United States
Environmental Protection
Agency

EPA/690/R-11/007F
Final
2-17-2011

Provisional Peer-Reviewed Toxicity Values for

9,10-Anthraquinone
(CASRN 84-65-1)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

AUTHORS, CONTRIBUTORS, AND REVIEWERS

CHEMICAL MANAGER

Jeff Swartout
National Center for Environmental Assessment, Cincinnati, OH

DRAFT DOCUMENT PREPARED BY

ICF International
9300 Lee Highway
Fairfax, VA 22031

PRIMARY INTERNAL REVIEWERS

Paul G. Reinhart, PhD, DABT
National Center for Environmental Assessment, Research Triangle Park, NC

Audrey Galizia, DrPH
National Center for Environmental Assessment, Washington, DC

This document was externally peer reviewed under contract to
Eastern Research Group, Inc.
110 Hartwell Avenue
Lexington, MA 02421-3136

Questions regarding the contents of this document may be directed to the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300).

TABLE OF CONTENTS

COMMONLY USED ABBREVIATIONS

BMC	benchmark concentration
BMD	benchmark dose
BMCL	benchmark concentration lower bound 95% confidence interval
BMDL	benchmark dose lower bound 95% confidence interval
HEC	human equivalent concentration
HED	human equivalent dose
IUR	inhalation unit risk
LOAEL	lowest-observed-adverse-effect level
$LOAEL_{ADJ}$	LOAEL adjusted to continuous exposure duration
$LOAEL_{HEC}$	LOAEL adjusted for dosimetric differences across species to a human
NOAEL	no-observed-adverse-effect level
$NOAEL_{ADJ}$	NOAEL adjusted to continuous exposure duration
$NOAEL_{HEC}$	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional reference concentration (inhalation)
p-RfD	provisional reference dose (oral)
POD	point of departure
RfC	reference concentration (inhalation)
RfD	reference dose (oral)
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_C	composite uncertainty factor
UF_D	incomplete-to-complete database uncertainty factor
UF_H	interhuman uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
WOE	weight of evidence

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR 9,10-ANTHRAQUINONE (CASRN 84-65-1)

BACKGROUND

HISTORY

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

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

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

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

DISCLAIMERS

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

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV document and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

QUESTIONS REGARDING PPRTVS

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

INTRODUCTION

9,10-Anthraquinone is a natural component of some plants (U.S. EPA, 1994a). Additionally, 9,10-Anthraquinone is an intermediate in the production of dyes and pigments, and it can also be formed during the kraft-pulping process conducted by the pulp and paper industry (Doi et al., 2005). It is also used as a catalyst in the isomerization of vegetable oils, as an accelerant in nickel electroplating (Doi et al., 2005), and as a bird repellant (Butterworth et al., 2001; Doi et al., 2005). 9,10-Anthraquinone is produced by three different methods: oxidation of anthracene, Diels-Adler reaction between 1,4-naphthoquinone and 1,3-butadiene, and Friedel-Craft reaction between benzene and phthalic anhydride with further treatment with concentrated sulfuric acid (Butterworth et al., 2004). Because anthracene is generally obtained from distilling coal tar, 9,10-anthraquinone obtained from oxidation of anthracene can contain varying amounts of polycyclic aromatic hydrocarbons (PAHs) (Butterworth et al., 2004). The empirical formula for 9,10-anthraquinone is $C_{14}H_8O_2$ (see Figure 1). A table of physicochemical properties is provided below (see Table 1). In this document, unless otherwise noted, "statistically significant" denotes a p-value < 0.05.

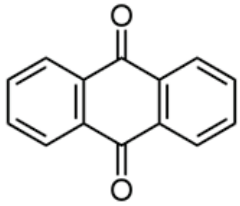

FIGURE 1. 9,10-Anthraquinone Structure

Table 1. Physicochemical Properties Table for 9,10-Anthraquinone (CASRN 84-65-1)[a]

Property (unit)	Value
Boiling point (ºC)	377
Melting point (ºC)	286
Density (g/cm^3)	-
Vapor pressure (Pa at 20ºC)	7.21×10^{-9}
pH (unitless)	-
Solubility in water (g/100 mL at 25ºC)	Estimated 3×10^{-7}
Relative vapor density (air = 1)	-
Molecular weight (g/mol)	208
Flash point (°C)	185
Log Octanol/water partition coefficient (unitless)	3.39

[a]Source: U.S. EPA (1994a)

No reference dose (RfD) or reference concentration (RfC) for 9,10-anthraquinone is included in the EPA IRIS database (U.S. EPA, 2010) or on the Drinking Water Standards and Health Advisories List (U.S. EPA, 2006). No RfD or RfC values have been reported in the HEAST (U.S. EPA, 2010). The Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1993, 1994b) does not include a Health and Environmental Effects Profile (HEEP) for 9,10-anthraquinone. Neither the Agency for Toxic Substances and Disease Registry (ATSDR, 2008) nor the World Health Organization (WHO, 2010) has reviewed the toxicity of 9,10-anthraquinone. CalEPA (2008a,b) has not derived toxicity values for exposure to 9,10-anthraquinone. No occupational exposure limits for 9,10-anthraquinone have been derived by the American Conference of Governmental Industrial Hygienists (ACGIH, 2010), the National Institute of Occupational Safety and Health (NIOSH, 2005), or the Occupational Safety and Health Administration (OSHA, 2010).

The EPA IRIS database does not include a cancer assessment for 9,10-anthraquinone (U.S. EPA, 2010) nor are cancer values included on the Drinking Water Standards and Health Advisories List (U.S. EPA, 2006). The International Agency for Research on Cancer (IARC, 2010) has not reviewed the carcinogenic potential of 9,10-anthraquinone. 9,10-Anthraquinone is not included in the *11th Report on Carcinogens* (National Toxicology Program [NTP], 2005a). However, NTP (2005b) has concluded that there is clear evidence of carcinogenicity in female F344/N rats and both male and female B6C3F$_1$ mice and some evidence of carcinogenicity in male F344/N rats. CalEPA (2008b) has not prepared a quantitative estimate of carcinogenic potential for anthraquinone. However, CalEPA's Office of Environmental Health Hazard (OEHHA), listed anthraquinone as a known carcinogen under Proposition 65, as of September 28, 2007 (CalEPA, 2007).

Literature searches were conducted on sources published from the 1950s through December 2010 for studies relevant to the derivation of provisional toxicity values for 9,10-anthraquinone, CAS No. 84-65-1. Searches were conducted using EPA's Health and Environmental Research Online (HERO) database of scientific literature. HERO searches the

following databases: AGRICOLA; American Chemical Society; BioOne; Cochrane Library; DOE: Energy Information Administration, Information Bridge, and Energy Citations Database; EBSCO: Academic Search Complete; GeoRef Preview; GPO: Government Printing Office; Informaworld; IngentaConnect; J-STAGE: Japan Science & Technology; JSTOR: Mathematics & Statistics and Life Sciences; NSCEP/NEPIS (EPA publications available through the National Service Center for Environmental Publications [NSCEP] and National Environmental Publications Internet Site [NEPIS] database); PubMed: MEDLINE and CANCERLIT databases; SAGE; Science Direct; Scirus; Scitopia; SpringerLink; TOXNET (Toxicology Data Network): ANEUPL, CCRIS, ChemIDplus, CIS, CRISP, DART, EMIC, EPIDEM, ETICBACK, FEDRIP, GENE-TOX, HAPAB, HEEP, HMTC, HSDB, IRIS, ITER, LactMed, Multi-Database Search, NIOSH, NTIS, PESTAB, PPBIB, RISKLINE, TRI; and TSCATS; Virtual Health Library; Web of Science (searches Current Content database among others); World Health Organization; and Worldwide Science. The following databases outside of HERO were searched for toxicity values: ACGIH, ATSDR, CalEPA, EPA IRIS, EPA HEAST, EPA HEEP, EPA OW, EPA TSCATS/TSCATS2, NIOSH, NTP, OSHA, and RTECS.

REVIEW OF POTENTIALLY RELEVANT DATA (CANCER AND NONCANCER)

Table 2 provides information for all of the potentially relevant toxicity studies. Entries for the principal studies are bolded and identified by the marking "PS."

Table 2. Summary of Potentially Relevant Data for 9,10-Anthraquinone (CASRN 84-65-1)

Notes[a]	Category	Number of Male/Female, Species, Study Type, Study Duration	Exposure Levels[b]	Critical Effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b,c]	Reference (Comments)
Human								
				1. Oral (mg/kg-day)[b]				
				None				
				2. Inhalation (mg/m^3)[b]				
	Acute	Number not specified, occupational exposure to anthraquinone dust, duration not specified	2−10 55−840 1650	Headache, general weakness, and skin and eye irritation (no quantitative information available).	-	-	-	Volodchenko et al. (1971)
	Subchronic	None						
	Chronic	Cause-specific mortality in 3266 (2859 men and 407 women) workers in a dye and resin manufacturing plant in New Jersey with at least 6 months employment	Not reported	Mortality, overall mortality was 10% lower than the general population.	-	-	-	Sathiakumar and Delzell (2000)
	Developmental	None						
	Reproductive	None						
	Carcinogenic	Cause-specific mortality in 3266 (2859 men and 407 women) workers in a dye and resin manufacturing plant in New Jersey with at least 6 months employment	Not reported	No difference in mortality due to all cancer; statistically significant increase in lung cancers in workers with anthraquinone dyes (South Dye workers, standardized mortality ratio [SMR] = 168; 95% CI: 115−237), but the study did not control for smoking or possible occupational exposure to other lung carcinogens (e.g., asbestos).	-	-	-	Sathiakumar and Delzell (2000)

Table 2. Summary of Potentially Relevant Data for 9,10-Anthraquinone (CASRN 84-65-1)

Notes[a]	Category	Number of Male/Female, Species, Study Type, Study Duration	Exposure Levels[b]	Critical Effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b,c]	Reference (Comments)
		1975 males, retrospective cohort, at least 6 months of employment in a dye factory in Scotland	Not reported	Cancer-related mortality was observed (not increased).	-	-	-	Gardiner et al. (1982) Stated to be exposed to substituted anthraquinones
		51 Caucasian male lung cancer cases (dye workers), 102 age-matched controls	Not reported	Lung cancer was observed (odds ratio [OR] of 2.4; 95% confidence interval [CI] of 1.1−5.2).	-	-	-	Barbone et al. (1992)
Animal								
				1. Oral (mg/kg-day)[b]				
PS	**Subchronic**	**10 Male/10 Female, F344 rat, dietary, 14 weeks**	**0, 135, 275, 555, 1130, or 2350[d]**	**Decreased body-weight gain (females), anemia (hematology changes), changes in clinical chemistry, increased estrous cycle, increased relative right kidney and liver weight, increased incidence of histopathological lesions in the liver, kidney, spleen, bone marrow, and thyroid, and urinary bladder effects were observed.**	**None**	**Not conducted**	**135**	**NTP (2005b)**

Table 2. Summary of Potentially Relevant Data for 9,10-Anthraquinone (CASRN 84-65-1)

Notes[a]	Category	Number of Male/Female, Species, Study Type, Study Duration	Exposure Levels[b]	Critical Effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b,c]	Reference (Comments)
		10 Male/10 Female, B6C3F$_1$ Mouse, dietary, 14 weeks	0, 250, 500, 1050, 2150, or 4300 in males 0, 300, 640, 1260, 2600, or 5300 in females[d]	Anemia (hematology changes), increased relative right kidney (males) and liver weight, increased incidence of histopathological lesions in the liver and spleen, and urinary bladder.	None	Not conducted	250	NTP (2005b)
PS	**Chronic**	**50 Male/50 Female, F344 rat, dietary, 2 years (105 weeks)**	**0, 20, 45, 90, or 180 in males 0, 25, 50, 100, or 200 in females[d]**	**Decreased body weight, increased incidence of nonneoplastic lesions in the kidney, liver, and spleen, and bone marrow.**	**None**	**Not conducted**	**20**	**NTP (2005b)**
		50 Male/50 Female, B6C3F$_1$ mouse, dietary, 2 years (105 weeks)	0, 90, 265, or 825 in males 0, 80, 235, or 745 in females[d]	Decreased body weight, increased incidence of nonneoplastic lesions in the liver and spleen, and urinary bladder.	None	Not conducted	80	NTP (2005b)
	Developmental	None						
	Reproductive	None						
	Carcinogenic	50 Male/50 Female, F344 rat, dietary, 2 years (105 weeks)	0, 5.4, 12.2, 24.4, or 48.9 in males 0, 6.0, 12.0, 23.9, or 47.8 in females[e]	Increased incidence of kidney and liver, and urinary bladder tumors were observed.	NA	None	NA	NTP (2005b)[f]
PS		**50 Male/50 Female, B6C3F$_1$ mouse, dietary, 2 years (105 weeks)**	**0, 13.7, 40.3, or 125.3 in males 0, 12.0, 35.2, or 111.6 in females[e]**	**Increased incidence of liver and thyroid tumors, males also had increased number of animals with malignant neoplasms.**	NA	**2.6**	NA	**NTP (2005b)[f]**

Table 2. Summary of Potentially Relevant Data for 9,10-Anthraquinone (CASRN 84-65-1)

Notes[a]	Category	Number of Male/Female, Species, Study Type, Study Duration	Exposure Levels[b]	Critical Effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b,c]	Reference (Comments)
		18/18 (C57BL/6 × C3H/Anf)F1 and (C57BL/6 × AKR)F1 mouse, gavage followed by dietary, 19 months	464[g]	Tumors were investigated (study was negative).	NA	Not conducted	NA	Innes et al. (1969)
2. Inhalation (mg/m^3)[b]								
	Subchronic	Number and sex not reported, mouse (strain not reported), inhalation, 4 months	0, 5.2 or 12.1 mg/m^3 [h]	Reduced body weight, hemoglobin, erythrocytes, vitamin C, relative reticulopenia, and increased incidence of lung lesions.	ND[i]	Not conducted	ND[i]	Volodchenko et al. (1971)
	Chronic	None						
	Developmental	None						
	Reproductive	None						
	Carcinogenic	None						

[a]Notes: PS = Principal study
[b]Dosimetry: All long-term exposure values (4 weeks and longer) are converted from a discontinuous to a continuous (weekly) exposure. Values for inhalation (cancer and noncancer), and oral (cancer only) are further converted to an HEC/D. Values from animal developmental studies are not adjusted to a continuous exposure.
[c]Not reported by the study author but determined from data
[d]Doses were reported in the study in mg/kg-day, presumably based on the concentrations in the diet (also provided in the study report), body weights, and food consumption (both of which were routinely measured)
[e]These doses are human equivalent doses (HED) that were calculated as follows: average daily dose in mg/kg-day × (average animal body weight ÷ average human body weight)$^{1/4}$ e.g., 180 mg/kg-day × (0.38 kg [male rat] ÷ 70 kg)$^{1/4}$= 48.9 mg/kg-day
[f]Butterworth et al. (2001) raised concern that the contaminant 9-nitroanthracene (9-NA) is responsible for the carcinogenicity observed in the NTP (2005b) studies. The NTP subcommittee stated that it was unlikely that 9-NA contributed to the carcinogenicity because of the low exposure levels, bioavailability, and relative mutagenicity. NTP concluded that there was clear evidence of carcinogenicity in female rats and mice of both genders
[g]Study states that animals were administered 464 mg/kg-day from 7 days of age until weaning at 28 days of age (dose was not adjusted for increase in body weight) via gavage followed by 1206 ppm in the diet (which was selected based on the maximum tolerated dose of 464 mg/kg-day)
[h]These exposure values are not adjusted to an HEC due to incomplete information in the report
[i]Not determined; insufficient detail

HUMAN STUDIES

Oral Exposures

No studies investigating the effects of subchronic or chronic oral exposure to 9,10-anthraquinone in humans have been identified.

