 ± 0.4	6.3 ± 0.2	103	4.0	6.1

[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] Feed consumption is expressed as grams of feed consumed per animal per day.
[d] Week of death: 13
[e] Week of death: 11
[f] Week of death: 2 (accidental)

Absolute and relative liver weights of 5,000, 10,000, 25,000, and 50,000 ppm male and female mice were significantly greater than those of the controls (Table H4). Absolute and relative kidney weights of 25,000 and 50,000 ppm males were significantly lower than those of the controls. Observations at necropsy included red staining of the gastrointestinal tract and its contents in all exposed male mice except those in the 2,500 ppm group and red staining in the kidney and urine. These findings were observed less frequently in females than in males.

Chemical-related lesions were present in the liver (Table 20). There were increased incidences of centrilobular hypertrophy in the 10,000, 25,000, and 50,000 ppm males with a dose-related increased severity. Minimal gold to brown pigment granules were present in the cytoplasm of hepatocytes of all exposed groups of males. Pigment was generally located in the centrilobular portion of the hepatic lobule. Pigment similar to that in male mice was present in just a few hepatocytes in the liver of one female in the 25,000-ppm group and one 50,000 ppm female.

TABLE 20
Incidences of Nonneoplastic Lesions of the Liver in Mice in the 13-Week Feed Study of 1-Amino-2,4-dibromoanthraquinone

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	25,000 ppm	50,000 ppm
Male						
Number Examined	10	10	10	10	10	10
Centrilobular Hypertrophy[a]	0	0	0	8** (1.8)[b]	8** (2.0)	10** (2.8)
Pigmentation	0	5* (1.0)	8** (1.0)	8** (1.0)	8** (1.0)	10** (1.1)
Female						
Number Examined	10	10	10	10	10	10
Pigmentation	0	0	0	0	1 (1.0)	1 (1.0)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected mice: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked

Dose Selection Rationale: As no 1-amino-2,4-dibromoanthraquinone-related adverse effects were observed in feed consumption, mean body weights, or survival, exposure concentrations chosen for the 2-year study were based mainly on the frequency and especially the severity of centrilobular hypertrophy of the liver in male mice. Because only lesions of mild severity were observed in the 10,000 and 25,000 ppm groups, and lesions of moderate severity were observed in the 50,000 ppm group, and these were not life-jeopardizing lesions, exposure concentrations selected for the 2-year study of 1-amino-2,4-dibromoanthraquinone in mice were 0, 10,000, and 20,000 ppm. Other considerations include consistency among males and females (since females could have been given higher exposure concentrations) and correspondence to the exposure concentrations selected for the start-stop, progression/regression experiments (stop-exposure evaluation) in rats.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 21 and in the Kaplan-Meier survival curves in Figure 6. Survival of exposed male mice was significantly lower than that of the controls; survival of exposed female mice was similar to that of the controls.

Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of exposed groups of male mice were lower than that of the controls after week 9; mean body weights of exposed groups of females were lower than that of the controls after week 17 (Figure 7, Tables 22 and 23). Final mean body weights of exposed groups of males were 15% to 17% lower than that of the controls; final mean body weights of exposed females were 14% to 19% lower than that of the controls. Feed consumption by exposed males and females was generally similar to that by the controls (Tables J5 and J6). Dietary levels of 10,000 and 20,000 ppm were estimated to deliver daily doses of approximately 1,700 and 3,500 mg 1-amino-2,4-dibromoanthraquinone/kg body weight to males and 2,000 and 4,400 mg/kg to females. Discoloration of the fur, urine, and feces was evident in all exposed groups as early as day 8.

TABLE 21
Survival of Mice in the 2-Year Feed Study of 1-Amino-2,4-dibromoanthraquinone

	0 ppm	10,000 ppm	20,000 ppm
Male			
Animals initially in study	60	60	60
15-Month interim evaluation[a]	10	9	10
Accidental death[a]	0	1	0
Moribund	7	23	21
Natural deaths	3	5	6
Animals surviving to study termination	40	22	23
Percent probability of survival at end of study[b]	81	45	47
Mean survival (days)[c]	656	620	609
Survival analyses[d]	P=0.001	P<0.001	P<0.001
Female			
Animals initially in study	60	60	60
15-Month interim evaluation[a]	10	10	10
Moribund	5	11	11
Natural deaths	6	5	6
Animals surviving to study termination	39[e]	34[e]	33
Percent probability of survival at end of study	78	69	66
Mean survival (days)	659	649	657
Survival analyses	P=0.234	P=0.381	P=0.259

[a] Censored from survival analyses
[b] Kaplan-Meier determinations based on the number of animals alive on first day of terminal sacrifice
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[d] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed columns.
[e] Includes one female that died during the last week of the study.

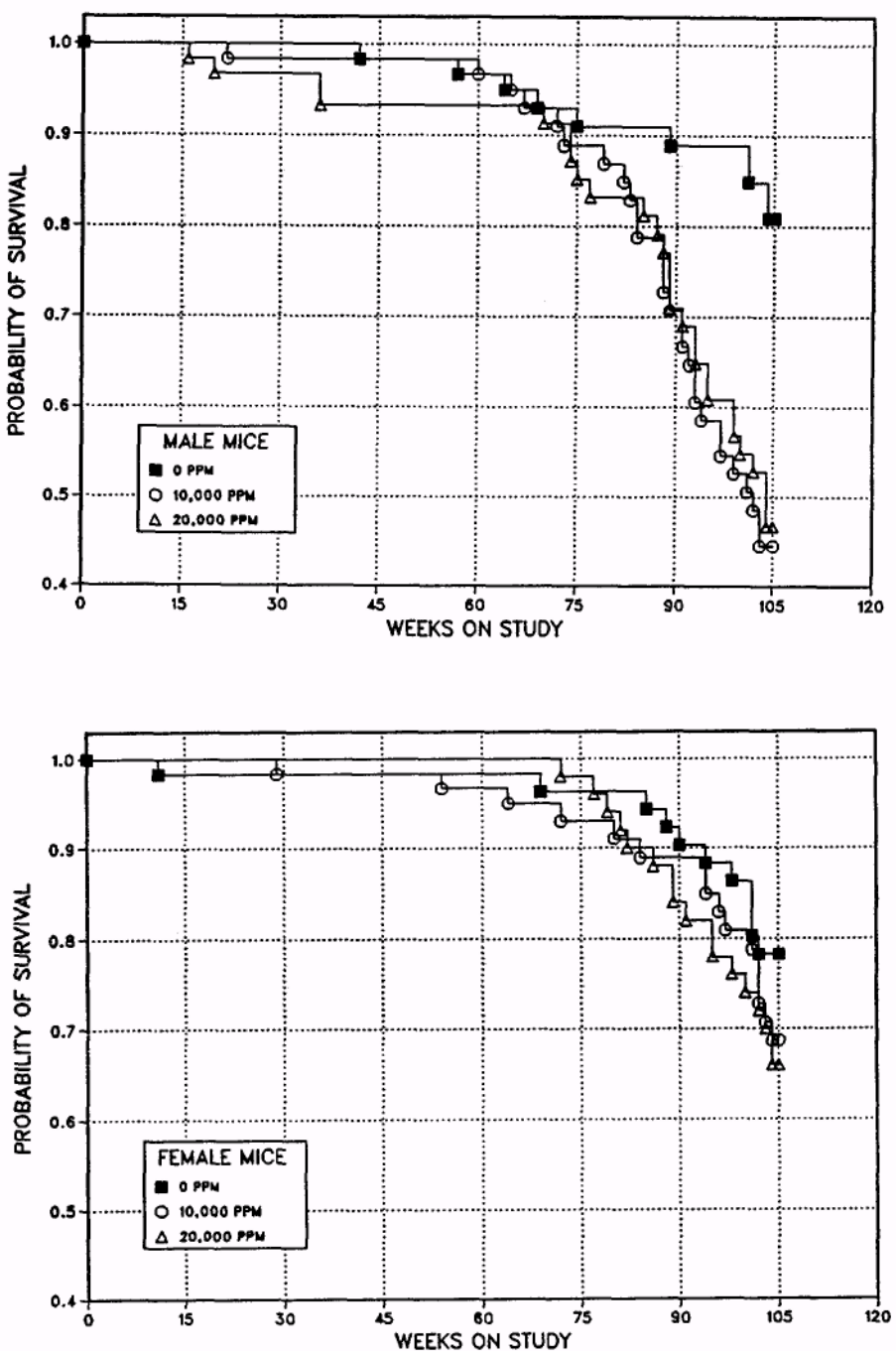


FIGURE 6
Kaplan-Meier Survival Curves for Mice Administered l-Amino-2,4-dibromoanthraquinone in Feed for 2 Years

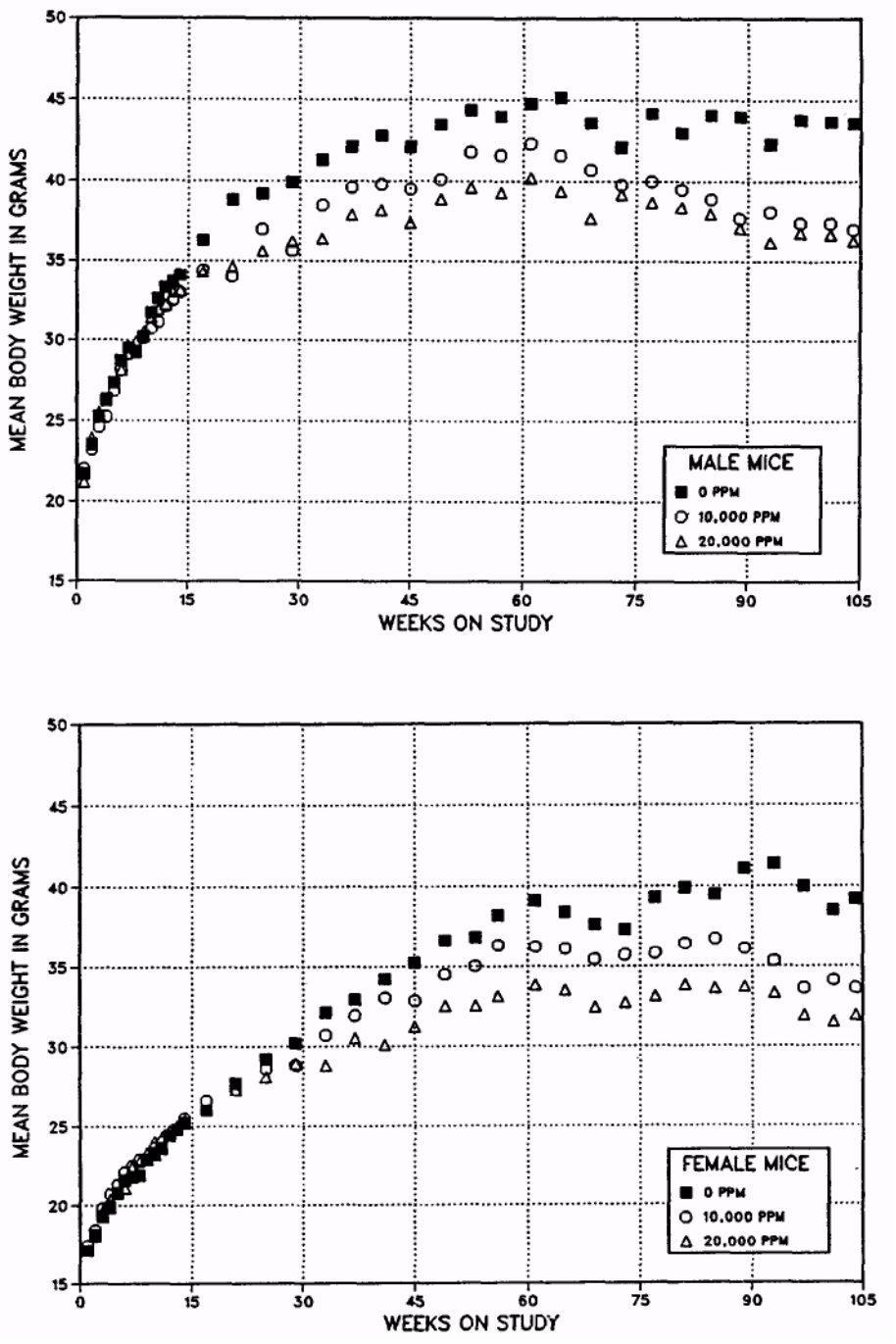


FIGURE 7
Growth Curves for Mice Administered l-Amino-2,4-dibromoanthraquinone in Feed for 2 Years