Inhalation Exposures

Little information is available regarding occupational exposure of humans to 9,10-anthraquinone, but a small number of epidemiology studies analyzing outcomes of workers exposed during manufacturing are available and are presented below. Although the majority of occupational exposure is likely via inhalation, dermal and oral exposures may have occurred.

ICI Americas (1985) provided a copy of Volodchenko et al. (1971) in the original Russian, as well as an English translation. In a production area where anthraquinone was produced by contact methods (specifics were not stated), the job area was stated to be contaminated with anthraquinone dust. The study authors reported the levels of dust in three areas as follows: 2−10 mg/m^3 near the contact apparatus, 55−840 mg/m^3 near scales, and as high as 1650 mg/m^3 when cleaning the gas conduits, with the majority (70%) of the dust reported to be 3.2 microns. No other specifics regarding any measurements were provided in the study report. The workers were reported to have complained of headache, general weakness, and irritation of skin (unprotected area) and eyes, though these effects were not analyzed quantitatively.

Sathiakumar and Delzell (2000) examined the cause-specific mortality rates in 3266 workers (2859 men and 407 women) who had worked for at least 6 months between 1952 to 1996 at a dye and resin manufacturing plant in New Jersey. Subjects were followed up for an average of 27 years, and the overall mortality rates were compared to the general population of New Jersey. Subjects were selected based on availability of dates of employment and Social Security numbers. Information was obtained from plant records and previous epidemiology investigations. In the plant, the South Dyes area produced anthraquinone dyes and intermediates, and the North Dyes area produced azo dyes and intermediates, as well as plastics and additives. Employment information was used to classify subjects according to eight major work areas (production, laboratories, maintenance, energy, waste treatment, warehouses, engineering, and administration) and four production subgroups (south dyes, north dyes, plastics, and additives, and other production). The overall mortality rate of the cohort was 10% lower than the general population of New Jersey. There was no difference in the overall cancer mortality. Although the overall cohort had slightly increased mortality from cancers of the colon,[1] the results were not statistically significant. There was, however, a statistically significant increase in deaths from lung cancers in South Dye workers (SMR: 168, 95% CI: 115−237), and North Dye workers had a statistically significant increase in deaths from stomach (SMR: 386, 95% CI: 125−901), bladder (SMR: 515, 95% CI: 140−1318), and central nervous system cancer (SMR: 517, 95% CI: 168−1206). The relative risk for lung cancer in South Dye workers compared to other workers was 1.7 (95% CI: 1.1−2.4). However, the results lacked a duration response, with increases noted in workers with <5 years and those with 20+ years but not in workers with interim years of employment. With regard to the increased incidence of bladder malignancies,

[1]Standardized mortality ratio [SMR]: 118, 95% CI: 78−172), lung (SMR: 122, 95% CI: 98−150), liver (SMR: 143, 95% CI: 53−311), bladder (SMR: 139, 95% CI: 60−273), central nervous system (SMR: 134, 95% CI: 58−264), and lymphosarcoma (SMR: 121, 95% CI: 25−354).

the study authors established that some employees had previously worked at the Cincinnati Chemical Works (CCW) in Ohio, which both produced and used benzidine, a known bladder carcinogen. In addition, the current study did not control for smoking and did not have information on occupational exposure to other possible carcinogens (e.g., asbestos).

Gardiner et al. (1982) conducted a retrospective cohort in 1975 workers of a dye factory in Scotland who had worked there for at least 6 months from January 1, 1956, to December 31, 1965. Mortality of these subjects was evaluated through June 30, 1980. Workers were stated to be exposed to substituted anthraquinones. All but 11 of the initial subjects were accounted for through June 30, 1980. There were a total of 470 deaths of these subjects. The age-adjusted mortality rate in Scotland as a whole was used as the control. There was no increase in overall mortality (SMR: 76.9; 470 observed ÷ 611.5 expected) or in mortality from all malignant neoplasms (SMR: 86.2; 129 observed ÷ 149.7 expected). An excess of esophageal cancer was noted in the engineering department (SMR: 139.5; 6 observed ÷ 4.3 expected), but no common cause could be identified. The study authors concluded that there was no increase in total or cancer-related mortality based on the age-adjusted SMRs.

Barbone et al. (1992) conducted a case-control study using 51 lung cancer cases and 102 controls from a cohort of Caucasian males employed at a dye and resin manufacturing plant in New Jersey. Workers in these cases developed lung cancer prior to October 1, 1988. Two controls meeting the following two criteria were selected for each case: (1) each control had the same birth year as the case; and (2) each control was still alive at the time of death or diagnosis of the case. Work histories were used to classify subjects by six work areas. Interviews were conducted to determine exposure assessment. The odds ratio (OR) for lung cancer in workers in the anthraquinone dye and epichlorohydrin production was 2.4 (95% CI: 1.1−5.2). There was no trend with duration of exposure. The ORs varied by building, with ORs of 12 (95% CI: 1.4−99) in the anthraquinone production building, 1.8 (95% CI: 0.6−5.1) in the anthraquinone intermediate dye building, 1.2 (95% CI: 0.5−2.9) in the anthraquinone dye synthesis building, and 3.3 (95% CI: 1.0−11) in the anthraquinone dye standardization building. The study was complicated because subjects had visited the infirmary for acute exposure to 48 different chemicals. Smoking histories were not available for about 20% of the subjects, but smoking was found to be a major cause of lung cancer among workers at this plant. In four of the six cases, workers employed in the anthraquinone dye and epichlorohydrin production area were also exposed to chlorine, which also had an elevated OR.

ANIMAL STUDIES

Oral Exposures

The effects of oral exposure of animals to anthraquinone have been evaluated in subchronic (NTP, 2005b), chronic (NTP, 2005b), and cancer (NTP, 2005b; Innes et al., 1969) studies. The National Toxicology Program (NTP, 2005b) conducted 14-week and 2-year chronic/carcinogenicity dietary studies in rats and mice. Because there are four studies (and genotoxicity studies discussed later), when discussing separate studies, the studies will be designated with different letters such that the subchronic rat study is NTP (2005b), etc., as is indicated in Tables 2 and 3, and when referring to the whole report throughout the document, it will be cited as NTP (2005b). The NTP (2005b) studies were conducted according to Good Laboratory Practice (GLP) regulations and were peer reviewed. There were no additional

subchronic or short-term oral studies with 9,10-anthraquinone. However, there was an additional chronic carcinogenicity study (Innes et al., 1969) using two strains of mice.

Subchronic Studies

The 14-week rat study by NTP (2005b) is selected as the principal study for derivation of a screening subchronic p-RfD. The NTP (2005b) administered 9,10-anthraquinone (99.8% pure) to male and female F344 rats (10/sex/treatment group) at dietary concentrations of 0, 1875, 3750, 7500, 15,000, or 30,000 ppm for 14 weeks. These concentrations were stated to be equivalent to average daily doses of 0, 135, 275, 555, 1130, and 2350 mg/kg-day, respectively, for both males and females. Dose formulations were prepared every 4 weeks by mixing 9,10-anthraquinone into the diet. Homogeneity analysis was conducted on 1875- and 30,000-ppm dose formulations, and stability analysis was conducted on a 230-ppm dose formulation. Dose formulations were found to be homogeneous and were stable for at least 35 days when stored at room temperature and protected from the air and light. Dose formulations were analyzed at the beginning and end of the 14-week study. Dose concentrations were within 10% of the nominal concentration. Animals were weighed, and clinical signs were recorded weekly. Food consumption was recorded twice a week (five animals were housed to a cage) and served as the basis for calculating the daily rates of compound consumption. Blood was collected on Days 4 and 22 and at study termination for hematology (erythrocyte, platelet, and leukocyte counts; hematocrit; hemoglobin, mean cell hemoglobin [MCH], mean cell volume [MCV], and mean cell hemoglobin concentration [MCHC]; erythrocyte and platelet morphology; and differential leukocyte counts) and clinical chemistry (urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase [ALT], alkaline phosphatase [ALP], creatine kinase, sorbitol dehydrogenase [SDH], and bile salts). Urinalysis (creatinine, glucose, total protein, aspartate aminotransferase, *N*-acetyl-β-D-glucosaminidase, γ-glutamyltransferase, total volume, and specific gravity) was conducted on urine collected in metabolism cages on Days 8, 26, and 89. Rats (10/sex/treatment group) specified for interim hematology, clinical chemistry, and urinalysis were administered the same concentrations and were euthanized without necropsy after urine collection on Day 26. At the end of the study, samples were collected from males for analysis of the reproductive organs (spermatid heads per testis and per gram testis, spermatid counts, and epididymal spermatozoal motility and concentration; left cauda epididymis, left epididymis, and left testis weight) and from females for vaginal morphology (estrous cycle length and relative frequency of the estrous stages) in controls and the three highest concentrations. Soluble protein and α2u-globulin were measured in the kidney homogenates at study termination in males. Necropsies were performed at terminal sacrifice and organs (heart, right kidney, liver, lungs, right testis, and thymus) were weighed. Complete histopathology was conducted on control and high-dose animals. Histopathology of the bone marrow, liver, kidney, spleen, thyroid gland, and urinary bladder (females only) were routinely examined in all groups.

All animals survived until study termination (NTP, 2005b). There were no changes in overall body-weight gain in males (weekly data were not provided). Although there was an initial decrease in food consumption in 275-mg/kg-day males, the food consumption was equal to or greater than the controls during the final week of treatment (see Table B.1). In female rats, there was a dose-dependent decrease in overall body-weight gain that was statistically different from the control even in the lowest treatment group. Although food consumption during the first week of treatment decreased in a dose-dependent manner, food consumption during the final week of treatment was 18−24% greater in all treatment groups compared to the control.

However, the study authors stated that the increased food consumption may have been due to scattering of the food and that the reduced food consumption was likely due to reduced palatability. There were no clinical signs related to treatment reported. By Day 4, animals had increased hematocrit, hemoglobin, and erythrocyte counts with doses ≥275 mg/kg-day, indicating erythrocytosis. However, this was followed by anemia that began on Day 22 and persisted through study termination (as measured by decreased hemoglobin, erythrocyte counts, and hematocrit). Hematology results were not related to dose, and while the decrease in hemoglobin and erythrocyte counts were statistically reduced in all treatment groups in both sexes (see Table B.2), hematocrit values were not. The lack of a decrease in hematocrit is likely due to the increase in mean cell volume. The statistically significant increase in reticulocytes in all treatment groups in both sexes indicates an erythropoietic response to the anemia. There were statistically significant increases in platelets, creatinine, total protein, and albumin at all doses generally in both sexes (see Table B.2). Urea nitrogen levels were generally increased but were only statistically significant in males with doses ≥555 mg/kg-day and in 275-mg/kg-day females. There were statistically significant decreases in ALP (dose dependent, both sexes) and bile salts (males only) and transient changes observed in the urine. Although there were no changes in sperm parameters or reproductive organs in the males, females administered 1130 or 2350 mg/kg-day had longer estrous cycles. There was a statistically significant increase in α2u-globulin (μg/g soluble protein) in the kidney homogenates in all treatment groups (only males evaluated) at study termination. There was no dose-response, with the increase similar in all treatment groups.

Necropsy body weight was significantly decreased in 2350-mg/kg-day males and in females treated with ≥275 mg/kg-day (NTP, 2005b). There was a dose-dependent increase in the relative weight of the right kidney and the liver with statistically significant increases observed at all doses in both sexes (see Table B.3). Histopathology of the liver revealed hypertrophy in all treated males and females, but none in the controls. In males, there was a dose-dependent increase in severity that was not observed in females. Histopathology of the kidney revealed hyaline droplet accumulation in all treated animals (both males and females) but not in the controls. Although all male rats (including the controls) had nephropathy, the severity was increased in treated rats. There was a statistically significant increase in nephropathy in females treated with ≥1130 mg/kg-day. Other histopathological findings included congestion (all treated rats), hematopoietic cell proliferation (all but one treated rat), and pigmentation (all treated rats) in the spleen; hyperplasia in the bone marrow (statistically significant increase in all groups except the lowest dose group in male rats); follicular cell hypertrophy in the thyroid (all rats exposed to ≥275 mg/kg-day); and inflammation and transitional epithelium hyperplasia in the urinary bladder (statistically significant increase in the highest female group only; males not examined) (see Table B.4). As all animals (i.e., 10/10) were affected in one endpoint or another of those listed in Table B.4, and at all doses including the lowest dose, no NOAEL can be derived from the data. These levels of response also preclude any meaningful modeling of these or of the organ-weight data (see Table B.3). However, a LOAEL of 135 mg/kg-day, the lowest dose tested, is established from the data, based on numerous changes in hematology, clinical chemistry, organ weights, body-weight gain (females), and histopathology.

In a separate study, NTP (2005b) administered 9,10-anthraquinone (99.8% pure) to male and female B6C3F$_1$ mice (10/sex/treatment group) at dietary concentrations of 0, 1875, 3750, 7500, 15,000, or 30,000 ppm for 14 weeks. These concentrations were stated to be equivalent to

average daily doses of 0, 250, 500, 1050, 2150, and 4300 mg/kg-day, respectively, in males and 0, 300, 640, 1260, 2600, and 5300 mg/kg-day, respectively, in females. Dose formulations were analyzed as in the rat study detailed above. Dose concentrations were within 10% of the nominal concentration. Methods were the same as those used in the rat study detailed above except male mice were individually housed, hematology and clinical chemistry were only conducted at study termination, and urinalysis was not conducted.

All animals survived until study termination (NTP, 2005b). There were no changes in body weight, body-weight gain, or food consumption in any of the treatment groups in either sex compared to the control group. There were no clinical signs related to treatment reported. Anemia, as measured by decreased hemoglobin, erythrocyte counts, and hematocrit, was noted at study termination with results more pronounced in female mice compared to male mice (see Table B.5). This was also associated with increases in mean cell volume and mean cell hemoglobin concentration. The increase in reticulocytes in all treatment groups in both sexes indicates an erythropoietic response to the anemia. There was also a statistically significant increase in platelets (see Table B.5). Although the methods indicate that clinical chemistry was conducted in the mice, there were no data reported. There were no changes in sperm parameters and reproductive organs in the males, and there were no effects on the estrous cycles in females.

Necropsy body weight was not statistically significantly affected in either gender (NTP, 2005b). There was a dose-dependent increase in the relative weight of the liver with statistically significant increases observed at all doses in both sexes (see Table B.6). High-dose males had a statistically significant increase in relative kidney weight. Histopathology of the liver revealed centrilobular hypertrophy in both sexes with statistically significant increases observed with doses ≥500 mg/kg-day. Severity increased in a dose-dependent manner in both genders. Other histopathological findings included (see Table B.7) hematopoietic cell proliferation (nearly all treated males), and pigmentation (all but one treated male) in the spleen; and transitional epithelium, cytoplasmic alteration in the urinary bladder (all treated mice) with a dose-dependent increase in severity in both genders. This study was used to determine concentrations for the 2-year study (NTP, 2005b); therefore, no NOAEL or LOAEL was reported. As with rats, all animals (10/10) were affected in at least one endpoint of those listed in Table B.7, and effects were observed at all doses including the lowest dose. These levels of response also preclude any meaningful modeling of these or of the organ-weight data (see Table B.6). No NOAEL can be derived from the data. However, a LOAEL of 250 mg/kg-day in male mice and 300 mg/kg-day in female mice is available from the data, based on numerous changes in hematology, organ weights, and histopathology.

Chronic/Carcinogenicity Studies

The 2-year rat study by NTP (2005b) is selected as the principal study for derivation of the screening chronic p-RfD. NTP (2005b) administered 9,10-anthraquinone (99.8% pure) to male and female F344 rats (50−60/sex/treatment group) at dietary concentrations of 0, 469, 938, 1875, or 3750 ppm for 2 years. These concentrations were stated to be equivalent to average daily doses of 0, 20, 45, 90, and 180 mg/kg-day, respectively, in males and 25, 50, 100, and 200 mg/kg-day, respectively, in females. Dose formulations were analyzed for homogeneity and stability as detailed above in the subchronic rat study. Dose formulations were analyzed for concentration every 8 or 12 weeks. Dose concentrations were within 10% of the nominal concentration. Food consumption was measured every four weeks (three males per cage and five females per cage) and served as the basis for calculating the daily rates of compound

consumption. Animals were examined twice daily with clinical signs recorded every four weeks. Body weight was recorded on Day 8, every 4 weeks, and at study termination. Five rats per sex in the control and high-dose groups were sacrificed for evaluation at 3 and 12 months. All animals were necropsied. Kidneys and livers were weighed at 12 months. Soluble protein and α2u-globulin were measured in the kidneys at 3 months for both males and females. At terminal sacrifice, animals were necropsied and a complete histopathological examination was conducted.

The survival in all exposed groups of male rats was similar to that of the controls (NTP, 2005b). Survival of all exposed groups of female rats was statistically significantly greater than that of the controls. Body weights were generally lower in both males and females during the later part of the study. Treated male rats had overall body-weight gains 5−10% lower than the controls, but the decrease was not correlated with increasing dose (see Table B.8). However, in females, there was a dose-dependent decrease in overall body-weight gain (see Table B.9) that ranged from 16−30% lower than the controls. α2u-Globulin (soluble protein) in kidney supernatant was reported for the control and high-dose group at 3 months with results indicating an increase in treated males and a decrease in treated females. There were extensive nonneoplastic lesions in the kidney, liver, spleen, and bone marrow (see Tables B.8 and B.9) as well as neoplastic lesions in the kidney, liver, and urinary bladder (see Tables B.10 and B.11) in both male and female rats. There was a dose-dependent increase in renal tubule adenoma or carcinoma in female rats—but not in male rats. Although there were increases in hepatocellular adenomas in female rats, their increase was not related to dose, and only 50 mg/kg-day females had a statistically significant increase above the controls. There was a statistically significant increasing trend for transitional epithelial papilloma or carcinoma in the urinary bladder, but the increase was small. The increase in males was greater, achieving a statistically significant increase in males administered 90 mg/kg-day, but there was only an apparent trend if high-dose males were dropped. Large fractions of treated animals were affected in one endpoint or the other, which were statistically significantly increased even at the lowest dose examined. However, the results generally were consistent across treatment groups and did not increase with increasing doses. The nature of the dose-response precludes any meaningful modeling of the data. Although there were some increases in lesions of the parathyroid gland, bone, stomach, and lung (mainly in the males), the study authors considered these to be secondary to the impaired renal function due to perturbations in calcium homeostasis, which is commonly observed in rats with severe nephropathy. The study authors did not specify a NOAEL or LOAEL but stated that there is some evidence for carcinogenicity in male rats and clear evidence for carcinogenicity in female rats. No NOAEL can be derived from the data; however, the LOAEL is 20 mg/kg-day in males and 25 mg/kg-day in females, based on the nonneoplastic lesions in the kidney, liver, and spleen in both male and female rats.

The 2-year mouse study by NTP (2005b) is selected as the principal study for derivation of the p-OSF. NTP (2005b) administered 9,10-anthraquinone (99.8% pure) to male and female B6C3F$_1$ mice (50/sex/treatment group) at dietary concentrations of 0, 833, 2500, or 7500 ppm for 2 years. The study authors stated these concentrations to be equivalent to average daily doses of 0, 90, 265, and 825 mg/kg-day, respectively, in males and 0, 80, 235, and 745 mg/kg-day, respectively, in females. Dose formulations were analyzed as detailed in the 2-year NTP (2005b) rat study. Dose concentrations were within 10% of the nominal concentration. food consumption was measured every 4 weeks (one male per cage and five females per cage), which served as the basis for calculating the daily rates of compound

consumption. Animals were examined twice daily with clinical signs recorded every 4 weeks Body weight was recorded on Day 8, every 4 weeks, and at study termination. At terminal sacrifice, animals were necropsied and complete histopathological examinations were conducted.

There was a statistically significant ($p < 0.001$) decrease in survival relative to controls in high-dose male mice that also followed a significant ($p < 0.001$) trend, but there was no difference in survival relative to the controls noted in female mice (NTP, 2005b). Body weights were generally lower in high-dose males and females during the later part of the study. Treated male mice had dose-dependent decreases in overall body weight, with gains 6−38% lower than the controls. However, in females, there were decreases in overall body-weight gain (see Table B.12) in only the mid- (235 mg/kg-day) and high-dose groups (745 mg/kg-day) compared to the controls. There were extensive nonneoplastic lesions in the liver, spleen, urinary bladder, and thyroid gland (see Tables B.12 and B.13) and neoplastic lesions in the liver and thyroid gland (see Tables B.14 and B.15) of both male and female rats. Statistically significant increases in nonneoplastic lesions included centrilobular hypertrophy in the liver (all groups in males and females); focal, fatty degeneration in the liver (low- and mid-dose males and high-dose females); erythrophagocytosis in the hepatocytes (all groups in males and females); eosinophilic focus in the liver (mid- and high-dose males and high-dose females); focal necrosis (high-dose males and females); hematopoietic cell proliferation in the spleen (high-dose males and females); follicular cell hyperplasia in the thyroid gland (high-dose males), and intracytoplasmic inclusion body in the urinary bladder (all treatment groups in both sexes). There was a dose-dependent increase in hepatocellular adenoma, carcinoma, and/or hepatoblastoma in both sexes with significant increases noted even with the low dose. There was also an increasing trend for follicular cell adenoma or carcinoma in the thyroid in males and females, but the results did not achieve statistical significance. However, male mice had a statistically significant increase in total malignant neoplasms at all doses. The study authors did not specify a NOAEL or LOAEL but stated that there is clear evidence for carcinogenicity in male and female mice. No NOAEL can be derived from the data; however, the LOAEL is 90 mg/kg-day in males and 80 mg/kg-day in females, based on the nonneoplastic lesions in the liver and bladder in both male and female rats.

Innes et al. (1969) present data on bioassays of 120 different chemicals including 9,10-anthraquinone. Female C57BL/6 mice were mated to C3H/Anf or AKR male mice to produce (C57BL/6 × C3H/Anf)F1 and (C57BL/6 × AKR)F1 mice. The maximum tolerated dose of 464 mg/kg was determined through a series of preliminary short-term studies that found no mortality after a single dose, 6 daily doses, and then 19 daily doses. Subsequently, starting at 7 days of age, 464 mg/kg 9,10-anthraquinone (purity not specified) in 0.5% gelatin was administered by daily gavage until weaning at 28 days of age. Although the dose was presented as mg/kg of body weight, the dose was not adjusted for weight gain during the 3-week period. At weaning, 18 mice of each sex per strain were retained and exposed to 1206 ppm mixed directly into the diet (this was stated to be based on the food consumption rate and body weight at weaning so that the animals continued to receive the maximum tolerated dose) for approximately 18 months. Ethyl carbamate was used as a positive control. Animals were sacrificed after 18 months (a range of termination times were noted due to the large number of animals in the ongoing study). Animals were necropsied and all major organs and gross lesions were examined histologically. 9,10-Anthraquinone was stated not to cause a significant increase in tumors, but specific results were not provided.

Inhalation Exposures

There is only a single subchronic inhalation study available (Volodchenko et al., 1971), and no chronic inhalation studies were available. ICI Americas (1985) provided a copy of the Volodchenko et al. (1971) Russian publication, as well as an English translation. Little useful information about the methods, the subjects, or the results is available from the report or the translation (reported exposure levels and effects given in Table 2). This study is not considered further for derivation of a p-RfC.

Chronic Studies

No studies investigating the effects of chronic inhalation of 9,10-anthraquinone in animals have been identified.

Reproductive and Developmental Studies

There are no formal developmental or reproductive studies available for 9,10-anthraquinone or its metabolites. However, some information for these endpoints is available from the NTP (2005b) studies. At the end of the 14-week study in rats (NTP, 2005b) and mice (NTP, 2005b), sperm samples were collected from all core study male rats and mice in the 0-, 7500-, 15,000-, and 30,000-ppm groups for sperm count and motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, spermatid counts, and epididymal spermatozoal motility and concentration. The left cauda epididymis, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from the core study female rats and mice in the 0-, 7500-, 15,000-, and 30,000-ppm dose groups. Vaginal cytology evaluations were used to evaluate estrous cycle length and relative frequency of the estrous cycle stages (i.e., diestrus, proestrus, estrus, and metestrus).

No differences in epididymal spermatozoal measurements were observed between exposed and control groups of rats (NTP, 2005b) or mice (NTP, 2005b). Estrous cycle lengths (in days) were longer in 15,000- and 30,000-ppm female rats compared to the controls. Results were also dose dependent with cycle lengths of 4.55 ± 0.17 days, 4.90 ± 0.15 days, 5.40 ± 0.31 days ($p < 0.05$), and 6.15 ± 0.33 days ($p < 0.01$) reported in the control, 7500-ppm, 15,000-ppm, and 30,000-ppm groups, respectively. No significant changes were observed in female mice.

OTHER DATA

Table 3 summarizes the toxicokinetic, mutagenicity, and genotoxicity studies conducted with 9,10-anthraquinone. Of particular note is the NTP (2005b) mutagenicity assays and the Butterworth et al. (2001) assessment of the mutagenicity of 2-nitroanthracene (2-NA), a reported contaminant of the anthraquinone formulation used in the NTP (2005b) bioassay. Butterworth et al. (2001) maintained that the tumorigenicity of 9,10-anthraquinone observed in the NTP (2005b)[2] bioassay was entirely due to the mutagenic 2-NA contaminant. NTP (2005b), however, found that the mutagenicity of 2-hydroxyanthracene, a major metabolite of 9,10-anthraquinone, was 7 times as mutagenic as 2-NA and would be a much more likely

[2]The results were apparently first reported in NTP Technical Report 494 (1999), which was not found on the NTP Web site. The bioassay was conducted even earlier, with results reported in Zeiger et al. (1988). The report was apparently updated in NTP, 2005b.

candidate for the causative agent, if mutagenicity was involved in the mode of action (a mutagenic MOA has not been established; see cancer oral descriptor section following).

A physiologically based pharmacokinetic (PBPK) model was developed to characterize rat tissue concentrations of 9,10-anthraquinone resulting from oral exposure in rats (NTP, 2005b). Plasma 9,10-anthraquinone concentrations estimated from this model may serve as a surrogate dosimeter for evaluating exposure-response data. However, the model is for rats only and cannot be extended to mice or humans for purposes of dosimetric adjustment.