TABLE 22
Mean Body Weights and Survival of Male Mice in the 2-Year Feed Study of 1-Amino-2,4-dibromoanthraquinone

Weeks on Study	0 ppm		10,000 ppm			20,000 ppm		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	21.7	60	22.0	101	60	21.2	98	60
2	23.5	60	23.2	99	60	23.9	102	60
3	25.2	60	24.6	98	60	25.5	101	60
4	26.3	60	25.2	96	60	26.2	100	60
5	27.3	60	26.9	99	60	27.3	100	60
6	28.7	60	28.1	98	60	28.1	98	60
7	29.5	60	29.2	99	60	29.6	100	60
8	29.2	60	29.3	100	60	29.9	102	60
9	30.2	60	30.1	100	60	30.5	101	60
10	31.7	60	30.7	97	60	31.4	99	60
11	32.6	60	31.1	95	60	31.9	98	60
12	33.3	60	32.1	96	60	32.2	97	60
13	33.7	60	32.5	96	60	33.0	98	60
14	34.1	60	33.0	97	60	33.1	97	60
17	36.3	60	34.4	95	60	34.3	95	59
21	38.8	60	34.0	88	60	34.6	89	58
25	39.2	60	37.0	94	59	35.6	91	58
29	39.9	60	35.6	89	59	36.2	91	58
33	41.3	60	38.5	93	59	36.4	88	58
37	42.1	60	39.6	94	59	37.9	90	56
41	42.8	60	39.8	93	59	38.2	89	56
45	42.1	59	39.5	94	59	37.4	89	56
49	43.5	59	40.1	92	59	38.9	89	56
53	44.4	59	41.8	94	59	39.6	89	56
57	44.0	59	41.6	95	59	39.3	89	56
61	44.8	58	42.3	94	58	40.2	90	56
65	45.2	57	41.6	92	58	39.4	87	56
69[a]	43.6	47	40.7	93	46	37.7	87	46
73	42.1	46	39.8	95	45	39.2	93	45
77	44.2	45	40.0	91	44	38.7	88	42
81	43.0	45	39.5	92	43	38.4	89	41
85	44.1	45	38.9	88	39	38.0	86	41
89	44.0	45	37.7	86	36	37.1	84	38
93	42.3	44	38.1	90	32	36.2	86	34
97	43.8	44	37.4	85	29	36.8	84	30
101	43.7	44	37.4	86	26	36.7	84	27
104	43.6	42	37.0	85	22	36.3	83	26
Mean for weeks								
1-13	28.7		28.1	98		28.5	99	
14-52	40.0		37.2	93		36.3	91	
53-104	43.8		39.6	90		38.1	87	

[a] Interim evaluation occurred during week 66.

TABLE 23
Mean Body Weights and Survival of Female Mice in the 2-Year Feed Study of 1-Amino-2,4-dibromoanthraquinone

Weeks on Study	0 ppm		10,000 ppm			20,000 ppm		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	17.1	60	17.4	102	60	17.1	100	60
2	18.1	60	18.4	102	60	18.0	99	60
3	19.3	60	19.8	103	60	19.6	102	60
4	19.9	60	20.7	104	60	20.4	103	60
5	20.8	60	21.3	102	60	20.8	100	60
6	21.6	60	22.1	102	60	21.1	98	60
7	21.8	60	22.5	103	60	22.4	103	60
8	21.9	60	22.9	105	60	22.8	104	60
9	22.9	60	22.9	100	60	23.3	102	60
10	23.2	60	23.6	102	60	24.0	103	60
11	23.6	60	24.0	102	60	24.3	103	60
12	24.4	59	24.4	100	60	24.7	101	60
13	24.8	59	24.8	100	60	25.0	101	60
14	25.2	59	25.5	101	60	25.4	101	60
17	26.0	59	26.6	102	60	26.0	100	60
21	27.7	59	27.3	99	60	27.3	99	60
25	29.2	59	28.6	98	60	28.1	96	60
29	30.2	59	28.8	95	60	28.9	96	60
33	32.1	59	30.7	96	59	28.8	90	60
37	32.9	59	31.9	97	59	30.5	93	60
41	34.2	59	33.0	97	59	30.1	88	60
45	35.2	59	32.8	93	59	31.2	89	60
49	36.6	59	34.5	94	59	32.5	89	60
53	36.8	59	35.0	95	59	32.5	88	60
56	38.2	59	36.3	95	58	33.1	87	60
61	39.1	59	36.2	93	58	33.8	86	60
65	38.4	59	36.1	94	57	33.5	87	60
69[a]	37.6	49	35.4	94	47	32.4	86	50
73	37.3	48	35.7	96	46	32.7	88	49
77	39.3	48	35.8	91	46	33.1	84	49
81	39.9	48	36.4	91	45	33.8	85	47
85	39.5	48	36.7	93	44	33.6	85	45
89	41.1	46	36.1	88	44	33.7	82	44
93	41.4	45	35.3	85	44	33.3	80	41
97	40.0	44	33.6	84	41	31.9	80	39
101	38.5	43	34.1	89	40	31.5	82	37
104	39.2	39	33.6	86	35	31.9	81	35
Mean for weeks								
1-13	21.5		21.9	102		21.8	101	
14-52	30.9		30.0	97		28.8	93	
53-104	39.0		35.5	91		32.9	84	

[a] Interim evaluation occurred during week 65.

Pathology and Statistical Evaluation

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the liver, forestomach, lung, kidney, uterus, and pituitary gland of mice. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one exposure group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

Liver: At the 15-month interim evaluation, absolute and relative liver weights of exposed groups of females were significantly greater than those of the controls (Table H5). Hepatocellular adenomas and carcinomas were observed in exposed groups of males and females; none were present in the controls (Tables 24, C1, and D1). At the end of the 2-year study, the incidences of hepatocellular adenoma and hepatocellular carcinoma in exposed groups of males and females were significantly increased (Tables 24, C3, and D3). The incidences of multiple hepatocellular adenomas or multiple hepatocellular carcinomas in exposed groups of males and females were also increased (Tables 24, C1, and D1). Incidences of hepatocellular adenomas or carcinomas (combined) in exposed groups of males and females exceeded the NTP historical ranges for feed study controls (Tables 24, C4a, and D4a). In addition to hepatocellular adenomas and carcinomas, a small number of hepatoblastomas occurred in exposed groups of males and females (Tables 24, C1, and D1). These malignant hepatocellular neoplasms contained areas resembling hepatocellular carcinoma; in addition, there were prominent lobules or nodular foci separated by vascular channels and composed of undifferentiated cells (Plates 9 and 10). The neoplastic cells were elongated with a scant amount of darkly staining cytoplasm and oval hyperchromatic nuclei. Cellular pleomorphism and mitotic figures were commonly present.

At the 15-month interim evaluation and at the end of the 2-year study, the incidences of centrilobular hypertrophy of hepatocytes in exposed groups of males were significantly increased, and the incidences of hepatocellular pigmentation were significantly increased in exposed groups of males and females (Tables 24, C5, and D5). This brown, granular pig-ment resembled that found in the 13-week studies of 1-amino-2,4-dibromoanthraquinone; histochemical procedures were not repeated during this 2-year study. The incidences of clear cell focus in exposed groups of female mice were significantly increased at the end of the 2-year study.

TABLE 24
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Feed Study of 1-Amino-2,4-dibromoanthraquinone

	0 ppm	10,000 ppm	20,000 ppm
Male			
15-Month Interim Evaluation			
Number Examined	10	9	10
Centrilobular Hepatocyte Hypertrophy[a]	0	9** (2.9)[b]	8** (2.9)
Pigmentation	0	9** (1.1)	10** (1.2)
Hepatocellular Adenoma	0	2	4*
Hepatocellular Adenoma or Carcinoma	0	3	4*
2-Year Study			
Number Examined	50	51	50
Basophilic Focus	0	4 (1.0)	3 (1.0)
Centrilobular Hepatocyte Hypertrophy	0	17** (2.0)	13** (2.0)
Clear Cell Focus	4 (1.3)	4 (1.0)	2 (1.0)
Eosinophilic Focus	0	6** (1.5)	1 (1.0)
Pigmentation	1 (3.0)	50** (1.1)	47** (1.4)
Hepatocellular Adenoma (Multiple)	6	29**	31**
Hepatocellular Adenoma (Single or Multiple)			
Overall rate[c]	10/50 (20%)	38/51 (75%)	39/50 (78%)
Terminal rate[d]	9/40 (23%)	20/22 (91%)	21/23 (91%)
Adjusted rate[e]	24.3%	94.7%	95.0%
First incidence (days)	723	451	484
Logistic regression test[f]	P<0.001	P<0.001	P<0.001
Hepatocellular Carcinoma (Multiple)	1	3	9**
Hepatocellular Carcinoma (Single or Multiple)			
Overall rate	9/50 (18%)	18/51 (35%)	21/50 (42%)
Terminal rate	7/40 (18%)	10/22 (45%)	9/23 (39%)
Adjusted rate	21.1%	58.1%	58.4%
First incidence (days)	445	505	535
Logistic regression test	P=0.002	P=0.017	P=0.003
Hepatocellular Adenoma or Carcinoma[g]			
Overall rate	18/50 (36%)	43/51 (84%)	42/50 (84%)
Terminal rate	15/40 (38%)	21/22 (95%)	22/23 (96%)
Adjusted rate	41.7%	97.7%	97.7%
First incidence (days)	445	451	484
Logistic regression test	P<0.001	P<0.001	P<0.001
Hepatoblastoma	0	3	5*

(continued)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test (15-month interim evaluation) or the logistic regression test (2-year study)
** $P \leq 0.01$
(T)Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected mice: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked
[c] Number of animals with neoplasm per number of animals with liver examined microscopically

TABLE 24
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Feed Study of 1-Amino-2,4-dibromoanthraquinone (continued)

	0 ppm	10,000 ppm	20,000 ppm
Female			
15-Month Interim Evaluation			
Number Examined	10	10	10
Pigmentation	0	10** (1.0)	9** (1.0)
Hepatocellular Adenoma (Multiple)	0	1	4*
Hepatocellular Adenoma (Single or Multiple)	0	2	7**
Hepatocellular Adenoma or Carcinoma	0	2	8**
2-Year Study			
Number Examined	50	50	50
Basophilic Focus	0	4 (1.3)	5* (1.2)
Clear Cell Focus	0	10** (1.2)	9** (1.6)
Eosinophilic Focus	0	4* (1.5)	2 (2.5)
Pigmentation	0	44** (1.1)	49** (1.6)
Hepatocellular Adenoma (Multiple)	0	40**	45**
Hepatocellular Adenoma (Single or Multiple)			
Overall rate	6/50 (12%)	45/50 (90%)	49/50 (98%)
Terminal rate	6/39 (15%)	32/34 (94%)	33/33 (100%)
Adjusted rate	15.4%	95.7%	100.0%
First incidence (days)	729 (T)	442	501
Logistic regression test	P<0.001	P<0.001	P<0.001
Hepatocellular Carcinoma (Multiple)	0	13**	13**
Hepatocellular Carcinoma (Single or Multiple)			
Overall rate	0/50 (0%)	23/50 (46%)	27/50 (54%)
Terminal rate	0/39 (0%)	17/34 (50%)	16/33 (48%)
Adjusted rate	0.0%	57.2%	60.8%
First incidence (days)	–[h]	503	538
Logistic regression test	P<0.001	P<0.001	P<0.001
Hepatocellular Adenoma or Carcinoma[i]			
Overall rate	6/50 (12%)	46/50 (92%)	50/50 (100%)
Terminal rate	6/39 (15%)	33/34 (97%)	33/33 (100%)
Adjusted rate	15.4%	97.9%	100.0%
First incidence (days)	729 (T)	442	501
Logistic regression test	P<0.001	P<0.001	P<0.001
Hepatoblastoma	0	0	2

[d] Observed incidence in animals surviving until the end of the study
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[f] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards these lesions as nonfatal.
[g] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 531/1,466 (36.2% ± 14.1%); range, 10%-68%
[h] Not applicable; no neoplasms in animal group
[i] Historical incidence: 247/1,462 (16.9% ± 10.7%); range, 3%-42%

Forestomach: Squamous cell papillomas were observed in 20,000 ppm males and in 10,000 and 20,000 ppm females at the 15-month interim evaluation (Tables 25, C1, and D1). At the end of the 2-year study, the incidences of squamous cell papilloma and squamous cell carcinoma in exposed groups of males and females were significantly increased (Tables 25, C3, and D3). The incidences of multiple squamous cell papilloma in 20,000 ppm males and females were also significantly increased in the 2-year study (Tables 25, C1, and D1). Incidences of squamous cell papilloma or carcinoma (combined) in exposed groups of males and females were significantly greater than those in the controls and exceeded the NTP historical ranges for feed study controls (Tables 25, C4b, and D4b). Compared to the exophytic masses with well-differentiated squamous epithelium typical of the squamous cell papillomas, the squamous cell carcinomas were locally invasive neoplasms that sometimes resulted in perforation of the forestomach (Plate 11). Frequently, a squamous cell carcinoma appeared to arise at the base of a squamous cell papilloma. Metastatic neoplasms arising from squamous cell carcinomas of the forestomach were observed in the coagulating glands, colon, duodenum, epididymis, gallbladder, glandular stomach, jejunum, kidney, liver, lung, ovary, pancreas, prostate gland, spleen, testis, and thymus of exposed mice (Tables C1 and D1).