Table 3. Other Studies for 9,10-Anthraquinone

Test	Materials and Methods	Results	Conclusions	Reference
Toxicokinetics	Blood was obtained on Day 8, and at 3, 6, 12, and 18 months (2−3 animals per time point) from male and female rats administered 3750 ppm during the core study.	Plasma concentrations were twice as high in females as males.	N/A	NTP (2005b)
Toxicokinetics	Male and female F344 rats and B6C3F$_1$ were administered ^{14}9,10-anthraquinone as a single administration either intravenously (2 mg/kg in rats, 4 mg/kg in mice) or via gavage (40, 100, or 400 mg/kg in rats and 100, 200, or 800 mg/kg in mice). Plasma concentrations were measured at various times.	After intravenous injection, plasma concentrations peaked initially and then decreased over time. After oral exposures, plasma concentrations peaked at around 8−12 hours in rats and at about 4 hours in mice. Although the plasma concentrations increased with dose, the plasma concentrations did not increase in a linear manner.	The data indicated first order absorption and elimination in a two-compartment open model.	NTP (2005b)
Toxicokinetics	24-hour urine samples were collected from male F344 rats administered different types of 9,10-anthraquinone samples; specifics on the test were unclear.	2-Hydroxyanthraquinone was the major metabolite excreted in the urine followed by 1-hydroxyanthraquinone. It was estimated that 2-hydroxyanthraquinone was present at a level 5.8 times greater than was theoretically possible for 9-NA.	2-Hydroxyanthraquinone is a major metabolite of 9,10-anthraquinone.	NTP (2005b)
Mutagenicity	Tested for reverse mutation in *Salmonella typhimurium* (preincubation assay) strains TA98 and TA100, with and without metabolic activation, at doses of 0 (DMSO only), 33, 100, 333, 1000, or 2500 µg/plate.	9,10-Anthraquinone caused an increase in reverse mutations, both in the presence and absence of metabolic activation, with the number greater than observed with the positive control.	9,10-Anthraquinone was considered mutagenic.	Zeiger et al. (1988)
Mutagenicity	Tested for reverse mutation in *Salmonella typhimurium* (Ames assay) strains TA98, TA100, TA1535, and TA1538, with metabolic activation, at doses of 0 (DMSO only), 4, 20, 100, 500, or 2500 µg/plate.	9,10-Anthraquinone was reported as negative.	9,10-Anthraquinone was not considered mutagenic.	Anderson and Styles (1978)
Mutagenicity	Tested for reverse mutation in *Salmonella typhimurium* (Ames assay) strains TA97, TA98, and TA100, with and without metabolic activation, at doses of 0 (DMSO only), 5, 10, 50, or 200 µg/plate.	There was no increase in the mutation frequency.	9,10-Anthraquinone was not considered mutagenic.	Sakai et al. (1985)

Table 3. Other Studies for 9,10-Anthraquinone

Test	Materials and Methods	Results	Conclusions	Reference
Mutagenicity	Tested for reverse mutation in *Salmonella typhimurium* (Ames assay) TA97, TA100, and TA2637 with metabolic activation with a maximum dose of 100 µg/plate; exact concentrations tested not reported.	9,10-Anthraquinone was reported as negative.	9,10-Anthraquinone was not considered mutagenic.	Tikkanen et al. (1983)
Mutagenicity	Tested for reverse mutation in *Salmonella typhimurium* (Ames assay) strains TA98, TA100, TA1535, TA1537, and TA1538, with and without metabolic activation, at doses of 0 (DMSO only), 0.2, 2, 10, or 20 µg/plate.	There was an increase in mutation frequency with TA98, TA1537, and TA1538, without metabolic activation.	9,10-Anthraquinone was considered mutagenic.	Liberman et al. (1982)
Mutagenicity	Tested for reverse mutation in *Salmonella typhimurium* (Ames assay) strains TA102 and TA1537, with and without metabolic activation, at doses of 0, 0.1, 0.3, 1, 3, 10, 30, 100, or 300 µg/plate.	There was no increase in mutation frequency.	9,10-Anthraquinone was not considered mutagenic.	Krivobok et al. (1992)
Mutagenicity	Tested for reverse mutation in *Salmonella typhimurium* (Ames assay) strains TA98, TA1537, and TA138, with and without metabolic activation, at doses of 0 (not specified if this was vehicle or untreated), 10, or 20 µg/plate.	Hydroxylated anthraquinones (which included 9,10-anthraquinone) were considered positive in the TA1537 strain, with and without metabolic activation at both concentrations, but not in the other two strains. However, the report did not specifically state results for 9,10-anthraquinone.	No conclusion is made. However, in a review, Brown (1980) stated that 9,10-anthraquinone was negative in all strains (TA98, TA100, TA1535, TA1537, and TA1538).	Brown and Brown (1976)
Mutagenicity	Tested for reverse mutation in *Salmonella typhimurium* (Ames assay) strains TA98 and TA100, with and without metabolic activation, at doses of 0, 33, 100, 333, 1000, or 2500 µg/plate of 97% pure 9,10-anthraquinone.	There was an increase in the number of revertants with and without metabolic activation.	The results were considered positive.	NTP (2005b)[a]
Mutagenicity	Tested for reverse mutation in *Salmonella typhimurium* (Ames assay) strains TA98, TA100, and TA102, with and without metabolic activation, at doses of 0, 100, 333, 1000, 3333, or 10,000 µg/plate of 100% pure 9,10-anthraquinone.	There was no increase in the number of revertants with and without metabolic activation.	The results were considered negative.	NTP (2005b)[a]

Table 3. Other Studies for 9,10-Anthraquinone

Test	Materials and Methods	Results	Conclusions	Reference
Mutagenicity	2-Hydroxyanthraquinone tested for reverse mutation in *Salmonella typhimurium* (Ames assay) strain TA98, with and without metabolic activation.	There was an increase in the number of revertants (2.2 per plate).	The results were considered positive.	NTP (2005b)[a]
Mutagenicity	2-NA tested for reverse mutation in *Salmonella typhimurium* (Ames assay) strain TA98, with and without metabolic activation.	There was an increase in the number of revertants (0.37 per plate).	The results were considered positive.	NTP (2005b)[a]
Mutagenicity	Tested for reverse mutation in *Salmonella typhimurium* (Ames assay) strains TA98, TA100, TA1535, and TA1537, and *Escheria coli* WP2uvrA, with and without metabolic activation, at doses of 0, 30, 60, 125, 250, 500, 1000 ,or 2000 µg/plate of 99% pure 9,10-anthraquinone derived from the oxidation of anthracene (this was the same anthracene used in the NTP, 2005b studies).	There was an increase in revertants without metabolic activation with the TA98, TA100, and TA1537 strains and in TA98 with metabolic activation.	It was concluded that the mutagenicity of the 9,10-anthraquinone used in the NTP (2005b)[b] study was due to the 9-NA contaminant, which was estimated to range from 0.04 to 2.4 µg/plate with the concentrations of 9,10-anthraquinone used.	Butterworth et al. (2001)
Mutagenicity	Tested for reverse mutation in *Salmonella typhimurium* (Ames assay) TA98, TA100, TA1535, and TA1537, and *Escheria coli* WP2uvrA, with and without metabolic activation, at doses of 0, 30, 60, 125, 250, 500, 1000, or 2000 µg/plate of purified 9,10-anthraquinone (the sample used in the NTP, 2005b studies was purified to remove contaminants).	There was no increase in revertants with or without metabolic activation in any of the strains at any of the concentrations.	It was concluded that the mutagenicity of the 9,10-anthraquinone used in the NTP (2005b)[b] study was due to the 9-NA contaminant, which was estimated to range from 0.04 to 2.4 µg/plate with the concentrations of 9,10-anthraquinone used.	Butterworth et al. (2001)
Mutagenicity	Tested for reverse mutation in *Salmonella typhimurium* (Ames assay) TA98, TA100, TA1535, and TA1537, and *Escheria coli* WP2uvrA, with and without metabolic activation, at doses of 0, 30, 60, 125, 250, 500, 1000, or 2000 µg/plate of 99% pure 9,10-anthraquinone derived using the Friedel-Crafts technology.	There was no increase in revertants in any of the strains tested.	9,10-Anthraquinone was considered negative for mutagenicity.	Butterworth et al. (2001)

Table 3. Other Studies for 9,10-Anthraquinone

Test	Materials and Methods	Results	Conclusions	Reference
Mutagenicity	Tested for reverse mutation in *Salmonella typhimurium* (Ames assay) TA98, TA100, TA1535, and TA1537, and *Escheria coli* WP2uvrA, with and without metabolic activation, at doses of 0, 30, 60, 125, 250, 500, 1000, or 2000 µg/plate of 99% pure 9,10-anthraquinone derived using Diels-Alder chemistry.	There was no increase in revertants in any of the strains tested.	9,10-Anthraquinone was considered negative for mutagenicity.	Butterworth et al. (2001)
Mutagenicity	L5178Y thymidine kinase (TK) +/- mouse lymphoma forward mutation assay was conducted, with or without metabolic activation, at concentrations of 0, 1.57 (with metabolic activation only), 3.13, 6.25, 12.5, 25.0, 37.5, or 50 µg/ml using 99% pure 9,10-anthraquinone derived using Diels-Alder chemistry.	There was no increase in the mutation frequency.	9,10-Anthraquinone was considered negative for mutagenicity.	Butterworth et al. (2001)
Genotoxicity	Chromosomal aberrations were evaluated in Chinese Hamster Ovary (CHO) cells after 20 and 44 hours of exposure to concentrations of 0, 12.5, 25, 37.5, or 50 µg/ml of 99% pure 9,10-anthraquinone derived using Diels-Alder chemistry with and without metabolic activation.	There was no increase in chromosomal aberrations.	The study was considered negative.	Butterworth et al. (2001)
Genotoxicity	The in vivo bone marrow mouse micronuclei assay was used. Crl:CD-1 (ICR)BR mice were administered 1250, 2500, or 5000 mg/kg 99% pure 9,10-anthraquinone derived using Diels-Alder chemistry in corn oil via gavage, and five males and five females were sacrificed at 24, 48, or 72 hours.	There was no increase in the micronucleated polychromatic erythrocytes in the bone marrow at any time point.	The study was considered negative.	Butterworth et al. (2001)

Table 3. Other Studies for 9,10-Anthraquinone

Test	Materials and Methods	Results	Conclusions	Reference
Genotoxicity	Male and female B6C3F$_1$ mice were fed 9,10-anthraquinone (99.8% pure) for 14 weeks at concentrations of 1875−30,000 ppm. Peripheral blood was collected and evaluated for micronucleated normochromatic erythrocytes.	In males, there was a statistically significant increase in micronucleated normochromatic erythrocytes in the 30,000-ppm group only but females had a statistically significant increase in all but the lowest dose group.	Results were considered positive.	NTP (2005b)
Genotoxicity	Male B6C3F$_1$ mice were injected i.p. with three doses of 9,10-anthraquinone 24 hours apart at doses of 500, 1000, or 2000 mg/kg, and micronucleated polychromatic erythrocytes in the bone marrow were evaluated.	There was no increase in micronucleated cells.	The results were considered negative.	NTP (2005b)
Genotoxicity	Syrian hamster embryo (SHE) cell transformation assay was conducted. Cells were exposed to 9,10-anthraquinone for 24 hours at concentrations of 0.5, 1, 3, 5, 6, 7, 8, or 9 µg/ml, then plated for 7 days or exposed to anthraquinone for 7 days at concentrations of 4.25, 4.5, 5, or 5.25 µg/ml and immediately evaluated.	After 24 hours of exposure, there were no effects on the relative plating efficiency, and there were no increases in morphological transformations. After 7 days of exposure, there was a dose-dependent decrease in relative plating efficiency with the highest dose 68% lower than the controls. However, there still was no increase in transformations.	Results were considered negative.	Kerckaert et al. (1996)

[a]It was stated in the NTP report that these results were also presented in Zeiger et al. (1988).
[b]Results apparently first reported in NTP Technical Report 494 (1999), which was not found; apparently superseded by NTP, 2005b.

DERIVATION OF PROVISIONAL VALUES

Tables 4 and 5 below present a summary of noncancer and cancer reference values, respectively.

DERIVATION OF ORAL REFERENCE DOSES

Derivation of Subchronic Provisional RfD (Subchronic p-RfD)

No subchronic p-RfD can be derived because doing so would require the application of a composite uncertainty factor of 10,000. However, a screening subchronic p-RfD is provided in Appendix A. NTP (2005b) studies in rats and mice were the only studies available to consider for derivation of the subchronic p-RfD. The study in rats (NTP, 2005b) is used for derivation of the screening subchronic p-RfD because the intake of test compound was lower in the rats, and both studies produced a LOAEL with the lowest dose tested.

Derivation of Chronic Provisional RfD (Chronic p-RfD)

No chronic p-RfD can be derived because doing so would require the application of a composite uncertainty factor of 10,000. However, a screening chronic p-RfD is provided in Appendix A. NTP (2005b) studies in rats and mice were the only studies available to consider for derivation of the chronic p-RfD. The study in rats (NTP, 2005b) is used for derivation of the screening chronic p-RfD because the intake of test compound was lower in the rats, and both studies produced a LOAEL with the lowest dose tested.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

The available data do not support derivation of any inhalation toxicity values. No subchronic or chronic p-RfC values can be derived for the following two reasons: there are no adequate animal inhalation studies, and the epidemiology studies do not provide any concentrations for 9,10-anthraquinone or demonstrate any definite relationship between 9,10-anthraquinone exposure and any toxic effect. The only inhalation animal study available was Volodchenko et al. (1971); a foreign publication with an English translation provided by ICI Americas (1985). Insufficient details on the study were provided (e.g., there were no specifics on the strain, sex, or number of animals used, the compound details, or the methods of inhalation exposure) to propose use of this study as a basis for derivation of any provisional reference value.

Table 4. Summary of Noncancer Reference Values for 9,10-Anthraquinone (CASRN 84-65-1)

Toxicity Type (units)	Species/Sex	Critical Effect	p-Reference Value	POD[a] Method	Pod	UF_C	Principal Study
Screening subchronic p-RfD	Rat/M+F	Liver, kidney, and spleen lesions	0.01 mg/kg-day	NOAEL/ LOAEL	135	10,000	NTP (2005b)
Screening chronic p-RfD (mg/kg-day)	Rat/M	Liver, kidney, and spleen lesions	0.002 mg/kg-day	NOAEL/ LOAEL	20	10,000	NTP (2005b)
Subchronic p-RfC (mg/m^3)	None	None	None	None	None	None	None
Chronic p-RfC (mg/m^3)	None	None	None	None	None	None	None

[a]POD = point of departure.

Table 5. Summary of Cancer Values for 9,10-Anthraquinone (CASRN 84-65-1)

Toxicity Type	Species/Sex	Tumor Type	Cancer Value	Principal Study
p-OSF	Mouse/M	Hepatocellular adenoma, carcinoma, or hepatoblastoma	$0.04\ (mg/kg\text{-}day)^{-1}$	NTP (2005b)
p-IUR	None	None	None	None

CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR (WOE)

Table 6 identifies the cancer weight-of-evidence (WOE) descriptor for 9,10-anthraquinone.

Table 6. Cancer WOE Descriptor for 9,10-Anthraquinone

Possible WOE Descriptor	Designation	Route of Entry (Oral, Inhalation, or Both)	Comments
"Carcinogenic to Humans"	N/A	N/A	Two occupational studies (Sathiakumar and Delzell, 2000; Barbone et al., 1992) found statistically significant increases in lung cancer in workers exposed to anthraquinone or anthraquinone dyes, but the results did not establish any definitive link with exposures, and the studies did not evaluate smoking status in the workers.
"Likely to Be Carcinogenic to Humans"	X	Oral	Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), there is enough evidence to suggest that 9,10-anthraquinone is likely to be carcinogenic to humans based on evidence of carcinogenicity in rats and mice in the NTP (2005b) oral bioassays. There was some controversy over the possibility that the contaminant 9-NA was responsible for the observed carcinogenicity in the NTP (2005b) studies. However, the NTP subcommittee did not believe the contaminant would be at a high enough concentration to account for the increase in tumors noted. In addition, there are no carcinogenicity studies available for 9-NA, and the primary metabolite, 2-hydroxyanthraquinone, was also found to be mutagenic. Occupational studies suggest carcinogenic potential via inhalation, although the doses and routes were not controlled or measured and other confounding factors were not considered.
"Suggestive Evidence of Carcinogenic Potential"	N/A	N/A	
"Inadequate Information to Assess Carcinogenic Potential"	N/A	N/A	
"Not Likely to Be Carcinogenic to Humans"	N/A	N/A	Positive cancer bioassay data exist.