Nonneoplastic lesions of the forestomach included acanthosis, hyperkeratosis, and basal cell hyperplasia (Tables 25, C5, and D5). At the 15-month interim evaluation, there were exposure-related increases in the incidences and severities of acanthosis and hyperkeratosis in exposed groups of males and females. At the end of the 2-year study, the incidences and severities of these lesions in exposed groups of males and females were generally greater than those in the controls.

TABLE 25
Incidences of Neoplasms and Nonneoplastic Lesions of the Forestomach in Mice in the 2-Year Feed Study of 1-Amino-2,4-dibromoanthraquinone

	0 ppm	10,000 ppm	20,000 ppm
Male			
15-Month Interim Evaluation			
Number Examined	9	9	10
Acanthosis (Hyperplasia)[a]	0	2 (1.0)[b]	3 (2.0)
Basal Cell Hyperplasia	0	1 (1.0)	1 (1.0)
Hyperkeratosis	1 (2.0)	2 (1.0)	3 (2.0)
Inflammation	0	0	3 (1.2)
Squamous Cell Papilloma	0	0	5*
2-Year Study			
Number Examined	50	50	50
Acanthosis	1 (1.0)	9** (1.1)	4 (2.0)
Basal Cell Hyperplasia	0	0	2 (1.5)
Hyperkeratosis	1 (1.0)	7* (1.0)	6 (1.8)
Inflammation	2 (1.5)	6 (1.2)	13** (1.5)
Squamous Cell Papilloma (Multiple)	0	2	5*
Squamous Cell Papilloma (Single or Multiple)			
Overall rate[c]	0/50 (0%)	13/51 (25%)	16/50 (32%)
Terminal rate[d]	0/40 (0%)	10/22 (45%)	11/23 (48%)
Adjusted rate[e]	0.0%	51.0%	55.6%
First incidence (days)	–[g]	613	606
Logistic regression test[f]	P<0.001	P<0.001	P<0.001
Squamous Cell Carcinoma			
Overall rate	0/50 (0%)	12/51 (24%)	13/50 (26%)
Terminal rate	0/40 (0%)	4/22 (18%)	4/23 (17%)
Adjusted rate	0.0%	36.5%	37.7%
First incidence (days)	–	505	523
Logistic regression test	P<0.001	P<0.001	P<0.001
Squamous Cell Papilloma or Carcinoma[h]			
Overall rate	0/50 (0%)	19/51 (37%)	27/50 (54%)
Terminal rate	0/40 (0%)	11/22 (50%)	14/23 (61%)
Adjusted rate	0.0%	61.2%	73.9%
First incidence (days)	–	505	523
Logistic regression test	P<0.001	P<0.001	P<0.001

(continued)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test (15-month interim evaluation) or the logistic regression test (2-year study)
** $P \leq 0.01$
(T)Terminal sacrifice

TABLE 25
Incidences of Neoplasms and Nonneoplastic Lesions of the Forestomach in Mice in the 2-Year Feed Study of 1-Amino-2,4-dibromoanthraquinone (continued)

	0 ppm	10,000 ppm	20,000 ppm
Female			
15-Month Interim Evaluation			
Number Examined	10	10	10
Acanthosis (Hyperplasia)	0	1 (1.0)	8** (2.0)
Basal Cell Hyperplasia	0	0	2 (1.5)
Hyperkeratosis	0	2 (1.0)	7** (2.0)
Inflammation	0	1 (1.0)	5* (1.6)
Squamous Cell Papilloma	0	4*	2
2-Year Study			
Number Examined	48	50	50
Acanthosis (Hyperplasia)	9 (1.7)	15 (1.7)	19* (1.6)
Basal Cell Hyperplasia	0	7* (1.4)	3 (1.7)
Hyperkeratosis	10 (1.4)	14 (1.4)	17 (1.5)
Inflammation	7 (1.4)	10 (1.4)	21** (1.7)
Squamous Cell Papilloma (Multiple)	0	4	14**
Squamous Cell Papilloma (Single or Multiple)			
Overall rate	2/50 (4%)	16/50 (32%)	27/50 (54%)
Terminal rate	2/39 (5%)	12/34 (35%)	23/33 (70%)
Adjusted rate	5.1%	41.7%	72.4%
First incidence (days)	729 (T)	671	538
Logistic regression test	P<0.001	P<0.001	P<0.001
Squamous Cell Carcinoma			
Overall rate	0/50 (0%)	12/50 (24%)	11/50 (22%)
Terminal rate	0/39 (0%)	8/34 (24%)	5/33 (15%)
Adjusted rate	0.0%	30.9%	27.3%
First incidence (days)	–	587	501
Logistic regression test	P=0.002	P<0.001	P<0.001
Squamous Cell Papilloma or Carcinoma[i]			
Overall rate	2/50 (4%)	25/50 (50%)	34/50 (68%)
Terminal rate	2/39 (5%)	18/34 (53%)	25/33 (76%)
Adjusted rate	5.1%	60.7%	80.5%
First incidence (days)	729	587	501
Logistic regression test	P<0.001	P<0.001	P<0.001

[a] Number of animals with lesion
[b] Average severity grade of lesions in affected mice: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked
[c] Number of animals with neoplasm per number of animals necropsied
[d] Observed incidence in animals surviving until the end of the study
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[f] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards these lesions as nonfatal.
[g] Not applicable; no neoplasms in animal group
[h] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 22/1,474 (1.5% ± 2.0%); range, 0%-6%
[i] Historical incidence: 33/1,470 (2.2% ± 3.1%); range, 0%-14%

Lung: Alveolar/bronchiolar adenomas were observed in exposed groups of males and females at the 15-month interim evaluation (Tables 26, C1, and D1). During the 2-year study, the incidences of alveolar/bronchiolar adenomas in exposed groups of males and females were significantly increased (Tables 26, C3, and D3). In male mice, the incidences of multiple alveolar/bronchiolar adenomas in exposed groups were significantly greater than that in the controls (Tables 26, C1, and D1). The incidences of alveolar/bronchiolar adenoma in exposed groups of males and females exceeded the NTP historical ranges for feed study controls (Tables 26, C4c, and D4c). The alveolar/bronchiolar adenomas were generally well-circumscribed, expansile masses that slightly compressed the surrounding normal pulmonary alveolar tissue (Plate 12). Well-differentiated cuboidal to columnar epithelial cells formed papillary structures or solid foci that filled alveolar spaces.

TABLE 26
Incidences of Neoplasms of the Lung in Mice in the 2-Year Feed Study of 1-Amino-2,4-dibromoanthraquinone

	0 ppm	10,000 ppm	20,000 ppm
Male			
15-Month Interim Evaluation			
Number Examined	10	9	10
Alveolar/bronchiolar Adenoma[a]	0	3	5*
2-Year Study			
Number Examined	50	51	50
Alveolar/bronchiolar Hyperplasia	1 (1.0)[b]	0	4 (1.3)
Alveolar/bronchiolar Adenoma (Multiple)	0	6**	9**
Alveolar/bronchiolar Adenoma (Single or Multiple)			
Overall rate[c]	7/50 (14%)	26/51 (51%)	24/50 (48%)
Terminal rate[d]	6/40 (15%)	12/22 (55%)	12/23 (52%)
Adjusted rate[e]	16.8%	71.0%	66.5%
First incidence (days)	445	578	248
Logistic regression test[f]	P<0.001	P<0.001	P<0.001
Alveolar/bronchiolar Carcinoma			
Overall rate	3/50 (6%)	4/51 (8%)	1/50 (2%)
Terminal rate	2/40 (5%)	2/22 (9%)	0/23 (0%)
Adjusted rate	6.9%	15.9%	3.0%
First incidence (days)	393	673	648
Logistic regression test	P=0.259N	P=0.512	P=0.251N
Alveolar/bronchiolar Adenoma or Carcinoma[g]			
Overall rate	10/50 (20%)	28/51 (55%)	25/50 (50%)
Terminal rate	8/40 (20%)	13/22 (59%)	12/23 (52%)
Adjusted rate	23.3%	75.0%	67.5%
First incidence (days)	393	578	248
Logistic regression test	P<0.001	P<0.001	P=0.002

(continued)

TABLE 26
Incidences of Neoplasms of the Lung in Mice in the 2-Year Feed Study of 1-Amino-2,4-dibromoanthraquinone (continued)

	0 ppm	10,000 ppm	20,000 ppm
Female			
15-Month Interim Evaluation			
Number Examined	10	10	10
Alveolar/bronchiolar Adenoma	0	3	2
2-Year Study			
Number Examined	50	50	49
Alveolar/bronchiolar Hyperplasia	0	0	1 (1.0)
Alveolar/bronchiolar Adenoma (Multiple)	0	2	1
Alveolar/bronchiolar Adenoma (Single or Multiple)			
Overall rate	4/50 (8%)	17/50 (34%)	13/49 (27%)
Terminal rate	3/39 (8%)	14/34 (41%)	9/33 (27%)
Adjusted rate	9.8%	45.6%	33.5%
First incidence (days)	685	587	538
Logistic regression test	P=0.017	P=0.001	P=0.015
Alveolar/bronchiolar Carcinoma	0	0	2
Alveolar/bronchiolar Adenoma or Carcinoma[h]			
Overall rate	4/50 (8%)	17/50 (34%)	15/49 (31%)
Terminal rate	3/39 (8%)	14/34 (41%)	10/33 (30%)
Adjusted rate	9.8%	45.6%	37.9%
First incidence (days)	685	587	538
Logistic regression test	P=0.006	P=0.001	P=0.005

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test (15-month interim evaluation) or the logistic regression test (2-year study)
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected mice: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked
[c] Number of animals with neoplasm per number of animals with lung examined microscopically
[d] Observed incidence in animals surviving until the end of the study
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[f] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards these lesions as nonfatal. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[g] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 265/1,469 (18.0% ± 7.6%); range, 4%-32%
[h] Historical incidence: 110/1,469 (7.5% ± 5.0%); range, 2%-26%

Kidney: Pigmentation was present in the kidneys of most mice after 2 years of exposure to 1-amino-2,4-dibromoanthraquinone (males: 0 ppm, 0/50; 10,000 ppm, 42/51; 20,000 ppm, 43/50; females: 0/50, 43/50, 43/50; Tables C5 and D5). This brown, granular pigment in the renal tubule epithelium and tubule lumina resembled the pigment described in the liver. There were no other chemical-related lesions in the kidney.

Uterus: There was a significant, but not exposure-related, increase in the incidence of uterine polyps or sarcomas (combined) (0/50, 5/50, 0/50; Table D1) in the 10,000 ppm females. Although this incidence (10%) was above the average for historical controls (3.5%), the combined incidence was within the historical control range (0%-16%; Table D4d). This marginal increase was not considered to be chemical related. There were no chemical-related nonneoplastic lesions in the reproductive tract (Table D5).

Pituitary Gland: A significant, but not exposure-related, increase in the incidence of adenoma of the pituitary gland (pars distalis) was observed in the 10,000 ppm females (1/43, 9/45, 4/43; Table D1). The incidence of adenoma (20%) in the 10,000 ppm females is slightly above the average for historical controls (15.2%), but is within the historical range (2%-36%; Table D4e). This marginal increase was not considered to be chemical related. The incidence of hyperplasia of the pars distalis of the pituitary gland was also increased in the 10,000-ppm females (7/43, 22/45, 7/43; Table D5).

DISPOSITION AND METABOLISM STUDIES

Adult male F344/N rats received [^{14}C]-labeled 1-amino-2,4-dibromoanthraquinone as a single intravenous dose of 0.4 mg 1-amino-2,4-dibromoanthraquinone/kg body weight or as a single oral dose of 2, 23, 118, 814, or 1,473 mg/kg. After excreta were collected for 72 hours, the animals were killed, and tissues were removed for analysis. Additional animals that received intravenous doses of 1-amino-2,4-dibromoanthraquinone were killed 0.25, 0.75, 2, 6, or 24 hours after chemical administration, and their tissues were analyzed. A 6-hour bile cannulation study was also performed.

From day 0 through day 3 after intravenous administration of [^{14}C]-1-amino-2,4-dibromoanthraquinone, about 50% of the ^{14}C was excreted in the feces, 15% in the urine, and 6% in expired air. Unmetabolized 1-amino-2,4-dibromoanthraquinone accounted for less than 3% of the excreted ^{14}C after intravenous administration. The amount of an oral dose that was absorbed was calculated from the percent of the dose that was excreted in expired air or urine after oral administration versus the percent of the dose excreted after intravenous administration. Excretion of ^{14}C in expired air yielded the most consistent results. For oral doses greater than or equal to 2 mg/kg, the amount of the dose that was absorbed fitted the equation: $absorbed\ dose = 6.6 \log(dose)$, with both doses expressed in mg/kg. While 90% of the 2 mg/kg dose was absorbed, only 2% of the 814 mg/kg dose was absorbed.

Two hours after intravenous administration, less than 3% of the circulating ^{14}C was attributed to the parent compound. The metabolites of 1-amino-2,4-dibromoanthraquinone in blood were primarily in the plasma fraction (blood:plasma ratio of approximately 0.5:1). The highest concentrations of ^{14}C in tissues 15 minutes after intravenous dosing were in excretory organs, lung, kidney, small intestine, liver, adipose tissue, and adrenal gland. Tissue:blood ratios (TBR) for these tissues were greater than or equal to 3:1. Only the liver and kidney had TBRs significantly greater than 1:1 at 72 hours. The terminal half-life of ^{14}C was approximately 40 hours in the liver and approximately 90 hours in the kidney. Adipose tissue contained primarily unmetabolized 1-amino-2,4-dibromoanthraquinone at 24 hours; liver, muscle, and skin contained mostly metabolites of 1-amino-2,4-dibromoanthraquinone. The elimination half-life of 1-amino-2,4-dibromoanthraquinone in adipose tissue was approximately 11 hours.

GENETIC TOXICOLOGY

1-Amino-2,4-dibromoanthraquinone (100 to 10,000 µg/plate) was tested for induction of gene mutations in four strains of *Salmonella typhimurium* in a preincubation protocol with and without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 (Table G1; Haworth *et al.*, 1983). 1-Amino-2,4-dibromoanthraquinone was

positive in the absence of S9 in the frameshift strains TA98 and TA1537; with S9, an equivocal response was obtained in TA1537, and TA98 was negative. In TA100, 1-amino-2,4-dibromoanthraquinone gave equivocal responses with and without S9, and all trials were negative with TA1535. The equivocal calls were the results of positive or weakly positive responses that were not duplicated in a second trial. Precipitation of 1-amino-2,4-dibromoanthraquinone occurred at concentrations of 100 µg/plate and above, and this may have been a factor in the nonreproducibility of the results.

1-Amino-2,4-dibromoanthraquinone was tested in two laboratories for induction of sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells, with and without Aroclor 1254-induced male Sprague-Dawley rat liver S9. In the sister chromatid exchange test, one laboratory observed a significant increase in sister chromatid exchanges only in the absence of S9, while the second laboratory recorded positive responses with and without S9 (Table G2; Loveday *et al.*, 1990). This discrepancy cannot be explained by a difference in dose levels employed at the two laboratories because the positive responses with S9 were observed at 3, 10, 15, and 30 µg/mL at the second laboratory, whereas negative trials resulted from testing doses up to 100 µg/mL at the first laboratory. In the chromosomal aberrations test, one laboratory observed a weakly positive response only in the absence of S9 (Table G3). Another laboratory obtained a positive response in the first trial without S9 but did not duplicate the positive response in the second trial, and the overall call without S9 was concluded to be equivocal (Loveday *et al.*, 1990). Neither laboratory observed an increase in chromosomal aberrations with 1-amino-2,4-dibromoanthraquinone in the presence of S9.

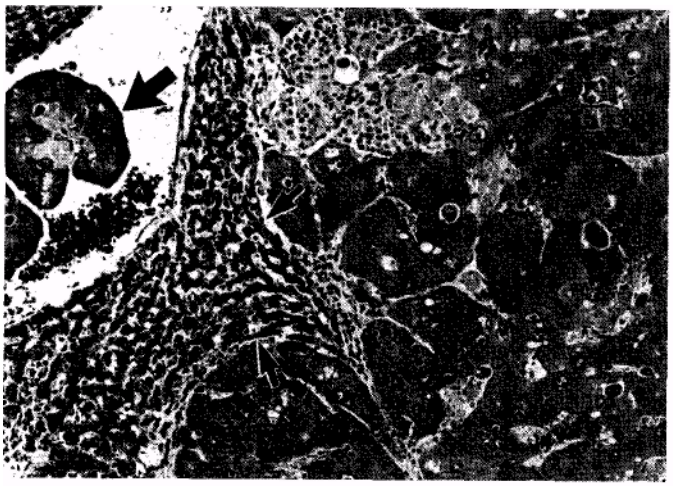


PLATE 1

An hepatocellular carcinoma in a female F344/N rat exposed to 10,000 ppm 1-amino-2,4-dibromoanthraquinone in feed for 2 years. Note compression of normal liver (small arrows) by neoplastic hepatocytes. Carcinoma embolus (large arrow) is in an hepatic vein. H&E; 90x

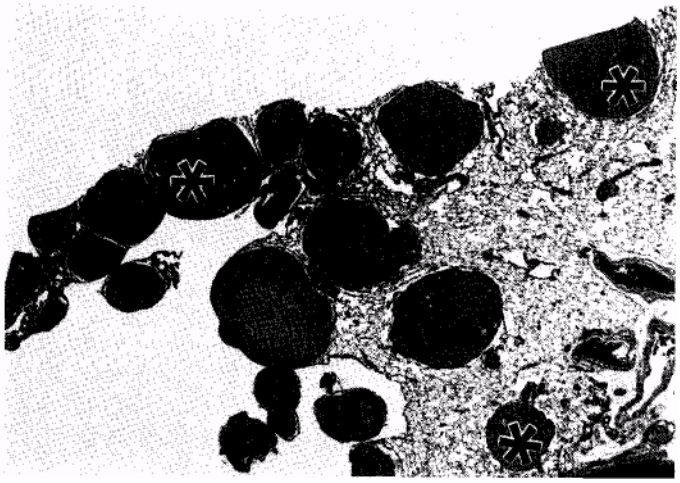


PLATE 2

Multiple metastatic foci (*) of an hepatocellular carcinoma in the lung of a female F344/N rat exposed to 5,000 ppm l-amino-2,4-dibromoanthraquinone in feed for 2 years. H&E; 15x

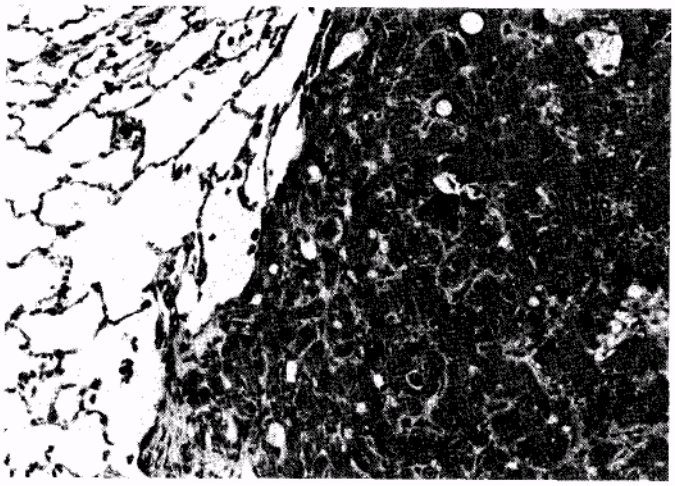


PLATE 3

Detail of a metastatic focus of the hepatocellular carcinoma shown in Plate 2 shows the solid and acinar growth patterns of the well-differentiated neoplastic hepatocytes. H&E; 90x

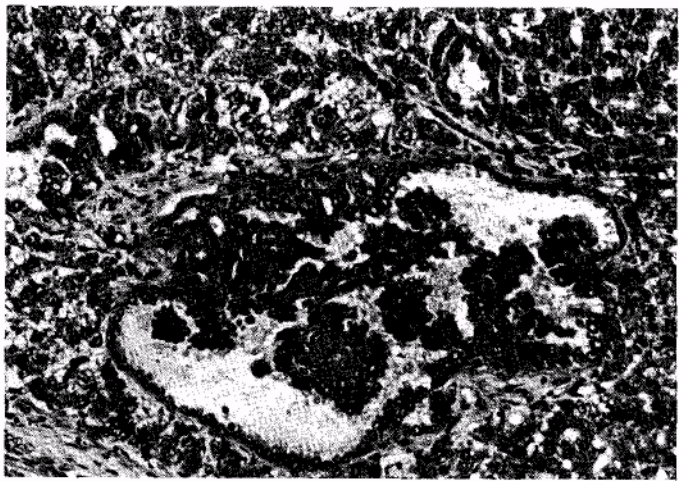


PLATE 4

An hepatocholangiocarcinoma in a female F344/N rat exposed to 10,000 ppm l-amino-2,4-dibromoanthraquinone in feed for 2 years. Note the well-differentiated hepatocyte (solid areas) and biliary components within the neoplasm. H&E; 140x

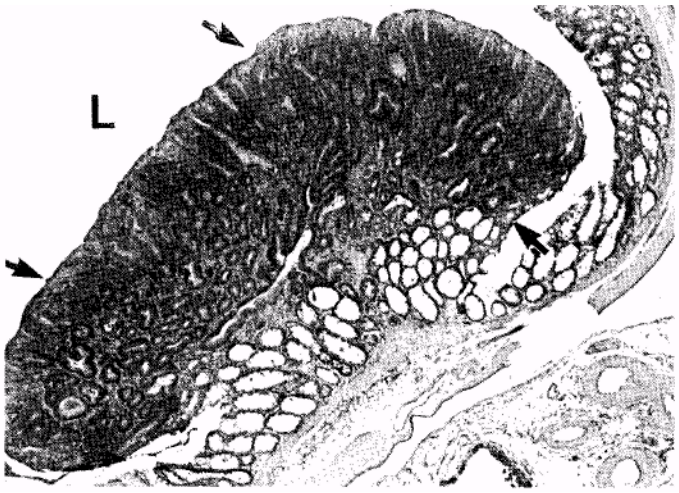


PLATE 5
An adenoma (adenomatous polyp) in the colon of a female F344/N rat exposed to 10,000 ppm l-amino-2,4-dibromoanthraquinone in feed for 2 years forms an exophytic mass (arrows) that partially occludes the intestinal lumen (L). H&E; 25*

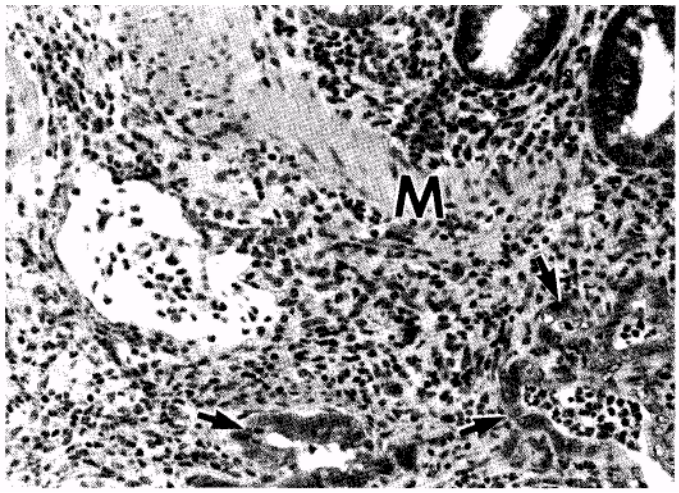


PLATE 6
Detail of a carcinoma in the colon of a female F344/N rat exposed to 10,000 ppm l-amino-2,4-dibromoanthraquinone in feed for 2 years. Note the disruption of the muscularis mucosa (M) layer at right with formation of irregular-shaped neoplastic glands (arrows), inflammation, and fibrosis in the submucosa. H&E; 160x