MODE-OF-ACTION DISCUSSION

The *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) define mode of action (MOA) as a sequence of key events and processes starting with the interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation. Examples of possible modes of carcinogenic action include mutagenic, mitogenic, antiapoptotic (inhibition of programmed cell death), cytotoxic with reparative cell proliferation, and immune suppression. The MOA of 9,10-anthraquinone-induced carcinogenicity has not yet been determined.

Mutagenic Mode of Action

The majority of data on 9,10-anthraquinone indicate that 9,10-anthraquinone is not mutagenic. 9,10-Anthraquinone may be clastogenic after longer-term exposure. 2-Hydroxyanthraquinone, a major metabolite of 9,10-anthraquinone, was found to be mutagenic in an Ames assay, but cannot be established as the causative agent for the tumorigenicity reported by NTP (2005b).

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

Because the mode of action of 9,10-anthraquinone is not known, the default linear quantitative methodology was applied under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005).

Derivation of Provisional Oral Slope Factor (p-OSF)

The carcinogenicity mouse study by NTP (2005b) is selected as the principal study for derivation of the p-OSF. The cancer endpoint is the incidence of hepatocellular adenoma, carcinoma, or hepatoblastoma in male mice. This study is well conducted, and the data from this study are able to support a quantitative cancer dose-response assessment. This study is a peer-reviewed technical report from the NTP, has been performed according to GLP principles, and otherwise meets the standards of study design and performance with numbers of animals, examination of carcinogenicity endpoints, and presentation of information. Details are provided in the "Selection of Potentially Relevant Studies" section. Although there were concerns raised about the presence of 9-NA and its possible contribution to the observed tumor incidence, the NTP subcommittee concluded that the levels of 9-NA exposure, bioavailability, and relative mutagenicity were not sufficient to cause the tumor incidence observed. The subcommittee also stated that 2-hydroxyanthraquinone, a major metabolite of 9,10-anthraquinone, could account for the pattern of tumorigenicity; 2-hydroxyanthraquinone was found to have a 7-fold greater mutagenicity than 9-NA and occurred at a greater level in the urine than was theoretically possible for 9-NA (NTP, 2005b). Therefore, NTP (2005b) concluded that the tumorigenicity observed in the study was a result of 9,10-anthraquinone exposure, rather than the 2-NA contaminant. The results from NTP (2005b) are used to derive the p-OSF.

NTP (2005b) provides the only acceptable carcinogenicity studies with increases in a number of different tumor endpoints in male and female rats and mice. All relevant tumor endpoints were modeled using EPA Benchmark Dose Software (BMDS version 2.1.1; U.S. EPA, 2009). The increase in hepatocellular adenoma, carcinoma, or hepatoblastoma in male mice provided the lowest POD ($BMDL_{10}$ = 2.61 mg/kg-day).

The following dosimetric adjustments were made for dietary treatment in adjusting doses for oral cancer analysis:

HED_{BMDL}	=	$BMDL_{10}$ × body-weight adjustment
Body-weight adjustment	=	$(BW_A \div BW_H)^{1/4}$
BW_H	=	70 kg (human reference body weight) (U.S. EPA, 1997)
BW_A	=	0.0373 kg (average body weight for male mice in chronic study) (U.S. EPA, 1988)
Body-weight adjustment	=	$(0.0373 \div 70)^{1/4} = 0.152$
HED_{BMDL}	=	0.395 mg/kg-day (2.61 × 0.152)

Table 7 presents BMD input data for incidence of hepatocellular adenoma, carcinoma, or hepatoblastoma in male mice administered 9,10-anthraquinone in the diet for 2 years.

Table 7. BMD Input for Incidence of Hepatocellular Adenoma, Carcinoma, or Hepatoblastoma in Male Mice Exposed to Dietary 9,10-Anthraquinone for 2 Years[a]

Administered Dose (mg/kg-day)	HED[b] (mg/kg-day)	Number of Animals	Response[c]
0	0	50	26 (52)
90	13.7	50	35 (70)*
265	40.3	50	43 (86)**
825	125.3	49	48 (98)**

[a]NTP (2005b)
[b]Human equivalent dose (administered dose × 0.152)
[c]Number of rats with tumors, ()= percentage of rats with tumors

*$p < 0.05$, **$p < 0.01$.

Table 8 shows the modeling results for hepatocellular adenoma, carcinoma, or hepatoblastoma in male mice. Table C.1 provides BMD modeling results for all cancer endpoints examined. Adequate model fit is obtained for the hepatocellular adenoma, carcinoma, or hepatoblastoma incidence data using the multistage-cancer model. A benchmark response of 10% extra risk above the control mean was used to estimate the benchmark dose (BMD), as recommended by EPA (2009). The BMD modeling results with 10% extra risk for hepatocellular adenoma, carcinoma, or hepatoblastoma in male mice yield a BMD_{10} of 3.8 mg/kg-day and a $BMDL_{10}$ of 2.6 mg/kg-day (see Table 8).

Table 8. BMD Values for Cancer Data from Dichotomous Multistage-Cancer BMD Model for 9,10-Anthraquinone for Derivation of the p-OSF[a]

Tumor Type	Species Sex	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)	Goodness-of-Fit *p*-Value[b]	Conclusions
Hepatocellular adenoma, carcinoma, or hepatoblastoma	Mouse/M	3.76	2.61	0.8591	Selected as POD for p-OSF

[a]NTP (2005b)
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria

Notes: BMD = benchmark dose; BMDL lower confidence limit (95%) on the benchmark dose; BMD_{10} and $BMDL_{10}$ = BMD and BMDL response rate of 10% incidence, extra risk

The BMDS output details for the selected model are provided in Appendix C. The $BMDL_{10}$ of 2.61 mg/kg-day from the 1-degree multistage model fit has been selected as the POD.

p-OSF = $0.1 \div BMDL_{10HED}$
= $0.1 \div 2.6$ mg/kg-day
= 0.0385 (mg/kg-day)$^{-1}$ or 3.85×10^{-2} per (mg/kg-day)$^{-1}$

The **p-OSF** rounds to **0.039 (mg/kg-day)$^{-1}$** or **4×10^{-2} per (mg/kg-day)$^{-1}$.**

Derivation of Provisional Inhalation Unit Risk (p-IUR)

No human or animal studies investigating the carcinogenicity of 9,10-anthraquinone following inhalation exposure have been identified. Therefore, derivation of a p-IUR is precluded.

APPENDIX A. PROVISIONAL SCREENING VALUES

For reasons noted in the main PPRTV document, it is inappropriate to derive a provisional subchronic or chronic p-RfD for 9,10-anthraquinone. However, information is available for this chemical which, although insufficient to support derivation of a provisional toxicity value, under current guidelines, may be of limited use to risk assessors. In such cases, the Superfund Health Risk Technical Support Center summarizes available information in an Appendix and develops a "screening value." Appendices receive the same level of internal and external scientific peer review as the PPRTV documents to ensure their appropriateness within the limitations detailed in the document. Users of screening toxicity values in an appendix to a PPRTV assessment should understand that there is considerably more uncertainty associated with the derivation of an appendix screening toxicity value than for a value presented in the body of the assessment. Questions or concerns about the appropriate use of screening values should be directed to the Superfund Health Risk Technical Support Center.

DERIVATION OF SCREENING CHRONIC AND SUBCHRONIC ORAL REFERENCE DOSES

Derivation of Screening Subchronic Provisional RfD (Subchronic p-RfD)

The subchronic study in rats by NTP (2005b) is selected as the principal study for derivation of the screening subchronic p-RfD. The critical endpoint is pathology in several organs, including the liver, kidney, and spleen, in male and female F344 rats. The lesions were described as of mild to moderate severity at the LOAEL. The presence of a 100% response in each of these organs at the lowest dose precludes conducting BMD modeling. This study is peer reviewed and performed according to GLP principles and otherwise meets the standards of study design and performance with numbers of animals, examination of potential toxic endpoints, and presentation of information. Details are provided in the "Review of Potentially Relevant Data" section. Among the available, acceptable studies, this study represents the lowest POD for developing a subchronic p-RfD.

The POD in this study is a LOAEL of 135 mg/kg-day for liver, kidney, and spleen lesions in male and female F344 rats.

No dosimetric adjustments were made for the dose in the principal study for dietary treatment because the study authors report the average daily doses, and no animal-to-human body-weight adjustment is used for oral noncancer assessments.

A screening subchronic p-RfD for 9,10-anthraquinone, based on 135 mg/kg-day in male and female rats, is derived as follows:

$$\textbf{Screening Subchronic p-RfD} = LOAEL_{ADJ} \div UF_C$$
$$= 135 \text{ mg/kg-day} \div 10{,}000$$
$$= 0.01 \text{ mg/kg-day}$$

The **screening subchronic p-RfD** rounds to **0.01 mg/kg-day** or **1×10^{-2} per mg/kg-day.**

Table A.1 summarizes the uncertainty factors for the screening subchronic p-RfD for 9,10-anthraquinone.

Table A.1. Uncertainty Factors for Screening Subchronic p-RfD of 9,10-Anthraquinone

UF	Value	Justification	Notes
UF_A	10	A UF_A of 10 is applied for interspecies extrapolation to account for potential toxicokinetic and toxicodynamic differences between rats and humans. There are no data to determine whether humans are more or less sensitive than rats to 9,10-anthraquinone.	
UF_D	10	A UF_D of 10 is selected because there are no acceptable two-generation reproduction studies or developmental studies. The available data do not suggest that additional studies may reveal sensitive effects not yet characterized.	NTP (2005b) examined and reported a lack of effects on various sperm parameters but some effects on the female estrous cycle.
UF_H	10	A UF_H of 10 is applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response to humans.	
UF_L	10	A UF_L of 10 is applied for using a POD based on a LOAEL because a NOAEL cannot be determined from the available database.	The doses employed in all relevant animal studies appear to be too high because lesions are present at high incidences at the lowest dose tested.
UF_C $\leq$3000	10,000		

Derivation of Screening Chronic Provisional RfD (Chronic p-RfD)

The study by NTP (2005b) is selected as the principal study for derivation of the screening chronic p-RfD. The critical endpoint is pathology in several organs, including the liver, kidney, and spleen, in male and female F344 rats. The presence of a near 100% response in each of these organs at the lowest dose and the absence of biological response data at lower doses preclude conducting a sensitivity analysis to distinguish dose-related responses in these various organs. This study is peer reviewed and performed according to GLP principles and otherwise meets the standards of study design and performance with numbers of animals, examination of potential toxic endpoints, and presentation of information. A LOAEL is evident at the lowest dose, with lesions noted in several organs of all treatment groups. Details are provided in the "Review of Potentially Relevant Data section." The available response levels, with near maximal response at the lowest dose tested for a number of systemic endpoints, precludes BMD dose-response modeling.

The POD in this study is a LOAEL of 20 mg/kg-day in male rats (the LOAEL in female rats is 25 mg/kg-day).

No dosimetric adjustments were made for the dose in the principal study for dietary treatment because the study authors reported the average daily doses, and no animal-to-human body-weight adjustment is used for oral noncancer assessments.

The **screening chronic p-RfD** for 9,10-anthraquinone, based on 20 mg/kg-day in male rats, is derived as follows:

Screening Chronic p-RfD = $LOAEL_{ADJ} \div UF_C$
= 20 mg/kg-day ÷ 10,000
= **0.002 mg/kg-day or 2×10^{-3} mg/kg-day**

Table A.2 summarizes the uncertainty factors for the screening chronic p-RfD for 9,10-anthraquinone.

Table A.2. Uncertainty Factors for Screening Chronic p-RfD of 9,10-Anthraquinone

UF	Value	Justification	Notes
UF_A	10	A UF_A of 10 is applied for interspecies extrapolation to account for potential toxicokinetic and toxicodynamic differences between rats and humans. There are no data to determine whether humans are more or less sensitive than rats to 9,10-anthraquinone.	
UF_D	10	A UF_D of 10 is selected because there are no acceptable two-generation reproduction studies or developmental studies. The available data do not suggest that additional studies may reveal sensitive effects not yet characterized.	NTP (2005b) examined and reported a lack of effects on various sperm parameters but some effects on the female estrous cycle.
UF_H	10	A UF_H of 10 is applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response to humans.	
UF_L	10	A UF_L of 10 is applied for using a POD based on a LOAEL because a NOAEL cannot be determined from the available database.	The doses employed in all relevant animal studies appear to be too high because lesions are present at high incidences at the lowest dose tested.
UF_S	1	A UF_S of 1 is applied because a chronic study was utilized as the principal study.	
UF_C ≤3000	10,000		

APPENDIX B. DATA TABLES

Table B.1. Body-Weight Gain and Food Consumption in Male and Female F344/N Rats Exposed to Oral (in Feed) 9,10-Anthraquinone for 14 Weeks[a]

Endpoint	Average Daily Dose (mg/kg-day)					
	0	135	275	555	1130	2350
Males						
Number of animals	10	10	10	10	10	10
Body-weight gain (g)	205 ± 4[b]	219 ± 3	222 ± 6	207 ± 5	212 ± 5	193 ± 5
Food consumption Week 1 (g/animal/day)	15.0	14.7	13.9	12.6	12.9	12.2
Food consumption Week 14 (g/animal/day)	15.8	16.0	15.9	16.7	16.8	16.9
Females						
Number of animals	10	10	10	10	10	10
Body-weight gain (g)	99 ± 2	90 ± 3**	80 ± 2**	74 ± 2**	74 ± 3**	65 ± 2**
Food consumption Week 1 (g/animal/day)	10.9	9.4	8.6	7.3	6.1	6.0
Food consumption Week 14 (g/animal/day)	8.2	9.8	9.8	9.7	10.1	10.2

[a]NTP (2005b)
[b]Mean ± standard error

**$p < 0.01$ (Williams or Dunnett's test)

Table B.2. Selected Hematology and Clinical Chemistry Parameters in Male and Female F344/N Rats Exposed to Oral (in Feed) 9,10-Anthraquinone for 14 Weeks[a]