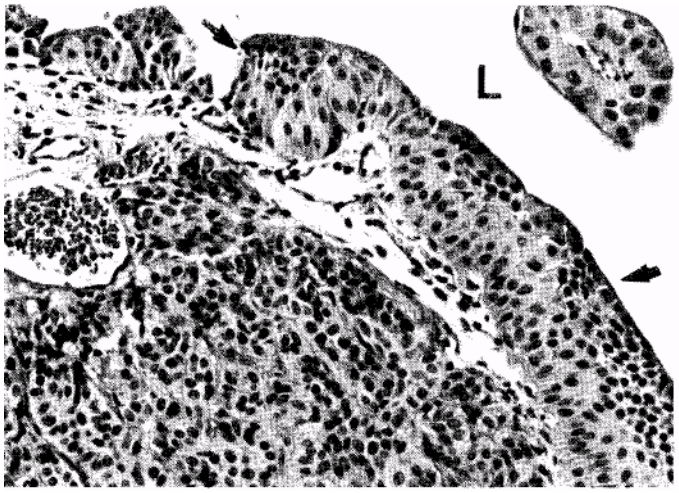


PLATE 7
A transitional cell carcinoma in the urinary bladder of a female F344/N rat exposed to 10,000 ppm l-amino-2,4-dibromoanthraquinone in feed for 2 years. Note the thickened neoplastic mucosal surface and a papillary projection of the neoplasm extending into the bladder lumen (L). The mucosal surface consists of a thickened layer of neoplastic transitional cells (arrows); a large nodule of transitional epithelium invades the wall of the urinary bladder. H&E; 160x

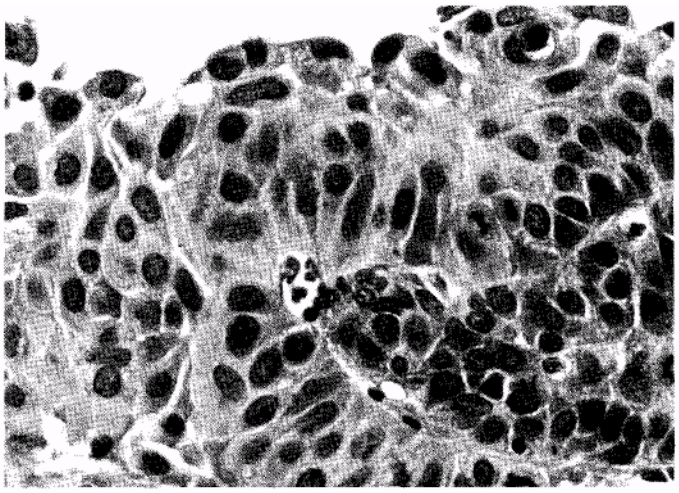


PLATE 8
Detail of the transitional cell carcinoma shown in Plate 7 shows cellular atypia and an increased number of mitotic cells. H&E; 320x

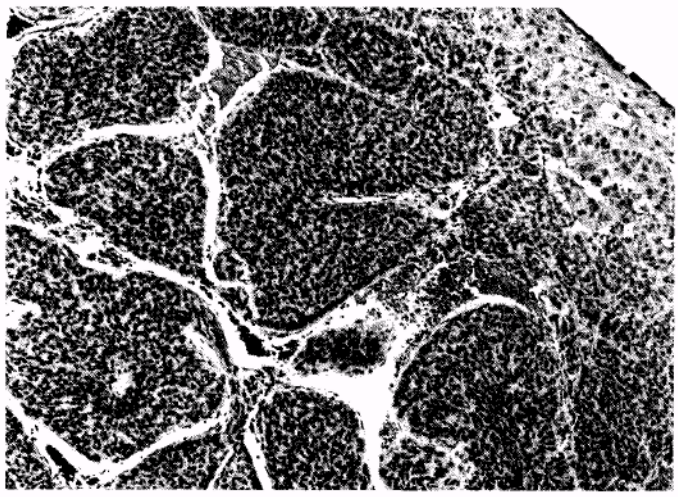


PLATE 9
An hepatoblastoma in the liver of a female B6C3F$_1$ mouse exposed to 20,000 ppm l-amino-2,4-dibromoanthraquinone in feed for 2 years consists of prominent neoplasm lobules separated by vascular channels. H&E; 80x

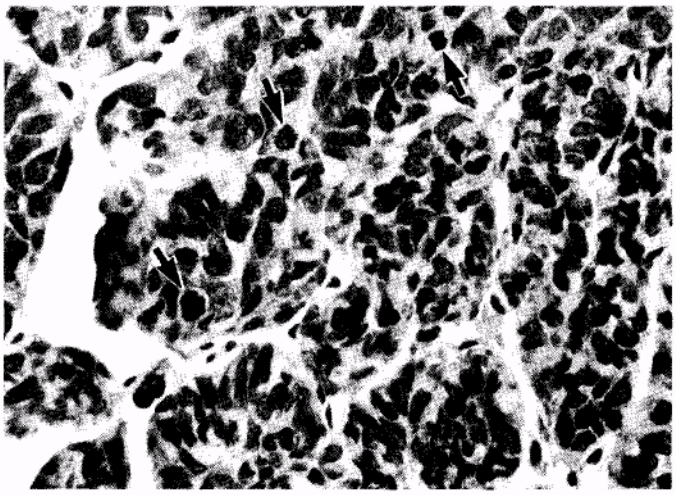


PLATE 10
Detail of the hepatoblastoma shown in Plate 9 shows closely packed undifferentiated neoplastic cells with scant cytoplasm, oval nuclei, and numerous mitotic cells (arrows). H&E; 320x

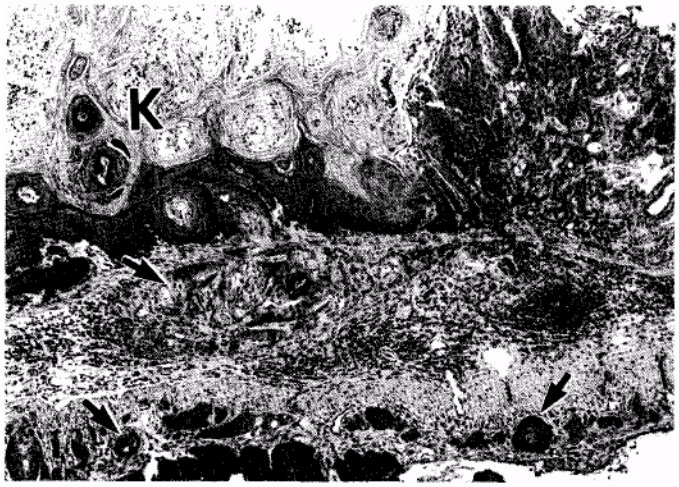


PLATE **11**
A squamous cell carcinoma of the forestomach in a male B6C3F$_1$ mouse exposed to 20,000 ppm l-amino-2,4-dibromoanthraquinone in feed for 2 years. Note the thickened keratin (K) layer on the mucosal surface and invasion of the wall by nodules (arrows) of neoplastic squamous cells that have extended through the peritoneal surface of the stomach. H&E; 40x

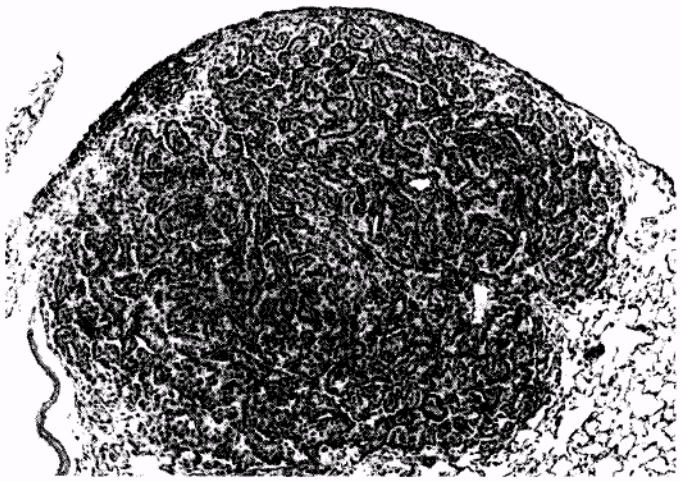


PLATE 12
An alveolar/bronchiolar adenoma of the lung in a male B6C3F$_1$ mouse exposed to 20,000 ppm l-amino-2,4-dibromoanthraquinone in feed for 2 years forms a non-encapsulated subpleural nodule. H&E; 60*

DISCUSSION AND CONCLUSIONS

Anthraquinones represent the largest group of naturally occurring quinones. Both natural and synthetic anthraquinones have been and continue to be widely used as colorants in food, drugs, cosmetics, hair dyes, and textiles. Dantron (1,8-dihydroxyanthraquinone) and emodin (1,3,8-trihydroxy-6-methylanthraquinone) are also used therapeutically as cathartics and purgatives. Chrysophanol (1,8-dihydroxy-3-methylanthraquinone) occurs in cascara sagrada, senna, and various species of *Rumex* and *Rheum* (rhubarb).

Anthraquinone and five substituted anthraquinones were selected for toxicologic characterization from a large group of amino-, alkyl-, nitro-, or halogen-containing anthraquinones. The basis for selection centered mainly on four criteria: 1) lack of available data on carcinogenicity, 2) magnitude of production and use patterns, 3) awareness of potential and actual human exposure, 4) and representation of as broad a spectrum of structural diversity within this class as possible. 1-Amino-2,4-dibromoanthraquinone was selected from a group of 36 environmentally significant aryl bromides. Since all other substituted anthraquinone chemicals already evaluated for long-term effects induced carcinogenic responses in laboratory animals (NCI, 1978a, 1978b, 1978c; IARC, 1982, 1987; NTP, 1986a), 1-amino-2,4-dibromoanthraquinone was predicted to be carcinogenic in laboratory animals as well (Fung *et al.,* 1993).

1-Amino-2,4-dibromoanthraquinone was studied for long-term toxicity and carcinogenesis using a "start-stop" experimental design. One of the first chemicals to be studied by the National Toxicology Program (NTP) with a start-stop protocol, 1-amino-2,4-dibromoanthraquinone was predicted to be carcinogenic, so the experimental design was selected in an attempt to gain some insight into the progression and/or regression of chemical-induced lesions as well as to perhaps gain knowledge about potential mechanisms of action.

Because 1-amino-2,4-dibromoanthraquinone caused significant carcinogenic responses in male and female rats and mice and in several organs and tissues, the discussion of lesions that follows has been grouped by organ.

Liver: 1-Amino-2,4-dibromoanthraquinone differs from other chemicals studied by the NTP because it induced greater than 90% incidences of multiple benign and malignant hepatocellular neoplasms in rats with frequent metastases (almost 50%) of the malignant liver neoplasms. Although other chemicals including 3,3′-dimethylbenzidine dihydrochloride (NTP, 1991) and furan (NTP, 1993a) have caused significant increases in benign and malignant liver neoplasms that approach a 100% incidence, metastases occurred in only one or two instances in a group of 50 rats. Only in the 18-month study of methyl carbamate (NTP, 1988) has a similarly high incidence of malignant, metastatic liver neoplasms occurred in male rats.

The liver lesions present after 13 weeks in the 25,000 and 50,000 ppm groups of rats included proliferative bile duct lesions (cholangiofibrosis) and foci of hepatocellular alteration. Based on morphological features of this proliferative bile duct lesion and results of transplantation and stop-exposure studies, cholangiofibrosis has been considered a "premalignant" lesion which is autonomous and progressive and not qualitatively different from cholangiocarcinoma (Maronpot *et al.,* 1991). While there is some disagreement on the biological behavior of cholangiofibrosis, this has generally been considered to be a preneoplastic lesion (Bannasch and Massner, 1976; Ohshima *et al*., 1984). Cholangiofibrosis has been described in toxicity studies of methapyrilene (Ohshima *et al.*, 1984), aflatoxin (Wilson *et al.,* 1985), and furan (NTP, 1993a) in rats.

Exposure concentrations of 1-amino-2,4-dibromoanthraquinone administered in the 2-year study were below those which produced cholangiofibrosis at 13 weeks; however, many of the benign and malignant liver neoplasms in the 2-year study in rats were composed of a mixed growth pattern of both hepatocytes and bile duct formation. The incidences of foci

of hepatocellular alteration were increased with 1-amino-2,4-dibromoanthraquinone exposure at 13 weeks and all scheduled intervals examined during the 2-year study. A detailed analysis of foci of hepatocellular alteration in the liver of rats from this study has been reported by Harada *et al.* (1989). In addition to overall exposure-related increased incidences of eosinophilic and clear cell foci of alteration, there were increases in size, number, and volume fraction of atypical eosinophilic, basophilic, and clear cell foci in rats that correlated with concentration and duration of 1-amino-2,4-dibromoanthraquinone exposure.