Endpoint	Average Daily Dose (mg/kg-day)					
	0	135	275	555	1130	2350
Males						
Number of animals	10	10	10	10	10	10
Hemoglobin (g/dL)	16.3 ± 0.1[b]	14.9 ± 0.2**	15.1 ± 0.1**	14.7 ± 0.2**	14.7 ± 0.2**	14.9 ± 0.2**
Erythrocytes (10^6/µL)	9.13 ± 0.10	8.02 ± 0.09**	8.12 ± 0.11**	8.00 ± 0.09**	8.03 ± 0.15 **	8.29 ± 0.10*
Reticulocytes (10^6/µL)	0.09 ± 0.01	0.15 ± 0.01**	0.15 ± 0.01**	0.15 ± 0.01**	0.15 ± 0.01**	0.17 ± 0.02**
Mean cell volume (fL)	53.7 ± 0.3	55.7 ± 0.2**	56.2 ± 0.3**	56.3 ± 0.2**	56.0 ± 0.4**	55.8 ± 0.3**
Mean cell hemoglobin concentration (g/dL)	33.3 ± 0.2	33.4 ± 0.2	33.1 ± 0.2	32.8 ± 0.2	32.9 ± 0.2	32.3 ± 0.1**
Platelets (10^3/µL)	668.3 ± 16.4	769.3 ± 21.4 **	789.9 ± 9.4 **	763.9 ± 15.1 **	824.4 ± 20.0 **	806.5 ± 24.5 **
Urea nitrogen (mg/dL)	20.3 ± 0.4	21.1 ± 0.5	21.3 ± 0.3	22.1 ± 0.6**	22.3 ± 0.4**	22.4 ± 0.5**
Creatinine (mg/dL)	0.67 ± 0.02	0.71 ± 0.01*	0.73 ± 0.02**	0.73 ± 0.02**	0.76 ± 0.02**	0.74 ± 0.02**
Total protein (g/dL)	6.7 ± 0.1	7.2 ± 0.1**	7.3 ± 0.1**	7.4 ± 0.1**	7.5 ± 0.2**	7.8 ± 0.1**
Albumin (g/dL)	4.7 ± 0.1	5.0 ± 0.1**	5.1 ± 0.1**	5.2 ± 0.1**	5.2 ± 0.1**	5.3 ± 0.1**
Alkaline phosphatase (IU/L)	624 ± 20	502 ± 16**	478 ± 14**	455 ± 19**	442 ± 21**	445 ± 17**
Bile salts (µmole/L)	20.3 ± 0.8	13.5 ± 1.1**	10.7 ± 0.5**	13.5 ± 0.9**	11.6 ± 0.8**	14.7 ± 2.7**
Females						
Number of animals	10	10	10	10	10	10
Hemoglobin (g/dL)	15.4 ± 0.2	14.1 ± 0.2**	14.4 ± 0.1**	14.3 ± 0.2**	14.3 ± 0.1**	13.9 ± 0.2**
Erythrocytes (10^6/µL)	7.92 ± 0.10	7.06 ± 0.12**	7.41 ± 0.04	7.39 ± 0.07*	7.41 ± 0.07*	7.24 ± 0.11**
Reticulocytes (10^6/µL)	0.10 ± 0.01	0.20 ± 0.02**	0.25 ± 0.02**	0.26 ± 0.02**	0.23 ± 0.01**	0.25 ± 0.02**
Mean cell volume (fL)	57.7 ± 0.03	60.5 ± 0.2**	59.3 ± 0.2*	59.5 ± 0.2*	59.6 ± 0.3**	59.4 ± 0.2**
Mean cell hemoglobin concentration (g/dL)	33.7 ± 0.3	33.0 ± 0.1	32.7 ± 0.1**	32.5 ± 0.1**	32.4 ± 0.2**	32.3 ± 0.2**
Platelets (10^3/µL)	740.6 ± 11.7	804.6 ± 12.0 **	848.4 ± 14.6 **	839.9 ± 10.7 **	870.4 ± 11.2 **	874.9 ± 18.8 **
Urea nitrogen (mg/dL)	18.6 ± 0.5	19.7 ± 0.6	20.6 ± 0.4*	18.5 ± 0.5	18.9 ± 0.4	19.5 ± 0.6
Creatinine (mg/dL)	0.66 ± 0.02	0.68 ± 0.01	0.70 ± 0.00*	0.72 ± 0.01**	0.70 ± 0.02*	0.71 ± 0.02*
Total protein (g/dL)	6.5 ± 0.1	7.2 ± 0.1**	7.5 ± 0.1**	7.9 ± 0.1**	7.9 ± 0.1**	8.1 ± 0.0**
Albumin (g/dL)	4.7 ± 0.1	5.1 ± 0.1**	5.4 ± 0.1**	5.5 ± 0.1**	5.6 ± 0.1**	5.7 ± 0.0**
Alkaline phosphatase (IU/L)	403 ± 20	330 ± 10**	321 ± 16**	293 ± 17**	282 ± 13**	274 ± 12**

[a]NTP (2005b)
[b]Values are presented as mean ± standard errors

*$p < 0.05$ (Dunn's or Shirley's test)
**$p < 0.01$

Table B.3. Selected Relative Organ Weights in Male and Female F344/N Rats Exposed to Oral (in Feed) 9,10-Anthraquinone for 14-Weeks[a,b]

Endpoint	Average Daily Dose (mg/kg-day)					
	0	**135**	**275**	**555**	**1130**	**2350**
Males						
Number of animals	10	10	10	10	10	10
Necropsy body weight	338 ± 5	349 ± 3	347 ± 5	331 ± 6	336 ± 6	322 ± 4*
Right kidney[c]	3.660 ± 0.055	3.966 ± 0.068 **	3.979 ± 0.087 **	4.203 ± 0.057 **	4.324 ± 0.092 **	4.537 ± 0.088 **
Liver[c]	39.712 ± 0.942	48.219 ± 1.008 **	53.535 ± 1.136 **	57.071 ± 0.982 **	62.268 ± 1.053 **	69.315 ± 0.755 **
Right testis[c]	4.335 ± 0.039	4.396 ± 0.066	4.501 ± 0.039	4.636 ± 0.092 **	4.605 ± 0.077 **	4.948 ± 0.075 **
Females						
Number of animals	10	10	10	10	10	10
Necropsy body weight	204 ± 3	198 ± 4	186 ± 3**	182 ± 2**	183 ± 3**	174 ± 1**
Right kidney[c]	3.476 ± 0.040	4.074 ± 0.048 **	4.378 ± 0.032 **	4.347 ± 0.068 **	4.526 ± 0.071 **	4.891 ± 0.076 **
Liver[c]	31.569 ± 0.612	45.272 ± 0.855 **	54.202 ± 1.282 **	60.189 ± 0.842 **	62.101 ± 1.221 **	74.840 ± 1.011 **

[a]NTP (2005b)
[b]Values are presented as mean ± standard error
[c]Relative organ weights are presented as mg organ weight/g body weight

*$p < 0.05$ (Williams or Dunnett's test)
**$p < 0.01$

Table B.4. Incidence of Selected Nonneoplastic Lesions in Male and Female F344/N Rats Exposed to Oral (in Feed) 9,10-Anthraquinone for 14 Weeks[a]

Endpoint	Average Daily Dose (mg/kg-day)					
	0	135	275	555	1130	2350
Males						
Liver						
Hypertrophy	0/10	10/10**	10/10**	10/10**	10/10**	10/10**
Severity[b]	0	1.0	1.8	2.0	2.0	2.9
Kidney						
Hyaline droplet accumulation	0/10	10/10**	10/10**	10/10**	10/10**	10/10**
Nephropathy severity[b,c]	1.0	1.7	1.6	1.7	2.0	2.2
Spleen						
Congestion	0/10	10/10**	10/10**	10/10**	10/10**	10/10**
Hematopoietic cell proliferation	0/10	10/10**	9/10**	10/10**	10/10**	10/10**
Pigmentation	0/10	10/10**	10/10**	10/10**	10/10**	10/10**
Bone Marrow						
Hyperplasia	0/10	3/10	5/10*	8/10**	6/10**	5/10*
Thyroid Gland						
Follicular cell hypertrophy	0/10	0/10	10/10**	10/10**	10/10**	10/10**
Females						
Liver						
Hypertrophy	0/9	10/10**	10/10**	10/10**	10/10**	10/10**
Severity[b]	0	1.0	2.0	1.8	2.0	2.0
Kidney						
Hyaline droplet accumulation	0/10	10/10**	10/10**	10/10**	10/10**	10/10**
Nephropathy	3/10	2/10	3/10	5/10	8/10*	10/10**
Spleen						
Congestion	0/9	10/10**	10/10**	10/10**	10/10**	10/10**
Hematopoietic cell proliferation	0/9	10/10**	10/10**	10/10**	10/10**	10/10**
Pigmentation	0/9	10/10**	10/10**	10/10**	10/10**	10/10**
Bone Marrow						
Hyperplasia	0/10	7/10**	7/10**	10/10**	9/10**	10/10*
Thyroid Gland						
Follicular cell hypertrophy	0/10	0/10	10/10**	10/10**	10/10**	10/10**
Urinary Bladder						
Inflammation	0/9	0/10	0/10	0/10	1/9	6/10**
Transitional epithelium hyperplasia	0/9	0/10	0/10	0/10	0/9	9/10**

[a]NTP (2005b)
[b]Average severity grade: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[c]All the animals including all of the controls developed nephropathy

*$p < 0.05$ (Fischer's exact test)
**$p < 0.01$

Table B.5. Selected Hematology Parameters in Male and Female B6C3F$_1$ Mice Exposed to Oral (in Feed) 9,10-Anthraquinone for 14-Weeks[a]

Males

Endpoint	Average Daily Dose (mg/kg-day)					
	0	**250**	**500**	**1050**	**2150**	**4300**
Number of animals	10	10	10	9	10	10
Hematocrit (%)	55.6 ± 1.2[b]	55.2 ± 1.1	53.3 ± 0.7	52.4 ± 0.9	52.5 ± 1.3	49.4 ± 1.2**
Hemoglobin (g/dL)	17.8 ± 0.2	17.9 ± 0.3	17.3 ± 0.2	17.1 ± 0.2	17.4 ± 0.3	16.7 ± 0.3*
Erythrocytes (10^6/μL)	11.45 ± 0.28	11.23 ± 0.25	10.79 ± 0.16	10.63 ± 0.17 *	10.70 ± 0.29 *	9.98 ± 0.28**
Reticulocytes (10^6/μL)	0.12 ± 0.02	0.17 ± 0.02	0.17 ± 0.01*	0.16 ± 0.01	0.20 ± 0.02 **	0.20 ± 0.03**
Mean cell volume (pg)	15.6 ± 0.2	16.0 ± 0.2	16.1 ± 0.2	16.1 ± 0.1	16.3 ± 0.2*	16.8 ± 0.2*
Mean cell hemoglobin concentration (g/dL)	32.0 ± 0.3	32.5 ± 0.4	32.5 ± 0.2	32.7 ± 0.3	33.3 ± 0.2 **	33.8 ± 0.3**
Platelets (10^3/μL)	844.1 ± 34.4	888.8 ± 35.6	951.5 ± 53.6	896.1 ± 34.0	1000.9 ± 53.8*	1005.5 ± 26.5 **

Females

Endpoint	Average Daily Dose (mg/kg-day)					
	0	**300**	**640**	**1260**	**2600**	**5300**
Number of animals	10	10	10	10	10	10
Hematocrit (%)	49.8 ± 0.3	48.3 ± 0.3**	46.7 ± 0.5**	47.8 ± 0.5**	46.6 ± 0.3**	45.6 ± 0.6**
Hemoglobin (g/dL)	16.6 ± 0.1	16.2 ± 0.1*	15.8 ± 0.1**	16.1 ± 0.1**	15.9 ± 0.2**	15.7 ± 0.1**
Erythrocytes (10^6/μL)	10.32 ± 0.05	9.77 ± 0.05 **	9.46 ± 0.10**	9.64 ± 0.11**	9.44 ± 0.06 **	9.09 ± 0.09**
Reticulocytes (10^6/μL)	0.10 ± 0.01	0.16 ± 0.02*	0.19 ± 0.02 **	0.20 ± 0.02 **	0.19 ± 0.02 **	0.26 ± 0.02**
Mean cell volume (fL)	48.2 ± 0.1	49.3 ± 0.2**	49.4 ± 0.2**	49.7 ± 0.2 **	49.3 ± 0.2**	50.3 ± 0.3**
Mean cell hemoglobin (pg)	16.1 ± 0.1	16.6 ± 0.1**	16.7 ± 0.1**	16.7 ± 0.1 **	16.9 ± 0.1**	17.3 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	33.3 ± 0.2	33.7 ± 0.1	33.8 ± 0.1	33.7 ± 0.2	34.1 ± 0.2**	34.5 ± 0.2**
Platelets (10^3/μL)	889.2 ±13.9	993.1 ± 16.5 **	971.9 ± 14.2 **	1012.1 ± 31 **	1065.3 ± 18.5 **	1096.6 ± 18.3 **

[a]NTP (2005b)
[b]Values are presented as mean ± standard error

*$p < 0.05$ (Dunn's or Shirley's test)
**$p < 0.01$

Table B.6. Selected Relative Organ Weights in Male and Female B6C3F$_1$ Mice Exposed to Oral (in Feed) 9,10-Anthraquinone for 14-Weeks[a,b]

Males						
	Average Daily Dose (mg/kg-day)					
Endpoint	**0**	**250**	**500**	**1050**	**2150**	**4300**
Number of animals	10	10	10	10	10	10
Necropsy body weight	38.7 ± 0.9	39.8 ± 0.8	39.5 ± 1.0	39.6 ± 0.7	37.0 ± 0.6	37.8 ± 0.6
Right kidney[c]	7.563 ± 0.122	7.355 ± 0.154	7.901 ± 0.336	7.548 ± 0.174	7.943 ± 0.174	8.518 ± 0.183**
Liver[c]	44.558 ± 0.469	49.669 ± 0.456**	53.105 ± 0.546**	59.312 ± 0.965**	69.203 ± 0.947**	80.206 ± 0.862**
Females						
	Average Daily Dose (mg/kg-day)					
Endpoint	**0**	**300**	**640**	**1260**	**2600**	**5300**
Number of animals	10	10	10	10	10	10
Necropsy body weight	29.8 ± 0.6	32.3 ± 0.8	31.3 ± 1.0	31.6 ± 0.8	31.2 ± 0.5	30.3 ± 0.8
Liver[c]	40.110 ± 1.029	44.497 ± 0.753*	49.332 ± 0.642**	51.365 ± 0.729**	60.412 ± 1.016**	74.799 ± 2.121**