Although foci of hepatocellular alteration in rats are believed to be precursors of liver neoplasms, their biological nature and potential for progression to neoplasms is uncertain (Popp and Goldsworthy, 1989; Squire, 1989). Some of this uncertainty results from a considerable variation in phenotypes of hepatocellular foci and the different biomarkers used in their classification. In many studies, the conversion rate of foci to neoplasms has been considered to be extremely low, and, in some instances, increases in the incidences of basophilic foci have not been associated with liver neoplasms (MacDonald *et al.,* 1988; Harada *et al.,* 1989; Squire, 1989). Clear and acidophilic cell foci have been suggested to be important in the development of some chemical-induced liver neoplasms (Bannasch *et al.*, 1989; Bannasch and Zerban, 1992). The atypical eosinophilic foci that occurred in rats administered 1-amino-2,4-dibromoanthraquinone were rarely observed in controls or in groups of rats receiving other hepatocarcinogens (Harada *et al.*, 1989). Adenomas in the livers of rats treated with 1-amino-2,4-dibromoanthraquinone often contained cells morphologically identical to those in the atypical eosinophilic foci, suggesting that some of these foci may have been precursors for the hepatic neoplasms.

Liver effects in male mice administered 1-amino-2,4-dibromoanthraquinone for 13 weeks consisted of pigmentation and hypertrophy that persisted throughout the 2-year study. Although hypertrophy did not occur in female mice during this early period, pigmentation in the liver was present by 15 months. Foci of hepatocellular alteration were not present in the 13-week study, and after 2 years, the incidences were only slightly increased in mice. After 2 years, there were increased incidences of liver neoplasms in all groups of mice exposed to 1-amino-2,4-dibromoanthraquinone in feed. This response was more prominent in females that also had a greater number of hepatocellular carcinomas and more multiple liver neoplasms than male mice. Unlike the highly metastatic liver neoplasms observed in rats, only a few neoplasms in mice had detectable metastatic foci. The incidences of hepatoblastomas were also increased in the exposed groups of male and female mice. These distinctive liver neoplasms rarely occur in control animals but have been induced in mice administered acetylaminofluorene (Nonoyama *et al.*, 1988) or *N*-nitrosodiethylamine (Ward *et al.*, 1983).

Large Intestine: Adenomatous polyps (adenomas) and carcinomas of the large intestine (distal colon and rectum) in rats were generally observed after 15 months of exposure to 1-amino-2,4-dibromoanthraquinone, although one adenomatous polyp (adenoma) was observed as early as 9 months in the 20,000 ppm group of male rats in the "stop-exposure" study. Further, these lesions were often grossly visible. Even when exposure was stopped after 9 months, the percentage of chemical-induced rectal neoplasms was equal to or greater than that observed with continuous exposure for 15 months. In many rats, these neoplasms were multiple and malignant, based upon local invasion and/or metastases. Neoplasms of the large intestine have not been observed for other previously tested anthraquinones. One other chemical studied by the NTP, bromodichloromethane (NTP, 1987a), resulted in similarly high incidences of benign and malignant neoplasms of the large intestine.

Kidney: Accumulation of pigment in the kidney was observed in both male and female rats by 13 weeks and throughout the 2-year study. 1-Amino-2,4-dibromoanthraquinone (or metabolite) pigment in the kidney of mice was not evident until after the 15-month evaluation; there were no increased incidences of other nonneoplastic lesions or neoplasms of the kidney in mice. In the kidney of rats, several changes in addition to pigment were present at 13 weeks. In male rats, increased accumulation of hyaline droplets was observed in the cytoplasm of the renal tubule epithelium, yet no chemical-related exacerbation of renal tubule epithelial regeneration was observed at 13 weeks. There was a slight enlargement (karyomegaly) of some nuclei in the renal tubule epithelium of male and female rats. At

the 15-month evaluation, this slight nuclear enlargement was still evident, and the severity of nephropathy (tubule epithelial regeneration; transitional cell hyperplasia of the renal pelvis) was increased in exposed male and female rats. Accumulation of hyaline droplets was not present in exposed male rats after the 9-month evaluation. The morphological appearance of hyaline droplets and their presence only in males is suggestive of accumulation of $\alpha_{2\mu}$-globulin in the renal tubule epithelium, although the identity of the protein droplets was not determined. Their absence in the kidney tubule cells of exposed male rats after the 9-month evaluation is consistent with the normally decreased production of $\alpha_{2\mu}$-globulin by the liver beginning at 5 months of age (Baetcke *et al.*, 1991). Chemicals that cause a hyaline droplet nephropathy syndrome are often empirically associated with increases in the incidences of benign and malignant renal tubule neoplasms, linear foci of mineralization of the renal medulla, and enhanced nephropathy in male rats after 2 years (Baetcke *et al.*, 1991); however, other alternative mechanistic explanations exist that do not show a dominant role for $\alpha_{2\mu}$-globulin. The key to this view centers on several chemicals that incite the "hyaline droplet syndrome," yet do not induce tubule cell neoplasms of the kidney (Barrett and Huff, 1991; Huff, 1992, 1993; Melnick, 1992). Another strong neoplastic response in the kidney of female rats shows that mechanisms other than those associated with hyaline droplet nephropathy were involved in the renal tubule neoplasm response in rats administered 1-amino-2,4-dibromoanthraquinone. Increased incidences of benign and malignant neoplasms of the kidney occurred in male and female rats in the NTP study of bromodichloromethane (NTP, 1987a).

Urinary Bladder: Chemical-related increased incidences of proliferative lesions (hyperplasia and neoplasia) of the transitional cell epithelium of the urinary bladder occurred in male and female rats with a greater number of neoplasms observed in female rats (45/146, 31%) than in male rats (16/146, 11%). In the stop-exposure groups, transitional cell hyperplasia was present in four female rats at 9 months, and, with the absence of continued chemical exposure, hyperplasia did not develop in male rats at the 15-month evaluation. A transitional cell carcinoma occurred in one female rat from the 15-month exposure group. With continuous exposure to 1-amino-2,4-dibromoanthraquinone, benign and malignant neoplasms of the urinary bladder developed by 15 months in female rats and in both male and female rats after 2 years of exposure. In rats following chronic administration of 1,4,5,8-tetraaminoanthraquinone, a spectrum of nonneoplastic lesions and neoplasms of the urinary bladder was observed with similar morphologic features including squamous metaplasia, squamous cell carcinoma, and proliferation of fat (fatty metaplasia) in the wall of the urinary bladder (NTP, 1986a). In that study, calculi were present in the urinary bladder of most rats, yet there was a significant increase in the incidence of smooth muscle neoplasms of the wall of the urinary bladder. The hypothesis of cell proliferation and development of urinary bladder neoplasms has been described (Greenfield *et al.*, 1984; Cohen *et al.*, 1991). The mechanism for formation of neoplasms of the urinary bladder is uncertain. Increased cell proliferation evidenced by transitional cell hyperplasia in the urinary bladder did not occur before 9 months in rats. Potential local irritant effects and associated neoplasm formation in the urinary bladder attributed to calculus formation (Okumara *et al.*, 1992) were not identified in this study. Most mice in that study had calculi of the urinary bladder, yet did not have any evidence of carcinogenic activity. No scientific consensus exists that endorses the notion that calculi or stones cause cancer; there may be some co-carcinogenic or promotion activity, yet even this does not occur consistently (Huff, 1992, 1993).

Forestomach: In both rats and mice, there were several nonneoplastic proliferative and inflammatory lesions in the forestomach at the end of the 2-year studies. These forestomach lesions were not observed in either species in the 13-week studies or in rats at the 9- and 15-month evaluations. In the stop-exposure evaluation, rats exposed to 20,000 ppm developed nonneoplastic lesions of the forestomach by 15 months. However, in the 9-month stop-exposure group, forestomach lesions were observed in a few males by the 15-month evaluation, but the incidences were higher than those observed in male rats with the continuous 15-month exposure. In female rats, forestomach lesions were present at 15 months with continuous exposure but were not observed at 9 months or after 6 months of nonexposure. Chemical-related lesions consisted of hyperplasia, hyperkeratosis and associated inflammation, and focal erosion or ulceration of the squamous mucosa. The inflammatory and ulcerative lesions were generally more severe and more common in

rats, but significant increases in the incidences of benign and malignant forestomach neoplasms were limited to mice. These data provide further evidence that inflammation or ulceration does not always result in neoplasia (Berenblum, 1944; Huff, 1992, 1993; Melnick *et al*., 1993a, 1993b). The absence of forestomach neoplasms in rats may have been related to lower exposure concentrations. Many of the malignant forestomach neoplasms of mice metastasized or invaded adjacent organs.

Increases in the incidences of forestomach neoplasms have not been observed in mice or rats following long-term administration of four other structurally related anthraquinones. However, administration of 1-amino-2-methylanthraquinone (NCI, 1978b) to rats for 62 weeks followed by a 6-month nonexposure period was associated with an increased incidence in hyperplasia of the forestomach. Administration of 2-methyl-1-nitroanthraquinone (NCI, 1978c) to rats for 78 weeks followed by a 6-month nonexposure period was also associated with an increased incidence of proliferative lesions of the forestomach. Marked increases in the incidences of forestomach neoplasms have been reported for some chemicals that caused a sustained proliferative response in the squamous mucosa that was evident within the first 2 to 13 weeks of chemical administration (NTP, 1987b). However, a number of other chemicals causing forestomach neoplasms in rodents have not been associated with an early, sustained increase in the incidence of hyperplasia (NTP, 1990a, 1990b, 1990c).

Lung: The incidences of alveolar/bronchiolar adenoma and multiple alveolar/bronchiolar adenoma (males only) of the lung were significantly increased in mice in the 10,000 and 20,000 ppm groups. Although there was no evidence for an increase in the incidence of hyperplasia or for a progression of the lung neoplasms to malignancy, the incidence of adenoma in all four exposure groups exceeded the NTP historical control ranges. Administration of a structurally related anthraquinone, C.I. Disperse Blue 1 (1,4,5,8-tetraaminoanthraquinone) (NTP, 1986a), resulted in a marginal increase in the incidence of alveolar/bronchiolar adenoma in male mice with no associated increase in the incidence of alveolar/bronchiolar hyperplasia. Other chemicals tested by the NTP have also caused increased incidences of lung neoplasms without increased incidences of alveolar/bronchiolar hyperplasia (NTP, 1994), but more commonly an increase in the incidence of alveolar/bronchiolar hyperplasia or inflammation is also present with increased incidences of lung neoplasms (NTP, 1986b, 1989, 1990a, 1992). Although the incidence of alveolar/bronchiolar carcinoma was not increased in the lungs of mice administered 1-amino-2,4-dibromoanthraquinone, a number of chemicals have induced both alveolar/bronchiolar adenomas and carcinomas (NTP, 1990b, 1990c, 1993b; Huff, 1994).

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *clear evidence of carcinogenic activity** of 1-amino-2,4-dibromoanthraquinone in male and female F344/N rats based on increased incidences of neoplasms in the liver, large intestine, kidney, and urinary bladder. There was *clear evidence of carcinogenic activity* of 1-amino-2,4-dibromoanthraquinone in male and female B6C3F$_1$ mice based on increased incidences of neoplasms in the liver, forestomach, and lung.

Exposure of male and female rats to 1-amino-2,4-dibromoanthraquinone for 2 years was associated with basophilic focus (males only), clear cell focus, eosinophilic focus, and pigmentation in the liver; renal tubule hyperplasia, renal tubule pigmentation, and transitional cell hyperplasia in the kidney; transitional cell hyperplasia, squamous metaplasia, and stromal metaplasia (females only) in the urinary bladder; squamous hyperplasia, hyperkeratosis, ulceration, and inflammation of the forestomach mucosa; and seminal vesicle atrophy. Exposure of male and female mice to 1-amino-2,4-dibromoanthraquinone for 2 years was associated with centrilobular hepatocellular hypertrophy (males only), basophilic focus, clear cell focus (females only), eosinophilic focus, and pigmentation in the liver; pigmentation in the kidney; and hyperplasia, basal cell hyperplasia, hyperkeratosis, and inflammation of the forestomach mucosa.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 13. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 15.

REFERENCES

Anderson, B.E., Zeiger, E., Shelby, M.D., Resnick, M.A., Gulati, D.K., Ivett, J.L., and Loveday, K.S. (1990). Chromosome aberration and sister chromatid exchange test results with 42 chemicals. *Environ. Mol. Mutagen.* **16** (Suppl. 18), 55-137.