[a]NTP (2005b)
[b]Values are presented as mean ± error
[c]Relative organ weights are presented as mg organ weight/g body weight

*$p < 0.05$ (Williams or Dunnett's test)
**$p < 0.01$

Table B.7. Incidence of Selected Nonneoplastic Lesions in Male and Female B6C3F$_1$ Mice Exposed to Oral (in Feed) 9,10-Anthraquinone for 14-Weeks[a]

Males						
	Average Daily Dose (mg/kg-day)					
Endpoint	**0**	**250**	**500**	**1050**	**2150**	**4300**
Liver						
Centrilobular hypertrophy	0/10	1/10	9/10**	10/10**	10/10**	10/10**
Severity[b]	0	1.0	1.6	2.8	3.0	3.1
Urinary Bladder						
Transitional epithelium, cytoplasmic alteration	0/10	10/10**	10/10**	10/10**	10/10**	10/10**
Severity[b]	0	1.1	2.5	3.1	3.2	3.8
Spleen						
Hematopoietic cell proliferation	0/10	6/10**	10/10**	10/10**	10/10**	9/10**
Pigmentation	0/10	10/10**	10/10**	10/10**	10/10**	9/10**
Females						
	Average Daily Dose (mg/kg-day)					
Endpoint	**0**	**300**	**640**	**1260**	**2600**	**5300**
Liver						
Centrilobular hypertrophy	0/10	2/10	5/10*	9/10**	7/10**	10/10**
Severity[b]	0	1.0	1.0	1.1	1.7	2.4
Urinary Bladder						
Transitional epithelium, cytoplasmic alteration	0/10	10/10**	10/10**	10/10**	10/10**	10/10**
Severity[b]	0	1.0	1.0	1.7	2.8	3.5

[a]NTP (2005b)
[b]Average severity grade 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
All the animals including all of the controls developed nephropathy

*$p < 0.05$ (Fischer's exact test)
**$p < 0.01$

Table B.8. Body-Weight Gain and Incidence of Selected Nonneoplastic Lesions in Male F344 Rats Exposed to Oral (in Feed) 9,10-Anthraquinone for 2 Years[a]

Endpoint	Average Daily Dose (mg/kg-day)				
	0	**20**	**45**	**90**	**180**
Body-weight gain	317[b]	293	286	301	289
Kidney					
Hyaline droplet accumulation	3/50[c]	14/50**	10/50	16/50**	16/50**
Medulla mineralization	30/50	42/50**	46/50**	47/50**	49/50**
Transitional epithelium hyperplasia	28/50	45/50**	44/50**	48/50**	48/50**
Liver					
Centrilobular hypertrophy	0/50	4/50	21/50**	13/50**	29/50**
Cystic degeneration	9/50	31/50**	36/50**	28/50**	29/50**
Inflammation	13/50	30/50**	28/50**	30/50**	27/50**
Eosinophilic focus	9/50	22/50**	30/50**	29/50**	20/50**
Mixed cell focus	4/50	12/50*	15/50**	13/50*	10/50
Cytoplasmic vacuolization	5/50	18/50**	23/50**	17/50**	23/50**
Spleen					
Congestion	6/50	35/50**	37/50**	30/50**	31/50**
Pigmentation	12/50	36/50 **	38/50**	33/50**	28/50**
Bone Marrow					
Hyperplasia	25/50	28/50	37/50*	36/50*	33/50

[a]NTP (2005b)
[b]Calculated from the weekly body-weight tables
[c]Number of animals with lesions/number of animals examined

*$p < 0.05$ (Fischer's exact test)
**$p < 0.01$

Table B.9. Body-Weight Gain and Incidence of Selected Nonneoplastic Lesions in Female F344 Rats Exposed to Oral (in Feed) 9,10-Anthraquinone for 2 Years[a]

Endpoint	Average Daily Dose (mg/kg-day)				
	0	25	50	100	200
Body-weight gain	242[b]	204	188	184	170
Kidney					
Hyaline droplet accumulation	33/50[c]	48/50**	45/50**	44/50**	44/50**
Nephropathy	39/50	49/50**	47/50*	49/50**	49/50**
Pigmentation	27/50	50/50**	48/50**	50/50**	47/49**
Medulla mineralization	17/50	25/50	27/50*	28/50*	20/49
Renal tubule hyperplasia	0/50	12/50**	13/50**	15/50**	11/49**
Transitional epithelium hyperplasia	0/50	5/50*	12/50**	3/50	10/50**
Liver					
Centrilobular hypertrophy	0/50	18/50**	23/50**	19/50**	26/50**
Cystic degeneration	0/50	5/50*	10/50**	10/50**	6/49*
Inflammation	25/50	46/50**	44/50**	38/50*	46/50**
Eosinophilic focus	8/50	32/50**	34/50**	39/50**	34/50**
Mixed cell focus	3/50	30/50**	20/50**	23/50**	13/49**
Angiectasis	3/50	15/50**	18/50**	15/50**	21/49**
Spleen					
Congestion	1/50	46/50**	42/50**	44/50**	45/50**
Pigmentation	33/50	45/50**	48/50**	48/50**	47/49**
Hematopoietic cell proliferation	39/50	50/50**	47/50*	47/50*	46/49*
Bone Marrow					
Hyperplasia	19/50	31/50*	28/50	19/50	23/50
Atrophy	4/50	13/50*	13/50*	11/50	13/50*

[a]NTP (2005b)
[b]Calculated from the weekly body-weight tables
[c]Number of animals with lesions/number of animals examined

*$p < 0.05$ (Fischer's exact test)
**$p < 0.01$

Table B.10. Incidence of Selected Neoplastic Lesions in Male F344 Rats Exposed to Oral (in Feed) 9,10-Anthraquinone for 2 Years[a]

Endpoint	Human Equivalent Dose (mg/kg-day)[b]				
	0	5.4	12.2	24.4	48.9
Kidney					
Renal tubule adenoma	1/50[c]	3/50	9/50*	5/50	3/50
Transitional epithelial papilloma	0/50	0/50	2/50	0/50	1/50
Urinary Bladder					
Transitional epithelial papilloma	0/50	1/50	3/50	7/50	3/49
Liver					
Hepatocellular adenoma or carcinoma	1/50	3/50	4/50	5/50	3/50

[a]NTP (2005b)
[b]Human Equivalent Dose = Average daily dose × body-weight adjustment, e.g., (180 mg/kg-day) × (0.38 kg ÷ 70 kg)$^{(0.25)}$
[c]Number of animals with lesions/number of animals examined

*p <0.05 (Fischer's exact test)
**p < 0.01

Table B.11. Incidence of Selected Neoplastic Lesions in Female F344 Rats Exposed to Oral (in Feed) 9,10-Anthraquinone for 2-Years[a]

Endpoint	Human Equivalent Dose (mg/kg-day)[b]				
	0	6.0	12.0	23.9	47.8
Kidney					
Renal tubule adenoma	0/50[c]	4/50	9/50**	7/50*	12/49**
Renal tubule adenoma or carcinoma	0/50	6/50*	9/50**	8/50**	14/49**
Liver					
Hepatocellular adenoma	0/50	2/50	6/50*	4/50	3/50
Urinary bladder					
Transitional epithelial papilloma or carcinoma	0/49	0/49	0/49	1/50	2/49

[a]NTP (2005b)
[b]Human Equivalent Dose = Average daily dose × body-weight adjustment, e.g., (200 mg/kg-day) × (0.229 kg ÷ 70 kg)$^{(0.25)}$
[c]Number of animals with lesions/number of animals examined

*p < 0.05 (Fischer's exact test)
**p < 0.01

Table B.12. Body-weight Gain and Incidence of Selected Nonneoplastic Lesions in Female B6C3F$_1$ Mice Exposed to Oral (in Feed) 9,10-Anthraquinone for 2 Years[a]

Endpoint	Average daily dose (mg/kg-day)			
	0	80	235	745
Body-weight gain	37.8[b]	38.2	36.2	33.9
Liver				
Centrilobular hypertrophy	1/49[c]	27/50**	22/50**	39/49**
Degeneration, fatty, focal	2/49	3/50	1/50	9/49*
Eosinophilic focus	6/49	15/50*	11/50	22/49**
Spleen				
Hematopoietic cell proliferation	9/45	17/49	17/48	26/48**
Urinary Bladder				
Intracytoplasmic inclusion body	0/44	40/48**	43/46**	46/48**

[a]NTP (2005b)
[b]Calculated from the weekly body-weight tables
[c]Number of animals with lesions/number of animals examined

*$p < 0.05$ (Fischer's exact test)
**$p < 0.01$

Table B.13. Body-Weight Gain and Incidence of Selected Nonneoplastic Lesions in Male B6C3F$_1$ Mice Exposed to Oral (in Feed) 9,10-Anthraquinone for 2-Years[a]

Endpoint	Average daily dose (mg/kg-day)			
	0	90	265	825
Body-weight gain	26.1[b]	24.5	24.2	16.2
Liver				
Centrilobular hypertrophy	24/50[c]	34/50*	41/50**	33/50**
Degeneration, fatty, focal	0/50	7/50**	6/50*	0/50
Hepatocyte, erythrophagocytosis	1/50	9/50**	13/50**	6/49*
Eosinophilic focus	14/50	17/50	24/50*	20/49*
Focal necrosis	2/50	3/50	3/50	8/49*
Spleen				
Hematopoietic cell proliferation	12/50	14/50	12/49	30/42
Urinary Bladder				
Intracytoplasmic inclusion body[d]	0/50	46/49**	46/49**	42/45**
Thyroid Gland				
Follicular cell hyperplasia	7/50	10/50	15/49	21/46**

[a]NTP (2005b)
[b]Calculated from the weekly body-weight tables
[c]Number of animals with lesions/number of animals examined
[d]Statistical analysis was not reported in the study report. Fischer's exact test was conducted on the data.

*$p < 0.05$ (Fischer's exact test)
**$p < 0.01$

Table B.14. Incidence of Selected Neoplastic Lesions in Male B6C3F$_1$ Mice Exposed to Oral (in Feed) 9,10-Anthraquinone for 2 Years[a]

Endpoint	Human Equivalent Dose (mg/kg-day)[b]			
	0	13.7	40.3	125.3
Liver				
Hepatocellular adenoma	21/50[c]	32/50*	38/50**	41/50**
Hepatocellular carcinoma	8/50	13/50	17/50*	21/50**
Hepatoblastoma	1/50	6/50	11/50**	37/49**
Hepatocellular adenoma, carcinoma, or hepatoblastoma	26/50	35/50*	43/50**	48/49**
Thyroid Gland				
Follicular cell adenoma	0/50	0/50	2/49	2/46
Total				
Animals with malignant neoplasms	18/50	28/50*	33/50**	45/50**

[a]NTP (2005b)
[b]Human Equivalent Dose = Average daily dose × body-weight adjustment, e.g.,
(825mg/kg-day) × (0.0373 kg ÷ 70 kg)$^{(0.25)}$
[c]Number of animals with lesions/number of animals examined

*$p < 0.05$ (Fischer's exact test)
**$p < 0.01$

Table B.15. Incidence of Selected Neoplastic Lesions in Female B6C3F$_1$ Mice Exposed to Oral (in Feed) 9,10-Anthraquinone for 2 Years[a]

Endpoint	Human Equivalent Dose (mg/kg-day)[b]			
	0	12.0	35.2	111.6
Liver				
Hepatocellular adenoma	6/49[c]	28/50**	27/50**	40/49**
Hepatocellular carcinoma	2/49	3/50	8/50	8/49*
Hepatocellular adenoma or carcinoma	6/49	30/50**	30/50**	41/49**
Thyroid Gland				
Follicular cell adenoma	1/45	1/48	2/48	2/48
Follicular cell carcinoma	0/45	0/48	0/48	2/48
Follicular cell adenoma or carcinoma	1/45	1/48	2/48	4/48

[a]NTP (2005b)
[b]Human Equivalent Dose = Average daily dose × body-weight adjustment, e.g.,
(745 mg/kg-day) × (0.0353 kg ÷ 70 kg)$^{(0.25)}$
[c]Number of animals with lesions/number of animals examined

*$p < 0.05$ (Fischer's exact test)
**$p < 0.01$

APPENDIX C. BMDS OUTPUTS

Table C.1. BMD Values for Cancer Data[a] from Dichotomous Multistage-Cancer Model for 9,10-Anthraquinone for Derivation of the p-OSF

Tumor Type	Species/ Sex	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)	Goodness-of-Fit *p*-Value[b]	Conclusions
Hepatocellular adenoma, carcinoma, or hepatoblastoma	Mouse/M	3.8	2.61	0.8591	Selected as POD for p-OSF
Hepatocellular adenoma or carcinoma	Mouse/F	6.0	4.5	0.0005	Failed to meet goodness of fit
All malignant neoplasms	Mouse/M	7.0	5.1	0.6492	Maximum order beta = 0
Hepatocellular adenoma	Mouse/M	10.3	6.8	0.0835	Failed to meet goodness of fit
Renal tubule adenoma or carcinoma	Rat/F	13.0	8.8	0.094	Failed to meet goodness of fit
Hepatoblastoma	Mouse/M	18.1	11.2	0.5119	
Hepatocellular carcinoma	Mouse/M	37.5	21.0	0.4782	

[a]NTP (2005b)
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria

BMD = benchmark dose; BMDL lower confidence limit (95%) on the BMD; BMD_{10} and $BMDL_{10}$ = BMD and BMDL for a BMR of 10% extra risk

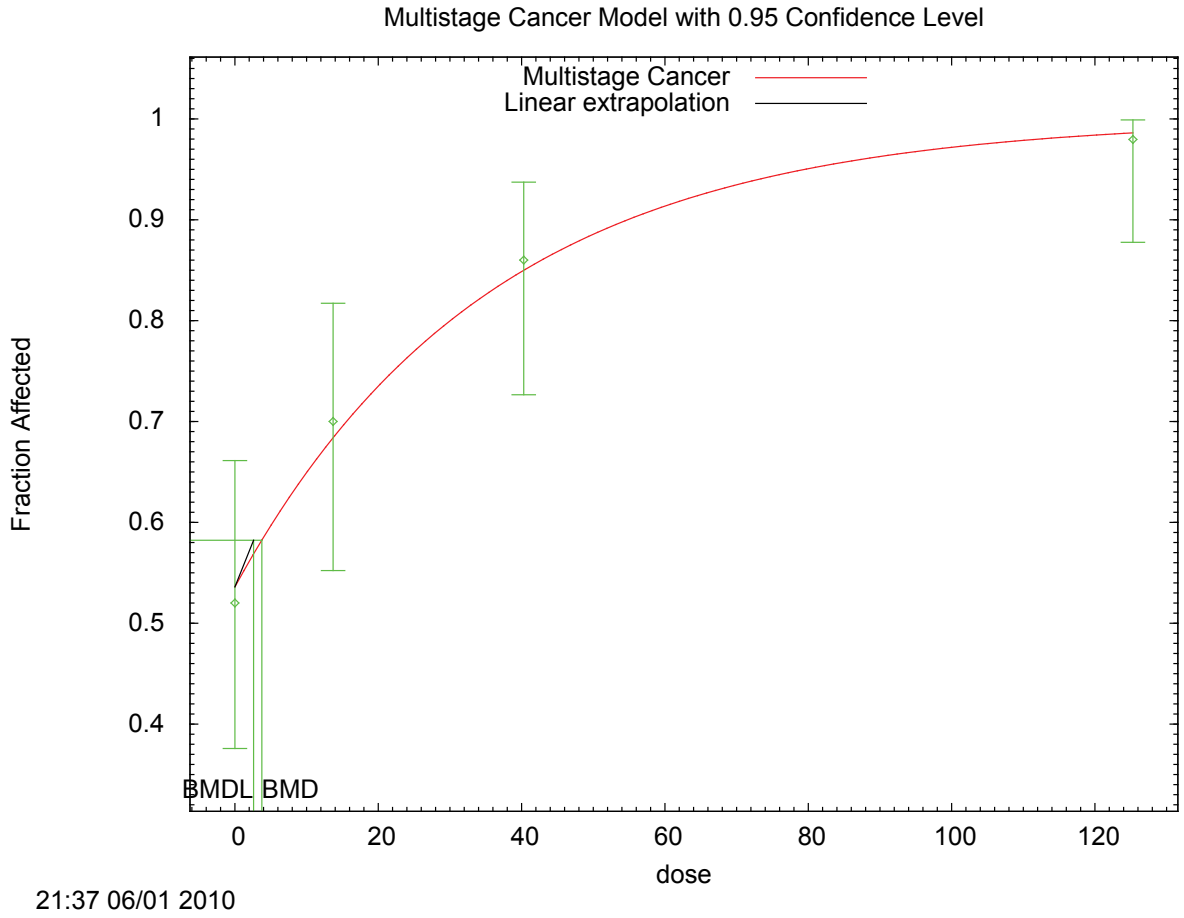

Figure C.1. Dichotomous Multistage-Cancer BMD Model for Hepatocellular Adenoma, Carcinoma, or Hepatoblastoma in Male Mouse Data (NTP, 2005b)

Text Output for Dichotomous Multistage-Cancer BMD Model for Hepatocellular Adenoma, Carcinoma, or Hepatoblastoma in Male Mouse Data (NTP, 2005b)

```
 ======================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\27\NTP_2005_2yr_adcarchepato_mice_m_MultiCanc_1.(d)
        Gnuplot Plotting File: C:\27\NTP_2005_2yr_adcarchepato_mice_m_MultiCanc_1.plt
                                            Tue Jun 01 21:37:25 2010
 ======================================================================

 Hepatocellular adenoma, carcinoma, or hepatoblastoma in Male B6C3F1 Mice at 2 yr
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive
```

```
   Dependent variable = DichPerc
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =     0.569271
                        Beta(1) =    0.0246892
                        Beta(2) =            0
                        Beta(3) =            0


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Beta(2)    -Beta(3)
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

             Background      Beta(1)

Background            1        -0.56

   Beta(1)        -0.56            1


                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background         0.535757           *                *                  *
        Beta(1)         0.028002           *                *                  *
        Beta(2)                0           *                *                  *
        Beta(3)                0           *                *                  *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -90.2903         4
   Fitted model        -90.4333         2      0.286058      2          0.8667
  Reduced model        -108.781         1       36.9812      3         <.0001

           AIC:         184.867
```

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.5358	26.788	26.000	50	-0.223
13.7000	0.6837	34.184	35.000	50	0.248
40.3000	0.8498	42.490	43.000	50	0.202
125.3000	0.9861	48.319	48.000	49	-0.389

Chi^2 = 0.30 d.f. = 2 P-value = 0.8591

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 3.76261

BMDL = 2.60971

BMDU = 8.06013

Taken together, (2.60971, 8.06013) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0383185

APPENDIX D. REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). (2010) Threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati, OH. As cited in HSDB (Hazardous Substances Data Bank) (2010). 625688

Anderson, D; Styles, JA. (1978) An evaluation of 6 short-term tests for detecting organic chemical carcinogens: appendix II - the bacterial mutation test. *Br J Cancer* 37(6):924−930. 594532

ATSDR (Agency for Toxic Substances and Disease Registry). (2008) Toxicological profile information sheet. U.S. Department of Health and Human Services, Public Health Service. Available online at http://www.atsdr.cdc.gov/toxprofiles/index.asp. Accessed on 4/8/2010. 595415

Barbone, F; Delzell, E; Austin, H; et al. (1992) A case-control study of lung cancer at a dye and resin manufacturing plant. *Am J Ind Med* 22(6):835−849. 625372

Brown, JP. (1980) A review of the genetic effects of naturally occurring flavonoids, anthraquinones and related compounds. *Mutat Res* 75(3):243−277. 625393

Brown, JP; Brown, RJ. (1976) Mutagenesis by 9,10-anthraquinone derivatives and related compounds in Salmonella typhimurium. *Mutat Re*s 40(3):203−224. 625392

Butterworth, BE; Mathre, OB; Ballinger, K. (2001) The preparation of anthraquinone used in the National Toxicology Program cancer bioassay was contaminated with the mutagen 9-nitroanthracene. *Mutagenesis* 16(2):169−177. 625510

Butterworth, BE; Mathre, OB; Ballinger, KE; et al. (2004) Contamination is a frequent confounding factor in toxicology studies with anthraquinone and related compounds. *Int J Toxicol* 23(5):335−344. 625383

CalEPA (California Environmental Protection Agency). (2007) Chemical listed effective September 28, 2007 as known to the state of California to cause cancer: Anthraquinone (CAS No. 84-65-1) [09/28/07]. Office of Environmental Health Hazard Assessment (OEHHA), Sacramento, California. Available online at http://oehha.ca.gov/Prop65/prop65_list/092807list.html. Accessed on 4/8/2010. 625842

CalEPA (California Environmental Protection Agency). (2008a) All OEHHA acute, 8-hour and chronic reference exposure levels (chRELs) as on December 18, 2008. Air Toxicology and Epidemiology, Office of Environmental Health Hazard Assessment (OEHHA). Available online at http://www.oehha.ca.gov/air/allrels.html. Accessed on 4/8/2010. 595416

CalEPA (California Environmental Protection Agency). (2008b) Hot spots unit risk and cancer potency values. Office of Environmental Health Hazard Assessment (OEHHA). Available online at http://www.oehha.ca.gov/air/hot_spots/pdf/TSDlookup2002.pdf. Accessed on 4/8/2010. 595417

Doi, AM; Irwin, RD; Bucher, JR. (2005) Influence of functional group substitutions on the carcinogenicity of anthraquinone in rats and mice: analysis of long-term bioassays by the National Cancer Institute and the National Toxicology Program. *J Toxicol Environ Health B Crit Rev* 8(2):109−126. 625361

HSDB (Hazardous Substances Data Bank). (2010) National Library of Medicine, National Institutes of Health, U.S. Department of Health and Human and Human Services, Bethesda, MD. Available online at http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB Accessed on 4/8/2010.

Gardiner, JS; Walker, SA; Maclean, AJ. (1982) A retrospective mortality study of substituted anthraquinone dyestuffs workers. *Br J Ind Med* 39(4):355−360. 625353

IARC (International Agency for Research on Cancer). (2010) IARC monographs on the evaluation of carcinogenic risks to humans. Available online at http://monographs.iarc.fr/index.php. Accessed on 4/10/2010. 597416

ICI Americas. (1985) Indirect food additive petition for anthraquinone (Vol. I & II) with attachments and cover letter dated 020185. ICI Americas, Wilmington, DE. NTIS No. OTS0521344; New Doc. ID 40-8580031. 624919

Innes, JRM; Ulland, BM; Valerio, MG; et al. (1969) Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: a preliminary note. *J Natl Cancer Inst* 42(6):1101−1114. 062632

Kerckaert, GA; Brauninger, R; LeBoeuf, RA; et al. (1996) Use of the Syrian hamster embryo cell transformation assay for carcinogenicity prediction of chemicals currently being tested by the National Toxicology Program in rodent bioassays. *Environ Health Perspect* 104(Suppl 5):1075−1084. 042055

Krivobok, S; Seigle-Murandi, F; Steiman, R; et al. (1992) Mutagenicity of substituted anthraquinones in the Ames/Salmonella microsome system. *Mutat Res* 279:1−8. 625123

Liberman, DF; Fink, RC; Schaefer, FL; et al. (1982) Mutagenicity of anthraquinone and hydroxylated anthraquinones in the Ames/Salmonella microsome system. *Appl Environ Microbiol* 43(6):1354−1359. 625115

NIOSH (National Institute for Occupational Safety and Health). (2005) NIOSH pocket guide to chemical hazards. Index of Chemical Abstracts Service Registry Numbers (CAS No.). Atlanta, Ga: Center for Disease Control and Prevention, U.S. Department of Health, Education and Welfare. Available online at http://www.cdc.gov/niosh/npg/npgdcas.html. Accessed on 4/8/2010. 070020

NTP (National Toxicology Program). (2005a) 11th Report on Carcinogens. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC. Available online at http://ntp-server.niehs.nih.gov/index.cfm?objectid=32BA9724-F1F6-975E-7FCE50709CB4C932. Accessed on 4/8/2010. 091126

NTP (National Toxicology Program). (2005b) NTP technical report on the toxicology and carcinogenesis studies of anthraquinone (CAS no. 84-65-1) in F344/N rats and B6C3F1 mice (feed studies). NTP TR 494; NIH Publication No. 05-3953. U.S. Department of Health and Human Services, Public Health Service, Research Triangle Park, NC. Available online at http://ntp.niehs.nih.gov/files/TR494web1.pdf. Accessed on 4/8/2010. 625586

OSHA (Occupational Safety and Health Administration). (2010) Air contaminants: occupational safety and health standards for shipyard employment, subpart Z, toxic and hazardous substances. U.S. Department of Labor, Washington, DC. OSHA Standard 1915.1000. Available online at http://www.osha.gov/pls/oshaweb/owadisp.show_ document?p_table=STANDARDS&p_id=10286. Accessed on 4/8/2010. 625691

Sakai, M; Yoshida, D; Mizusaki, S. (1985) Mutagenicity of polycyclic aromatic hydrocarbons and quinones on Salmonella typhimurium TA97. *Mutat Res* 156(1−2):61−67. 625011

Sathiakumar, N; Delzell, E. (2000) An updated mortality study of workers at a dye and resin manufacturing plant. *J Occup Environ Med* 42(7):762−771. 625513

Tikkanen, L; Matsushima, T; Natori, S. (1983) Mutagenicity of anthraquinones in the Salmonella preincubation test. *Mutat Res* 116(3−):297−304. 625013

U.S. EPA (Environmental Protection Agency). (1988) Recommendations for and documentation of biological values for use in risk assessment. Environmental Criteria and Assessment Office, Cincinnati, OH. EPA/600/6-87/008. Available online at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=34855. Accessed on 4/26/2010. 064560

U.S. EPA (Environmental Protection Agency). (1993) Chemical assessments and related activities (CARA). Office of Health and Environmental Assessment, Washington, DC. OHEA-I-127. Available online at nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=90140400.txt 625693

U.S. EPA (Environmental Protection Agency). (1994a) Technical support document 9,10-anthraquinone (draft final),Washington, DC. New Doc. ID 408580018; NTIS No. OTS0521336. 624913

U.S. EPA (Environmental Protection Agency). (1994b) Chemical assessments and related activities (CARA). Office of Health and Environmental Assessment, Washington, DC. EPA/600/R-94/904. Available online at http:/.nepis.epa.gov/Exe/ZyPURL.cgi?Dockey= 60001G8L.txt. 596444

U.S. EPA (Environmental Protection Agency). (1997) Exposure factors handbook (final report). National Center for Environmental Assessment, Washington, DC; EPA/600/P-95/002F a-c. Available online at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=12464. Accessed on 5/14/2010. 594981

U.S. EPA (Environmental Protection Agency). (2010) Health effects assessment summary tables (HEAST). Prepared by the Office of Research and Development, National Center for Environmental Assessment, Cincinnati OH for the Office of Emergency and Remedial Response, Washington, DC. Available online at http://epa-heast.ornl.gov/. Accessed on 4/8/2010. 595422

U.S. EPA (Environmental Protection Agency). (2005) Guidelines for carcinogen risk assessment. Risk Assessment Forum, Washington, D.C.; EPA/630/P-03/001F. Federal Register 70(66):17765–17817. Available online at http://www.epa.gov/raf/publications/pdfs/CANCER_GUIDELINES_FINAL_3-25-05.PDF. Accessed on 4/8/2010. 086237

U.S. EPA (Environmental Protection Agency). (2006) 2006 Edition of the drinking water standards and health advisories. Office of Water, Washington, DC; EPA 822/R-06/013. Available online at http://www.epa.gov/waterscience/criteria/drinking/dwstandards.pdf. Accessed on 4/8/2010. 091193

U.S. EPA (Environmental Protection Agency). (2009) Benchmark dose software (BMDS) version 2.1.1 [build: 11/06/2009]. Available online at http://www.epa.gov/NCEA/bmds. Accessed on 12/19/2009. 200772

U.S. EPA (Environmental Protection Agency). (2010) Integrated risk information system (IRIS). Available online at http://www.epa.gov/iris/. Accessed on 4/8/2010. 595423

Volodchenko,VA; Gudz, ZA; Tumchenko, AN. (1971) Fixing the maximum permissible concentration of anthraquinone in the atmosphere of the working area. *Gig Tr Prof Zabol* 15:58−59. As provided in ICI Americas (1985).

WHO (World Health Organization). (2010) Online catalogs for the Environmental Health Criteria Series. Available online at http://www.who.int/ipcs/publications/ehc/en/. Accessed on 4/8/2010. 595424

Zeiger, E; Anderson, B; Haworth, S; et al. (1988) Salmonella mutagenicity tests IV Results from the testing of 300 chemicals. *Environ Mol Mutagen* 11(Suppl 12):1−157. 024516