Armitage, P. (1971). *Statistical Methods in Medical Research*, pp. 362-365. John Wiley and Sons, New York.

Arneson, D.W., Siemann, L.G., and Huff, J.E. (1996). Carcinogenesis bioassays and the question of chemical purity: Chemical characterization and carcinogenicity of several substituted anthraquinone dyes. *Toxicology* (in press).

Ashby, J., and Tennant, R.W. (1991). Definitive relationships among chemical structure, carcinogenicity, and mutagenicity for 301 chemicals tested by the U.S. NTP. *Mutat. Res.* **257**, 229-306.

Baetcke, K.P., Hard, G.C., Rodgers, I.S., McGaughy, R.E., and Tahan, L.M. (1991). Alpha$_{2\mu}$-globulin: Association with chemically induced renal toxicity and neoplasia in the male rat. (EPA/625/3-91/019F). Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC.

Bannasch P., and Massner B. (1976). Histogenese und cytogenese von cholangiofibromen und cholangiocarcinomen bei nitrosomorpholin-vergifteten ratten. *Z. Krebsforsch*. **87**, 239-255.

Bannasch, P., and Zerban, H. (1992). Predictive value of hepatic preneoplastic lesions as indicators of carcinogenic response. In *Mechanisms of Carcinogenesis in Risk Identification* (H. Vainio, P.N. Magee, D.B. McGregor, and A.J. McMichael, Eds.), pp. 389-427. IARC, Lyon, France.

Bannasch, P., Enzmann, H., Klimek, F., Weber, E., and Zerban, H. (1989). Significance of sequential cellular changes inside and outside foci of altered hepatocytes during hepatocarcinogenesis. *Toxicol. Pathol.* **17**, 617-629.

Barrett, J.C., and Huff, J. (1991). Cellular and molecular mechanisms of chemically induced renal carcinogenisis. *Ren. Fail.* **13**, 211-225.

Berenblum, I. (1944). Irritation and carcinogenesis. *Arch. Pathol.* **38**, 233-244.

Boorman, G.A., Montgomery, C.A., Jr., Eustis, S.L., Wolfe, M.J., McConnell, E.E., and Hardisty, J.F. (1985). Quality assurance in pathology for rodent carcinogenicity studies. In *Handbook of Carcinogen Testing* (H.A. Milman and E.K. Weisburger, Eds.), pp. 345-357. Noyes Publications, Park Ridge, NJ.

Brown, J.P., and Brown, R.J. (1976). Mutagenesis by 9,10-anthraquinone derivatives and related compounds in *Salmonella typhimurium*. *Mutat. Res.* **40**, 203-224.

Bucher, J.R., Alison, R.H., Montgomery, C.A., Huff, J., Haseman, J.K., Farnell, D., Thompson, R., and Prejean, J.D. (1987). Comparative toxicity and carcinogenicity of two chlorinated paraffins in F344/N rats and B6C3F1 mice. *Fundam. Appl. Toxicol.* **9**, 454-468.

Cesarone, C.F., Bolognesi, C., and Santi, L. (1982). Evaluation of damage to DNA after in vivo exposure to different classes of chemicals. *Arch. Toxicol.* **5**, 355-359.

Chung, R.H. (1978). Anthraquinone derivatives. In *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 2, 3rd ed. (M. Grayson and D. Eckroth, Eds.), pp. 708-757. John Wiley and Sons, Inc., New York.

Chung, R.H., and Farris, R.E. (1979). Dyes, anthraquinone. In *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 8, 3rd ed. (M. Grayson and D. Eckroth, Eds.), pp. 212-279. John Wiley and Sons, Inc., New York.

Code of Federal Regulations (CFR) **21**, Part 58.

Cohen, S.M., Ellwein, L.B., Okamura, T., Masui, T., Johansson, S.L., Smith, R.A., Wehner, J.M., Khachab, M., Chappel, C.I., Shoenig, G.P., Emerson, J.L., and Garland, E.M. (1991). Comparative bladder tumor promoting activity of sodium saccharin, sodium ascorbate, related acids, and calcium salts in rats. *Cancer Res.* **51**, 1766-1777.

Cox, D.R. (1972). Regression models and life-tables. *J. R. Stat. Soc.* **B34**, 187-220.

Crawford, B.D. (1985). Perspectives on the somatic mutation model of carcinogenesis. In *Advances in Modern Environmental Toxicology: Mechanisms and Toxicity of Chemical Carcinogens and Mutagens* (M.A. Mehlman, W.G. Flamm, and R.J. Lorentzen, Eds.), pp. 13-15. Princeton Scientific Publishing Co., Inc., Princeton, NJ.

Dietz, D.D., Abdo, K.M., Haseman, J.K., Eustis, S.L., and Huff, J.E. (1991). Comparative toxicity and carcinogenicity studies of tetracycline and oxytetracycline in rats and mice. *Fundam. Appl. Toxicol.* **17**, 215-224.

Dinse, G.E., and Haseman, J.K. (1986). Logistic regression analysis of incidental-tumor data from animal carcinogenicity experiments. *Fundam. Appl. Toxicol.* **6**, 44-52.

Dinse, G.E., and Lagakos, S.W. (1983). Regression analysis of tumour prevalence data. *Appl. Statist.* **32**, 236-248.

Dixon, W.J., and Massey, F.J., Jr. (1951). *Introduction to Statistical Analyses*, 1st ed., pp. 145-147. McGraw-Hill Book Company, Inc., New York.

Dunkel, V.C., Zeiger, E., Brusick, D., McCoy, E., McGregor, D., Mortelmans, K., Rosenkranz, H.S., and Simmon, V.F. (1985). Reproducibility of microbial mutagenicity assays: II. Testing of carcinogens and noncarcinogens in *Salmonella typhimurium* and *Escherichia coli*. *Environ. Mol. Mutagen.* **7** (Suppl. 5), 1-248.

Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1096-1121.

Dunnick, J.K., Eustis, S.L., Huff, J.E., and Haseman, J.K. (1989). Two-year toxicity and carcinogenicity studies of ampicillin trihydrate and penicillin VK in rodents. *Fundam. Appl. Toxicol.* **12**, 252-257.

Fleischman, R.W., Esber, H.J., Hagopian, M., Lilja, H.S., and Huff, J. (1986). Thirteen-week toxicology studies of 1-amino-2,4-dibromoanthraquinone in Fischer 344/N rats and B6C3F$_1$ mice. *Toxicol. Appl. Pharmacol.* **82**, 389-404.

Fung, V.A., Huff, J., Weisburger, E.K., and Hoel, D.G. (1993). Predictive strategies for selecting 379 NCI/NTP chemicals evaluated for carcinogenic potential: Scientific and public health impact. *Fundam. Appl. Toxicol.* **20**, 413-436.

Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B., and Zeiger, E. (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. *Environ. Mol. Mutagen.* **10** (Suppl. 10), 1-175.

Gart, J.J., Chu, K.C., and Tarone, R.E. (1979). Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. *J. Natl. Cancer Inst.* **62**, 957-974.

Greenfield, R.E., Ellwein, L.B., and Cohen, S.M. (1984). A general probabilistic model of carcinogenesis: Analysis of experimental urinary bladder cancer. *Carcinogenesis* **5**, 437-445.

Harada, T., Maronpot, R.R., Morris, R.W., and Boorman, G.A. (1989). Observations on altered hepatocellular foci in National Toxicology Program two-year carcinogenicity studies in rats. *Toxicol. Pathol.* **17**, 690-708.

Haseman, J.K. (1984). Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. *Environ. Health Perspect.* **58**, 385-392.

Haseman, J.K., Huff, J., and Boorman, G.A. (1984). Use of historical control data in carcinogenicity studies in rodents. *Toxicol. Pathol.* **12**, 126-135.

Haseman, J.K., Huff, J.E., Rao, G.N., Arnold, J.E., Boorman, G.A., and McConnell, E.E. (1985). Neoplasms observed in untreated and corn oil gavage control groups of F344/N rats and (C57BL/6N × C3H/HeN)F_1 (B6C3F_1) mice. *JNCI* **75**, 975-984.

Hawley, G.G., Ed. (1981). *The Condensed Chemical Dictionary,* 10th ed. Van Nostrand Reinhold Company, New York.

Haworth, S., Lawlor, T., Mortelmans, K., Speck, W., and Zeiger, E. (1983). *Salmonella* mutagenicity test results for 250 chemicals. *Environ. Mutagen.* **5** (Suppl. 1), 3-142.

Hollander, M., and Wolfe, D.A. (1973). *Nonparametric Statistical Methods*, pp. 120-123. John Wiley and Sons, New York.

Huff, J.E. (1992). Chemical toxicity and chemical carcinogenesis. Is there a causal connection? A comparative morphological evaluation of 1500 experiments. In *Mechanisms of Carcinogenesis in Risk Identification* (H. Vainio, P.N. Magee, D.B. McGregor, and A.J. McMichael, Eds.), pp. 437-475. IARC, Lyon, France.

Huff, J.E. (1993). Absence of morphologic correlation between chemical toxicity and chemical carcinogenesis. *Environ. Health Perspect.* **101** (Suppl. 5), 45-54.

Huff, J. (1994). Chemically associated respiratory carcinogenesis in rodents and in humans. In *Carcinogenesis* (M.P. Waalkes and J.M. Ward, Eds.), pp. 199-214. Raven Press, Ltd., New York.

Huff, J.E., and Haseman, J.K. (1991). Exposure to certain pesticides may pose real carcinogenic risk. *Chem. Eng. News* **69**, 33-37.

Huff, J.E., and Kluwe, W.M. (1984). Phthalate esters carcinogenicity in F344/N rats and B6C3F_1 mice. In *Industrial Hazards of Plastics and Synthetic Elastomers* (J. Järvisalo, P. Pfäffli, and H. Vainio, Eds.), pp. 137-154. Alan R. Liss, Inc., New York.

Huff, J.E., McConnell, E.E., Haseman, J.K., Boorman, G.A., Eustis, S.L., Schwetz, B.A., Rao, G.N., Jameson, C.W., Hart, L.G., and Rall, D.P. (1988). Carcinogenesis studies: Results of 398 experiments on 104 chemicals from the U.S. National Toxicology Program. *Ann. N.Y. Acad. Sci.* **534**, 1-30.

Huff, J.E., Cirvello, J., Haseman, J.K., and Bucher, J.R. (1991). Chemicals associated with site-specific neoplasia in 1394 long-term carcinogenesis experiments in laboratory rodents. *Environ. Health Perspect.* **93**, 247-271.

International Agency for Research on Cancer (IARC) (1982). *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Some Aromatic Amines, Anthraquinones and Nitroso Compounds, and Inorganic Fluorides Used in Drinking-water and Dental Preparations*, Vol. 27, pp. 191-212. IARC, Lyon, France.

International Agency for Research on Cancer (IARC) (1987). *IARC Mongraphs on the Evaluation of Carcinogenic Risks of Chemicals to Humans. Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs, Volumes 1 to 42*. (Suppl. 7). IARC, Lyon, France.

Jonckheere, A.R. (1954). A distribution-free *k*-sample test against ordered alternatives. *Biometrika* **41**, 133-145.

Kaplan, E.L., and Meier, P. (1958). Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.* **53**, 457-481.

Kluwe, W.M., McConnell, E.E., Huff, J.E., Haseman, J.K., Douglas, J.F., and Hartwell, W.V. (1982). Carcinogenicity testing of phthalate esters and related compounds by the National Toxicology Program and the National Cancer Institute. *Environ. Health Perspect.* **45**, 129-133.

Kluwe, W.M., Huff, J.E., Matthews, H.B., Irwin, R., and Haseman, J.K. (1985). Comparative chronic toxicities and carcinogenic potentials or 2-ethylhexyl-containing compounds in rats and mice. *Carcinogenesis* **6**, 1577-1583.

Lamb, J.C., IV, Huff, J.E., Haseman, J.K., Murthy, A.S., and Lilja, H. (1986). Carcinogenesis studies of 4,4′-methylenedianiline dihydrochloride given in drinking water to F344/N rats and B6C3F1 mice. *J. Toxicol. Environ. Health* **18**, 325-337.

Loveday, K.S., Anderson, B.E., Resnick, M.A., and Zeiger, E. (1990). Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro. V: Results with 46 chemicals. *Environ. Mol. Mutagen.* **16**, 272-303.

McConnell, E.E., Solleveld, H.A., Swenberg, J.A., and Boorman, G.A. (1986). Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. *JNCI* **76**, 283-289.

MacDonald, J.S., Gerson, R.J., Kornbrust, D.J., Kloss, M.W., Prahalada, S., Berry, P.H., Alberts, A.W., and Bokelman, D.L. (1988). Preclinical evaluation of lovastatin. *Am. J. Cardiol.* **62**, 16J-27J.

McKnight, B., and Crowley, J. (1984). Tests for differences in tumor incidence based on animal carcinogenesis experiments. *J. Am. Stat. Assoc.* **79**, 639-648.

Maronpot, R.R., and Boorman, G.A. (1982). Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. *Toxicol. Pathol.* **10**, 71-80.

Maronpot, R.R., Giles, H.D., Dykes, D.J., and Irwin, R.D. (1991). Furan-induced hepatic cholangiocarcinomas in Fischer 344 rats. *Toxicol. Pathol.* **19**, 561-570.

Melnick, R.L. (1992). Critique does not validate assumptions in the model on $\alpha_{2\mu}$-globulin and renal carcinogenesis. An alternative hypothesis on the role of chemically induced protein droplet ($\alpha_{2\mu}$-globulin) nephropathy in renal carcinogenesis. *Regul. Toxicol. Pharmacol.* **16**, 111-125.

Melnick, R.L., and Huff, J. (1992). 1,3-Butadiene: Toxicity and carcinogenicity in laboratory animals and in humans. *Rev. Environ. Contam. Toxicol.* **124**, 111-139.

Melnick, R.L., Huff, J., Haseman, J.K., Dieter, M.P., Grieshaber, C.K., Wyand, D.S., Russfield, A.B., Murthy, A.S.K., Fleischman R.W., and Lilja, H.S. (1983). Chronic effects of agar, guar gum, gum arabic, locust-bean gum, or tara gum in F344 rats and $B6C3F_1$ mice. *Food Chem. Toxicol.* **21**, 305-311.

Melnick, R.L., Huff, J.E., Barrett, J.C., Maronpot, R.R., Lucier, G., and Portier, C.J. (1993a). Meeting report: Cell proliferation and chemical carcinogenesis. *Mol. Carcinog.* **7**, 135-138.

Melnick, R.L., Huff, J., Barrett, J.C., Maronpot, R.R., Lucier, G., and Portier, C.J. (1993b). Cell proliferation and chemical carcinogenesis: Symposium overview. *Environ. Health Perspect.* **101** (Suppl. 5), 3-8.

The Merck Index (1989). 11th ed. (S. Budavari, Ed.), p. 721. Merck and Co., Inc., Rahway, NJ.

Miller, J.A., and Miller, E.C. (1977). Ultimate chemical carcinogens as reactive mutagenic electrophiles. In *Origins of Human Cancer* (H.H. Hiatt, J.D. Watson, and J.A. Winsten, Eds.), pp. 605-627. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Morgan, D.L., Dunnick, J.K., Goehl, T., Jokinen, M.P., Matthews, H.B., Zeiger, E., and Mennear, J.H. (1994). Summary of the National Toxicology Program benzidine dye initiative. *Environ. Health Perspect.* **102** (Suppl. 2), 63-78.

National Cancer Institute (NCI) (1976). Guidelines for Carcinogen Bioassay in Small Rodents. Technical Report Series No. 1. NIH Publication No. 76-801. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Cancer Institute (NCI) (1978a). Bioassay of 2-Aminoanthraquinone for Possible Carcinogenicity (CAS No. 117-79-3). Technical Report Series No. 144. NIH Publication No. 78-1399. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Cancer Institute (NCI) (1978b). Bioassay of 1-Amino-2-methylanthraquinone for Possible Carcinogenicity (CAS No. 82-28-0). Technical Report Series No. 111. NIH Publication No. 78-1366. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Cancer Institute (NCI) (1978c). Bioassay of 2-Methyl-1-nitroanthraquinone for Possible Carcinogenicity (CAS No. 129-15-7). Technical Report Series No. 29. NIH Publication No. 78-829. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Institutes of Health (NIH) (1978). Open Formula Rat and Mouse Ration (NIH-07). Specification NIH-11-1335. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Toxicology Program (NTP) (1986a). Toxicology and Carcinogenesis Studies of C.I. Disperse Blue I (CAS No. 2475-45-8) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies). Technical Report Series No. 299. NIH Publication No. 86-2555. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1986b). Toxicology and Carcinogenesis Studies of Benzene (CAS No. 71-43-2) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies). Technical Report Series No. 289. NIH Publication No. 86-2545. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1987a). Toxicology and and Carcinogenicity Studies of Bromodichloromethane (CAS No. 75-27-4) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies). Technical Report Series No. 321. NIH Publication No. 88-2577. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1987b). Carcinogenesis Studies of Ethyl Acrylate (CAS No. 140-88-5) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies). Technical Report Series No. 259. NIH Publication No. 87-2515. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1988). Toxicology and Carcinogenesis Studies of Methyl Carbamate (CAS No. 598-55-0) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies). Technical Report Series No. 328. NIH Publication No. 88-2584. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1989). Toxicology and Carcinogenesis Studies of *N*-Methylolacrylamide (CAS No. 924-42-5) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies). Technical Report Series No. 352. NIH Publication No. 89-2807. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1990a). Toxicology and Carcinogenesis Studies of Benzofuran (CAS No. 271-89-6) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies). Technical Report Series No. 370. NIH Publication No. 90-2825. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1990b). Toxicology and Carcinogenesis Studies of Glycidol (CAS No. 556-52-5) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies). Technical Report Series No. 374. NIH Publication No. 90-2829. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1990c). Toxicology and Carcinogenesis Studies of Benzaldehyde (CAS No. 100-52-7) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies). Technical Report Series No. 378. NIH Publication No. 90-2833. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1991). Toxicology and Carcinogenesis Studies of 3,3′-Dimethylbenzidine Dihydrochloride (CAS No. 612-82-8) in F344/N Rats (Drinking Water Studies). Technical Report Series No. 390. NIH Publication No. 91-2845. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1992). Toxicology and Carcinogenesis Studies of Naphthalene (CAS No. 91-20-3) in B6C3F$_1$ Mice (Inhalation Studies). Technical Report Series No. 410. NIH Publication No. 92-3141. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1993a). Toxicology and Carcinogenesis Studies of Furan (CAS No. 110-00-9) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies). Technical Report Series No. 402. NIH Publication No. 93-2857. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1993b). Toxicology and Carcinogenesis Studies of 1,3-Butadiene (CAS No. 106-99-0) in B6C3F$_1$ Mice (Inhalation Studies). Technical Report Series No. 434. NIH Publication No. 93-3165. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1994). Toxicology and Carcinogenesis Studies of Ozone (CAS No. 10028-15-6) and Ozone/NNK (CAS No. 10028-15-6/64091-91-4) in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies). Technical Report Series No. 440. NIH Publication No. 95-3371. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

Nonoyama, T., Fullerton, F., Reznik, G., Bucci, T.J., and Ward, J.M. (1988). Mouse hepatoblastomas: A histologic, ultrastuctural, and immunohistochemical study. *Vet. Pathol.* **25**, 286-296.

Ohshima, M., Ward, J.M., Brennan, L.M., and Creasia, D.A. (1984). A sequential study of methapyrilene hydrochloride-induced liver carcinogenesis in male F344 rats. *JNCI* **72**, 759-765.

Okumara, M., Hasegawa, R., Shirai, T., Ito, M., Yamada, S., and Fukushima, S. (1992). Relationship between calculus formation and carcinogenesis in the urinary bladder of rats administered the non-genotoxic agents, thymine or melamine. *Carcinogenesis* **13**, 1043-1045.

Popp, J.A., and Goldsworthy, T.L. (1989). Defining foci of cellular alteration in short-term and medium-term rat liver tumor models. *Toxicol. Pathol.* **17**, 561-568.

Sendelbach, L.E. (1989). A review of the toxicity and carcinogenicity of anthraquinone derivatives. *Toxicology* **57**, 227-240.

Squire, R.A. (1989). Evaluation and grading of rat liver foci in carcinogenicity tests. *Toxicol. Pathol.* **17**, 685-689.

Straus, D.S. (1981). Somatic mutation, cellular differentiation, and cancer causation. *JNCI* **67**, 233-241.

Tarone, R.E. (1975). Tests for trend in life table analysis. *Biometrika* **62**, 679-682.

Tennant, R.W., Margolin, B.H., Shelby, M.D., Zeiger, E., Haseman, J.K., Spalding, J., Caspary, W., Resnick, M., Stasiewicz, S., Anderson, B., and Minor, R. (1987). Prediction of chemical carcinogenicity in rodents from in vitro genetic toxicity assays. *Science* **236**, 933-941.

United States International Trade Commission (USITC) (1993). Synthetic organic chemicals: United States production and sales, 1991. USITC Publication 2607. U.S. Government Printing Office, Washington, DC.

Ward, J.M., Rice, J.M., Creasia, D., Lynch, P., and Riggs, C. (1983). Dissimilar patterns of promotion by di(2-ethylhexyl)pthalate and phenobarbital of hepatocellular neoplasia initiated by diethylnitrosamine in B6C3F1 mice. *Carcinogenesis* **4**, 1021-1029.

Weisburger, E.K., Murthy, A.S.K., Lilja, H.S., and Lamb, J.C., IV (1984). Neoplastic response of F344 rats and B6C3F$_1$ mice to the polymer and dyestuff intermediates 4,4′-methylenebis(*N*,*N*-dimethyl)-benzenamine, 4,4′-oxydianiline, and 4,4′-methylenedianiline. *JNCI* **72**, 1457-1463.

Williams, D.A. (1971). A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* **27**, 103-117.

Williams, D.A. (1972). The comparison of several dose levels with a zero dose control. *Biometrics* **28**, 519-531.

Wilson, T.M., Nelson, P.E., and Knepp, C.R. (1985). Hepatic neoplastic nodules, adenofibrosis, and cholangiocarcinomas in male Fischer 344 rats fed corn naturally contaminated with *Fusarium moniliforme*. *Carcinogenesis* **6**, 1155-1160.

Yang, R.S.H., Huff, J., Germolec, D.R., Luster, M.I., Simmons, J.E., and Seely, J.C. (1989). Biological issues in extrapolation. In *Carcinogenicity and Pesticides. Principles, Issues, and Relationships* (N.N. Ragsdale and R.E. Menzer, Eds.), pp. 142-163. American Chemical Society (ACS) Symposium Series 414. ACS, Washington, DC.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K. (1988). *Salmonella* mutagenicity tests. IV. Results from the testing of 300 chemicals. *Environ. Mol. Mutagen.* **11** (Suppl. 12), 1-158.

Zeiger, E., Haseman, J.K., Shelby, M.D., Margolin, B.H., and Tennant, R.W. (1990). Evaluation of four in vitro genetic toxicity tests for predicting rodent carcinogenicity: Confirmation of earlier results with 41 additional chemicals. *Environ. Mol. Mutagen.* **16** (Suppl. 18), 1-14.

Appendix B: Tissues examined for histopathology in mice and rats in the NTP bioassays of ADBAQ

Tissues examined for histopathology in mice and rats in the NTP bioassays of ADBAQ

13-Week studies	2-Year studies	Stop-exposure evaluation
Complete histopathologic examinations were performed on all animals that died during the study, control animals, and 50,000 ppm animals. In addition to tissue masses, gross lesions, and associated lymph nodes, the tissues examined included: adrenal gland, bone and marrow, brain, clitoral gland, epididymis, esophagus, gallbladder (mice), heart, kidney, large intestine (cecum, colon, rectum), liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, small intestine (duodenum, jejunum, ileum), spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, and uterus. The kidney, liver, spleen (rats), thymus (rats), and uterus (rats) of all other exposed animals were examined.	Complete histopathologic examinations were performed on all animals. In addition to tissue masses, gross lesions, and associated lymph nodes, the tissues examined included: adrenal gland, bone and marrow, brain, clitoral gland, epididymis, esophagus, gallbladder (mice), heart, kidney, large intestine (cecum, colon, rectum), liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, small intestine (duodenum, jejunum, ileum), spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, and uterus.	Complete histopathologic examinations were performed on all animals. In addition to tissue masses, gross lesions, and associated lymph nodes, the tissues examined included: adrenal gland, bone and marrow, brain, clitoral gland, epididymis, esophagus, heart, kidney, large intestine (cecum, colon, rectum), liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, small intestine (duodenum, jejunum, ileum), spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, and uterus.